

Sex Differences and Sex Steroids in Lung Health and Disease

Elizabeth A. Townsend, Virginia M. Miller, and Y. S. Prakash

Departments of Physiology and Biomedical Engineering (E.A.T., V.M.M., Y.S.P.), Surgery (V.M.M.), and Anesthesiology (Y.S.P.), College of Medicine, Mayo Clinic, Rochester, Minnesota 55905

Sex differences in the biology of different organ systems and the influence of sex hormones in modulating health and disease are increasingly relevant in clinical and research areas. Although work has focused on sex differences and sex hormones in cardiovascular, musculoskeletal, and neuronal systems, there is now increasing clinical evidence for sex differences in incidence, morbidity, and mortality of lung diseases including allergic diseases (such as asthma), chronic obstructive pulmonary disease, pulmonary fibrosis, lung cancer, as well as pulmonary hypertension. Whether such differences are inherent and/or whether sex steroids play a role in modulating these differences is currently under investigation. The purpose of this review is to define sex differences in lung structure/function under normal and specific disease states, with exploration of whether and how sex hormone signaling mechanisms may explain these clinical observations. Focusing on adult age groups, the review addresses the following: 1) inherent sex differences in lung anatomy and physiology; 2) the importance of certain time points in life such as puberty, pregnancy, menopause, and aging; 3) expression and signaling of sex steroid receptors under normal vs. disease states; 4) potential interplay between different sex steroids; 5) the question of whether sex steroids are beneficial or detrimental to the lung; and 6) the potential use of sex steroid signaling as biomarkers and therapeutic avenues in lung diseases. The importance of focusing on sex differences and sex steroids in the lung lies in the increasing incidence of lung diseases in women and the need to address lung diseases across the life span. (*Endocrine Reviews* 33: 1–47, 2012)

- I. Introduction
- II. Sex Differences in Lung Structure and Function
 - A. Measurement of lung structure and function
 - B. Historical studies
 - C. Sex differences in prenatal and early postnatal lung
 - D. Sex differences in puberty and beyond
- III. Sex Differences in Lung Diseases
 - A. Asthma
 - B. Atopy and allergic rhinitis
 - C. COPD and lung cancer
 - D. Fibrotic diseases
 - E. Pulmonary hypertension
 - F. Other conditions
- IV. Sex Steroids in Lung Physiology and Pathophysiology
 - A. Sex steroid signaling
 - B. Upper and lower airways
 - C. Lung parenchyma
 - D. Pulmonary vasculature
 - E. Immune cells and function
- V. Clinical Implications of Sex Differences and Sex Steroid Signaling
- VI. Future Directions
- VII. Conclusions

I. Introduction

Sex differences in health and disease as clinical and research issues have long been topics of interest, especially in cardiovascular structure and function (1–5), metabolism (6–9), and cognition (10–15). Despite such recognition, in 2001, the Institute of Medicine (IOM) published an enlightening report (“Exploring Biological Contributions to Human Health—Does Sex Matter?”; <http://www.nap.edu/openbook.php?isbn=0309072816>), which highlighted the importance of sex as a biological variable (rather than an observational feature) with recommendations for including sex as a factor in clinical practice norms as well as a topic of bench and clinical research.

Abbreviations: ACh, Acetylcholine; AHR, airway hyperresponsiveness; AR, androgen receptor; ASM, airway smooth muscle; BEC, bronchial epithelial cell; BPD, bronchopulmonary dysplasia; $[Ca^{2+}]_i$, intracellular calcium; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; DC, dendritic cell; DHEA, dehydroepiandrosterone; DHT, 5 α -dihydrotestosterone; DLCO, diffusing capacity for carbon monoxide; EGF, epidermal growth factor; EGFR, EGF receptor; eNO, exhaled NO; eNOS, endothelial NOS; ER, estrogen receptor; FEF, forced expiratory flow; FEV1, forced expiratory volume in 1 sec; FRC, functional residual capacity; FVC, forced VC; GPCR, G protein-coupled receptor; IFN, interferon; iNOS, inducible NOS; LPS, lipopolysaccharide; 2ME, 2-methoxy estradiol; MHT, menopausal hormone therapy; MMP, matrix metalloproteinase; NK, natural killer; NO, nitric oxide; NOS, NO synthase; NSCLC, non-small cell lung cancer; OC, oral contraceptives; PAH, pulmonary arterial hypertension; PBMC, peripheral blood mononuclear cell; Penh, enhanced pause; PF, pulmonary fibrosis; PH, pulmonary hypertension; PR, progesterone receptor; RDS, respiratory distress syndrome; Th1, T helper 1; Th2, T helper 2; TLC, total lung capacity; VC, vital capacity.

ISSN Print 0021-972X ISSN Online 1945-7197

Printed in U.S.A.

Copyright © 2012 by The Endocrine Society

doi: 10.1210/er.2010-0031 Received December 20, 2010. Accepted August 15, 2011.

First Published Online January 12, 2012

These recommendations are reiterated in an article in the journal *Nature* (497). Although the IOM report focused on sex differences in several organ systems (other than the reproductive system), there was surprisingly little attention paid to the respiratory system, *i.e.*, the upper and lower conducting airways and the lung parenchyma.

Inherent sex differences in the lung are apparent from early in life and throughout the human life span. Both clinical and basic research studies have examined sex differences in lung structure and function in both health and disease. Although some studies have focused on the likely role of hormones in sex differences, others have explored inherent physiological differences as well as sociocultural factors. There is also considerable epidemiological evidence for a role for sex in the incidence, susceptibility and severity of a variety of lung diseases. Here, the healthcare and financial burden of lung disease is certainly not trivial (~35 million persons with chronic lung disease and 350,000 deaths in the United States alone in 2006; American Lung Association 2006 lung disease data), and it highlights the importance of recognizing and studying sex differences in lung anatomy and physiology. As an example, Fig. 1 compares the average number of publications per year that focus on “sex” and “heart” (or other terms

relating to the cardiovascular system) to “sex” and “lung” (or other terms relating to the respiratory system). Although research regarding influence of sex on lung diseases is not as well studied as in the cardiovascular realm, there appears to be a steady increase from year to year. Accordingly, the major goal of this review is to highlight this growth in research relating to sex and the lung. We will largely address research on the adult lung, where most of the past work has focused, with brief backgrounds on prenatal, early postnatal, and prepubertal sex differences in lung anatomy/physiology and the influence of sex steroids. The reader will be referred to excellent and detailed reviews by other investigators on these topics.

This review briefly introduces modern techniques and parameters for clinical and bench-research assessment of lung structure and function (defining common terms and abbreviations; *Section II*). With a brief historical perspective, we will describe what is currently known regarding inherent lung structure and function in the two sexes (focusing largely on the postnatal time period), and highlighting key life events involving large hormonal changes such as puberty, pregnancy, and menopause. Subsequently, clinical data on sex differences in specific lung diseases will be summarized (*Section III*), setting the stage to address the roles of inherent sex differences, as well as sex steroids in lung health and disease. We have focused on diseases where sex differences have been recognized, with the understanding that absence of sex difference does not necessarily rule out a role for sex steroids influencing a particular disease. However, considering the limited data, we felt it important to at least highlight diseases where sex steroid signaling could be implicated. Furthermore, these diseases span different cellular and extracellular components of the lung, and thus provide a rationale for dividing the subsequent section (*Section IV*) along these lines. Here, we discuss controversies not only in the epidemiology, but also in the potential role of sex steroids as being beneficial *vs.* detrimental. Nonetheless, *Sections II and III* will establish the importance of researching the lung from a sex-specific perspective. *Section IV* will then systematically examine what is known regarding expression and signaling of steroid receptors in lung components, introducing *in vitro* cellular studies, animal work, as well as examination of human samples. The impact of sex differences and steroid signaling on specific lung components in the disease process will be discussed with the understanding that many lung diseases involve multiple cell types. Finally, we will provide commentaries on the importance of sex differences and sex steroids in lung health and disease and identify areas where more basic and clinical research is needed (*Section V*). Given the increasing recognition of the importance of sex steroids in the lung, it is only a matter

Figure 1.

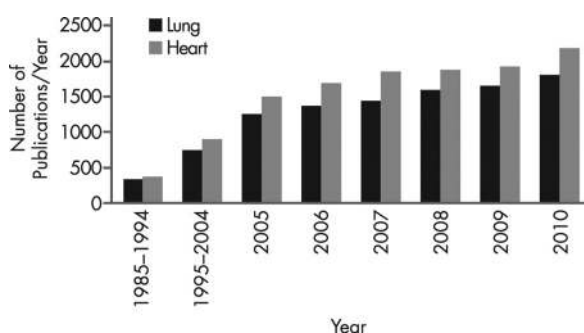


Figure 1. Publications relating to sex and the lung. A PubMed search was conducted to compare the number of articles published per year related to sex differences in the lung *vs.* heart. Search terms (title, abstract, and keywords) used were “sex” or “gender” along with “heart, cardiac, vascular (not pulmonary)” to define the broad term “heart” represented in the figure. The search was repeated with “sex” or “gender” and search terms “respiratory, lung, airway, and pulmonary,” which comprise the term “lung.” Searches were conducted annually from 2005 to 2010 (for recent trends), and decade searches 1985–1994 and 1995–2004 (with average numbers of articles per year presented). Over the last two decades, there has been a substantial increase in the number of publications related to sex and lung, with tremendous increases in recent years. However, these numbers remain substantially smaller than those involving sex and heart. Nonetheless, the encouraging upward trend in studies illustrates the recognition that visibility of and research on sex differences in the lung needs to be enhanced. Within this subset is the additional recognition of the role of sex steroids in lung physiology and pathophysiology.

of time before modulation of their signaling is considered a viable therapeutic option. Furthermore, if sex differences in specific diseases are related to expression or function of steroid signaling pathways, then perhaps these pathways could serve as markers of disease risk, severity, and/or outcome. These issues will be briefly discussed (*Section VI*).

II. Sex Differences in Lung Structure and Function

A. Measurement of lung structure and function

Evaluation of lung structure and function is an important aspect of pulmonary research and medicine. Clinically and in the human-based laboratory setting, several methods are commonly used: spirometry, measurement of lung volumes, and quantification of diffusing capacity. Spirometry is the most common pulmonary function test for measurement of volume and air flow rate during inhalation and exhalation. Pneumotachographs generated via spirometry are used to assess clinical conditions such as obstructive pulmonary diseases [*e.g.*, asthma, chronic obstructive pulmonary disease (COPD), and restrictive diseases, *e.g.*, pulmonary fibrosis (PF), respiratory muscle weakness]. Two important measurements in spirometry are forced expiratory volume in 1 sec (FEV1) and forced vital capacity (FVC). The ratio of FEV1 to FVC is a useful parameter for distinguishing obstructive *vs.* restrictive lung diseases. In general, decreased FEV1/FVC suggests an obstructive condition, whereas a normal or even increased ratio with substantial reduction in FVC is seen as a restrictive problem (16–18). Flow-volume loops, which include forced inspiratory and expiratory maneuvers, are useful in determining static and dynamic obstructions to airflow. Here, larger, less compliant airways with rigid structures such as cartilage contribute significantly to static resistance to airflow, whereas smaller more compliant airways within the lung can dynamically vary in resistance. Other parameters such as forced expiratory flow (FEF) are calculated using flow-volume loops, and these additional parameters are used to differentiate between effort-dependent and effort-independent expiratory airflow. Contractility or reactivity of the airways is tested standardly using the methacholine challenge wherein increasing concentrations of this bronchoconstrictor agonist are provided by nebulization, and changes in resistance to positive pressure inspiratory and expiratory airflow are measured. Concurrently, bronchodilator testing is useful in determining the reversibility of airway obstruction in diseases such as asthma or COPD. Measurement of maximal inspiratory and expiratory pressures

is performed to determine causes of decreased vital capacity (VC) or muscle strength. Measurement of lung volumes complements spirometry. Common measurements include total lung capacity (TLC), functional residual capacity (FRC), and residual volume, which are used to distinguish between disease types. Measurement of diffusing capacity for carbon monoxide (DLCO) assesses gas exchange. Considering the long-standing testing and use of these measurement techniques and parameters, scales for males and females of different age groups are available, allowing for comparisons between studies and equally effective detection of disease conditions in both sexes.

Within the bench laboratory setting, many of the tests and parameters used in humans can also be applied to a range of test animals. For example, a commonly used technique is the noninvasive unrestrained (or restrained) whole body plethysmography where animals are enclosed in rigid chambers and their breathing patterns, tidal volumes, and other parameters are measured using pneumotachometers. A useful derived parameter commonly used to report resistance to inspiratory/expiratory airflow is enhanced pause (Penh) (19, 20), with the caveat that there is disagreement regarding the physiological variables that are represented by Penh (some investigators believe that Penh may reflect sensory nervous activity or airway irritability rather than actual changes in airway resistance). A second, and complementary but invasive technique to measure lung function is by direct measurement of resistance and compliance using an endotracheal tube or tracheostomy in an anesthetized (sometimes paralyzed) animal. Both the noninvasive and invasive approaches are amenable to the methacholine challenge test with bronchodilator response. In small animal studies, a common procedure after invasive or noninvasive measurement of airway mechanics is the collection of bronchoalveolar cells and fluid using lavage, which can then be analyzed for relative amounts of immune cells (neutrophils *vs.* macrophages *vs.* eosinophils) or cytokines and other inflammation mediators.

At the tissue and cell levels, gross structures can be evaluated in lungs that are inflated at standardized pressures [*e.g.*, 25 mm Hg in the mouse (21)] and fixed (*e.g.*, formalin or paraformaldehyde) or rapidly frozen. Changes in airway or pulmonary vascular structures are typically determined in sections processed via immunohistochemistry, hematoxylin-eosin staining, or other histological procedures to identify specific cell types. Here, standards have been introduced for measurement of airway epithelial *vs.* smooth muscle dimensions, and alveolar branching and septation, thus allowing comparison across studies (21). Immunostaining allows for semiquantitative evaluation of expression and localization of proteins within different parts of the lung

(22). Many studies use gel electrophoresis or PCR of whole lung samples to determine changes in protein or mRNA. However, the heterogeneity in cell type and cell-specific differences in expression of the same protein within the lung frequently limit interpretation. Other techniques such as fluorescence immunohistochemistry followed by digital imaging (*e.g.*, confocal microscopy) can be used to provide semiquantitative estimation of expression and localization of proteins within specific parts of the lung (22). A relatively new technique that may be helpful is laser capture microdissection (23, 24) wherein specific areas (*e.g.*, epithelium *vs.* smooth muscle *vs.* endothelium) or even single cells can be excised from lung sections using a laser and then analyzed for mRNA or protein (23, 24).

B. Historical studies

In the mid 19th century, a study by Hutchinson (25) identified sex differences in breathing mechanics using basic spirometry. In the few women studied, breathing maneuvers equivalent to VC measurements were attributed to rib muscle contractions in women, compared to a diaphragm-based effort in men. Rib-based breathing in women was attributed to accommodation for impaired diaphragmatic function in the pregnant woman. In contrast, Ellis (26) found that VC in women, when corrected for height, was smaller, attributing this to presumed lower metabolic rate of women and thus less need for larger breaths. In perhaps the first systematic study of hormonal effects on respiratory parameters (although not the primary goal of the study), Ott (27) performed daily measurements of VC, respiratory muscle force, and other characteristics in healthy women over several months and menstrual cycles. A complex index representing “functional energy” was found to be at approximately 50% midcycle, peaking to 75% before menstruation, and then ebbing substantially after menstrual bleeding. These early studies are described in more detail in a recent review by Becklake and Kauffmann (28).

C. Sex differences in prenatal and early postnatal lung

The lung is one of the few organs that continue to develop after birth, up to 2–4 yr of age in humans (28–30), with continued growth and changes in lung complexity well into adolescence (28, 29, 31). Sex differences in lung development and maturation have been observed as early as 16–24 wk gestation (32, 33). For example, the number of bronchi is fewer in female fetuses compared with males, but female fetuses mature faster (34). Later in gestation, surfactant is produced earlier by female fetuses and is thought to help maintain patency of small airways (35, 36). Neonatal females have higher expiratory flow rates

compared with male neonates when corrected for size (37), a comparison that remains true throughout their life span (see *Section II.D*). Table 1 summarizes sex differences in lung anatomy and physiology over the course of prenatal lung development and postnatal growth into adulthood. The reader is referred to other in-depth reviews on this fascinating topic (28, 30, 38–42). In addition to inherent sex differences in the lung *in utero*, maternal and/or fetal sex steroids can also have effects. The reader is referred to excellent in-depth reviews on the complex role of steroids in the developing lung (28, 39, 43, 44).

The clinical relevance of intrinsic and steroid-induced sex differences in lung development lies in the greater susceptibility of premature male infants to respiratory distress syndrome (RDS; *Section III.F*) (36, 45), presumably due to decreased surfactant production and larger lung size compared with the premature female infant. Premature male infants are also more susceptible to bronchopulmonary dysplasia (BPD; see *Section III.F*). Furthermore, early establishment of differences in lung structure and function may underlie or influence the course of diseases such as asthma in childhood and in adults (see *Section III.A*).

Early postnatal lung development and maturity occurs predominantly via exponential increases in number and size of alveoli (29, 46). At birth, the female lung is smaller than that of the male, with fewer respiratory bronchioles (30, 47), but not the number of alveoli per unit area. Accordingly, the total number of alveoli and surface area are actually greater in males throughout childhood.

D. Sex differences in puberty and beyond

During childhood and adolescence, female airways and lung parenchyma grow proportionally, whereas in males airway growth lags behind the growth of the lung, thereby exhibiting disproportionately fewer alveoli for the number of airways in boys (43, 48, 49), a concept called dysanapsis (for details, see Refs. 28, 40, 47, 50, and 51). Briefly, dysanapsis suggests that airway length, not diameter, determines FEF rates. Thus, larger lungs (*e.g.*, male lungs) would have longer conducting airways and would thus be at a disadvantage during expiration [reflected by a higher dysanapsis index as determined by Mead (50)]. This concept has been confirmed by several lines of evidence based in lung morphometry, physiological measurements, and clinical evaluations (28, 40, 47, 51). The clinical and functional significance lies in methods to normalize lung function for differences in lung size, especially in the prepubertal period when growth rates for boys and girls differ substantially.

The proportional growth of the airways and lungs in females results in lower specific airway resistance and fa-

TABLE 1. Influence of sex steroids on the lung at different life stages in humans

Age/time frame	Lung anatomy and physiology	Observed sex difference	Refs.
16–26 wk gestational age (canalicular phase of lung development)	Fetal breathing movements; bronchial lumens enlarge; vascularization of lung tissue. Respiratory bronchioles and alveolar ducts develop from terminal bronchioles (wk 24).	Females exhibit mouth movements earlier than males.	32
26–36 wk gestational age (saccular phase of lung development)	Establishment of blood-air interface (respiratory epithelium with type I and II alveolar cells); surfactant production; terminal sacs form and expand; overall increase in lung volume.	Females produce surfactant earlier than males.	35, 36
36 wk (first postnatal week)	Alveoli form and multiply; increased FEF and decreased airway resistance.	Females have smaller lungs and lower specific airway resistance than males.	37, 41, 42
Up to 1 yr of age	Lung grows linearly with age and airway resistance increases rapidly; alveolar multiplication continues.	Females have higher FEF rates than males.	32, 37, 38, 42
Prepubertal childhood years (1–10 yr)	Alveolar multiplication continues (up to 2 yr); lung growth is dysanaptic.	Females have larger airways in relation to lung size and lower specific resistance than males. Female lungs are smaller overall as compared to males. Large airways grow proportionately to lung in females, this growth lags in males. Smaller airways grow faster in females as compared to males.	30, 34, 491 37, 42 48
Adolescence, puberty (10–18 yr)	Lung growth increases with age (FVC and TLC).	Female growth velocity for FVC peaks sooner than males. Specific airway resistance decreases in females up to 18 yr but not in males. Higher effort independent flows in women than men.	37, 42, 52, 492
Adults (20–70 yr)	Peak expiratory flow, FEV ₁ , and transpulmonary pressure decrease with age; decrease in lung elastic recoil, vital capacity, chest wall compliance; increased residual volume and FRC; overall increase in airway conductance with age.	Males have higher PEF than women; higher airway conductance in males than females; no observed differences in elastic recoil or chest wall compliance.	56, 57, 87, 493, 494
Pregnancy (8–36 wk)	No changes in FVC, FEV ₁ , VC, airway resistance, or lung compliance throughout term (12 wk to 4 months postpartum).		53, 54
Pregnancy (third trimester)	TLC and FRC decreased at 36 wk gestation; increase in inspiratory capacity in third trimester; increase in specific airway conductance (returns to normal 5 wk postpartum); 50% reduction in total pulmonary resistance.		53, 54

PEF, Peak expiratory flow.

cilitates larger FEF rates (37). However, when somatic growth has ceased, VC, TLC, and peak flows are larger in males compared with females of equal height, although females still have larger expiratory flow rates (28, 52). Assuming that the original finding of Hutchinson (25) (*Section II.B*) of greater diaphragmatic contribution in men is valid, the contribution of respiratory muscles could be a confounding factor. During adolescence, males generate higher respiratory pressures than females at all lung volumes, which is attributed to the influence of testosterone and the changing shape of the thorax and respiratory muscles during puberty (52). However, such effects cannot compensate for the higher specific airway resistance in men.

Sex differences in lung size of neonates and prepubertal children, normalized for height, are maintained through the greater pubertal growth spurt of girls (41, 47). However, the greater effort-independent expiratory flow rates observed in prepubertal girls are less obvious in young women where the ratio of residual volume to TLC is increased (42) such that expiratory flow rates normalized to TLC are comparable between the sexes. Age-related increases in FVC occur more in men in their early postpubertal years, partly due to the greater respiratory pressures generated by males via a male sex steroid effect (52). Nonetheless, sex differences in the dysanaptic growth of the airways *vs.* lungs are maintained such that in adult-

hood, FEV1/FVC is higher in women (52), which should place them at an advantage in terms of respiratory mechanics and airflow. The importance of these differences lies in sex differences in diseases such as asthma in the prepubertal period and changes in incidence and severity that occurs after puberty (discussed in *Section III.A*).

Although sex differences in lung function noted in the postpubertal period are maintained during adulthood, pregnancy marks a unique situation in women where anatomical changes of the uterus affect the diaphragm and thorax, while resulting physiological changes also take place. TLC and FRC both decrease in the third trimester, due in part to fetal orientation compressing the abdominal contents into the thorax (53, 54). Despite these changes, FVC, FEV1, or VC are not decreased due in part to increases in inspiratory capacity and a 50% decrease in pulmonary resistance (54). The relevance of these changes lies in the modulation of asthma symptoms and severity during pregnancy, although such anatomical and functional changes alone are not entirely contributory (see *Section III.A*).

As adults age, independent of disease pathology, there is a loss of lung function. Lung elastic recoil and elastic recoil of the large airways decreases, along with a decrease in alveolar air volume with age, whereas the relative amount of connective tissue in the lungs increases (55, 56). These anatomical changes lead to a decrease in maximal expiratory flow rate for both men and women; however, the rate of decrease in lung elastic recoil and maximal expiratory flow rates tends to be greater in men compared with women (57, 58). Additionally, women are older at the onset of these decreases in lung function. The observed sex difference in declining lung function may be a result of smaller overall airways of women having a beneficial effect on lung mechanics or a negative aftermath of dysanaptic growth in boys from the prepubertal period. Thus, throughout adulthood, the female lung should be situated to function better than the male lung. However, this simplistic expectation does not appear to hold true, as discussed in *Section III.A* with reference to diseases such as asthma.

III. Sex Differences in Lung Diseases

As discussed in *Sections II.C and II.D*, from infancy through adulthood, intrinsic sex differences in lung anatomy and physiology are present. Although such intrinsic differences could contribute to the pathophysiology of lung disease of prematurity (*e.g.*, RDS and BPD; *Section III.F*), lung diseases of children and adults are modulated by socioeconomic, cultural, environmental, and behav-

ioral factors. Regardless, clear sex differences in the presentation of diseases such as asthma are apparent throughout the human life span. Before puberty, more boys than girls are diagnosed with asthma, whereas the opposite is true after the onset of puberty during the reproductive years in females. Additionally, there is a staggering increase in the number of females that are being diagnosed with COPD, whereas the number of men being diagnosed has stabilized. Although much of this change in COPD statistics may be a result of increased or maintained numbers of female smokers (compared with a decrease in the number of male smokers), there are data suggesting that women may also be more susceptible to cigarette smoke, additionally contributing to this sex disparity in COPD as well as lung cancer. Overall, this collection of epidemiological data underscores sex differences in the lung and raises the issue of what role inherent differences in lung structure *vs.* sex hormones play in modulating lung health and disease.

In *Section III*, we will summarize the current state of clinical knowledge on sex differences in several important lung diseases (especially in adults), providing a basis for considering both intrinsic differences in lung structure and function in males *vs.* females as well as the potential contribution of sex steroids. Here, it is important to emphasize that it is not always possible to clearly separate intrinsic *vs.* sex steroid-induced differences; indeed, in the adult lung, it is likely that there is a complex and incompletely understood interaction between these two aspects. Furthermore, considering the multifactorial nature of most lung diseases and the involvement of multiple cell types, differing sex steroid effects on specific cell types in the lung make the issue even more complex. Therefore, in *Section III*, we focus on aspects of lung diseases that are likely due to intrinsic sex differences in lung structure or function, as well as aspects likely involving the overall effect of sex steroids (where known). In *Section IV*, we proceed to dissect out the potential contribution of sex steroid effects on specific lung cells contributing to the diseases of *Section III*.

A. Asthma

Asthma is an inflammatory disease of the airways that is likely multifactorial in origin and involves both intrinsic and environmental factors. Hallmark symptoms of asthma include exaggerated airway narrowing in response to endogenous bronchoconstrictors [*e.g.*, acetylcholine (ACh)] as well as environmental stimuli (*e.g.*, pollen, cigarette smoke), resulting in expiratory airflow limitation and accompanying dyspnea and wheezing (59–65). Despite substantially improved understanding of the pathogenesis of asthma, as well as development and

implantation of a large variety of drugs targeting different mechanisms, there continues to be a global increase in the prevalence, morbidity, and even mortality of asthma over the past few decades (~300 million people worldwide; World Health Organization, Asthma fact sheet no. 307), with the prevalence and severity of pediatric asthma increasing at even higher rates compared with adults or the overall population (66–68). There is now epidemiological evidence for sex differences in asthma and related diseases that may be due to biological susceptibility, age-related changes in the hormonal milieu, environmental exposures, as well as healthcare and socioeconomic factors (69, 70).

Although a number of factors can result in asthma, early events in life may be contributory. With the rates of preterm birth rising, but survival increasing, respiratory morbidity of children born prematurely is high, with poor respiratory and pulmonary function persisting through adulthood. Children with classical BPD (typically born before 1990) who may now be adults demonstrate both structural and functional lung problems (71–77). For example, preterm birth at less than 26 wk is associated with reduced expiratory flow, increased respiratory symptoms, and medication use at 6 yr of age and, as recently demonstrated, to 11 yr of age (prepubertal) (78, 79). Although the use of prenatal steroids and surfactant have reduced the incidence of RDS and classical BPD, a newer form of BPD involving alveolar and vascular dysmorphogenesis still occurs (see *Section III.F*). It appears that both populations (classical and new BPD) may be at the same risk for childhood respiratory problems including asthma. The reader is referred to several recent reviews on prenatal, perinatal, and early postnatal causes of childhood and adult asthma (71, 80–86).

Regardless of gestational age at birth, in prepubertal children, male airway growth lags behind that of female airway growth, suggesting an inherent risk factor for greater asthma in male children (43, 49, 87). During childhood, more boys than girls are diagnosed with asthma. A confounding factor may be the relative underdiagnosis and undertreatment of girls with asthma (88, 89). Although large-scale systematic studies have not been performed to determine the reasons underlying the underdiagnosis of asthmatic girls, lower activity levels compared with age-matched boys (and thus less frequent triggering of asthma exacerbations) may be contributory. Furthermore, a single study in Swiss children (90) suggested a sex-based bias in recognition of symptoms in girls by healthcare providers, unless the severity and reporting of symptoms was comparable to that of boys [colloquially referred to as Yentl syndrome in the context of coronary artery disease (91)]. Nonetheless, it is likely that asthma

symptoms are indeed greater in boys. The disease presents in infancy within the first few years of life. Male gender is a major risk factor for the development of asthma at this early age (92, 93) and the ratio of boys to girls is approximately 2:1 before the age of 5 yr (94, 95), with the risk of chronic asthma being 4-fold greater for boys until the age of 14. Here, it is important to emphasize that both male sex (biological factors) and male gender (socio-constructs relating to outdoor play and indoor pet exposure) are factors for the development of asthma. Thus, sex *vs.* gender as factors may be difficult to separate in this instance. Nonetheless, these epidemiological data are consistent with the concept of structural dysanapsis and suggest that, in the prepubertal setting of low sex steroid levels, inherent structural differences may be more important to sex differences in asthma in childhood.

Male predominance in asthma persists until the onset of puberty (ages 10–14) when the number of boys and girls with this disease is approximately equal (88, 96). Interestingly, puberty results in a switch in the sex distribution of asthma. After puberty, during the reproductive years, approximately twice as many women as men have asthma (93, 97, 98). The switch in sex ratio for asthma at the time of puberty may be partially attributable to pubertal growth patterns such that female airway and lung sizes are smaller than for males. However, size (*i.e.*, inherent structural differences) alone is not a sufficient explanation, and there is now clear evidence that sex hormones influence asthma development and severity. Girls who undergo early menarche (before the age of 12), have twice the risk of developing asthma after puberty compared with women in whom menarche occurs later (99, 100). The underlying mechanisms for this increased risk or incidence have not been examined. However, as discussed in *Section IV*, the pleiotropic effects of female sex steroids (particularly estrogens) on airway cells may be contributory.

In adult women, the cyclical variations in sex steroid levels with the menstrual cycle may also influence asthma symptoms. However, the temporal correlation between asthma symptoms and steroid levels does not provide a simple answer to whether estrogen and/or progesterone improve or worsen asthma. Approximately 40% of women with asthma will experience premenstrual asthma, *i.e.*, changes in severity at specific times during their menstrual cycle (101–103). These changes have been documented to occur in the luteal and late-luteal phase of the menstrual cycle when there are large fluctuations in the levels of progesterone and estrogen. However, it should be noted that if anything, worsening of symptoms usually occurs when estrogen levels are on the downswing, suggesting that estrogens may be normally protective for asthma symptoms. Of course,

an alternative explanation is that elevated estrogen levels in the preceding days (*i.e.*, midcycle) may manifest as worsening of symptoms after considerable delay, which would make estrogens detrimental to airway function (see *Section IV*). Regardless of the underlying mechanisms, changes in symptoms often lead to increased bronchodilator use and increased hospitalization (93, 101, 104). Some women with mild to moderate asthma, but not with severe asthma, have found relief of premenstrual asthma exacerbations with the use of oral contraceptives (OC) which suppress large fluctuations in circulating hormones (105, 106).

These effects of exogenous estrogens modulating premenstrual asthma negate a simple explanation of beneficial *vs.* detrimental effects of sex steroids on asthma, underscoring the multifactorial nature of such diseases and a potentially complex short- or long-term interplay between sex steroids and underlying pathophysiological mechanisms. Some studies suggest that cyclical variations in asthma severity are partially attributable to abnormal or absent up-regulation of β_2 -adrenoceptors in circulating lymphocytes of menstruating asthmatic women during the late luteal phase; however, increased responsiveness of lymphocytes to cAMP may compensate for the lack of receptor up-regulation in these women (107). Furthermore, exhaled nitric oxide (eNO) levels (a marker of inflammation) increase in nonasthmatic women (108) during the luteal phase, suggesting that premenstrual asthma involves increased airway inflammation (102, 109, 110). A number of other mechanisms have been proposed including altered airway contractility, progesterone effects on bronchodilation, and altered immune function. Current knowledge on these important topics is presented in greater detail in *Section IV*.

Women with asthma face another challenge of sex steroid influence with pregnancy. Asthma is the most frequent respiratory disease experienced by pregnant women [as many as 8% of all pregnant women have asthma (111, 112)]. Estrogen and progesterone concentrations rise steadily during pregnancy and reach peak levels during the third trimester (93). Despite the number of pregnant women with a diagnosis of asthma, only approximately 10% experience acute asthmatic exacerbations during pregnancy as compared with the near 40% of women experiencing premenstrual worsening (94, 113). It has been reported that approximately one third of women with asthma show an improvement of symptoms in their third trimester, whereas another third demonstrate worsening of their asthma, and a final third show no changes (114, 115). Here, women with severe asthma predominantly show worsening of asthma symptoms during pregnancy compared with women with mild to moderate asthma

(115, 116). It is interesting that in both premenstrual variations and during pregnancy, severely asthmatic women appear to have worsening of symptoms, are less responsive to bronchodilator therapy than mild to moderate asthmatic women, and do not benefit from OC in alleviating premenstrual exacerbations (106). Whether female sex steroids contribute to this recalcitrant situation, or whether severe asthma is a different entity altogether (117–119) remains to be determined. An interesting caveat here is the limited data suggesting that the sex of the fetus can influence severity of maternal asthma symptoms. Androgens have antiinflammatory properties, and thus male fetuses may protect women prone to asthma exacerbations compared with female fetuses (93, 112). However, other studies have found that the sex of the fetus has no effect on maternal asthma severity (120). Separately, progesterone is known to be a respiratory stimulant and may have bronchodilatory properties (121, 122) (see *Section IV*). However, the lack of consistent alleviation in asthma symptoms across pregnant women with preexisting asthma suggests the need to explore other mechanisms that could be contributory.

After the shift toward increased incidence and severity of asthma in women after puberty, the next major change occurs at menopause. Menopause is generally characterized by a hormonal shift consisting of high levels of FSH and LH and low levels of estrogen and progesterone (123, 124). The sex ratio for asthma development decreases around the age of menopause and approaches unity, indicating no sex difference in asthma presentation in this age range. Studies have shown that the peak in adult asthma exacerbations in women with preexisting disease occurs at approximately 50 yr of age, the median for onset of menopause (125, 126). In women with preexisting asthma, menopause generally leads to more frequent exacerbations (suggesting that in premenopausal years sex steroids may have been protective); however, there is a slight decrease in the number of reported asthma-related hospital admissions in women more than 50 yr old as compared with women 20–50 yr of age (124). Furthermore, postmenopausal women have a decreased risk of developing *de novo* asthma compared with age-matched, premenopausal women (127). Although these data would again suggest a role for sex steroids on changes in asthma exacerbations and severity at the time of menopause, as with pregnancy, a simple deleterious *vs.* protective role for female sex steroids cannot be easily assigned.

Occurrence of asthma after menopause is generally very severe in nature and requires aggressive therapy including high doses of oral corticosteroids (128). Postmenopausal asthma also generally presents without a family history and without atopy, although chronic sinusitis is

often observed in conjunction with exacerbations (129). In postmenopausal women with asthma, low serum levels of FSH and LH with high levels of 17β -estradiol were detected, compared with nonasthmatic postmenopausal women (130) as well as age-matched women with preexisting disease (131).

Menopausal hormone therapy (MHT) in women [used by up to 60% of postmenopausal women (132)] has confounding effects on asthma development and airway disease (133). In general, use of MHT, specifically estrogen, increases the risk of developing asthma, as well as increasing asthma symptoms, in a dose-dependent manner (127, 134). One of the largest studies, the U.S. Nurses Health Study (1995–2004), prospectively examined MHT use and postmenopausal asthma onset. In this study, estrogen and estrogen-progesterone combination posed similar risks to the development of asthma, but not COPD (135). A similar, more recent study, although corroborative for the estrogen MHT effects, found no increased risk for new asthma onset in the combined estrogen-progesterone group (134). Taken together, these data suggest that increasing levels of estrogen, either endogenously or exogenously, can increase asthma symptoms and the risk of asthma onset.

The above discussion largely focused on age-related changes in asthma in women. However, it is important to recognize that incidence/severity of asthma in men can also change, albeit not to the same extent. Indeed, in men, severity of asthma is relatively stable from puberty until later in life (*e.g.*, >50 yr) when decreasing serum testosterone levels may contribute to an increase in asthma (136, 137). Taken in combination with the reversal in male:female ratio for asthma at puberty, these data would suggest that testosterone may be beneficial in asthma. On the other hand, a recent study found no correlation between hypogonadism in aged men and asthma (138). Although much research on asthma has focused on the effects of female sex steroids, little has been reported on the effects of exogenous androgens in alleviating asthmatic symptoms. In a small study, testosterone (10 mg/d for 5 d) was found to improve symptoms in 88% of women suffering from premenstrual asthma (139). Female patients suffering from status asthmaticus found rapid, acute relief (within 20 min) with a combined testosterone-gonadotropin injection (12.5 mg, 500 IU, respectively) (139). Although these limited data are from relatively old studies, they do suggest that increased androgen levels may be beneficial in both males and females. Further work is needed to determine the underlying mechanisms and the potential for androgen therapy as an acute alternative therapeutic option in cases of severe asthma. In *Section IV*, we discuss current understanding of androgen effects on

different cellular components of the lung that may contribute to diseases such as asthma.

B. Atopy and allergic rhinitis

Atopy is defined as the tendency to exhibit an adverse IgE response to specific allergens or to show increased responsiveness, measured in wheal size, to skin prick tests (140) (with the caveat that skin reactivity does not always correlate with elevated IgE levels). The relevance of atopy lies in the evidence that skin prick tests correlate with allergic rhinitis, whereas elevated IgE levels are a predictor for allergic asthma (141, 142). Furthermore, allergic sensitization to food can be an underrecognized risk factor for asthma (143).

In general, atopy is more prevalent in prepubertal boys compared with girls, as are asthma and allergic rhinitis (140, 144). This sex difference may be due in part to differences in environmental exposure between girls and boys, reflecting outdoor and recreational activity patterns (145). However, even after puberty, when there is a sex reversal in the prevalence of asthma (*Section III.A*), men continue to exhibit a similar, if not greater, prevalence of atopy compared with women (146, 147). In contrast, some reports suggest that more women present with atopy after puberty when compared with men (148). This discrepancy in the prevalence of atopy may be attributable to differences in population selection criteria between studies, or to menstrual status of women included in the later study. There is a notable decrease in atopy with age for both males and females, but this is most pronounced in women with preexisting disease after the onset of menopause (149).

At birth, both boys and girls have relatively similar levels of cord blood IgE. However, serum IgE levels rise more rapidly in boys at an early age, contributing to the increased incidence of prepubescent atopy (150). Conversely, women show a sharp decline in serum IgE levels during puberty (141). Males continue to have higher IgE levels than women for every decade (141), although these levels decline steadily in both men and women with age (151). Although males and females with allergic asthma have higher IgE levels than nonasthmatics, male asthmatics have consistently higher IgE levels compared with females (149, 150). Interestingly, women who use OC have significantly higher IgE levels than nonusers, albeit significantly less than those detected in males (149). Women in the periovulatory phase (cycle d 10–20) also show significantly lower IgE levels than women in other phases of the menstrual cycle (141). Increased IgE levels are associated with increased risk of eczema, which carries a female predominance in adults (152) and correlates strongly with the incidence of asthma and allergic rhinitis (153). Accord-

ingly, these patterns in atopy and IgE may reflect sex differences in allergic diseases such as rhinitis and provide clues to underlying mechanisms not only for rhinitis but for asthma as well.

Allergic airway inflammation is marked by increases in eNO and may be a surrogate measure for airway eosinophilia (154). eNO has been found to be generally greater in men compared with women, in both normal subjects and those with atopy (155), corresponding to lower eosinophil counts in women with allergies (156). However, estrogen enhances adhesion of eosinophils to nasal mucosa vasculature as well as eosinophil degranulation, whereas testosterone attenuates this response (157). Additionally, there is a stronger correlation of airway eosinophil count and asthma in women than in men, suggesting that women may be generally more sensitive than men to a given level of eosinophilia (149).

It is thought that estrogens are involved in the production of cytokines as well as triggering T helper 2 (Th2)-dominant immune response (158, 159) (see *Section IV.E* for more detailed discussion). Progesterone may also produce Th2-dominant cytokines including IL-4 (160). Estrogen and progesterone are both implicated in degranulation of eosinophils (161). In contrast, testosterone has an opposing effect on the immune system (162, 163). It is well-recognized that a majority of immune diseases exhibit a female predominance; however, the role of sex steroids in the incidence and number of exacerbations in atopy and other diseases is still unclear. Regardless, the above, albeit complex, summary highlights the need for recognition and identification of sex-specific factors in atopy and allergic diseases relevant to the airways and lungs.

From the discussion above, it appears likely that in addition to inherent sex differences, sex steroids play a modulatory role in a range of allergic airway diseases. The role of sex steroids has been examined in animal models of asthma (44, 164–168). However, data from these studies are not entirely consistent with the clinical data in humans. Female mice are more susceptible to airway disease (*e.g.*, induced using ovalbumin sensitization and challenge) compared to males (169), but they display higher IgE levels, greater bronchiolar inflammation, and relative resistance to glucocorticoid (166, 170, 171). In these animal models, androgens appear to have a protective role, with estrogens being actually proinflammatory. For example, castration in males exacerbates disease (171), whereas ovariectomy or estrogen receptor (ER) blockade in females alleviates it (reversed by exogenous estrogens) (172). However, in contrast to the expected bronchodilatory effects, exogenous progesterone worsens allergic airway disease in mice (173). An important aspect of these

findings that only complicates interpretation is the seemingly opposing effect of estrogens on airway inflammation *vs.* airway reactivity (the two elements of diseases such as asthma). In contrast to the findings of enhanced inflammation, estrogens appear to protect against airway hyperresponsiveness (AHR) (174, 175) with the absence of estrogens (ovariectomy) or ER signaling (knockout mouse) resulting in lower responsiveness to bronchoconstrictor stimuli (methacholine challenge). In rats, estradiol blunts ACh-induced bronchoconstriction, potentially via enhanced epithelial acetylcholinesterase activity or enhanced nitric oxide (NO) signaling (176). ER seem to be involved here because mice lacking ER show spontaneous AHR, with increased neuronally derived ACh (168). Conversely, AHR is greater in male mice but may be due to androgen effects on neuronally mediated airway reflexes rather than direct effects on the airway itself (177). Overall, these animal data verify a role for sex steroids in asthma as observed in humans. However, the discrepancy between airway inflammation *vs.* responsiveness suggests that comparison of animal *vs.* human data, acute *vs.* chronic alterations in sex steroid levels or action, and the parameters being measured (reactivity *vs.* inflammation) should be done with caution. Furthermore, it is important to consider the differential effects of sex steroids on specific cell types (*e.g.*, epithelium *vs.* smooth muscle) and species differences therein that could contribute to discrepant results in the overall effects of steroids on airway function. These issues are discussed in more depth in *Section IV*.

C. COPD and lung cancer

Despite the recognized harm of both active smoking and passive exposure (secondhand smoke), diseases caused by environmental tobacco smoke exposure are on the rise worldwide. In this regard, secondhand smoke can affect all age groups from the fetus (via placental transfer of toxic components in cigarette smoke) through the aging adult. *In utero* exposure to cigarette smoke toxins due to maternal smoking carries independent risk for reduced postnatal lung function (178–181) as well as continued respiratory problems such as asthma, wheezing, and respiratory infections in young children (182). Whether sex differences exist in the effect of antenatal cigarette smoke exposure is not clear. However, if cigarette smoke impairs bronchiolar development early in gestation, this will be limiting to airway performance because the number of bronchi are set by birth (unlike postnatal growth of alveoli; Table 1). Considering sex differences in the number of bronchi and the concept of dysanapsis (*Section II.D*), males may be particularly susceptible. Indeed, maternal smoking during pregnancy, which is associated with sig-

nificantly reduced FRC and expiratory flow rates in the newborn, has greater effects on male infants (180). Such reduced expiratory flow rates can persist throughout childhood and even early adulthood (183). Thus, it is likely that early exposure to cigarette smoke may set the stage for lifelong lung disease, especially in males. However, in the absence of longitudinal cohort studies in animals or in humans from early postnatal development through senescence, a definite link between fetal exposure to cigarette smoke components and COPD has not been established. Here, it would be of interest to explore sex differences in the progression toward COPD in offspring of mothers who smoked (or were exposed to secondhand smoke) during pregnancy or during the postnatal period.

COPD is a disease characterized by symptoms including progressive dyspnea upon exertion that does not reverse with bronchodilator therapy and is mostly unresponsive to steroids (184–186). It involves emphysematous destruction of lung parenchyma and narrowing of the airways (187). COPD has long been thought of as a disease of the male smoker. However, there has been an alarming rise in the number of women diagnosed with COPD every year compared with men (185, 188), with the number of deaths from COPD being higher in women than in men in both the United States and Canada for the first time (185, 189). Social and environmental factors are likely contributors in this change in diagnosis rate. Women are smoking more than ever, whereas more men are quitting smoking. Approximately 25% of the cigarettes sold in the United States were sold to women in the 1980s, and this number has stayed relatively stable; however, the number of adolescent females who are smoking is increasing steadily (188, 190, 191). Furthermore, physicians are becoming increasingly comfortable in diagnosing COPD in women, whereas previously women were commonly given the diagnosis of asthma, even when decreased lung function did not resolve with bronchodilator therapy (192).

Although socioeconomic and cultural factors may partially explain the sex difference in COPD at present, women may have an inherently higher susceptibility to cigarette smoke than men (193–195). Women typically develop symptoms of the disease at a younger age than men and also have a substantially smaller pack-year smoking history when compared to men. When normalized for pack-years smoked, the rate of lung function decline in women is faster than that of men, with women losing as much as 10 ml/pack-year and men losing 8 ml/pack year (193, 196). In this regard, it is interesting that whereas the female infant displays less susceptibility to maternal smoking (180), the adult female displays greater susceptibility. Here, it is not known whether female sex steroids play a

role in modulating susceptibility, or alternatively whether male sex steroids are protective.

In addition to smokers with COPD, women are the majority in a subsection of COPD cases involving non-smokers or never-smokers (excluding those with α -1 antitrypsin deficiency, a genetic risk factor for early development of COPD). For example, one study found that nearly 80% of those with early-onset COPD were non-smoking females (197). This corresponded with another study which found that more than 85% of nonsmoking COPD cases were women (198). Because nonsmokers represent only 12% of all COPD cases, these data suggest that women have a higher predisposition to the disease. An alternative hypothesis here is that these data represent more secondhand smoke exposure in women with male partners who smoke.

Women have a greater risk of being hospitalized for COPD-related reasons compared with men. This could be due, in part, to an increased perception of symptoms and a greater tendency to seek healthcare treatment than men (199). Regardless, there was no increased risk of death for women during their hospital stay. Conversely, recent studies suggest that once discharged, women have better outcomes than men when on long-term oxygen therapy (200). Additionally, in patients with COPD who have successfully quit smoking, women had a 2.5-fold greater improvement in lung function compared with men who quit (201).

A diagnosis of COPD encompasses both emphysema and chronic bronchitis with airway narrowing. There exists a sex bias in the type of COPD diagnosed even when normalized for smoking history and severity of disease. Men typically have more emphysematous deterioration of the lung, whereas women tend to have more reactive airways and more pronounced airway narrowing (202, 203). Analysis of computed tomography data from the National Emphysema Treatment Trial showed statistically less emphysema in women despite similar FEV1 compared with their male cohorts. Although MHT could have varying effects on airway function, hormone replacement has been reported to be associated with higher FEV1 in elderly women (204). Interestingly, this may explain why there is a greater bronchodilator response in female, but not male, smoking relatives of early-onset COPD probands.

Overall, the above clinical evidence indicates a clear difference between men and women in the presentation and diagnosis of COPD. Sex, hormonal state, and environmental factors, which include the extent and intensity of cigarette smoke exposure, may all play a role in the observed differences, but much more research is necessary to elucidate the mechanisms underlying these differences.

Furthermore, it is important to consider these mechanisms in the context of lung cancer, which can be a devastating consequence of cigarette smoke exposure.

Lung cancer is the leading cause of cancer-related death in both men and women in the United States (205). Sex differences in both clinical and pathological outcomes of patients with lung cancer have been examined extensively (205–210). Lung cancer kills more women each year than breast, uterine, and ovarian cancer combined (211, 212). Although the incidence of lung cancer in men is declining, the incidence in women is continuing to rise, and the observed gender gap in this disease is narrowing (212). The increased incidence in women is partially attributable to increased smoking habits in women, which peaked in 1960 (213). Although the current smoking rate for men is still significantly higher than in women (22.3 vs. 17.4%), women tend to smoke fewer pack-years than men but are diagnosed at an earlier age. Taken together, these data would suggest that women have a higher susceptibility to cigarette smoke (as is also suggested by the COPD statistics) (214–217). Independent of smoking status, however, nonsmokers diagnosed with lung cancer are approximately three times more likely to be female, suggesting an additional hormonal component (210, 216). Fortunately, women diagnosed with lung cancer have better prognoses and 5-yr survival than men at all stages and subtypes of disease including small cell lung cancer, which characteristically carries a poor prognosis (205, 217, 218).

Non-small cell lung cancer (NSCLC; includes adenocarcinoma, squamous cell carcinoma, and large cell carcinoma) is the leading lung cancer histology, accounting for approximately 85% of cases. Among smokers, men have more squamous cell carcinoma, whereas women have more adenocarcinoma (218, 219). When considering nonsmokers, adenocarcinoma is the predominant histology, with women forming the majority of presenters with this disease (206, 217).

The above clinical and epidemiological data would suggest that sex hormones may contribute to lung cancer. However, data on estrogen, progesterone, and MHT prevent clear conclusions from being drawn. Menstrual and reproductive factors related to lung cancer risk were evaluated in multiple studies (220–222). The Shanghai Women's Health Study evaluated the risk of lung cancer in never-smoking females and found reduced risk with increased number of births, later age of menopause, and a longer reproductive period (*i.e.*, greater hormonal exposure). These data were contradicted by others noting that early onset menopause (age 40 yr or younger) increased the risk of adenocarcinoma in women (221), and that longer reproductive periods, defined as early-age menarche

and late-age onset of menopause, increased the risk of lung cancer in Japanese never-smokers (222). Furthermore, limited data relating specifically to MHT are mixed, showing increased lung cancer risk, no effect, or decreased lung cancer risk in MHT users (209, 223–226). For NSCLC, women diagnosed at older ages have better survival (227), and higher circulating estrogen levels in general correlate with shorter survival (228). These results suggest that menopausal women not on MHT should have better survival rates, as evidenced by one study (226). Conflicting reports suggest that MHT alone does not alter lung cancer risk in women but increases the risk if women use MHT and continue to smoke, whereas others have found MHT to be protective against lung cancer in female smokers (209). Finally, one of the largest trials evaluating MHT in the United States was the Women's Health Initiative. Lung cancer was not a primary outcome of the study; however, *post hoc* analysis revealed that there was no significant increase in lung cancer incidence in women receiving hormone therapy.

Overall, the above seemingly conflicting and complex data suggest that the dose, timing, and duration of sex steroids (*e.g.*, number of births, MHT) *vs.* those of cigarette smoke exposure (*e.g.*, total pack-years, smoking cessation during pregnancy/lactation) are modulating factors in susceptibility and course of lung cancer in women. Although it is not clear how sex steroids influence responses to inhaled tobacco smoke, tumor biology and growth, or response to therapies, it is known that ER are present in normal lung tissue and lung cancer tumors. In fact, ER β -positive lung tumors are a positive predictor for survival, whereas patients with ER α -positive tumors have decreased survival rates (229, 230). Given this and other sex differences in incidence and survival in lung cancer, further research exploring the role of sex steroids in lung cancer therapies should be pursued.

D. Fibrotic diseases

PF is a progressive disease characterized by inflammation and scarring of the lungs, it affects approximately 5 million persons worldwide, and it is more common in men than women (231). This restrictive lung disease is characterized by progressive decline in FVC and FEV1 as well as a reduction in TLC. The incidence of PF ranges from 1.4:1 to 2.1:1 (male:female) (231, 232). Environmental factors including cigarette smoking increase the odds ratio of developing PF by 2-fold. Median survival is 2–5 yr from diagnosis, and the only viable treatment option is lung transplantation. Mortality rates from PF have been reported to be higher in men compared with women in the United States from 1992–2003 (233). Interestingly, the mortality rates are increasing more rapidly in women

than in men, suggesting that the observed sex differences in PF would be abolished or even reversed in the near future (233).

Sex differences in interstitial lung diseases such as PF have been studied in animal models. Cigarette smoke exposure leads to greater emphysematous changes of alveoli of female mice (234). In bleomycin models of PF, higher mortality and greater fibrosis (collagen deposition, cytokine expression) have been observed in female rats compared with males: effects diminished by ovariectomy, but exacerbated by estrogen (235). However, the opposite sex difference has been observed in mouse models of bleomycin injury. The role of sex steroids in these models is not entirely clear. *In vitro*, estrogens are known to enhance release of profibrotic factors in lung fibroblasts (see *Section IV.C*). A single study has further suggested that airway fibrosis is controlled by both relaxin and estrogen with the latter having a protective effect in the absence of relaxin (236). *In vitro*, data regarding development and progression of PF are lacking, and many studies on sex steroid effects on fibroblasts and extracellular matrix proteins have not been lung specific. Given that mortality rates are increasing more rapidly in women, detailed investigation on the relationships between sex hormones and fibrosis is warranted.

E. Pulmonary hypertension

Pulmonary hypertension (PH) is a relatively rare disease that affects approximately one or two people per million, with approximately 300 new cases every year in the United States. However, PH leads to more than 15,000 deaths and more than 250,000 hospital visits per year (237, 238). PH is characterized by mean pulmonary arterial pressure of at least 25 mm Hg, increased vascular proliferation and remodeling, intimal fibrosis, plexiform lesions, right ventricular hypertrophy, and ultimately right heart failure (237, 239, 240). There is female predominance of both idiopathic and familial (heritable) PH, with female:male prevalence ratio from 2:1 to as high as 4:1 (239–241). Additionally, PH generally presents in women in their 30s, approximately 10 yr earlier than in men (242, 243). With the observed female predominance in all types of PH—idiopathic, familial, pulmonary arterial hypertension (PAH), and portopulmonary hypertension—the influence of gender and sex steroids has been a focus of ongoing research and has been reviewed recently (239).

PAH occurs in two distinct age groups of women: those of childbearing age generally in their 30s, and postmenopausal women (240, 244). Early PAH was at first attributed to the now well-recognized adverse effects of appetite suppressants (including aminorex fumarate and fenflu-

ramine) in young women (245, 246); however, despite the removal of such harmful agents from the market, the increased prevalence in women has persisted, suggesting a possible hormonal link. Here, OC, specific to younger women, may be a risk factor (247); however, some epidemiological data do not support this idea (246). The use of MHT in postmenopausal females suggests a protective effect from development of PH (248). Experimental evidence, as well as data from systemic hypertension research, suggests that estrogens have a protective effect on pulmonary vasculature; however, this does not appear to be the case in the predominantly female patient population (240). Several autoimmune diseases associated with the development of PH such as lupus, scleroderma, and rheumatoid arthritis (238, 249) have a female predominance. Estrogen and progesterone have been shown to exacerbate immune responses, whereas androgens are protective (250–252). Given that the majority of PH presents without preexisting autoimmune disorders, the correlation between these diseases, although interesting, does not fully explain the observed sex differences in PH.

Although the role of estrogens in PH is recognized clinically, the direct effects of estrogen and its metabolites on the pulmonary vasculature are still under investigation. Animal data and traditional models of PH show that estrogens have protective effects, yet women are consistently diagnosed more often than men. The role of estrogens and the development of PH have been reviewed extensively (240, 253, 254). Briefly, estrogen is known to stimulate both NO and prostacyclin production in the pulmonary vasculature (255, 256). NO is one of the most effective endogenous vasodilators. Furthermore, continuous iv infusion of epoprostenol has been shown to have beneficial effects in patients with PH, increasing both short- and long-term survival (239). Additionally, estrogen downregulates gene expression of endothelin-1, which is a potent vasoconstrictor and vascular smooth muscle mitogen (257). Preliminary results employing endothelin-1 receptor antagonist, sitaxsentan, suggest beneficial effects in patients with PH (239). 2-Methoxy estradiol (2ME), an estrogen metabolite, has been shown to inhibit endothelin-1 and stimulate prostacyclin production in vasculature (253). Estrogens themselves have also been shown to be mitogenic (endothelial and vascular smooth muscle cells) and prothrombotic. The development of *in situ* thromboses is a hallmark of idiopathic PH and may be a result of the hormonal milieu in women who develop this disease. Additionally, effects of estrogen on endothelial growth and dysfunction are unclear; however, data suggest that estrogens negatively regulate endothelial cell plexiform lesion development. Taken together, the above data suggest that sex steroids likely contribute (either positively or neg-

atively) to sex differences in PH pathogenesis, progression, and response to therapy. It is also clear, however, that it is not simply estrogen that makes women more susceptible. Further research into the underlying mechanisms, the roles of progesterone in women, and perhaps a protective role in men is necessary. Here, the differential effects of sex steroids on different cell types of the pulmonary vasculature (endothelium *vs.* smooth muscle) are also important. *Section IV.D* explores current understanding of these effects in more detail. Finally, the development of animal models more representative of the human disease will also be essential in discovering novel therapies for the treatment of PH.

F. Other conditions

1. RDS

Advances in neonatal intensive care have greatly increased survival in babies as young as 24 wk gestation. Considering the time course of fetal lung development, the premature infant is at great disadvantage from a respiratory and survival perspective. Indeed, many infants weighing less than 1000 g at birth have RDS (hyaline membrane disease) with initial hypoxia and the need for supplemental oxygen, as well as requiring intubation and mechanical ventilation (258). Male fetuses are at higher risk for premature birth (~55% of premature infants are boys) and for developing RDS (relative risk, ~1.6) (36, 259–261). Administration of antenatal corticosteroids to mothers at risk for premature delivery reduces RDS more in female premature infants (262, 263) (however, see recent meta-analysis in Ref. 264 suggesting equal benefit of corticosteroids in males and females). Earlier surfactant production in the female fetus, compared with the males, may play a role in the maintenance of airway patency (28).

2. Bronchopulmonary dysplasia

A chronic disease of the immature lung (265), BPD occurs in more than 20% of the approximately 50,000 premature births in the United States of less than 1500 g weight (266, 267). BPD is a risk factor for childhood asthma and other respiratory illnesses that may persist into adulthood. There is now considerable evidence that preterm male infants are also at higher risk for BPD (268, 269). Although the mechanisms underlying BPD are still under investigation, they include RDS itself, inflammation, exposure to increased oxygen concentration, and (if applicable) barotrauma resulting from mechanical ventilation. In this regard, the levels of TGF β , known to be involved in tissue repair, are higher in tracheal aspirates of preterm male infants who went on to develop BPD than in preterm males who did not develop BPD (268). The fetus

is exposed to high levels of estradiol and progesterone, which decreases by several orders of magnitude after parturition. Premature birth deprives the newborn of these female sex hormones at an earlier stage of lung development. Accordingly, there has been recent interest in the replacement of estrogen and/or progesterone in extremely premature infants who are at risk for BPD (270–272), although the results are not conclusive. Other mechanisms such as sex steroid modulation of NO may also play a role in regulation of airway and vascular tone and remain to be examined.

3. Cystic fibrosis (CF)

Although many of the diseases described above suggest a role for sex steroids, the contribution of inherent sex differences is exemplified by CF, a systemic disease caused by a mutation in the CF transmembrane conductance regulator gene that manifests in airway destruction from repeated infections and impaired mucus clearance. There is no sex-linked difference in the incidence of CF (273); however females aged 1–20 yr have a significantly higher mortality (274, 275) and reduced life span (276) compared with males. In CF, girls have lower FEV1 than males, contrary to what is found in healthy children and adolescents (273, 275). Anatomically, the smaller lungs of females in combination with impaired mucus clearance may explain increased risk of infection in females, another predictor of mortality (275, 276). Interestingly, the relative risk of death for females remains roughly constant at 1.6 throughout childhood, adolescence, and young adulthood, suggesting that hormonal influences at puberty may not be contributory (275, 276). However, recent studies have reported cyclical decreases in lung function in female patients with CF, which may increase their susceptibility to bacterial infection when estrogen levels are low (277). Alternatively, periovulatory (peak) concentrations of estradiol can decrease uridine triphosphate-induced chloride secretion, which may contribute to impaired mucus clearance (278).

IV. Sex Steroids in Lung Physiology and Pathophysiology

A. Sex steroid signaling

The synthesis pathway for sex steroids is thoroughly established (for recent reviews, see Refs. 279 and 280). Both gonadally derived and locally produced sex steroids (281–283) determine the eventual concentration systemically and in various tissues. In this regard, studies evaluating the effects of sex steroids in the lung report values in a variety of formats that range from exogenous molar

concentrations to endogenous serum levels, which makes drawing comparisons between studies somewhat difficult. Table 2 provides normal ranges of testosterone, estradiol, and progesterone for men and women (nonpregnant, pregnant, and postmenopausal) in both nanograms per milliliter and molarity and is derived from a number of established sources (284–286).

Both genomic (11, 287–292) and nongenomic (289–295) aspects of sex steroid signaling (albeit in tissues other than the lung) have been extensively reviewed. Classical sex steroid receptors include two ER (ER α and ER β), two progesterone receptors (PR-A and PR-B), and the androgen receptor (AR). As members of the superfamily of nuclear receptors (296, 297), these receptors bind their respective hormones, resulting in receptor translocation from cytoplasm to nucleus, where homodimerization and in some cases heterodimerization can occur. Classically, ER α is a better transcriptional activator than ER β , with the suggestion that ER β antagonizes ER α (298, 299). Nuclear heterodimers can form between ER α and ER β , and ER α with AR (287). For progesterone, PR-B is the main activator of gene transcription, whereas PR-A acts as a repressor of PR-B and ER transcription (290, 300). The AR binds both testosterone and the more active metabolite, 5 α -dihydrotestosterone (DHT) (301, 302).

Rapid sex steroid signaling events involve membrane-localized steroid receptors, either full-length or truncated, as well as G protein-coupled receptors (GPCR) that are capable of binding sex steroids [e.g., the GPCR30 or GPER that is estrogen-sensitive (303, 304)]. Nongenomic effects of steroids have been reviewed extensively by several authors, and we refer readers to these reviews for more detailed explanations (289–295). A major aspect of nongenomic signaling of sex steroids is modulation of intracellular calcium ([Ca²⁺]_i) (253, 292, 305–307). Modulation of [Ca²⁺]_i can involve increasing Ca²⁺ via the phospholipase C-diaclyglycerol-inositol trisphosphate signaling cascade (305) or more commonly reducing Ca²⁺ by inhibiting a variety of influx mechanisms such as L-type

Ca²⁺ channels (306, 308), K⁺ channels (309), or chloride currents (310, 311).

Downstream effectors of GPCR including cyclic nucleotides, protein kinase C, protein kinase A, and protein kinase G, are also modulated by sex steroids (312, 313). Steroid receptor activation can also induce a myriad of intracellular signaling pathways including MAPK, tyrosine kinases, and lipid kinases. In turn, activation of these pathways can alter subsequent steroid receptor activation, including ligand-independent activation or direct phosphorylation of these receptors by MAPK (314–316). Estradiol has been shown to activate ERK1/2, p38, and JNK pathways leading to both c-Jun and c-Fos gene transcription (290, 291, 317, 318). Thus, rapid, nongenomic actions of estrogens can indeed exhibit genomic downstream effects as well. PR-B exhibits cross talk with ER whereby it primes ER to activate the Src-Ras-ERK pathways (319). Additionally, PR can activate p42 MAPK and phosphatidylinositol-3-kinase in *Xenopus* oocytes (315). In vascular endothelial cells, ER α activation leads to phosphatidylinositol-3-kinase-Akt-eNOS activation producing the vasodilator NO (291, 320). Activation of AR involves the c-Src, Raf-1, and ERK-2 pathways leading to downstream involvement of MAPK (321, 322).

In addition to signaling intermediates, the action of steroid receptors (especially genomic effects) can be further modulated by a large number of coregulators that fine-tune enhancement (coactivator) or suppression (corepressor) of steroid-responsive gene expression. In the absence of ligand, the receptors are associated with heat shock proteins and other chaperones that prevent unwanted activity. With ligand binding, receptor activation, and nuclear translocation, binding to hormone-responsive elements of target genes results in recruitment of coactivators to help up-regulate transcription. In contrast, corepressors interact with steroid receptors in the absence of hormone or in the presence of antihormone. Thus, coregulators can greatly influence sex steroid function. A number of coactivators and corepressors have been identified to date,

TABLE 2. Serum sex steroid values in humans

	Males	Nonpregnant females			Pregnant females	Menopausal females
		Follicular	Preovulatory	Luteal		
Testosterone	2–15 ng/ml 6–50 nM	200–800 pg/ml 0.7–2.5 nM	200–800 pg/ml 0.7–2.5 nM	200–800 pg/ml 0.7–2.5 nM	1–1.4 ng/ml 3.5–5 nM	200–800 pg/ml 0.7–2.5 nM
Estradiol	15–50 pg/ml 50–200 pM	20–100 pg/ml 80–500 pM	150–400 pg/ml 0.5–1.5 nM	60–200 pg/ml 0.2–0.8 nM	1–40 ng/ml 1–150 nM	10–30 pg/ml 40–120 pM
Progesterone	250–900 pg/ml 0.8–2.8 nM	0.3–1.2 ng/ml 0.3–1.5 nM	0.7–2.5 ng/ml 2–10 nM	1–18 ng/ml 10–60 nM	9–300 ng/ml 25–1000 nM	<0.2–1.1 ng/ml 0.6–3.5 nM

An important aspect in terms of research on sex steroid effects in the lung or experimental use of sex steroids is the need to approximate physiological serum levels of hormones when assessing the contribution of sex steroid signaling to disease pathogenesis or amelioration. Hormone levels vary greatly in women after puberty, but are more stable in men. Hormonal changes with the menstrual cycle, changes with pregnancy, and menopause can correlate to changes in disease state and symptoms in a variety of lung disease (see text).

although the tissue-specific distribution, regulation, and function of such coregulators are still under investigation. Accordingly, more detailed review of this important, but emerging topic is beyond the scope of this article. The reader is referred to a few recent reviews in this area (323–326).

The above brief description of sex steroid signaling highlights the fact that depending on the cell type, steroid concentration, relative receptor expression, coregulators, and the relative importance of these signaling pathways, sex steroids have the potential for wide-ranging and complex modulation of cellular function. Cross talk between receptor signaling pathways may allow for even more nuanced regulation. These issues have not been specifically examined in lung or the cells therein but are likely to be present in this organ as well.

B. Upper and lower airways

1. Nasal epithelium

The majority of the work to date (albeit limited) on sex steroid signaling in nasal epithelium has largely been in the context of diseases such as rhinitis. From these data, a role for sex steroids (particularly estrogens) in nasal epithelium can probably be assumed. The relevance of such a role lies in the modulation of nasal epithelial function during specific phases of adulthood, such as pregnancy where sex steroid levels can change substantially.

ER and PR are present in the nasal mucosa. Interestingly, although AR mRNA has been detected, no protein has been found in human turbinate samples (327). Accordingly, it is likely that the female sex steroids are the ones of relevance in the nasal epithelium. ER and PR expression are substantially up-regulated in the presence of increased circulating levels of estrogen and progesterone, for example as occurs with pregnancy (328). Histological changes consistent with increased edema and mucus secretion in the nasal epithelium of women have been reported to occur with different phases of the menstrual cycle (329–331). These changes may underlie altered rhinitis symptoms in women during the menstrual cycle and with pregnancy. However, the relationship between protein expression and function is less clear. For example, only ER β (but not ER α or PR isoforms) is expressed in the nasal mucosa of both males and females (332). Some studies suggest that ER β may actually serve to inhibit action of other steroid receptors (294, 333), and thus estrogen effects on nasal epithelium may occur only in the presence of up-regulated ER α expression (*e.g.*, during pregnancy). Interestingly, progesterone has little to no effect on the nasal mucosa (334). These data would suggest that estrogen rather than progesterone is likely involved in rhinitis ex-

acerbations in women; however, functional studies on sex steroid signaling in allergic rhinitis are few.

Some studies suggest that innate allergy and hypersensitivity to endogenous sex steroids or OC explain changes in rhinitis symptoms in women (335, 336). In one study, nasopharynx-nostril pressure gradients were used to assess nasal mucosal reactivity. Approximately 33% of women who suffered from allergic rhinitis and were also taking OC had a positive nasal response to topical administration of OC. There was no response in control groups, which consisted of males without atopy, females without atopy on OC, or females with atopy not on OC (336). Although there was a positive correlation of rhinitis symptoms in a subset of allergic women on OC in both studies, a large percentage of the cohort in Ref. 336 were unreactive to the sex hormone stimulation *per se*. However, older studies have reported thickening of nasal mucosa in a variety of mammals subsequent to exogenous estrogen treatment (334, 337). Although actual ER isoform expression was not examined in these studies, these data suggest a more complex influence of sex hormones on allergic rhinitis than a simple increase in mucus production by the epithelium (157, 338, 339). For example, in nasal biopsies from pregnant women suffering from nasal symptoms, there was an up-regulation of cholinergic nerve activity around nasal blood vessels (338). Estrogen also increased the density of muscarinic receptors in nasal mucosa of pregnant guinea pigs (339). Histamine receptor mRNA in cultured human nasal epithelial cells and human mucosal microvascular endothelial cells was increased by estrogen and progesterone (but not testosterone) (340). Histamine is released in response to allergen stimuli, and hyperreactivity of nasal mucosa to histamine is a hallmark of allergic rhinitis.

The clinical and limited basic research data suggest that in the nasal epithelium, a number of mechanisms can be modulated, largely by estrogens. In addition to effects on mucus production, estrogen may affect cholinergic and histaminergic signaling in the nasal passageways, resulting in altered blood flow and mucosal thickening. The relevance of these findings lies in identifying targets (epithelium *vs.* vasculature *vs.* innervation) to alleviate nasal symptoms during the menstrual cycle or pregnancy and provide relief to rhinitis sufferers. Furthermore, studies are needed to determine whether mechanisms underlying rhinitis exacerbation in males are similar and involve sex steroid signaling due to local conversion of testosterone. Finally, with the understanding that rhinitis and similar diseases involve a large inflammatory component, it will be important to establish the effect of sex steroids on immune cell function and inflammatory signaling within the epithelium.

2. Bronchial epithelium

The bronchial epithelium serves as a barrier within the airway, an early recipient of and responder to external stimuli including pathogens, allergens, and pollutants, and as a modulator of airway tone. Bronchial epithelial inflammation is a key factor in the pathogenesis of asthma, bronchitis, and a host of other “lung” diseases. Lung cancers (*e.g.*, squamous cell carcinomas, NSCLC) are derived from bronchial epithelial cells (BEC). Accordingly, understanding sex differences in BEC structure/function or the effects of sex steroids on BEC is important. However, few studies have examined the effect of sex steroids on BEC.

In immortalized airway epithelial lines, both ER α (341, 342) and ER β (342) were present, with ER β being the predominant isoform. In a recent study, we found that freshly isolated human bronchial epithelial tissue, as well as single BEC express abundant ER α and ER β in fairly comparable and abundant quantities (343). Whether PR or AR is expressed in BEC is not known, although expression is likely. Regardless, it is possible that as with nasal epithelium, only ER are of relevance in the bronchial epithelium.

The relevance of sex steroid signaling in BEC lies, at least in part, in the potential regulation of NO in the airway. Studies in vascular endothelium have already established that estrogens can facilitate dissociation of endothelial NO synthase (eNOS) from caveolae of the plasma membrane, resulting in activation of the NO pathway and potentiation of vasodilation (344). Akin to endothelium, NO produced by the bronchial epithelium can be a potent bronchodilator (345, 346). Furthermore, with inflammation, eNO is used as an indicator of airway inflammation (110, 154), although the source of such NO may actually be inducible NO synthase (iNOS) (347), with BEC being the predominant source. In an immortalized BEC line H441, estradiol has been shown to acutely increase the conversion of [3 H]L-arginine to [3 H]L-citrulline through eNOS activation, an effect inhibited by the ER antagonist fulvestrant (ICI 182,780) (341). In a recent study, we found that both ER α and ER β activation [achieved using receptor-specific ligands (R,R)-5,11-diethyl-5,6,11,12-tetrahydro-2,8-chrysenediol ((R,R)-THC), diaryl-propionitrile] acutely (within minutes) increases NO production in nonimmortalized, enzymatically dissociated human BEC (343). Such effects are accompanied by eNOS phosphorylation. Furthermore, in human bronchial rings from females, physiologically relevant concentrations of estrogens (<10 nM) produce potent bronchodilation, which is substantially blunted by epithelial denudation. These limited data suggest that, similar to endothelium, estrogens are capable of inducing NO in bronchial epithelium via

nongenomic mechanisms, thus potentially modulating bronchodilation.

In humans, eNO fluctuates in menstruating women compared with women on OC such that eNO decreased when estrogen levels were high, whereas increased progesterone levels correlated with increased eNO. Additionally, women on OC did not exhibit fluctuations in eNO (110). Other studies on the relationship of menstrual status and eNO have yielded conflicting results (108, 109, 348, 349). eNO is thought to affect activity of iNOS and inflammatory cells in the lung. No work to date has examined the effect of sex steroids on iNOS regulation in BEC. Nonetheless, menstrual fluctuations in eNO suggest the contribution of both genomic (considering the baseline levels of estrogens or progesterone) and possibly nongenomic (during specific points of the hormonal cycle) mechanisms.

In addition to altered production of NO, BEC growth and proliferation are effected in diseases such as bronchitis and asthma. In other tissues, estrogens, progesterone, and testosterone are all known to modulate cell proliferation (291, 350, 351). There is currently limited information on the effect of sex steroids on BEC proliferation. Effects of estrogen on proliferation of immortalized BEC were conflicting (342). Estradiol increased proliferation in three of five cell lines; however, only two of the three lines exhibited ER activation. To date, no work on the effects of androgens or progesterone on BEC has been reported.

Finally, sex steroid signaling in bronchial epithelium may also be important in CF. Although the role of sex steroids is not well established in CF, cyclical decreases in lung function in female CF patients (277) and estradiol effects on uridine triphosphate-induced chloride secretion (278) suggest that susceptibility to infection and impaired mucus clearance may both be modulated by estrogens. Considering the importance of the airway epithelium, these are certainly topics that should be pursued from a perspective of understanding sex steroid signaling in the airway, as well as for development of new avenues for drug therapy targeting airway diseases.

3. Alveolar epithelium

Sex steroid signaling in alveolar epithelium is relevant across the life span. Sex steroids, specifically androgens, affect the maturation of alveolar type II epithelial cells and the subsequent production of surfactant in male fetuses (352–354). Changes in the histopathology of type II cells in adults show a sex difference, with men developing more emphysematic COPD than women (202, 203) and with women having a greater predominance of lung adenocarcinoma (217–219, 355). The presence of ER α and ER β as well as PR and AR has been reported in the alveolar epithelial

layer of normal lung biopsies as well as in tumors (355–357) and immortalized alveolar epithelial cell lines including NCI-A549 and NCI-H23 (355, 357, 358). Furthermore, sex steroid enzymes aromatase and 17β -hydroxysteroid dehydrogenase have also been detected (358, 359). Thus, the potential exists for a range of sex steroid effects within the airway epithelium. However, as with nasal and bronchial epithelium, studies to date have largely focused on these effects in the context of disease.

Studies on the actions of sex steroid in emphysema are lacking. Early research in rats suggested that administration of progesterone or medroxyprogesterone is able to reverse emphysematic changes in lung air spaces (360, 361). A similar effect of progesterone was reported in an experimental model of papain-induced emphysema (362, 363). Interestingly, administration of progesterone to patients suffering from emphysema reduced hypercapnia and improved symptoms (364); however, mechanisms underlying such effects were not studied, and it is not entirely clear whether there is actual improvement in lung structure. Furthermore, it is not known whether these effects can be attributed to signaling in alveolar epithelium *per se*. Nonetheless, these limited data would suggest that female sex hormones may be protective against the development of emphysema, although much more work on this topic is warranted before clinical implementation of sex steroids in emphysematic COPD.

Estrogen has been implicated in the predominance of NSCLC in females as well in the progression of tumors. Women are generally younger than men and develop adenocarcinoma with less pack-year smoking history than men. It is thought that female sex hormones play a role in these observations, especially given the well-documented effect of estrogen on ER-positive breast cancer progression. Although the selective ER modulator tamoxifen has proven efficacy in preventing breast cancer proliferation, it is suspected to promote lung tumor growth (365). Indeed, estradiol-induced enhancement of NSCLC proliferation was reported in the NCI-H23 cell line, an effect attenuated with small interfering RNA directed against ER α or ER β or the pharmacological ER antagonist fulvestrant (ICI 182,780) (366). The combination of epidermal growth factor (EGF) and estradiol increased p42/p44 MAPK activity in NCI-H23 cells above EGF or estradiol alone. Functional interactions between ER and EGF receptor (EGFR) were found in lung cancer cells (367). EGFR activation subsequently activates ER independent of ligand binding (366). Accordingly, a combination of fulvestrant and the EGFR kinase inhibitor erlotinib has been shown to prevent xenograft tumor growth better than either intervention alone (366). This correlates with the clinical observation that patients with tumors overex-

pressing EGFR and ER α have poorer outcomes (368). Additionally, favorable outcomes are negatively correlated to serum estrogen levels (228). In addition to gonadally derived estrogens, local production may also be relevant in the case of lung cancers. Aromatase, the enzyme that converts testosterone to estrogen, was found in more than 85% of NSCLC from male and female patients (369), and aromatase expression negatively correlates with long-term survival in postmenopausal women (370). Overall, if anything, these data suggest a detrimental role for estrogens in the alveolar epithelium when it comes to cancer, which appears to be in sharp contrast to a potentially beneficial role in COPD or even in the bronchial epithelium. This only emphasizes the need for more careful examination of sex steroid signaling within specific lung cell types and exploration of targeted delivery of drugs to modulate these effects within cells of interest.

Contrary to the pro-proliferative effects of estrogen, progesterone inhibits proliferation in a dose-dependent manner in NSCLC cell lines and reduces tumor volume in nude mice (355). Nearly 50% of NSCLC patient specimens stain positive for PR (371), which predicts better outcomes. Local progesterone synthesis (via 3β hydroxysteroid dehydrogenase) may also be important (372, 373). Thus, in women, the relative amounts of circulating and local levels, of estrogen *vs.* progesterone may be important determinants of tumor progression and patient prognosis. However, much more work is needed to understand the potential interaction between ER and PR signaling within tumors and the relative influence of estrogens *vs.* progesterone on these receptors, especially in women.

Androgen effects in lung cancer have been barely studied. Androgens promote proliferation in AR-positive small cell lung cancer cells (374). AR are present in lung cancer tissue samples and the NCI-A549 cell line, with testosterone up-regulating AR in these cells (357). AR expression is usually low in the NCI-A549 cell line but is significantly up-regulated with the androgen DHT, which works with EGF via p38 MAPK to enhance cellular growth (375). In addition to proliferative effects, testosterone may also up-regulate genes such as CYP1A1 (357), which encodes proteins that convert estradiol to more potent metabolites (253), catalyze elements of cigarette smoke and other carcinogens, and have been associated with increased susceptibility to bronchogenic carcinoma (376). These limited data generally suggest a deleterious effect of androgens in lung cancer. Although much more work is needed here, it is important to consider whether local aromatase-induced conversion of testosterone to estrogen could potentiate cancer cell proliferation, especially via interactions with the EGFR.

In addition to lung cancer, there has been considerable recent interest in the role of sex steroids in acute lung injury. A beneficial and protective role for estrogens, but a potentially detrimental role for progesterone, has been reported in cardiac and renal ischemia/reperfusion injury (377–380) in both animals and humans. These data set the stage for examining the impact of sex steroids on acute lung injury, although to date such effects have been examined only in animal experiments. In general, it appears that male sex and androgens are both detrimental to AHR and inflammation after aspiration of bacterial lipopolysaccharide (LPS) (167). Indeed, even in females, testosterone worsens the inflammatory response. Furthermore, even in lung injury secondary to injury in other organs (*e.g.*, to the gut), androgens are detrimental whereas estrogens are protective (381–383). Consistent with a protective role, administration of exogenous estrogen to previously ovariectomized mice reverses LPS-induced lung injury (384), potentially through modulation of cytokine profiles and cell adhesion molecules (384), as well as modulation of cellular apoptosis (385). Furthermore, estrogens, acting via ER β may modulate iNOS and thus reduce the level of inflammation (386). These exciting data provide a basis for work in humans to explore both sex differences in the response to (and recovery from) acute lung injury and the potential protective role of estrogens.

4. Airway smooth muscle (ASM)

Research regarding the role of sex steroids in modulation of ASM is rapidly progressing given clinically observed sex differences in asthma and airway-predominant COPD. Both increased AHR to cholinergic agents, and ASM hypertrophy and hyperplasia (increased ASM mass) contribute to the pathogenesis of asthma (43, 387). Additionally, increased ASM mass and decreasing lumen diameter are hallmarks of chronic bronchitis and COPD (388). An important caveat here is that considering the multifactorial nature of diseases such as asthma or COPD, sex differences in airway structure and function may reflect a net effect of sex steroids on multiple cell types within the airway. We will focus on BEC *vs.* ASM *vs.* immune cells in the current review. However, it is important to recognize the potential role of other mechanisms including airway innervation that may regulate airway irritability (*e.g.*, in the presence of environmental triggers) as well as airway tone (by release of bronchoconstrictors such as ACh *vs.* bronchodilators such as NO). Another consideration is the contribution of genomic *vs.* nongenomic mechanisms. Here, we will review what is known on the direct role of sex steroids on ASM reactivity and proliferation.

The general consensus from *in vitro* work, although limited, is that estrogens are bronchodilatory. However,

attention should be paid to the concentrations being used as well as whether acute or chronic effects are being considered. An early study (122) on the relatively acute (60 min) effects of estradiol, testosterone, and progesterone reported potentiation of isoprenaline-induced relaxation in isolated pig bronchus. Here, supraphysiological concentrations of estradiol were found to be the strongest potentiator of the bronchodilatory response to isoprenaline (122). Another set of studies found that chronic (21 d), low doses of estradiol (10 $\mu\text{g}/\text{kg}$) increased the concentration of inhaled ACh necessary to double airway resistance in ovariectomized rats (176) (an epithelium-dependent effect), whereas rats receiving high-dose estradiol (100 $\mu\text{g}/\text{kg}$) had increased responsiveness to ACh (389) (an epithelium-independent effect). Although species differences may explain the discrepancies between these studies, the acute exposure in one study (122) *vs.* chronic exposure in the others (176, 389) should be noted.

Studies on estradiol-induced relaxation of tracheal strips from rabbits have been consistent with the rat studies but have implicated a different mechanism involving direct effects on ASM (390, 391). For example, 100 μM estradiol has been found to relax rabbit tracheal strips precontracted with ACh, an effect not reduced by the NO synthase (NOS) inhibitor N^G-nitro-L-Arginine or removal of the epithelium (391). Although the estradiol concentration in this study was substantially higher than the physiological range, estradiol effects were attributed (at least in part) to prostaglandin synthesis and cGMP modulation of ASM tone (391). This is significant because both cGMP and estrogens can influence mechanisms such as Ca²⁺ influx channels, and thus estrogens can potentiate the effects of epithelially derived NO. Separately, prostaglandins can modulate cAMP levels, which may provide an additional avenue for estrogens to potentiate bronchodilation.

The mechanisms by which estrogens directly influence ASM contractility (*i.e.*, not via epithelially derived NO or innervation) have not been systematically examined. As with other cell types, estrogens could modulate membrane potential (*e.g.*, via K⁺ channels) as well as other Ca²⁺ regulatory mechanisms. In the mouse, it has been reported that estrogens enhance activity of Ca²⁺ activated K⁺ channels, thus lowering membrane potential and indirectly reducing Ca²⁺ (174). In human ASM cells, physiological concentrations of estrogens as low as 100 pM substantially decrease Ca²⁺ responses to agonists such as ACh (392). These effects appear to predominantly involve ER α , with only a minor contribution of ER β despite relatively equivalent expression of both receptors in human ASM. These effects also appear to involve inhibition of L-type channels as well as store-operated calcium channels, which are im-

portant in regulating $[Ca^{2+}]_i$. Regulation of $[Ca^{2+}]_i$ in ASM involves both calcium influx and calcium release from intracellular stores (392–394). Estrogens do not appear to have a significant effect on $[Ca^{2+}]_i$ stores in human ASM, whereas in human BEC, we recently found that the same concentrations of estrogens can induce sarcoplasmic reticulum Ca^{2+} release via inositol trisphosphate receptor channels (343). Overall, these limited data suggest that a major mechanism by which estrogens can produce bronchodilation is by reduction of $[Ca^{2+}]_i$ in ASM in a non-genomic fashion. Genomic effects of estrogens on Ca^{2+} regulation in ASM have not been examined but could potentially involve altered expression of Ca^{2+} regulatory proteins or intracellular signaling mechanisms that may indirectly modulate both Ca^{2+} and the contractile apparatus of ASM.

A potential, but obvious reason for sex differences in airway reactivity may be differences in ER expression of ASM derived from male *vs.* female humans and/or animals. In pilot studies, we have found that ASM derived from male *vs.* female patients express full-length ER α and ER β to comparable extents (E. A. Townsend, M. A. Thompson, C. M. Pabelick, and Y. S. Prakash, unpublished observations), whereas others have found ER expression in lungs from both male and female mice (395). Accordingly, the potential exists for ER activation in both males and females. In our previous study on calcium regulation, we examined ASM cells derived only from female patients (392). Therefore, sex differences in airway reactivity at the cellular or whole animal level may involve differences in the activation of signaling pathways downstream of the ER and should be examined systematically.

Although the *in vitro* work in tracheal or bronchial rings is consistent with the idea of estrogen-induced bronchodilation, *in vivo* studies in mice on sex differences in asthma are less clear. A potential problem here is that it is difficult to isolate the effects of sex steroid on ASM alone in the setting of elevated presence and activity of inflammatory cells and cytokines, as well as steroid effects on other airway elements (especially epithelium or airway innervation). Several murine models of allergic asthma exhibit sex differences in airway responsiveness *vs.* airway inflammation, but the data are conflicting (44, 167, 169, 171, 174, 175, 177). For example, male C57BL/6 mice show more AHR than females, indicating a protective effect of estrogen (167). However, an inherent sex difference in AHR does not necessarily suggest a constrictive or dilatory effect of sex steroids on ASM. Furthermore, female mice actually exhibit more airway inflammation (166, 170). Again, it is not clear whether estrogen and/or progesterone are actually involved. Importantly, these studies highlight the importance of distinguishing between AHR

vs. inflammation. In these models, ovalbumin, LPS, or tobacco smoke was used to induce an allergic phenotype resulting in subsequent AHR.

Focusing on AHR, estradiol substantially blunts carbachol-induced airway constriction via the NO-cGMP-PKG pathway, resulting in increased activation of Ca^{2+} -activated potassium channels (174). This effect may be sex-specific because only male mice exhibited methacholine-AHR, and administration of estrogen to males attenuated this AHR (175). Female mice lacking the ER α receptor display enhanced airway responsiveness to methacholine, thought to be related to M2 muscarinic receptor dysfunction (168). Furthermore, in response to electrical field stimulation (a common technique used to identify the contribution of neurally derived signals), tracheal rings from these knockout mice released more ACh from airway innervation compared with their wild-type controls. These limited data suggest at least partial involvement of ER signaling within airway nerves as well as NO in sex differences in AHR. Interestingly, these animal studies would suggest a lack of direct effect of estrogens on ASM, which appears to be in sharp contrast to the *in vitro* work (especially in human cells). Several reasons may underlie this discrepancy, including species differences in estrogen action on ASM; the interaction between ASM and other cells including epithelium, immune, and nerves *in vivo* that is absent *in vitro*; and the lack of current *in vitro* data on how estrogens affect ASM in the presence of inflammation. Further work is required to determine the true importance of ASM in mediating estrogen effects in the airway, especially in the presence of inflammation.

The effects of progesterone on ASM contractility have been less studied. Progesterone has been reported to potentiate the relaxant properties of isoprenaline in isolated, constricted pig bronchial rings (122). Here, 40 μ M progesterone had a greater potentiating effect than testosterone, but it was less effective than estradiol. Again, the extremely high concentration of progesterone should be noted (Table 2). Another study reported that progesterone and 5 β -pregnenolone prevented agonist-induced contraction of guinea pig trachea, with a greater effect of progesterone (396). These effects were proposed to involve direct inhibition of Ca^{2+} entry but not via γ -aminobutyric acid $_A$ -receptors (396).

Overall, the limited *in vitro* data suggest that, similar to estrogens, progesterone has a bronchodilatory effect. However, further study is required to determine whether such effects are maintained in the presence of inflammation and can thus be used as a potentially new avenue to target AHR. However, in male mice sensitized with ovalbumin, progesterone actually exacerbates AHR (173), but only in sensitized animals. Direct effects of pro-

gesterone on ASM are hard to assess in this context because only male mice were used without confirmation of PR expression. Additionally, there was the confounding presence of inflammation in sensitized mice (173).

The effects of oxytocin on airway responsiveness were recently reported (397). Oxytocin was found to increase cytosolic Ca^{2+} in human ASM cells and promote force generation and airway narrowing in murine tracheal rings and precision cut lung slices (397). Progesterone is known to inhibit oxytocin binding to the oxytocin receptor in uterine smooth muscle to maintain quiescence during pregnancy (398). As such, progesterone may prevent oxytocin-induced airway narrowing through competitive binding. Whether such an effect also contributes to airway tone remains to be determined.

The nongenomic effects of androgens on ASM have been examined recently (399, 400). Physiological concentrations of testosterone have been found to relax precontracted rabbit tracheal smooth muscle in a dose-dependent manner (399). Removal of the epithelium attenuates the testosterone response; however, inhibition of AR with flutamide does not alter the relaxant effects of testosterone. This group also used BSA-conjugated, membrane-impermeant testosterone and saw similar relaxant effects in epithelium-intact strips, but not upon removal of the epithelium (399). Similarly, in male guinea pig and bovine tracheal rings and strips, DHT produces substantial relaxation (400). These relaxations required pharmacological concentrations of the androgen, and the effects were epithelium independent, in contrast to the work of Koulomenta *et al.* (399). In agreement with previous studies, however, AR inhibition with flutamide did not affect the DHT-induced relaxation. These relaxant effects were attributed to voltage-gated Ca^{2+} channel antagonism (400). Again, these limited *in vitro* data do not provide any clear indication as to whether ASM is actually involved in mediating androgen effects on bronchodilation and stress the need for further examination of this topic. Here, as with progesterone, the *in vivo* animal data are confounding. As stated earlier, male C57BL/6 mice are more hyperresponsive than female mice (167) and appear to involve testosterone-mediated vagal effects on the airway. Castrated male mice have lower airway responsiveness to methacholine than intact males, similar to that of female mice. Exogenous testosterone therapy to castrated males or females increased responsiveness. Furthermore, bilateral cervical vagotomy abrogated methacholine responsiveness in intact male, but not female mice (177). These data suggest that airway responsiveness is controlled through different physiological mechanisms in male *vs.* female mice. Another study indicated that male mice treated with LPS developed more airway responsiveness than female

mice (167). This sex difference may be due to the effects of LPS on Th2 inflammatory cytokines (401) and their role in allergic airway disease (discussed in *Section IV.E*). Again, direct effects of testosterone or androgens on ASM *per se* are difficult to determine in these *in vivo* models and warrant further work.

In addition to altered ASM Ca^{2+} , increased smooth muscle mass and hyperplasia can also lead to increased contraction and force generation. In this regard, ASM hyperplasia is an important aspect of airway remodeling in diseases such as asthma. Here, work regarding sex steroid effects on ASM proliferation is lacking. Although some cues may be taken from steroid effects on vascular smooth muscle, other effects have been proven to differ between the airway and vasculature, and further investigation on this topic specifically in the airway is warranted. Pretreatment of ASM with physiological concentrations of testosterone, estradiol, and progesterone (1 nM–1 μM) has been reported to have no effect on thrombin-induced cell proliferation (402). In this study, EGF was also used to stimulate ASM proliferation, but sex steroid effects in combination with this mitogen were not evaluated. Given the potentiating effect of estrogen and EGF signaling in alveolar epithelium, these effects in smooth muscle should be considered further. Furthermore, the testosterone and estrogen precursor dehydroepiandrosterone (DHEA) significantly and in a dose-dependent manner inhibited rat tracheal smooth muscle proliferation in response to fetal bovine serum or platelet-derived growth factor. This was attributed to the prevention of DNA binding with activator protein-1 (403). The effects of DHEA metabolites were not examined in this study. Because DHEA is a precursor to sex steroids, this strengthens the argument for examining sex steroid effects on ASM proliferation. A very recent study has indeed found that physiological concentrations of both testosterone and 17 β -estradiol substantially enhance proliferation of rabbit ASM cells to comparable levels (404). The contrast in the data that sex steroids potentially induce bronchodilation (at least acutely) but with chronic exposure can worsen airway remodeling by enhancing cell proliferation should be noted, especially for future work in determining whether sex steroids (especially estrogens) are actually beneficial or detrimental to airway function. This question is only further complicated by the role of inflammation in airway diseases and sex steroid effects on the immune system (*Section IV.E*).

C. Lung parenchyma

Although PF is more common in males, mortality is actually higher in women, suggesting a need to investigate sex hormone effects on collagen and fibrotic deposition as well as enzymes responsible for the breakdown of the ex-

tracellular matrix, including matrix metalloproteinases (MMPs). Bleomycin treatment is the most common rodent model of PF. It induces similar histology in rodents as in humans developing PF after treatment with this chemotherapeutic drug in a dose-dependent fashion (405). One study (235) reported higher mortality with greater lung collagen deposition in female rats, an effect blunted substantially by ovariectomy, but enhanced by estradiol replacement. Furthermore, a positive correlation between estradiol dose and TGF- β mRNA suggested a possible mechanism for the observed increase in fibrosis (235). However, another study (406) found that male mice had a tendency toward greater fibrosis adjacent to the airways compared with female mice, demonstrating decreased lung function with increased static lung compliance. This decline in lung function subsequent to bleomycin treatment closely approximates that observed in humans. Castration of male mice protected them from decline in lung function; however, replacement with DHT exacerbated the decline. These data suggest that androgens are detrimental in bleomycin-induced lung fibrosis (406). Conversely, a protective effect for estrogens has been reported in rats where ovariectomy was found to exacerbate PF and ASM thickening, effects reversed by 2ME replacement (407). Taken together, these animal data indicate that female sex hormones and metabolites may be beneficial in retarding fibroblast growth and progression of pulmonary fibrosis. This has been verified by the few studies performed in lung fibroblasts *in vitro* where growth of human lung fibroblasts decreased upon exposure to 2ME (407), consistent with the *in vivo* data. Early studies in fetal lung fibroblasts showed that high concentrations of testosterone, estrogen, and progesterone (5 $\mu\text{g}/\text{ml}$) all decrease cell density in culture (408). In lung myofibroblasts, incubation with 20 nM estradiol inhibited proliferation via a nongenomic, rapid estradiol effect involving phosphorylation of the Raf1-ERK-MAPK pathway (409). Additionally, peroxisome proliferator-activated receptor- γ activation inhibited lung fibroblast proliferation in a bleomycin model of PF (410). DHEA, the testosterone and estradiol precursor, may be an agonist for peroxisome proliferator-activated receptor- γ . Thus, the presence of enzymes that metabolize this steroid in lung cells would greatly influence the effect of sex steroids on lung fibroblast proliferation. This concept remains to be verified.

Increased secretion of collagen, fibronectin, laminin, and other profibrotic proteins is not the sole mechanism responsible for development of PF. MMPs degrade collagen and extracellular matrix components, and changes in the activity of these endopeptidases can lead to various pathologies. Decreased MMP activity is implicated in preventing carcinoma metastasis and decreasing tumor an-

giogenesis, while exacerbating lung fibrosis. As such, effects of sex steroid on MMPs are equally important in balancing proper synthesis and degradation of fibrotic proteins in the lung. Again, data in a variety of tissues are conflicting regarding sex steroid effects on MMPs (and are not reviewed here), with almost no data relating to the lung. This represents an important aspect of sex steroid signaling in the lung that is ripe for research exploration, with the potential for modifying the process of diseases such as asthma and PF. Here, particular attention should be paid to MMP-2 and MMP-9, which are thought to be highly relevant to lung disease.

D. Pulmonary vasculature

Potential beneficial effects of estrogens on the systemic cardiovascular system with regard to hypertension would suggest a protective contribution of female sex steroids against the development or progression of PH. Indeed, in experimental animal models of PH, estradiol attenuates or inhibits development of the disease. However, this protective nature is not realized clinically, thus forming the basis for an “estrogen paradox” in PH. Furthermore, compared with the systemic vasculature, less is known on the role of other steroids (progesterone, testosterone, and DHEA) in the development or modulation of PH. Several excellent reviews regarding sex steroids in the pulmonary vasculature cover these topics (239, 240, 253, 256, 411). Here, we briefly summarize what is known, and not known, and highlight the need for further consideration of sex steroid signaling in regard to PH.

Estrogen has been the most extensively studied sex hormone in regard to the pulmonary vasculature, and most studies show a vasodilatory effect. The acute vasodilatory and protective effects of estradiol are present in both hypoxia- and monocrotaline-induced PH models (253). Female rats and pigs develop less severe PH when exposed to hypoxia compared with males (412, 413). Additionally, ovariectomy exacerbates hypoxia-induced PH, whereas estradiol replacement reverses this exacerbation (412, 414). In isolated rings of mouse pulmonary arteries, estradiol relaxes precontracted vessels from both males and females (415). Overall, these data indicate a protective role for estradiol in attenuating hypoxic pulmonary vasoconstriction (416). In monocrotaline-induced models of PH, female rats develop less severe disease than either male rats or ovariectomized female rats (417). Estradiol treatment in both males and ovariectomized female rats attenuates PH and approximates that of intact females (418, 419). These data would also be consistent with a protective role for estrogens. However, in other animal models of PH, estrogens were not found to be beneficial in preventing disease development, with female animals devel-

oping more severe PH than males animals (420, 421). These latter models employ occlusion and serotonin receptor overexpression in the development of PH. The occlusive model more closely approximates the human condition compared to hypoxia and monocrotaline-induced PH, whereas overexpression of serotonin may approximate endothelial dysfunction. The discrepancies in estrogen effects in these different models cannot be explained currently.

Estrogenic effects on the pulmonary vasculature are regulated in part by endothelial production of NO, which is mediated through both ER α and ER β (197, 256). This production appears to be in contrast to estradiol effects in the systemic circulation, where ER α is primarily involved (422, 423). In addition to rapid activation of eNOS in pulmonary endothelium, estrogen stimulates transcriptional up-regulation of NOS (424), along with down-regulation of the potent vasoconstrictor and smooth muscle mitogen endothelin-1 (257). Additionally, estrogen stimulates release of prostacyclin and also modulates vasodilatory enzymes, cyclooxygenase-1 and -2 (255, 425).

In addition to the endothelium, estradiol can directly affect vascular smooth muscle. Estradiol has been shown to acutely relax endothelium-denuded vascular smooth muscle from coronary arteries by opening the hyperpolarizing BK_{Ca} via a cGMP-dependent mechanism (312, 426). Other studies have shown estrogen effects on the voltage-sensitive L-type calcium channels (427, 428). Estrogen also inhibits the RhoA/Rho-kinase pathway (429), wherein RhoA translocation to the membrane induces smooth muscle contraction and proliferation, and thus inhibition of this pathway should enhance relaxation. Although these effects of estrogens have been extensively demonstrated in the systemic vasculature, far fewer studies have reported similar effects in the pulmonary circuit (430–432). Here, it is important to emphasize that despite overlap between vascular smooth muscle of systemic *vs.* pulmonary arteries in mechanisms of [Ca²⁺]_i and force regulation, their relative contribution and thus the overall effect of estrogens are different. Furthermore, regulatory mechanisms can differ substantially between pulmonary arteries of different species. Accordingly, it is important to determine the mechanisms of estrogen action in pulmonary arterial smooth muscle of species and the disease models of interest.

Prolonged exposure to estradiol can be pro-proliferative or proapoptotic, depending on concentration, and is tissue-specific. Estradiol stimulates proliferation of pulmonary artery smooth muscle cells, measured by thymidine incorporation, in a dose-dependent manner (418). However, others have shown that estradiol prevents proliferation of vascular smooth muscle cells derived from the systemic circulation, possibly by inhibiting the Rho-kinase

cascade. This effect has yet to be demonstrated in the pulmonary system (429). Additionally, estrogen can regulate serum levels of vascular endothelial growth factor, thereby indirectly affecting cell proliferation. These studies suggest that even if estrogens have a beneficial effect on pulmonary vascular tone, more chronic effects of estrogens may be detrimental in changing vascular structure.

Effects of progesterone on the pulmonary vasculature have not been studied as extensively as estrogen. Progesterone receptors are present in pulmonary endothelium of patients with PH (433); however, their function has not been examined. In isolated rat pulmonary arteries, progesterone was the most potent hormone vasodilator when compared with testosterone and estrogen in both male and female rats (434). The vasodilatory properties of progesterone have been found to involve inhibition of both voltage-gated and receptor-operated Ca²⁺ channels (435). In addition, treatment with the NOS inhibitor N^G-nitro-L-Arginine reduced progesterone-induced dilation, implying an endothelial-dependent NO mechanism (435). Progesterone has been found to blunt PH and right ventricular hypertrophy in monocrotaline-induced PH of ovariectomized female rats (436). In systemic vasculature, progesterone inhibits both endothelial cell and vascular smooth muscle cell proliferation (437–439). Whether a similar alleviating effect of chronic progesterone treatment occurs in the pulmonary vasculature is not known. Nonetheless, one study in patients with PH suggests efficacy of progesterone therapy in improving the PH phenotype where patients with more severe disease had lower serum progesterone levels and treatment with progesterone reduced pulmonary artery pressures (440). Considering the fact that estrogens and progesterone are simultaneously present in women, much more research is needed to understand the interactions between these sex steroids vis-à-vis pulmonary artery structure and function. This may be of particular relevance in conditions such as women patients with preexisting PH who become pregnant and are at high risk of exacerbated PH during and after pregnancy.

As with progesterone, testosterone has been largely understudied in the context of PH. Treatment of rat pulmonary arteries with testosterone results in an acute vasodilation more potent than estradiol in arteries of both male and female rats (434). Testosterone effects do not appear to involve prostaglandins or NO, in contrast to female sex steroids, and also do not involve the AR (441). However, similar to progesterone, testosterone inhibits both voltage-gated and receptor operated Ca²⁺ channels (441). Interestingly, testosterone effects are more pronounced in the human systemic circulation. An *in vitro* study of human pulmonary and mesenteric arteries showed that tes-

tosterone was only half as effective in producing vasodilation of pulmonary vessels as in systemic vasculature (442), whereas an *in vivo* study showed no vasodilatory effects of testosterone in the pulmonary circulation (443). DHEA, which has the highest circulating levels of all steroids, can produce vasodilation in a hypoxic model of PH in male rats (444). These vasodilatory properties have been proposed to involve BK_{Ca} channels (445) as well as the up-regulation of soluble guanylate cyclase (446). DHEA has been found to stimulate endothelial proliferation in bovine aortic endothelial cells (447). Because proliferation of endothelial cells and the formation of plexiform lesions exacerbate PH, the effects of DHEA on cellular proliferation as well as relaxation need further investigation. Additionally, previous studies were conducted solely in male animals, but the combination of DHEA with estrogen and progesterone should be considered, given the female predominance of PH and the fact that DHEA is a precursor to both testosterone and estrogen.

E. Immune cells and function

Allergic airway diseases involve a substantial inflammatory component with key players such as dendritic cells (DCs), CD4+ lymphocytes, regulatory T cells, and B lymphocytes. The complex interactions between these elements (along with an increasingly recognized contribution of structural cells within the lung) results in an overall increase in the level of Th2 cytokines such as IL-4, IL-5, and IL-13, leading to increased IgE levels (which may further enhance mast cell activation), eosinophilia, and AHR.

The classic thinking was that cytokines produced from respective cells of the T helper 1 (Th1) *vs.* Th2 response reciprocally inhibit each other, and disease manifestations represent a Th1/Th2 imbalance; however, as our understanding of interactions between immune cells and other components has improved, this concept has proven far more complex. Nonetheless, based on the idea of differential immune regulation, several studies have examined the relationship between autoimmune and allergic diseases in predominantly female cohorts, hypothesizing that individuals suffering from autoimmune diseases should be less susceptible to allergic diseases and vice versa. These studies have shown both positive (448, 449) and inverse (450–452) correlations between autoimmune and allergic diseases. Nonetheless, the overwhelming female predominance in autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis and a higher female:male ratio for allergic asthma in adults suggest that sex hormones likely play a role in modulating immunological inflammation. The reader is referred to several comprehensive reviews regarding sex hormones,

inflammation, and various immune cells (295, 453) for more detailed discussions. The following discussion is limited to exploring the role of immune cells relevant to lung diseases (where data are available) and the effects of sex steroids. Figure 2 schematically summarizes current knowledge of sex steroid effects on specific types of immune cells, and illustrates the likely overall complex effect of sex steroids on immune function. An important caveat, as described elegantly by Straub (453) is that sex steroid effects on immune cell and function is dependent on concentration, timing, duration, and context of exposure, which is difficult to control and/or evaluate in most clinical settings as well as in many experimental protocols.

1. T cells

The ability of immune cells to bind estrogens and androgens has been known for several decades. Both peripheral blood mononuclear cells (PBMCs) and thymic cells bind estradiol, whereas in humans, only thymic cells have been found to express binding sites for androgens. Although estrogens do modulate PBMC function, the underlying mechanisms are not clear. A recent study showed intracellular expression of both ER α (albeit truncated ER α -46 being abundant) and ER β in all types of lymphocytes including peripheral natural killer (NK) cells (454), with estrogen activation of intracellular signaling cascades. Although these limited data suggest that estrogens can modulate T cells, whether such effects are detrimental or alleviating for lung disease is not entirely clear.

The potential relevance of estrogen effects on T cells lies in exacerbation of asthma during the luteal phase and during pregnancy, when the high estrogen levels shift the female immune system toward a Th2-type response (455). Increased levels of IL-4 and IL-10 secretion (Th2-specific cytokines) occur during pregnancy and in *in vitro* experiments involving CD4+ T cells from humans (453). Additionally, TNF α (Th1-specific cytokine) secretion is attenuated at higher physiological concentrations of these estrogens (~40–400 nM) (456) [although another Th1 cytokine, interferon (IFN) γ is actually up-regulated]. These effects appear to be mediated via ER α (453, 457). Relevant to allergic airway disease, in NK cells, estrogens can promote IFN γ , which can up-regulate iNOS and cyclooxygenase-2, subsequently contributing to inflammation.

Progesterone effects on immune cells similar to those of estrogen have been documented, although the presence of PR in various immune cells is not clear (295, 458–461). Progesterone inhibits generation of Th1 type cells in both humans and mice (160, 462) and induces both IL-4 (160) and IL-10 (463) cytokine production while antagonizing nuclear factor- κ B activation, preventing TNF α action (464). Accordingly, in both pregnant and nonpregnant

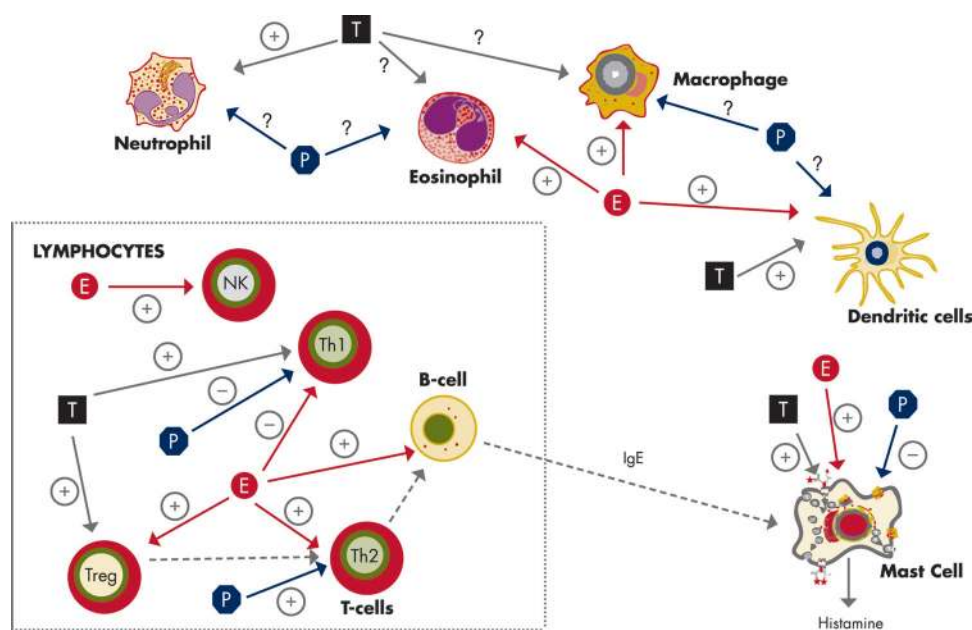
Figure 2.

Figure 2. Sex steroid effects on immune cells. Many lung diseases involve a substantial inflammatory component with key players such as DC and monocytes/macrophages that are particularly important in the initial response to antigens, CD4+ lymphocytes, regulatory T cells (Tregs), B lymphocytes, and other immune cells. In diseases such as asthma, mast cells, CD4+ T lymphocytes, and eosinophils are particularly important. Interactions between antigen-presenting cells and naive CD4 T lymphocytes induces the generation of polarized T lymphocytes characterized at Th1 (predominantly $\text{INF-}\gamma$ secreting) or Th2 (predominantly IL-4 secreting); Th2 polarized cells subsequently have additional downstream effects on B-cell antibody secretion that may further enhance mast cell activation. The effects of estrogen (E), progesterone (P), or testosterone (T) on different types of immune cells have been examined to varying and incomplete extents (see Section IV.E), mostly in the context of autoimmune diseases. This figure schematically summarizes current knowledge of sex steroid effects on specific types of immune cells that are particularly important in lung diseases and illustrates the likely overall complex effect of sex steroids on immune function. An important caveat not represented here (see Ref. 453) is that sex steroid effects on immune cell and function is dependent on concentration, timing and duration, and context of exposure.

women, progesterone and estrogen may synergize, or act additively, to alter the Th1/Th2 balance and thus contribute to asthma exacerbation and its sequelae. A potential confounder is the relative concentration of either sex steroid, as well as their additional effects on resident airway cells such as epithelium. There are currently no data on these topics, but they should clearly be a focus for future research in suppressing airway inflammation, especially in the pregnant asthmatic.

In contrast to female sex steroids, androgens favor a Th1 immune response. Testosterone increases $\text{INF}\gamma$ secretion in CD4+ T cells (251, 465, 466). Lymphocytes from males stimulated with phytohemagglutinin show higher secretion of Th1 cytokines $\text{INF}\gamma$ and IL-2 (163). DHEA, precursor to estrogen and testosterone, circulates as DHEA 3 β -sulfate and is higher in males than females. Data regarding DHEA effects on Th1/Th2 cytokine balance are contradictory, stemming in part from the fact that rodents (where many studies have been conducted) do not secrete DHEA from the gonads or the adrenals. Human T cells treated with DHEA followed by mitogens or im-

munogens secrete more IL-2 (Th1) (466, 467), a finding also reported for mice (468). DHEA attenuates allergic airway inflammation in mice, which may involve Th1 up-regulation by DHEA (469). Interestingly, male patients with asthma and atopic dermatitis have lower circulating levels of DHEA than healthy controls. PBMC isolated from asthmatics and treated with DHEA reduced both Th1 and Th2 cytokine responses compared with controls (470). These studies, however, were conducted primarily in males due to limited enrollment of women with AHR in the study (470).

Overall, these data, although not entirely consistent, suggest that female sex steroids may tilt the immune response toward Th2, whereas male sex steroids either tilt the response to Th1 or suppress inflammation, but more attention needs to be paid to the sex of the cells being studied and the sex ratio of patient populations involved in the trials. These differences are very important in understanding sex differences in allergic airway diseases as well as their exacerbation in the setting of altered hor-

monal status such as pregnancy, menopause, and even aging.

2. Dendritic cells

DCs are initiators of the immune response, and they induce T-cell responses to antigens and other signals after migration to lymphoid tissue. Additionally, macrophages residing in the lung may activate DC migration and maturation. Macrophages implicated in allergic asthma promote Th2 immune responses and are referred to as alternatively activated macrophages. Female mice sensitized with ovalbumin show increased numbers of migrating myeloid DC as well as lung macrophages compared with males (471). DC have been shown to express PR-A and both ERs, whereas macrophages derived from different regions express all steroid receptors (295). However, there are currently no data on how female sex steroids may modulate DC activation, migration, or their triggering of the immune response in lung diseases. In studies not involving lung, estrogen has been shown to promote functional DC formation from bone marrow precursors, which is blunted by ER modulators such as tamoxifen (472), and to promote DC stimulation of T cells (471). In human DCs, DHEA enhances expression of Th1 markers (473) and induces maturity of DCs, leading to Th1 immune response. These very limited data suggest that sex steroids modulate the very early steps in allergic airway disease. However, their role in prepubertal asthma (in both males and females) as well as at puberty, where the male:female ratio switches (or women develop asthma *de novo*), remains to be determined. It would also be of relevance to determine whether sex steroid modulation of DC function underlies sex differences in asthma.

3. Eosinophils

Eosinophilia is a hallmark of allergic asthma. Increased eosinophilia has been observed in ovalbumin sensitized BALB/c female mice compared to males, an effect prevented by depletion of female sex hormones before sensitization (474). Eosinophils are known to bind estradiol (475, 476); however, no PR or AR expression has been reported. In response to estrogen, eosinophils degranulate and increase in adhesiveness (157, 295, 476). Despite lacking binding sites, progesterone has been shown to increase eosinophilia-related AHR in ovalbumin-sensitized BALB/c mice (173). This may be attributed to the conversion of progesterone to estrogen. These limited data would suggest that female sex steroid-induced eosinophilia, in combination with steroid effects on T cells, would help to exacerbate the inflammatory process in favor of allergic asthma. Whether an opposing role for androgens is present in males is not known.

4. B cells

Activated Th2 cells influence B-cell activation, leading to increased serum levels of IgE. Here, female sex steroids may be important because testosterone stimulates mast cell degranulation (477); however, this effect appears to be indirect. It is well-documented that women have higher serum antibody concentrations compared to men. Furthermore, IgE binds mast cells causing degranulation and the release of histamine, IL-4, and IL-13. IgE levels in women have been shown to vary with hormonal status (141), which may play a part in premenstrual asthma exacerbations. Mast cells express both PR subtypes A and B as well as ER α and ER β , but do not express AR. Progesterone inhibits mast cell migration and proliferation and histamine release (295, 478, 479). Conversely, estrogen stimulates degranulation and increases histamine secretion in primed mast cells. This is a case where progesterone antagonizes the effects of estrogen in immune response. Furthermore, depending on the relative effects of estrogen *vs.* progesterone on T cells, eosinophils *vs.* B cells, the overall inflammatory response may vary, as may occur in premenstrual asthma or in pregnancy. There is currently no information examining these latter issues.

V. Clinical Implications of Sex Differences and Sex Steroid Signaling

In *Sections II, III and IV*, we presented an overview of some of the current knowledge on sex differences in lung structure and function across the life span in health and disease and the potential contribution of sex steroids to such differences. Although much remains to be established regarding the complex roles of sex steroids in the body in general, it is clear that sex steroid signaling in lung tissues should be a major focus of bench and clinical research. Basic understanding of the mechanisms of sex differences might not yet be complete, but the physiological importance of sex differences in lung structure and function have already been incorporated to a certain extent into clinical medicine via the use of sex-specific values for lung function corrected for size and age (*i.e.*, predicted values for common variables in clinical lung function tests). Unfortunately, the correction of these variables for age and size somewhat minimizes the intrinsic sex differences that exist in the lung at any given age, although the need for such corrections is understandable for the purpose of simplifying clinical interpretations (*e.g.*, comparing the growth of boys *vs.* girls in the setting of different activity levels, nutrition, and racial characteristics). An important caveat is that the statistical models used to produce these parameters, while well-fitting in the midrange of age and size, can

deviate from the actual situation because they do not always consider the variations in lung parameters at the onset of puberty and in the timeframes of somatic *vs.* lung growth, or the nonlinear relationship between the two (*i.e.*, dysanapsis). Furthermore, models in adults also work well in the midrange of age and size (*e.g.*, height), but not necessarily at the extremes. These caveats result in overestimation of predicted lung function at the higher extremes of age but underestimation in the young. Finally, a majority of the models do not consider race or ethnic origin in developing the nomograms. Nonetheless, such nomograms have helped incorporate sex differences in lung function in clinical medicine and form a platform to examine variations across individuals as well as changes with disease. Here, in terms of sex differences, studies are needed to include factors such as smoking histories (especially in adolescents, where the female airway may be more susceptible), reproductive history, family history (including genetic factors), and individual variations. Furthermore, age and physical characteristic limits of the predictive models and the need for their update are particularly relevant today where human life span has been substantially extended, and there is a growing worldwide problem of obesity along with its associated comorbidities.

Factors that contribute to decreased or impaired lung structure and function early in development can contribute to respiratory diseases throughout the life span. Accordingly, by virtue of their effects on fetal and postnatal lung development, sex steroids can contribute to fetal, childhood, pubertal, and adult lung in terms of development, growth, and aging (see *Section III.F* for respiratory diseases of the newborn, *Section III.A* for asthma, and *Section III.B* for potential contribution to COPD). Alterations in sex steroid levels can influence lung structural development with significant postnatal consequences, manifested by diseases such as RDS and BPD early in childhood (*Section III.F*), and asthma in childhood and beyond (*Section III.A*). Here, the effect of *in utero* and childhood tobacco smoke exposure may be significant. Clinical interventions to minimize maternal smoking during pregnancy and postpartum should be encouraged. Indeed, cigarette smoking is undoubtedly detrimental to the lung at all ages. This is only further emphasized by the limited, yet worrisome, data that there may be a link between *in utero* exposure to cigarette smoke components and adult diseases such as COPD (*Section III.B*). And even here, males and females appear to differ in susceptibility, as suggested by sex differences in age-related changes in lung function and the incidence and outcomes of diseases such as COPD and lung cancer. In this regard, the Lung Health Study (195) demonstrated the importance of early interventions in smoking to potentially reverse the declines in flow rates,

even in patients with COPD, with substantially greater effectiveness in women compared with men [albeit with some data suggesting greater difficulty for women in abstaining from smoking (480)]. Considering the ongoing problem of smoking in women (especially adolescents) worldwide, studies should focus on abstinence and smoking cessation programs in the school and social environments.

One clinically important area where sex differences and/or sex steroid effects are highly relevant is allergic inflammation of the upper and lower airways. As discussed in *Section III.B*, atopy can correlate with allergic rhinitis and asthma. Here, the greater incidence of atopy, allergic rhinitis, and asthma in prepubertal boys (140, 144) may be intrinsic or due in part to differences in environmental exposure between girls and boys reflecting outdoor and recreational activity patterns (145). However, after puberty, the complex effects of sex steroids come into play, such that some studies find that the prevalence of atopy can remain higher in adult men compared with women (146), whereas others find a switch in this pattern (148) and further changes with menopause (149) that are consistent with the peripubertal and perimenopausal changes in male:female ratio for asthma. Based on animal data, these patterns are consistent with a protective role for androgens but a proinflammatory effect of estrogens. However, it is important to recognize that interpretation is complicated by the opposing effect of estrogens on inflammation *per se* (*e.g.*, see *Section IV.E*) *vs.* bronchoconstriction and bronchodilation (see discussion on bronchial epithelium and ASM in *Section IV.B*), the two elements of diseases such as asthma. These dual effects may be particularly important in women during periods of large and/or sustained changes in sex steroid levels, as exemplified by changes in IgE levels with OC use (*Section III.B*) and by changes in asthma symptoms during the menstrual cycle (premenstrual asthma) and during pregnancy (*Section III.A*). Furthermore, estrogens and progesterone seem to sometimes produce similar effects (*e.g.*, eosinophil degranulation relevant to rhinitis) and could thus synergize in their overall effect, whereas in some instances the two sex steroids can have opposing effects (as in cell proliferation; *Section IV.B*). Accordingly, depending on their relative levels, receptor expression, and the context of exposure, interactions between these steroids may help explain premenstrual asthma and changes during pregnancy. Individual genetic and physiological variations in the asthma phenotype, responsiveness to sex steroids, and ongoing medications (*e.g.*, steroids or β -adrenoceptor agonists) may further color the overall symptomatology. Finally, sex steroid effects on airway cells may be further modulated by the presence and extent

of inflammation. These complex issues are yet to be systematically examined. Here, the clinical realm may be ideal due to the possibility of long-term monitoring and thus at least a correlative examination of sex steroids and airway diseases.

The clinical relevance of sex steroids also lies in several other lung diseases that show a female predominance, especially PH. Although PH is multifactorial in origin, its incidence early in women and its exacerbation with pregnancy suggest both sex differences and a role for female sex steroids. Clearly, understanding how these two aspects contribute to PH will be critical to management strategies, including informing patients regarding the risks of pregnancy.

In contrast to diseases such as asthma in adults and PH with a female predominance, fibrotic lung diseases such as PF have been considered more common in men (231). However, the protective *vs.* detrimental roles of male or female sex steroids in such diseases are not all clear (*Section III.D*). Given that mortality rates for PF are increasing more rapidly in women (233), further investigation on sex hormones and fibrosis is warranted.

The themes of sex differences and the role of sex steroids raise the question of whether (and how) our understanding of sex steroids can be used to clinically and physiologically develop biomarkers to diagnose, monitor, and perhaps treat lung diseases. Measurement of circulating hormonal levels would certainly be one option, with the obvious caveat that corrections will need to be made for age, comorbidities, and in the case of women of childbearing age menstrual or pregnancy status. Furthermore, circulating levels may not necessarily reflect those at the site of action, where local tissue hormone production may also play a role. Accordingly, it may be important to test for enzymes such as aromatase, especially in the lung, where locally derived hormones may be cell-specific. In addition to hormone levels, receptor expression and functionality within the tissue of interest (*e.g.*, bronchial epithelium, smooth muscle) will be key, especially considering the variety of receptors and the myriad of pathways they can activate. Finally, it will be important to determine whether genetic variations and polymorphisms of receptors (or downstream signaling pathways) play a role in sex differences in disease presentation and progression. For example, there is currently no single nucleotide polymorphism of ER associated with asthma, but an association with ER α gene variants has been reported (481). In NSCLC, ER β , aromatase, and EGFR expression may be predictive of survival (229, 230). These multimodal clinical testing approaches are currently available but have not been widely or systematically implemented for lung diseases.

The therapeutic potential of sex steroids in lung disease has been barely examined. Here, a simplistic approach of administering any of these steroids is not feasible due to their pleiotropic effects. Administration of estradiol and progesterone to neonates has been attempted for treatment of BPD but was found to be without benefit (270). Male sex is a risk factor for sepsis and organ failure after trauma where lung injury is a major complication, whereas estradiol is protective for women (379, 380, 383, 482–486). In animal studies, estrogen administration to males or ovariectomized females prevents hemorrhagic shock-induced sepsis and organ failure and also promotes wound healing (483, 487–489). Here, estrogen bolsters the immune response, which is depressed after trauma, leading to improved survival (490). These estrogen effects are observed acutely, with estrogen supplementation given 1 h before experimental injury providing protection (377, 453). Whether a similar therapy can be used in humans has never been tested, and the underlying mechanisms of estrogen protection are unknown. However, these initial studies underline growing interest in the use or inhibition of sex steroids in the clinical arena.

VI. Future Directions

There are intrinsic differences in the lungs of males *vs.* females; however, there is no doubt that right from the fetal stage, sex steroids (be they locally produced or circulating) have a profound influence on lung development and etiology of respiratory diseases. These influences change in extent, target, and effectiveness over the life span. Current understanding of sex hormone contribution to lung disease is summarized in Table 3; sex steroid influences on individual cells in the lung are schematically represented in Fig. 3. Although much remains to be established regarding sex steroid signaling, it is clear that their role in lung tissues should be a major focus of bench and clinical research. Here, the goal should be to better understand the mechanisms by which sex steroids (acting either genomically or nongenomically) modulate the structure and function of individual cell types within the lung under normal conditions and in the presence of other factors that contribute to lung diseases (*e.g.*, inflammation, infection, fibrosis). In many situations, estrogen and progesterone may interact to either enhance or suppress the other's action. A specific example of such cooperative *vs.* opposing interactions between sex steroids is their effects on airway cells, relevant to asthma. As schematically illustrated in Fig. 4 using current (but limited) knowledge in this area, effects of individual sex steroids on one cell type may be detrimental to airway structure/function (*e.g.*,

TABLE 3. Sex differences and sex steroid effects in lung diseases

Disease	Clinical sex difference ^a	Hormone effects				Ref.
		Clinical		Experimental		
		Alleviates	Exacerbates	Alleviates	Exacerbates	
Allergic rhinitis	Boys > girls Women ¹ ≠ men, controversial	T (Th1 shift)	E2 (Th2 shift), P4?			140, 495 146–148
Asthma	Boys > girls Women > men	T P4	T (dysanapsis) E2 (Th2 shift)	E2,P4	T	92–94, 100 43, 93, 98, 99, 101, 102, 122, 174, 175, 177, 396 185, 188
COPD						
Airway	Women > men		E2?			203
Emphysema	Men > women			P4		202, 360, 364
PH	Women > men		E2, P4	E2,P4	T	237, 238, 241–243, 256, 412, 414, 434, 441, 496
PF	Men > women			E2, T	E2,T	231, 232, 235, 406
CF	Boys≈girls ^b and men≈women				E2	273–275, 277, 278
Lung cancer						
Adenocarcinoma	Women > men	P4	T, E2			228, 355, 356, 375
Squamous cell	Men > women					

E2, Estradiol; P4, progesterone; T, testosterone; ?, clinical data is limited but suggestive.

^a Refers to what is observed clinically in human patients and to distinguish this difference from animal models of disease using male vs. female specimens, because they do not always correspond with what is observed clinically.

^b Although CF incidence shows little sex difference, girls are usually at higher risk for infection and mortality.

estrogen effects on inflammation or epithelial and ASM proliferation), whereas the same steroid could help alleviate symptoms via its effects on another cell type (e.g., reducing $[Ca^{2+}]_i$ in ASM cells and enhancing bronchodilation). Complicating these effects is the sometimes additive effect of other sex steroids (e.g., progesterone enhancement of inflammation; Fig. 4; also see Fig. 2). This has particular relevance to lung diseases in women where cyclical variations in the levels of these steroids as well as rapid and large changes with pregnancy may explain aspects of premenstrual asthma and pregnancy-associated exacerbations. Furthermore, such interactions may also be important in the pulmonary vasculature in the development of PH and in the interstitium relevant to PF. Even in males, age-related change in the relative levels of testosterone *vs.* estradiol may contribute individually or via interactions to preexisting lung diseases.

Basic research will help clarify the potential contribution of sex steroids to lung diseases in men *vs.* women and form the basis for the potential development of clinical therapies that target sex steroid signaling either in a stimulatory or an inhibitory fashion. Conversely, more clinical work will help drive the basic research to improve on current therapies and development of new ones. These areas of research present an exciting and timely opportunity to improve, enhance, and individualize the health care provided to women and men at different times in their life span.

In terms of mechanistic understanding of sex differences in lung structure or function, a critical stumbling

block is identifying the relative importance of genomic *vs.* nongenomic sex steroid signaling in lung tissue. Certainly, in the area of cancers or cardiovascular disease, it is the myriad of genomic effects of sex steroids, especially estrogens, that are considered important, with the rapid aspects of nongenomic effects being essentially of intellectual curiosity (with the understanding that nongenomic effects could lead to eventual changes at the nuclear level). With regard to the lung, we believe that both aspects of sex steroid signaling may be important, as is the case in the vasculature. Rapid, nongenomic effects may serve to modulate airway or vascular tone, alter the profile of immune cell-derived factors such as cytokines or chemokines, and by virtue of triggering a host of signaling pathways (e.g. calcium, MAPK, cAMP response element-binding) set the stage of further genomic effects. Genomic effects may occur on a background of sex steroid concentrations in both males and females, with variations in steroid levels (e.g., menstrual cycle, pregnancy, menopause) leading to initial nongenomic effects as well as enhancement of ongoing genomic effects. In this regard, responsiveness of different components of the lung (epithelium *vs.* smooth muscle *vs.* immune cells, for example) may differ, depending on the receptor profile, as well as modulating factors such as inflammation. These complex issues remain to be well-examined.

With the development of rapid automated assay systems, it may be possible to design and implement bio-

Figure 3.

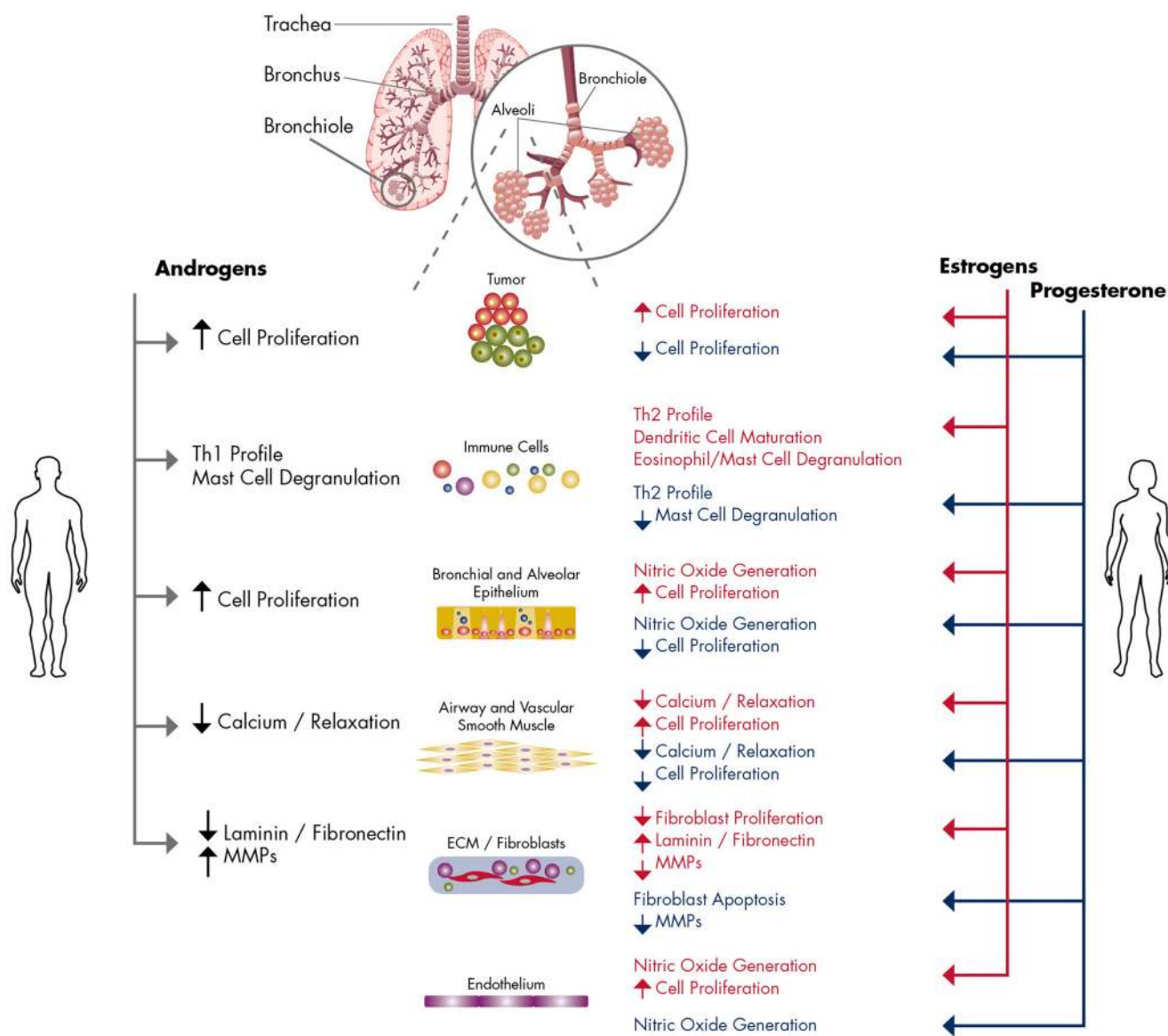


Figure 3. Summary of sex steroid effects on individual cell types of the lung. Many cell types are involved in the pathogenesis of lung disease. Therefore, individual effects of sex steroids must be integrated to achieve a better understanding of sex hormone effects in the manifestation, exacerbation, or alleviation of these diseases as a whole. Androgens, estrogen, and progesterone can have different effects at the cellular level that are concentration-, dose-, and time-dependent (which are described in the text). In general, progesterone and estrogen can either enhance or counterbalance their mutual effects in the female, whereas androgens generally have opposing effects, compared with estrogen, in the male.

marker assays for circulating and tissue hormone levels as well as receptor expression (or even their variants) and downstream signaling pathways. Responsiveness of cells and tissues from individuals to sex steroids, either alone or in combination, may then be examined to tailor therapies.

The lung is a unique organ for drug delivery, in that inhalation of pharmacological agents or other drug delivery systems can help achieve high local levels, but not necessarily high systemic levels. Accordingly, targeted delivery of agents that modulate sex steroid signaling to spe-

cific cell types is an attractive option. With advances in elucidating the genomic and nongenomic mechanisms of sex steroids on lung cell physiology, possible therapeutics involving these avenues are entirely possible, including modulation of eNOS-induced vaso- and bronchodilation by estrogen, immune response Th1/Th2 switching by androgens, calcium-induced smooth muscle relaxation via progesterone and testosterone, and inhibition of proliferation via ER antagonists or progesterone therapy. Indeed, modulation of estrogen signaling is being explored for PH

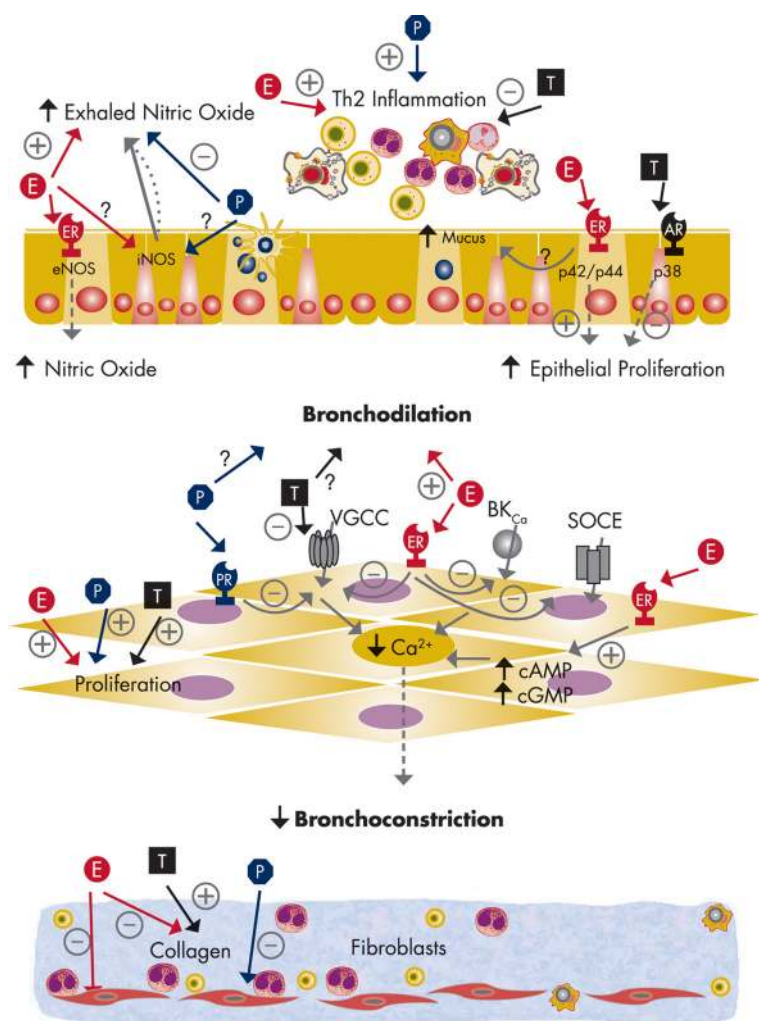
Figure 4.

Figure 4. Sex steroid effects in asthma. The complex effects of individual sex steroids on specific cell types of the lung, as well as cooperative vs. opposing effects of different sex steroids within a cell type, are illustrated in the case of asthma (with the caveat that current knowledge of sex steroid effects in asthma is limited). Asthma represents an inflammation-driven response of the airway to environmental or intrinsic allergens, pollutants, and other factors such as cigarette smoke. In response, the airway undergoes structural changes represented by epithelial thickening with increased mucous production, ASM proliferation, and hyperreactivity, as well as remodeling of the extracellular matrix. Sex steroids, especially estrogens, may on the one hand have detrimental effects on airway structure/function by enhancing inflammation or epithelial and ASM proliferation, while also alleviating symptoms via reducing $[Ca^{2+}]_i$ in ASM and thus enhancing bronchodilation. Complicating these effects is the sometimes additive, sometimes opposing, effect of other sex steroids. For example, progesterone may also enhance Th2 inflammation and cell proliferation but further bronchodilation. On the other hand, estrogen and progesterone have opposing effects on eNO. In general, testosterone has effects opposite to those of female sex steroids (but note the enhancement of ASM proliferation).

and lung cancer (208, 217, 239, 253) where therapies involving predominantly estrogen signaling have examined the role of this hormone in aberrant cellular proliferation.

The use of OC in attenuating premenstrual asthma exacerbations has had varying results, but it highlights the

potential of sex steroid pathways as an alternative therapeutic pathway beyond standard approaches such as glucocorticoids and β -adrenoceptor agonists. For example, in ongoing studies in human ASM, we have found that estrogens can potentiate β -adrenoceptor signaling in terms of reducing $[Ca^{2+}]_i$, thus facilitating bronchodilation (E. A. Townsend and Y. S. Prakash, unpublished observations). Although these preliminary explorations suggest a novel approach to bronchodilation, these data need to be verified, and a number of hurdles still need to be overcome before the potential for use in humans is even considered. Obviously, targeting of cells such as bronchial and alveolar epithelium, innervation, smooth muscle and pulmonary vasculature requires different approaches. Furthermore, due to the somewhat ubiquitous expression of sex steroid receptors within the lung, targeting of specific cell types relevant to a disease is not straightforward. However, novel technologies such as nanoparticles and promoter-driven expression systems may be helpful. Another important caveat is the complex interactions between sex steroids within a cell type (illustrated in Fig. 2 for immune cells, Fig. 3 across different cell types, and Fig. 4 as such interactions relate to asthma), as well as interactions with factors such as inflammatory mediators or infectious agents, which may limit efficacy of therapeutic agents or result in unintended effects. Furthermore, from studies in the cardiovascular system, it is clear that the effects of estrogens can vary by individual due to genetic variations in receptor structure/function, downstream signaling, and metabolism of the drug. Finally, studies in humans and animals on the vascular effects of estrogens suggest that timing of hormone treatment is important to achieve preventive and treatment goals. All of these important, complex issues remain to be examined in the lung.

VII. Conclusions

There is increasing evidence that sex differences in the lung exist at every level from intracellular signaling through whole organ structure and function. Sex differences occur not only under normal conditions, but also in a variety of lung diseases. Furthermore, sex differ-

ences arise as a result of intrinsic differences as well as complex effects of sex steroids. Modulation of lung structure and function by sex steroids appears to be not just a feature of the postpubertal period, but also occurs even during lung development and continues throughout the life span, albeit in different, complex, and incompletely understood ways. Here, the type of sex steroid, concentration, duration of exposure, and a myriad of factors appear to be involved in determining the overall effect on lung structure or function. Although much remains to be established regarding intrinsic sex differences or the effects of sex steroids, what is now clear is that, consistent with the 2001 IOM report on the importance of sex in human health (“Exploring Biological Contributions to Human Health—Does Sex Matter?” <http://www.nap.edu/openbook.php?isbn=0309072816>), sex is clearly a biological variable rather than an observational feature to consider both in bench and clinical research, as well as in medical practice. Here, the study and implementation of sex differences in the respiratory system clearly lags behind current knowledge in other nongonadal organ systems such as the heart, the systemic vasculature, the brain, and the endocrine system. However, there is much hope that the increasing attention to this topic, as evidenced by the numbers of publications in this area over the past 10 yr, will bring about a change in the research and clinical culture as it relates to lung disease.

Acknowledgments

Address all correspondence and requests for reprints to: Y. S. Prakash, M.D., Ph.D., Professor of Anesthesiology and Physiology, Mayo Clinic, 4-184 West Joseph Saint Marys Hospital, 200 First Street SW, Rochester, Minnesota 55905. E-mail: prakash.ys@mayo.edu.

This work was supported in part by the Mayo Graduate School (to E.A.T.), National Institutes of Health Grants HL088029 and HL056470 (to Y.S.P.), the Flight Attendants Medical Research Institute (to Y.S.P.), and the Mayo Clinic Departments of Physiology and Biomedical Engineering (to V.M.M. and Y.S.P.), Anesthesiology (to Y.S.P.), and Surgery (to V.M.M.).

Disclosure Summary: The authors have nothing to disclose.

References

1. Pérez-López FR, Larrad-Mur L, Kallen A, Chedraui P, Taylor HS 2010 Gender differences in cardiovascular disease: hormonal and biochemical influences. *Reprod Sci* 17:511–531
2. Konhilas JP 2010 What we know and do not know about sex and cardiac disease. *J Biomed Biotechnol* 2010: 562051
3. Arain FA, Kuniyoshi FH, Abdalrhim AD, Miller VM 2009 Sex/gender medicine. The biological basis for personalized care in cardiovascular medicine. *Circ J* 73: 1774–1782
4. Miller VM 2010 Sex-based differences in vascular function. *Womens Health (Lond Engl)* 6:737–752
5. Leuzzi C, Sangiorgi GM, Modena MG 2010 Gender-specific aspects in the clinical presentation of cardiovascular disease. *Fundam Clin Pharmacol* 24:711–717
6. Bigos KL, Pollock BG, Stankevich BA, Bies RR 2009 Sex differences in the pharmacokinetics and pharmacodynamics of antidepressants: an updated review. *Gend Med* 6:522–543
7. Beierle I, Meibohm B, Derendorf H 1999 Gender differences in pharmacokinetics and pharmacodynamics. *Int J Clin Pharmacol Ther* 37:529–547
8. Wang X, Magkos F, Mittendorfer B 2011 Sex differences in lipid and lipoprotein metabolism: it’s not just about sex hormones. *J Clin Endocrinol Metab* 96:885–893
9. Greenhill C 2011 Metabolism: sex differences in fatty liver disease. *Nat Rev Endocrinol* 7:313
10. McEwen BS 2010 Stress, sex, and neural adaptation to a changing environment: mechanisms of neuronal remodeling. *Ann NY Acad Sci* 1204(Suppl):E38–E59
11. Gillies GE, McArthur S 2010 Estrogen actions in the brain and the basis for differential action in men and women: a case for sex-specific medicines. *Pharmacol Rev* 62:155–198
12. Manson JE 2008 Prenatal exposure to sex steroid hormones and behavioral/cognitive outcomes. *Metabolism* 57(Suppl 2):S16–S21
13. Hines M 2010 Sex-related variation in human behavior and the brain. *Trends Cogn Sci* 14:448–456
14. Reddy DS 2010 Neurosteroids: endogenous role in the human brain and therapeutic potentials. In: Ivanka S, ed. *Progress in brain research*. New York: Elsevier; 113–137
15. Janicki SC, Schupf N 2010 Hormonal influences on cognition and risk for Alzheimer’s disease. *Curr Neurol Neurosci Rep* 10:359–366
16. Fry DL, Hyatt RE 1960 Pulmonary mechanics. A unified analysis of the relationship between pressure, volume and gasflow in the lungs of normal and diseased human subjects. *Am J Med* 29:672–689
17. Milic-Emili J, Koulouris NG, D’Angelo E 1999 Spirometry and flow-volume loops. *Eur Respir Mon* 12:20–32
18. Hayes Jr D, Kraman SS 2009 The physiologic basis of spirometry. *Respir Care* 54:1717–1726
19. Lomask M 2006 Further exploration of the Penh parameter. *Exp Toxicol Pathol* 57(Suppl 2):13–20
20. Hoymann HG 2007 Invasive and noninvasive lung function measurements in rodents. *J Pharmacol Toxicol Methods* 55:16–26
21. Hsia CC, Hyde DM, Ochs M, Weibel ER 2010 An official research policy statement of the American Thoracic Society/European Respiratory Society: standards for quantitative assessment of lung structure. *Am J Respir Crit Care Med* 181:394–418
22. Seaborn T, Khan PA, Cloutier M, Maltais F, Piedboeuf B 2007 Short-term response to tracheal occlusion during perinatal lung development in mice. *Exp Lung Res* 33:441–457
23. Espina V, Heiby M, Pierobon M, Liotta LA 2007 Laser

- capture microdissection technology. *Expert Rev Mol Diagn* 7:647–657
24. Espina V, Wulfkühle JD, Calvert VS, VanMeter A, Zhou W, Coukos G, Geho DH, Petricoin 3rd EF, Liotta LA 2006 Laser-capture microdissection. *Nat Protoc* 1:586–603
 25. Hutchinson J 1846 On the capacity of the lungs, and on the respiratory functions, with a view of establishing a precise and easy method of detecting disease by the spirometer. *Med Chir Trans* 29:137–252
 26. Ellis H 1894 Man and woman: a study of human secondary sexual characters. New York: Charles Scribner's Sons
 27. Ott O 1890 Les lois de la périodicité de la fonction physiologique dans l'organisme féminin. *Nouvelles archives d'obstétrique et de gynécologie* 502–506
 28. Becklake MR, Kauffmann F 1999 Gender differences in airway behaviour over the human life span. *Thorax* 54:1119–1138
 29. Dezateux C, Stocks J 1997 Lung development and early origins of childhood respiratory illness. *Br Med Bull* 53:40–57
 30. Thurlbeck WM 1975 Postnatal growth and development of the lung. *Am Rev Respir Dis* 111:803–844
 31. Thurlbeck WM, Angus GE 1975 Growth and aging of the normal human lung. *Chest* 67:3S–6S
 32. Hepper PG, Shannon EA, Dornan JC 1997 Sex differences in fetal mouth movements. *Lancet* 350:1820
 33. Boddy K, Dawes GS 1975 Fetal breathing. *Br Med Bull* 31:3–7
 34. Thurlbeck WM 1982 The pathology of small airways in chronic airflow limitation. *Eur J Respir Dis Suppl* 121:9–18
 35. Fleisher B, Kulovich MV, Hallman M, Gluck L 1985 Lung profile: sex differences in normal pregnancy. *Obstet Gynecol* 66:327–330
 36. Torday JS, Nielsen HC 1987 The sex difference in fetal lung surfactant production. *Exp Lung Res* 12:1–19
 37. Doershuk CF, Fisher BJ, Matthews LW 1974 Specific airway resistance from the perinatal period into adulthood. Alterations in childhood pulmonary disease. *Am Rev Respir Dis* 109:452–457
 38. Langston C, Kida K, Reed M, Thurlbeck WM 1984 Human lung growth in late gestation and in the neonate. *Am Rev Respir Dis* 129:607–613
 39. Seaborn T, Simard M, Provost PR, Piedboeuf B, Tremblay Y 2010 Sex hormone metabolism in lung development and maturation. *Trends Endocrinol Metab* 21:729–738
 40. Schwartz J, Katz SA, Fegley RW, Tockman MS 1988 Sex and race differences in the development of lung function. *Am Rev Respir Dis* 138:1415–1421
 41. Merkus PJ, ten Have-Opbroek AA, Quanjer PH 1996 Human lung growth: a review. *Pediatr Pulmonol* 21:383–397
 42. Hibbert M, Lannigan A, Raven J, Landau L, Phelan P 1995 Gender differences in lung growth. *Pediatr Pulmonol* 19:129–134
 43. Carey MA, Card JW, Voltz JW, Arbes Jr SJ, Germolec DR, Korach KS, Zeldin DC 2007 It's all about sex: gender, lung development and lung disease. *Trends Endocrinol Metab* 18:308–313
 44. Carey MA, Card JW, Voltz JW, Germolec DR, Korach KS, Zeldin DC 2007 The impact of sex and sex hormones on lung physiology and disease: lessons from animal studies. *Am J Physiol Lung Cell Mol Physiol* 293:L272–L278
 45. Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Kirmeyer S 2006 Births: final data for 2004. *Natl Vital Stat Rep* 55:1–101
 46. Stocks J, Quanjer PH 1995 Reference values for residual volume, functional residual capacity and total lung capacity. ATS workshop on lung volume measurements. Official Statement of The European Respiratory Society. *Eur Respir J* 8:492–506
 47. Thurlbeck WM 1982 Postnatal human lung growth. *Thorax* 37:564–571
 48. Pagtakhan RD, Bjelland JC, Landau LI, Loughlin G, Kaltenborn W, Seeley G, Taussig LM 1984 Sex differences in growth patterns of the airways and lung parenchyma in children. *J Appl Physiol* 56:1204–1210
 49. Hoffstein V 1986 Relationship between lung volume, maximal expiratory flow, forced expiratory volume in one second, and tracheal area in normal men and women. *Am Rev Respir Dis* 134:956–961
 50. Mead J 1980 Dysanapsis in normal lungs assessed by the relationship between maximal flow, static recoil, and vital capacity. *Am Rev Respir Dis* 121:339–342
 51. Taussig LM, Cota K, Kaltenborn W 1981 Different mechanical properties of the lung in boys and girls. *Am Rev Respir Dis* 123:640–643
 52. Schrader PC, Quanjer PH, Olivier IC 1988 Respiratory muscle force and ventilatory function in adolescents. *Eur Respir J* 1:368–375
 53. García-Río F, Pino-García JM, Serrano S, Racionero MA, Terreros-Caro JG, Alvarez-Sala R, Villasante C, Villamor J 1997 Comparison of helium dilution and plethysmographic lung volumes in pregnant women. *Eur Respir J* 10:2371–2375
 54. Gee JB, Packer BS, Millen JE, Robin ED 1967 Pulmonary mechanics during pregnancy. *J Clin Invest* 46:945–952
 55. Turner JM, Mead J, Wohl ME 1968 Elasticity of human lungs in relation to age. *J Appl Physiol* 25:664–671
 56. Gibson GJ, Pride NB, O'cain C, Quagliato R 1976 Sex and age differences in pulmonary mechanics in normal non-smoking subjects. *J Appl Physiol* 41:20–25
 57. Pelzer AM, Thomson ML 1966 Effect of age, sex, stature, and smoking habits on human airway conductance. *J Appl Physiol* 21:469–476
 58. Brody JS 1985 Cell-to-cell interactions in lung development. *Pediatr Pulmonol* 1:S42–S48
 59. James A 2005 Remodelling of airway smooth muscle in asthma: what sort do you have? *Clin Exp Allergy* 35:703–707
 60. Hirota S, Helli PB, Catalli A, Chew A, Janssen LJ 2005 Airway smooth muscle excitation-contraction coupling and airway hyperresponsiveness. *Can J Physiol Pharmacol* 83:725–732
 61. Joubert P, Hamid Q 2005 Role of airway smooth muscle in airway remodeling. *J Allergy Clin Immunol* 116:713–716
 62. Lazaar AL, Panettieri Jr RA 2005 Airway smooth muscle: a modulator of airway remodeling in asthma. *J Allergy Clin Immunol* 116:488–495; quiz 496
 63. Lukacs NW, Hogaboam CM, Kunkel SL 2005 Chemo-

- kines and their receptors in chronic pulmonary disease. *Curr Drug Targets Inflamm Allergy* 4:313–317
64. **Frieri M** 2005 New concepts in asthma pathophysiology. *Curr Allergy Asthma Rep* 5:339–340
 65. **Frieri M** 2005 Inflammatory issues in allergic rhinitis and asthma. *Allergy Asthma Proc* 26:163–169
 66. **Visness CM, London SJ, Daniels JL, Kaufman JS, Yeatts KB, Siega-Riz AM, Calatroni A, Zeldin DC** 2010 Association of childhood obesity with atopic and nonatopic asthma: results from the National Health and Nutrition Examination Survey 1999–2006. *J Asthma* 47:822–829
 67. **Michelson PH, Williams LW, Benjamin DK, Barnato AE** 2009 Obesity, inflammation, and asthma severity in childhood: data from the National Health and Nutrition Examination Survey 2001–2004. *Ann Allergy Asthma Immunol* 103:381–385
 68. **Joseph SP, Borrell LN, Shapiro A** 2010 Self-reported lifetime asthma and nativity status in U.S. children and adolescents: results from the National Health and Nutrition Examination Survey 1999–2004. *J Health Care Poor Underserved* 21:125–139
 69. **McHugh MK, Symanski E, Pompeii LA, Delclos GL** 2009 Prevalence of asthma among adult females and males in the United States: results from the National Health and Nutrition Examination Survey (NHANES), 2001–2004. *J Asthma* 46:759–766
 70. **McHugh MK, Symanski E, Pompeii LA, Delclos GL** 2010 Prevalence of asthma by industry and occupation in the U.S. working population. *Am J Ind Med* 53:463–475
 71. **Kwinta P, Pietrzyk JJ** 2010 Preterm birth and respiratory disease in later life. *Expert Rev Respir Med* 4:593–604
 72. **Greenough A** 2008 Long-term pulmonary outcome in the preterm infant. *Neonatology* 93:324–327
 73. **Nickerson BG** 1985 Bronchopulmonary dysplasia. Chronic pulmonary disease following neonatal respiratory failure. *Chest* 87:528–535
 74. **Doyle LW, Anderson PJ** 2009 Long-term outcomes of bronchopulmonary dysplasia. *Semin Fetal Neonatal Med* 14:391–395
 75. **Bhandari A, Panitch HB** 2006 Pulmonary outcomes in bronchopulmonary dysplasia. *Semin Perinatol* 30:219–226
 76. **Bhutani VK, Abbasi S** 1992 Long-term pulmonary consequences in survivors with bronchopulmonary dysplasia. *Clin Perinatol* 19:649–671
 77. **Maritz GS, Morley CJ, Harding R** 2005 Early developmental origins of impaired lung structure and function. *Early Hum Dev* 81:763–771
 78. **Kirkby J, Welsh L, Lum S, Fawke J, Rowell V, Thomas S, Marlow N, Stocks J** 2008 The EPICure study: comparison of pediatric spirometry in community and laboratory settings. *Pediatr Pulmonol* 43:1233–1241
 79. **Fawke J, Lum S, Kirkby J, Hennessy E, Marlow N, Rowell V, Thomas S, Stocks J** 2010 Lung function and respiratory symptoms at 11 years in children born extremely preterm: the EPICure study. *Am J Respir Crit Care Med* 182:237–245
 80. **Sly PD** 2011 The early origins of asthma: who is really at risk? *Curr Opin Allergy Clin Immunol* 11:24–28
 81. **Brown MA, Halonen M** 1999 Perinatal events in the development of asthma. *Curr Opin Pulm Med* 5:4–9
 82. **Pole JD, Mustard CA, To T, Beyene J, Allen AC** 2009 Antenatal steroid therapy for fetal lung maturation: is there an association with childhood asthma? *J Asthma* 46:47–52
 83. **Kumar R** 2008 Prenatal factors and the development of asthma. *Curr Opin Pediatr* 20:682–687
 84. **Prescott SL, Clifton V** 2009 Asthma and pregnancy: emerging evidence of epigenetic interactions in utero. *Curr Opin Allergy Clin Immunol* 9:417–426
 85. **Saglani S, Bush A** 2007 The early-life origins of asthma. *Curr Opin Allergy Clin Immunol* 7:83–90
 86. **Le Souëf PN** 2005 Can asthma be predicted from an early age? *Curr Opin Allergy Clin Immunol* 5:71–75
 87. **Boezen HM, Jansen DF, Postma DS** 2004 Sex and gender differences in lung development and their clinical significance. *Clin Chest Med* 25:237–245
 88. **Sennhauser FH, Kühni CE** 1995 Prevalence of respiratory symptoms in Swiss children: is bronchial asthma really more prevalent in boys? *Pediatr Pulmonol* 19:161–166
 89. **Siersted HC, Boldsen J, Hansen HS, Mostgaard G, Hyldebrandt N** 1998 Population based study of risk factors for underdiagnosis of asthma in adolescence: Odense School-child Study. *BMJ* 316:651–655; discussion 655–656
 90. **Kühni CE, Sennhauser FH** 1995 The Yentl syndrome in childhood asthma: risk factors for undertreatment in Swiss children. *Pediatr Pulmonol* 19:156–160
 91. **Orth-Gomer K** 2000 New light on the Yentl syndrome. *Eur Heart J* 21:874–875
 92. **Myers TR** 2000 Pediatric asthma epidemiology: incidence, morbidity, and mortality. *Respir Care Clin N Am* 6:1–14
 93. **Melgert BN, Ray A, Hylkema MN, Timens W, Postma DS** 2007 Are there reasons why adult asthma is more common in females? *Curr Allergy Asthma Rep* 7:143–150
 94. **Caracta CF** 2003 Gender differences in pulmonary disease. *Mt Sinai J Med* 70:215–224
 95. **Bjornson CL, Mitchell I** 2000 Gender differences in asthma in childhood and adolescence. *J Gend Specif Med* 3:57–61
 96. **Vink NM, Postma DS, Schouten JP, Rosmalen JG, Boezen HM** 2010 Gender differences in asthma development and remission during transition through puberty: the TRacking Adolescents' Individual Lives Survey (TRAILS) study. *J Allergy Clin Immunol* 126:498–504.e1–e6
 97. **Redline S, Gold D** 1994 Challenges in interpreting gender differences in asthma. *Am J Respir Crit Care Med* 150:1219–1221
 98. **Schatz M, Camargo Jr CA** 2003 The relationship of sex to asthma prevalence, health care utilization, and medications in a large managed care organization. *Ann Allergy Asthma Immunol* 91:553–558
 99. **Salam MT, Wenten M, Gilliland FD** 2006 Endogenous and exogenous sex steroid hormones and asthma and wheeze in young women. *J Allergy Clin Immunol* 117:1001–1007
 100. **Postma DS** 2007 Gender differences in asthma development and progression. *Gend Med* 4(Suppl B):S133–S146
 101. **Vrieze A, Postma DS, Kerstjens HA** 2003 Perimenstrual asthma: a syndrome without known cause or cure. *J Allergy Clin Immunol* 112:271–282
 102. **Chhabra SK** 2005 Premenstrual asthma. *Indian J Chest Dis Allied Sci* 47:109–116
 103. **Farha S, Asosingh K, Laskowski D, Hammel J, Dweik RA, Wiedemann HP, Erzurum SC** 2009 Effects of the menstrual

- cycle on lung function variables in women with asthma. *Am J Respir Crit Care Med* 180:304–310
104. Gibbs CJ, Coutts II, Lock R, Finnegan OC, White RJ 1984 Premenstrual exacerbation of asthma. *Thorax* 39:833–836
 105. Dratva J, Schindler C, Curjuric I, Stolz D, Macsali F, Gomez FR, Zemp E 2010 Perimenstrual increase in bronchial hyperreactivity in premenopausal women: results from the population-based SAPALDIA 2 cohort. *J Allergy Clin Immunol* 125:823–829
 106. Forbes L, Jarvis D, Burney P 1999 Do hormonal contraceptives influence asthma severity? *Eur Respir J* 14:1028–1033
 107. Tan KS, McFarlane LC, Lipworth BJ 1997 Loss of normal cyclical β_2 adrenoceptor regulation and increased premenstrual responsiveness to adenosine monophosphate in stable female asthmatic patients. *Thorax* 52:608–611
 108. Kharitonov SA, Logan-Sinclair RB, Busset CM, Shinebourne EA 1994 Peak expiratory nitric oxide differences in men and women: relation to the menstrual cycle. *Br Heart J* 72:243–245
 109. Oguzulgen IK, Turktas H, Erbas D 2002 Airway inflammation in premenstrual asthma. *J Asthma* 39:517–522
 110. Mandhane PJ, Hanna SE, Inman MD, Duncan JM, Greene JM, Wang HY, Sears MR 2009 Changes in exhaled nitric oxide related to estrogen and progesterone during the menstrual cycle. *Chest* 136:1301–1307
 111. Schatz M, Dombrowski MP 2009 Clinical practice. Asthma in pregnancy. *N Engl J Med* 360:1862–1869
 112. Kwon HL, Triche EW, Belanger K, Bracken MB 2006 The epidemiology of asthma during pregnancy: prevalence, diagnosis, and symptoms. *Immunol Allergy Clin North Am* 26:29–62
 113. Pereira A, Krieger BP 2004 Pulmonary complications of pregnancy. *Clin Chest Med* 25:299–310
 114. Schatz M, Dombrowski MP, Wise R, Thom EA, Landon M, Mabie W, Newman RB, Hauth JC, Lindheimer M, Caritis SN, Leveno KJ, Meis P, Miodovnik M, Wapner RJ, Paul RH, Varner MW, O'sullivan MJ, Thurnau GR, Conway D, McNellis D 2003 Asthma morbidity during pregnancy can be predicted by severity classification. *J Allergy Clin Immunol* 112:283–288
 115. Juniper EF, Daniel EE, Roberts RS, Kline PA, Hargreave FE, Newhouse MT 1989 Improvement in airway responsiveness and asthma severity during pregnancy. A prospective study. *Am Rev Respir Dis* 140:924–931
 116. Schatz M, Harden K, Forsythe A, Chilingar L, Hoffman C, Sperling W, Zeiger RS 1988 The course of asthma during pregnancy, post partum, and with successive pregnancies: a prospective analysis. *J Allergy Clin Immunol* 81:509–517
 117. Hermosa JL, Sánchez CB, Rubio MC, Mínguez MM, Walther JL 2010 Factors associated with the control of severe asthma. *J Asthma* 47:124–130
 118. Wenzel SE 2005 Severe asthma in adults. *Exp Lung Res* 31(Suppl 1):22
 119. Louis R 2009 Severe asthma: how can we differentiate phenotypes? *Swiss Med Wkly* 139:274–277
 120. Firoozi F, Ducharme FM, Lemièrre C, Beauchesne MF, Perreault S, Forget A, Blais L 2009 Effect of fetal gender on maternal asthma exacerbations in pregnant asthmatic women. *Respir Med* 103:144–151
 121. Slatkowska L, Jensen D, Davies GA, Wolfe LA 2006 Phasic menstrual cycle effects on the control of breathing in healthy women. *Respir Physiol Neurobiol* 154:379–388
 122. Foster PS, Goldie RG, Paterson JW 1983 Effect of steroids on β -adrenoceptor-mediated relaxation of pig bronchus. *Br J Pharmacol* 78:441–445
 123. Kohler PC, Trump DL 1986 Ectopic hormone syndromes. *Cancer Invest* 4:543–554
 124. Balzano G, Fuschillo S, Melillo G, Bonini S 2001 Asthma and sex hormones. *Allergy* 56:13–20
 125. Skobeloff EM, Spivey WH, St Clair SS, Schoffstall JM 1992 The influence of age and sex on asthma admissions. *JAMA* 268:3437–3440
 126. Bonner JR 1984 The epidemiology and natural history of asthma. *Clin Chest Med* 5:557–565
 127. Troisi RJ, Speizer FE, Willett WC, Trichopoulos D, Rosner B 1995 Menopause, postmenopausal estrogen preparations, and the risk of adult-onset asthma. A prospective cohort study. *Am J Respir Crit Care Med* 152:1183–1188
 128. Balzano G, Fuschillo S, De Angelis E, Gaudiosi C, Mancini A, Caputi M 2007 Persistent airway inflammation and high exacerbation rate in asthma that starts at menopause. *Monaldi Arch Chest Dis* 67:135–141
 129. Bellia V, Augugliaro G 2007 Asthma and menopause. *Monaldi Arch Chest Dis* 67:125–127
 130. Della Torre F, Cassani L, Segale M 1988 Asma ed orticaria nell'anziano: ruolo degli ormoni ipofiso-gonadici in menopausa. *Rassegna Geriatrica* 24:165–171
 131. Farina F, Colombi S, Cantone R, Pastore M, Centanni S, Galimberti M 1986 [Study of hypophyseal and gonadal hormones and cases of postmenopausal occurrence of bronchial asthma]. *Minerva Med* 77:243–247
 132. Ballard K 2002 Women's use of hormone replacement therapy for disease prevention; results of a community survey. *Br J Gen Pract* 52:835–837
 133. Hepburn MJ, Dooley DP, Morris MJ 2001 The effects of estrogen replacement therapy on airway function in postmenopausal, asthmatic women. *Arch Intern Med* 161:2717–2720
 134. Romieu I, Fabre A, Fournier A, Kauffmann F, Varraso R, Mesrine S, Leynaert B, Clavel-Chapelon F 2010 Postmenopausal hormone therapy and asthma onset in the E3N cohort. *Thorax* 65:292–297
 135. Barr RG, Camargo Jr CA 2004 Hormone replacement therapy and obstructive airway diseases. *Treat Respir Med* 3:1–7
 136. Canguven O, Albayrak S 2011 Do low testosterone levels contribute to the pathogenesis of asthma? *Med Hypotheses* 76:585–588
 137. Zannolli R, Morgese G 1997 Does puberty interfere with asthma? *Med Hypotheses* 48:27–32
 138. Guay A, Seftel AD, Traish A 2010 Hypogonadism in men with erectile dysfunction may be related to a host of chronic illnesses. *Int J Impot Res* 22:9–19
 139. Wulfsohn NL, Politzer WM, Henrico JS 1964 Testosterone therapy in bronchial asthma. *S Afr Med J* 38:170–172
 140. Sears MR, Burrows B, Flannery EM, Herbison GP, Holdaway MD 1993 Atopy in childhood. I. Gender and allergen

- related risks for development of hay fever and asthma. *Clin Exp Allergy* 23:941–948
141. Vellutini M, Viegi G, Parrini D, Pedreschi M, Baldacci S, Modena P, Biavati P, Simoni M, Carrozzi L, Giuntini C 1997 Serum immunoglobulins E are related to menstrual cycle. *Eur J Epidemiol* 13:931–935
 142. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG 1989 Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 320:271–277
 143. Liu AH, Jaramillo R, Sicherer SH, Wood RA, Bock SA, Burks AW, Massing M, Cohn RD, Zeldin DC 2010 National prevalence and risk factors for food allergy and relationship to asthma: results from the National Health and Nutrition Examination Survey 2005–2006. *J Allergy Clin Immunol* 126:798–806.e13
 144. Bertelsen RJ, Carlsen KC, Carlsen KH, Granum B, Doekes G, Håland G, Mowinckel P, Løvik M 2010 Childhood asthma and early life exposure to indoor allergens, endotoxin and $\beta(1,3)$ -glucans. *Clin Exp Allergy* 40:307–316
 145. Bousquet J, Fokkens W, Burney P, Durham SR, Bachert C, Akdis CA, Canonica GW, Dahlen SE, Zuberbier T, Bieber T, Bonini S, Bousquet PJ, Brozek JL, Cardell LO, Cramer R, Custovic A, Demoly P, van Wijk RG, Gjomarkaj M, Holland C, Howarth P, Humbert M, Johnston SL, Kauffmann F, Kowalski ML, Lambrecht B, Lehmann S, Leynaert B, Lodrup-Carlsen K, Mullol J, Niggemann B, Nizankowska-Mogilnicka E, Papadopoulos N, Passalacqua G, Schünemann HJ, Simon HU, Todo-Bom A, Toskala E, Valenta R, Wickman M, Zock JP 2008 Important research questions in allergy and related diseases: nonallergic rhinitis: a GA2LEN paper. *Allergy* 63:842–853
 146. Senthilselvan A, Rennie D, Chénard L, Burch LH, Babiuk L, Schwartz DA, Dosman JA 2008 Association of polymorphisms of toll-like receptor 4 with a reduced prevalence of hay fever and atopy. *Ann Allergy Asthma Immunol* 100:463–468
 147. Pausjensen ES, Cockcroft DW 2003 Sex differences in asthma, atopy, and airway hyperresponsiveness in a university population. *Ann Allergy Asthma Immunol* 91:34–37
 148. Fagan JK, Scheff PA, Hryhorczuk D, Ramakrishnan V, Ross M, Persky V 2001 Prevalence of asthma and other allergic diseases in an adolescent population: association with gender and race. *Ann Allergy Asthma Immunol* 86:177–184
 149. Siroux V, Curt F, Oryszczyn MP, Maccario J, Kauffmann F 2004 Role of gender and hormone-related events on IgE, atopy, and eosinophils in the Epidemiological Study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy. *J Allergy Clin Immunol* 114:491–498
 150. Bottema RW, Reijmerink NE, Koppelman GH, Kerkhof M, Postma DS 2005 Phenotype definition, age, and gender in the genetics of asthma and atopy. *Immunol Allergy Clin North Am* 25:621–639
 151. Barbee RA, Halonen M, Lebowitz M, Burrows B 1981 Distribution of IgE in a community population sample: correlations with age, sex, and allergen skin test reactivity. *J Allergy Clin Immunol* 68:106–111
 152. Harrop J, Chinn S, Verlato G, Olivieri M, Norbäck D, Wjst M, Janson C, Zock JP, Leynaert B, Gislason D, Ponzio M, Villani S, Carosso A, Svanes C, Heinrich J, Jarvis D 2007 Eczema, atopy and allergen exposure in adults: a population-based study. *Clin Exp Allergy* 37:526–535
 153. Anderson HR, Pottier AC, Strachan DP 1992 Asthma from birth to age 23: incidence and relation to prior and concurrent atopic disease. *Thorax* 47:537–542
 154. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ 1998 Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 53:91–95
 155. Taylor DR, Mandhane P, Greene JM, Hancox RJ, Filsell S, McLachlan CR, Williamson AJ, Cowan JO, Smith AD, Sears MR 2007 Factors affecting exhaled nitric oxide measurements: the effect of sex. *Respir Res* 8:82
 156. Barrenäs F, Andersson B, Cardell LO, Langston M, Mobini R, Perkins A, Soini J, Ståhl A, Benson M 2008 Gender differences in inflammatory proteins and pathways in seasonal allergic rhinitis. *Cytokine* 42:325–329
 157. Hamano N, Terada N, Maesako K, Numata T, Konno A 1998 Effect of sex hormones on eosinophilic inflammation in nasal mucosa. *Allergy Asthma Proc* 19:263–269
 158. Sekigawa I, Naito T, Hira K, Mitsuishi K, Ogasawara H, Hashimoto H, Ogawa H 2004 Possible mechanisms of gender bias in SLE: a new hypothesis involving a comparison of SLE with atopy. *Lupus* 13:217–222
 159. Talal N 1992 Sjogren's syndrome: historical overview and clinical spectrum of disease. *Rheum Dis Clin North Am* 18:507–515
 160. Piccinni MP, Giudizi MG, Biagiotti R, Beloni L, Giannarini L, Sampognaro S, Parronchi P, Manetti R, Annunziato F, Livi C 1995 Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. *J Immunol* 155:128–133
 161. Hamano N, Terada N, Maesako K, Hohki G, Ito T, Yamashita T, Konno A 1998 Effect of female hormones on the production of IL-4 and IL-13 from peripheral blood mononuclear cells. *Acta Otolaryngol Suppl* 537:27–31
 162. Araneo BA, Dowell T, Diegel M, Daynes RA 1991 Dihydrotestosterone exerts a depressive influence on the production of interleukin-4 (IL-4), IL-5, and γ -interferon, but not IL-2 by activated murine T cells. *Blood* 78:688–699
 163. Girón-González JA, Moral FJ, Elvira J, García-Gil D, Guerrero F, Gavilán I, Escobar L 2000 Consistent production of a higher TH1:TH2 cytokine ratio by stimulated T cells in men compared with women. *Eur J Endocrinol* 143:31–36
 164. Allen JE, Bischof RJ, Suec Chang HY, Hirota JA, Hirst SJ, Inman MD, Mitzner W, Sutherland TE 2009 Animal models of airway inflammation and airway smooth muscle remodelling in asthma. *Pulm Pharmacol Ther* 22:455–465
 165. Chang HY, Mitzner W 2007 Sex differences in mouse models of asthma. *Can J Physiol Pharmacol* 85:1226–1235
 166. Corteling R, Trifilieff A 2004 Gender comparison in a murine model of allergen-driven airway inflammation and the response to budesonide treatment. *BMC Pharmacol* 4:4

167. Card JW, Carey MA, Bradbury JA, DeGraff LM, Morgan DL, Moorman MP, Flake GP, Zeldin DC 2006 Gender differences in murine airway responsiveness and lipopolysaccharide-induced inflammation. *J Immunol* 177:621–630
168. Carey MA, Card JW, Bradbury JA, Moorman MP, Haykal-Coates N, Gavett SH, Graves JP, Walker VR, Flake GP, Voltz JW, Zhu D, Jacobs ER, Dakhama A, Larsen GL, Loader JE, Gelfand EW, Germolec DR, Korach KS, Zeldin DC 2007 Spontaneous airway hyperresponsiveness in estrogen receptor- α -deficient mice. *Am J Respir Crit Care Med* 175:126–135
169. Melgert BN, Postma DS, Kuipers I, Geerlings M, Luinge MA, van der Strate BW, Kerstjens HA, Timens W, Hylkema MN 2005 Female mice are more susceptible to the development of allergic airway inflammation than male mice. *Clin Exp Allergy* 35:1496–1503
170. Seymour BW, Friebertshauer KE, Peake JL, Pinkerton KE, Coffman RL, Gershwin LJ 2002 Gender differences in the allergic response of mice neonatally exposed to environmental tobacco smoke. *Dev Immunol* 9:47–54
171. Hayashi T, Adachi Y, Hasegawa K, Morimoto M 2003 Less sensitivity for late airway inflammation in males than females in BALB/c mice. *Scand J Immunol* 57:562–567
172. Ligeiro de Oliveira AP, Oliveira-Filho RM, da Silva ZL, Borelli P, Tavares de Lima W 2004 Regulation of allergic lung inflammation in rats: interaction between estradiol and corticosterone. *Neuroimmunomodulation* 11:20–27
173. Hellings PW, Vandekerckhove P, Claeys R, Billen J, Kasran A, Ceuppens JL 2003 Progesterone increases airway eosinophilia and hyper-responsiveness in a murine model of allergic asthma. *Clin Exp Allergy* 33:1457–1463
174. Dimitropoulou C, White RE, Ownby DR, Contreras JD 2005 Estrogen reduces carbachol-induced constriction of asthmatic airways by stimulating large-conductance voltage and calcium-dependent potassium channels. *Am J Respir Cell Mol Biol* 32:239–247
175. Matsubara S, Swasey CH, Loader JE, Dakhama A, Joetham A, Ohnishi H, Balhorn A, Miyahara N, Takeda K, Gelfand EW 2008 Estrogen determines sex differences in airway responsiveness after allergen exposure. *Am J Respir Cell Mol Biol* 38:501–508
176. Degano B, Prévost MC, Berger P, Molimard M, Pontier S, Rami J, Escamilla R 2001 Estradiol decreases the acetylcholine-elicited airway reactivity in ovariectomized rats through an increase in epithelial acetylcholinesterase activity. *Am J Respir Crit Care Med* 164:1849–1854
177. Card JW, Voltz JW, Ferguson CD, Carey MA, DeGraff LM, Peddada SD, Morgan DL, Zeldin DC 2007 Male sex hormones promote vagally mediated reflex airway responsiveness to cholinergic stimulation. *Am J Physiol Lung Cell Mol Physiol* 292:L908–L914
178. Moshhammer H, Neuberger M 2006 Smoking in pregnancy. *Environ Health Perspect* 114:A150
179. Moshhammer H, Hoek G, Luttmann-Gibson H, Neuberger MA, Antova T, Gehring U, Hrubá F, Pattenden S, Rudnai P, Slachtova H, Zlotkowska R, Fletcher T 2006 Parental smoking and lung function in children: an international study. *Am J Respir Crit Care Med* 173:1255–1263
180. Young S, Sherrill DL, Arnott J, Diepeveen D, LeSouëf PN, Landau LI 2000 Parental factors affecting respiratory function during the first year of life. *Pediatr Pulmonol* 29:331–340
181. Gilliland FD, Li YF, Peters JM 2001 Effects of maternal smoking during pregnancy and environmental tobacco smoke on asthma and wheezing in children. *Am J Respir Crit Care Med* 163:429–436
182. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ 1995 Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 332:133–138
183. Li YF, Gilliland FD, Berhane K, McConnell R, Gauderman WJ, Rappaport EB, Peters JM 2000 Effects of in utero and environmental tobacco smoke exposure on lung function in boys and girls with and without asthma. *Am J Respir Crit Care Med* 162:2097–2104
184. Celli BR 1995 Pathophysiology of chronic obstructive pulmonary disease. *Chest Surg Clin N Am* 5:623–634
185. Chapman KR 2004 Chronic obstructive pulmonary disease: are women more susceptible than men? *Clin Chest Med* 25:331–341
186. Pauwels R 2001 Global initiative for chronic obstructive lung diseases (GOLD): time to act. *Eur Respir J* 18:901–902
187. Davis RM, Novotny TE 1989 The epidemiology of cigarette smoking and its impact on chronic obstructive pulmonary disease. *Am Rev Respir Dis* 140:S82–S84
188. Varkey AB 2004 Chronic obstructive pulmonary disease in women: exploring gender differences. *Curr Opin Pulm Med* 10:98–103
189. Mannino DM, Homa DM, Akinbami LJ, Ford ES, Redd SC 2002 Chronic obstructive pulmonary disease surveillance—United States, 1971–2000. *MMWR Surveill Summ* 51:1–16
190. Gritz ER 1984 Cigarette smoking by adolescent females: implications for health and behavior. *Women Health* 9:103–115
191. Gold DR, Wang X, Wypij D, Speizer FE, Ware JH, Dockery DW 1996 Effects of cigarette smoking on lung function in adolescent boys and girls. *N Engl J Med* 335:931–937
192. Chapman KR, Tashkin DP, Pye DJ 2001 Gender bias in the diagnosis of COPD. *Chest* 119:1691–1695
193. Prescott E, Osler M, Andersen PK, Hein HO, Borch-Johnsen K, Lange P, Schnohr P, Vestbo J 1998 Mortality in women and men in relation to smoking. *Int J Epidemiol* 27:27–32
194. Carter R, Nicotra B, Huber G 1994 Differing effects of airway obstruction on physical work capacity and ventilation in men and women with COPD. *Chest* 106:1730–1739
195. Anthonisen NR, Connett JE, Kiley JP, Altose MD, Bailey WC, Buist AS, Conway Jr WA, Enright PL, Kanner RE, O'Hara P 1994 Effects of smoking intervention and the use of an inhaled anticholinergic bronchodilator on the rate of decline of FEV1. The Lung Health Study. *JAMA* 272:1497–1505
196. Gan WQ, Man SF, Postma DS, Camp P, Sin DD 2006 Female smokers beyond the perimenopausal period are at increased risk of chronic obstructive pulmonary disease: a systematic review and meta-analysis. *Respir Res* 7:52
197. Silverman EK, Weiss ST, Drazen JM, Chapman HA, Carey V, Campbell EJ, Denish P, Silverman RA, Celedon JC,

- Reilly JJ, Ginns LC, Speizer FE 2000 Gender-related differences in severe, early-onset chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 162:2152–2158
198. Biring SS, Brightling CE, Bradding P, Entwisle JJ, Vara DD, Grigg J, Wardlaw AJ, Pavord ID 2002 Clinical, radiologic, and induced sputum features of chronic obstructive pulmonary disease in nonsmokers: a descriptive study. *Am J Respir Crit Care Med* 166:1078–1083
 199. Prescott E, Lange P, Vestbo J 1997 Effect of gender on hospital admissions for asthma and prevalence of self-reported asthma: a prospective study based on a sample of the general population. Copenhagen City Heart Study Group. *Thorax* 52:287–289
 200. Miyamoto K, Aida A, Nishimura M, Aiba M, Kira S, Kawakami Y 1995 Gender effect on prognosis of patients receiving long-term home oxygen therapy. The Respiratory Failure Research Group in Japan. *Am J Respir Crit Care Med* 152:972–976
 201. Connett JE, Murray RP, Buist AS, Wise RA, Bailey WC, Lindgren PG, Owens GR 2003 Changes in smoking status affect women more than men: results of the Lung Health Study. *Am J Epidemiol* 157:973–979
 202. Camp PG, Coxson HO, Levy RD, Pillai SG, Anderson W, Vestbo J, Kennedy SM, Silverman EK, Lomas DA, Paré PD 2009 Sex differences in emphysema and airway disease in smokers. *Chest* 136:1480–1488
 203. Martinez FJ, Curtis JL, Sciruba F, Mumford J, Giardino ND, Weinmann G, Kazerooni E, Murray S, Criner GJ, Sin DD, Hogg J, Ries AL, Han M, Fishman AP, Make B, Hoffman EA, Mohsenifar Z, Wise R 2007 Sex differences in severe pulmonary emphysema. *Am J Respir Crit Care Med* 176:243–252
 204. Carlson CL, Cushman M, Enright PL, Cauley JA, Newman AB 2001 Hormone replacement therapy is associated with higher FEV1 in elderly women. *Am J Respir Crit Care Med* 163:423–428
 205. Shafer D, Albain K 2009 Lung cancer outcomes in women. *Semin Oncol* 36:532–541
 206. Egleston BL, Meireles SI, Flieder DB, Clapper ML 2009 Population-based trends in lung cancer incidence in women. *Semin Oncol* 36:506–515
 207. Ramchandran K, Patel JD 2009 Sex differences in susceptibility to carcinogens. *Semin Oncol* 36:516–523
 208. Siegfried JM, Hershberger PA, Stabile LP 2009 Estrogen receptor signaling in lung cancer. *Semin Oncol* 36:524–531
 209. Chlebowski RT 2009 Menopausal hormone therapy, hormone receptor status, and lung cancer in women. *Semin Oncol* 36:566–571
 210. Harichand-Herdt S, Ramalingam SS 2009 Gender-associated differences in lung cancer: clinical characteristics and treatment outcomes in women. *Semin Oncol* 36:572–580
 211. Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ 2003 Cancer statistics, 2003. *CA Cancer J Clin* 53:5–26
 212. Rivera MP, Stover DE 2004 Gender and lung cancer. *Clin Chest Med* 25:391–400
 213. Fiore MC, Novotny TE, Pierce JP, Hatziaandreu EJ, Patel KM, Davis RM 1989 Trends in cigarette smoking in the United States. The changing influence of gender and race. *JAMA* 261:49–55
 214. Henschke CI, Yip R, Miettinen OS 2006 Women's susceptibility to tobacco carcinogens and survival after diagnosis of lung cancer. *JAMA* 296:180–184
 215. Risch HA, Howe GR, Jain M, Burch JD, Holowaty EJ, Miller AB 1993 Are female smokers at higher risk for lung cancer than male smokers? A case-control analysis by histologic type. *Am J Epidemiol* 138:281–293
 216. Kabat GC 1996 Aspects of the epidemiology of lung cancer in smokers and nonsmokers in the United States. *Lung Cancer* 15:1–20
 217. Belani CP, Marts S, Schiller J, Socinski MA 2007 Women and lung cancer: epidemiology, tumor biology, and emerging trends in clinical research. *Lung Cancer* 55:15–23
 218. Ferguson MK, Wang J, Hoffman PC, Haraf DJ, Olak J, Masters GA, Vokes EE 2000 Sex-associated differences in survival of patients undergoing resection for lung cancer. *Ann Thorac Surg* 69:245–249; discussion 249–250
 219. Siegfried JM 2001 Women and lung cancer: does oestrogen play a role? *Lancet Oncol* 2:506–513
 220. Weiss JM, Lacey Jr JV, Shu XO, Ji BT, Hou L, Yang G, Li H, Rothman N, Blair A, Gao YT, Chow WH, Zheng W 2008 Menstrual and reproductive factors in association with lung cancer in female lifetime nonsmokers. *Am J Epidemiol* 168:1319–1325
 221. Taioli E, Wynder EL 1994 Re: Endocrine factors and adenocarcinoma of the lung in women. *J Natl Cancer Inst* 86:869–870
 222. Liu Y, Inoue M, Sobue T, Tsugane S 2005 Reproductive factors, hormone use and the risk of lung cancer among middle-aged never-smoking Japanese women: a large-scale population-based cohort study. *Int J Cancer* 117:662–666
 223. Schabath MB, Wu X, Vassilopoulou-Sellin R, Vaporciyan AA, Spitz MR 2004 Hormone replacement therapy and lung cancer risk: a case-control analysis. *Clin Cancer Res* 10:113–123
 224. Baik CS, Strauss GM, Speizer FE, Feskanich D 2010 Reproductive factors, hormone use, and risk for lung cancer in postmenopausal women, the Nurses' Health Study. *Cancer Epidemiol Biomarkers Prev* 19:2525–2533
 225. Ramnath N, Menezes RJ, Loewen G, Dua P, Eid F, Alkhaddo J, Paganelli G, Natarajan N, Reid ME 2007 Hormone replacement therapy as a risk factor for non-small cell lung cancer: results of a case-control study. *Oncology* 73:305–310
 226. Ganti AK, Sahnoun AE, Panwalkar AW, Tendulkar KK, Potti A 2006 Hormone replacement therapy is associated with decreased survival in women with lung cancer. *J Clin Oncol* 24:59–63
 227. Albain KS, Crowley JJ, LeBlanc M, Livingston RB 1991 Survival determinants in extensive-stage non-small-cell lung cancer: the Southwest Oncology Group experience. *J Clin Oncol* 9:1618–1626
 228. Albain KS, Belani CP, Bonomi P, O'Byrne KJ, Schiller JH, Socinski M 2006 PIONEER: a phase III randomized trial of paclitaxel poliglumex versus paclitaxel in chemotherapy-naïve women with advanced-stage non-small-cell lung cancer and performance status of 2. *Clin Lung Cancer* 7:417–419
 229. Sun HB, Zheng Y, Ou W, Fang Q, Li P, Ye X, Zhang BB, Yang H, Wang SY 2011 Association between hormone receptor expression and epidermal growth factor receptor

- mutation in patients operated on for non-small cell lung cancer. *Ann Thorac Surg* 91:1562–1567
230. Mah V, Marquez D, Alavi M, Maresh EL, Zhang L, Yoon N, Horvath S, Bagryanova L, Fishbein MC, Chia D, Pietras R, Goodglick L 19 April 2011 Expression levels of estrogen receptor β in conjunction with aromatase predict survival in non-small cell lung cancer. *Lung Cancer* doi:10.1016/j.lungcan.2011.03.009
 231. Meltzer EB, Noble PW 2008 Idiopathic pulmonary fibrosis. *Orphanet J Rare Dis* 3:8
 232. Gribbin J, Hubbard RB, Le Jeune I, Smith CJ, West J, Tata LJ 2006 Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. *Thorax* 61:980–985
 233. Olson AL, Swigris JJ, Lezotte DC, Norris JM, Wilson CG, Brown KK 2007 Mortality from pulmonary fibrosis increased in the United States from 1992 to 2003. *Am J Respir Crit Care Med* 176:277–284
 234. March TH, Wilder JA, Esparza DC, Cossey PY, Blair LF, Herrera LK, McDonald JD, Campen MJ, Mauderly JL, Seagrave J 2006 Modulators of cigarette smoke-induced pulmonary emphysema in A/J mice. *Toxicol Sci* 92:545–559
 235. Gharaee-Kermani M, Hatano K, Nozaki Y, Phan SH 2005 Gender-based differences in bleomycin-induced pulmonary fibrosis. *Am J Pathol* 166:1593–1606
 236. Lekgabe ED, Royce SG, Hewitson TD, Tang ML, Zhao C, Moore XL, Tregear GW, Bathgate RA, Du XJ, Samuel CS 2006 The effects of relaxin and estrogen deficiency on collagen deposition and hypertrophy of nonreproductive organs. *Endocrinology* 147:5575–5583
 237. Runo JR, Loyd JE 2003 Primary pulmonary hypertension. *Lancet* 361:1533–1544
 238. Robles AM, Shure D 2004 Gender issues in pulmonary vascular disease. *Clin Chest Med* 25:373–377
 239. Pugh ME, Hemnes AR 2010 Development of pulmonary arterial hypertension in women: interplay of sex hormones and pulmonary vascular disease. *Womens Health (Lond Engl)* 6:285–296
 240. Sakao S, Tanabe N, Tatsumi K 2010 The estrogen paradox in pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol* 299:L435–L438
 241. Badesch DB, Raskob GE, Elliott CG, Krichman AM, Farber HW, Frost AE, Barst RJ, Benza RL, Liou TG, Turner M, Giles S, Feldkircher K, Miller DP, McGoon MD 2010 Pulmonary arterial hypertension: baseline characteristics from the REVEAL Registry. *Chest* 137:376–387
 242. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, Yaici A, Weitzenblum E, Cordier JF, Chabot F, Dromer C, Pison C, Reynaud-Gaubert M, Haloun A, Laurent M, Hachulla E, Simonneau G 2006 Pulmonary arterial hypertension in France: results from a national registry. *Am J Respir Crit Care Med* 173:1023–1030
 243. Rich S, Dantzker DR, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Koerner SK, Levy PC, Reid LM, Vreim CE, Williams GW 1987 Primary pulmonary hypertension. A national prospective study. *Ann Intern Med* 107:216–223
 244. Taraseviciute A, Voelkel NF 2006 Severe pulmonary hypertension in postmenopausal obese women. *Eur J Med Res* 11:198–202
 245. Kay JM, Smith P, Heath D 1971 Aminorex and the pulmonary circulation. *Thorax* 26:262–270
 246. Abenheim L, Moride Y, Brenot F, Rich S, Benichou J, Kurz X, Higenbottam T, Oakley C, Wouters E, Aubier M, Simonneau G, Bégaud B 1996 Appetite-suppressant drugs and the risk of primary pulmonary hypertension. International Primary Pulmonary Hypertension Study Group. *N Engl J Med* 335:609–616
 247. Masi AT 1976 Editorial: pulmonary hypertension and oral contraceptive usage. *Chest* 69:451–453
 248. Beretta L, Caronni M, Origgi L, Ponti A, Santaniello A, Scorza R 2006 Hormone replacement therapy may prevent the development of isolated pulmonary hypertension in patients with systemic sclerosis and limited cutaneous involvement. *Scand J Rheumatol* 35:468–471
 249. Opravil M, Pechère M, Speich R, Joller-Jemelka HI, Jenni R, Russi EW, Hirschel B, Lüthy R 1997 HIV-associated primary pulmonary hypertension. A case control study. Swiss HIV Cohort Study. *Am J Respir Crit Care Med* 155:990–995
 250. Cutolo M, Capellino S, Sulli A, Seriola B, Secchi ME, Villaggio B, Straub RH 2006 Estrogens and autoimmune diseases. *Ann NY Acad Sci* 1089:538–547
 251. Martin JT 2000 Sexual dimorphism in immune function: the role of prenatal exposure to androgens and estrogens. *Eur J Pharmacol* 405:251–261
 252. Rubtsov AV, Rubtsova K, Kappler JW, Marrack P 2010 Genetic and hormonal factors in female-biased autoimmunity. *Autoimmun Rev* 9:494–498
 253. Tofovic SP 2010 Estrogens and development of pulmonary hypertension: interaction of estradiol metabolism and pulmonary vascular disease. *J Cardiovasc Pharmacol* 56:696–708
 254. Smith AM, Jones RD, Channer KS 2006 The influence of sex hormones on pulmonary vascular reactivity: possible vasodilator therapies for the treatment of pulmonary hypertension. *Curr Vasc Pharmacol* 4:9–15
 255. Sherman TS, Chambliss KL, Gibson LL, Pace MC, Mendelsohn ME, Pfister SL, Shaul PW 2002 Estrogen acutely activates prostacyclin synthesis in ovine fetal pulmonary artery endothelium. *Am J Respir Cell Mol Biol* 26:610–616
 256. Lahm T, Crisostomo PR, Markel TA, Wang M, Weil BR, Novotny NM, Meldrum DR 2008 The effects of estrogen on pulmonary artery vasoreactivity and hypoxic pulmonary vasoconstriction: potential new clinical implications for an old hormone. *Crit Care Med* 36:2174–2183
 257. Earley S, Resta TC 2002 Estradiol attenuates hypoxia-induced pulmonary endothelin-1 gene expression. *Am J Physiol Lung Cell Mol Physiol* 283:L86–L93
 258. Sweet DG, Carnielli V, Greisen G, Hallman M, Ozek E, Plavka R, Saugstad OD, Simeoni U, Speer CP, Halliday HL 2010 European consensus guidelines on the management of neonatal respiratory distress syndrome in preterm infants—2010 update. *Neonatology* 97:402–417
 259. Farrell PM, Avery ME 1975 Hyaline membrane disease. *Am Rev Respir Dis* 111:657–688
 260. Ingemarsson I 2003 Gender aspects of preterm birth. *BJOG* 110(Suppl 20):34–38
 261. Khoury MJ, Marks JS, McCarthy BJ, Zaro SM 1985 Factors affecting the sex differential in neonatal mortality: the

- role of respiratory distress syndrome. *Am J Obstet Gynecol* 151:777–782
262. Heljić S, Maksić H, Kalkan I, Krdalić B 2009 The effects of antenatal corticosteroids and surfactant replacement on neonatal respiratory distress syndrome. *Bosn J Basic Med Sci* 9:225–228
 263. Jobe AH, Bancalari E 2001 Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 163:1723–1729
 264. Roberge S, Lacasse Y, Tapp S, Tremblay Y, Kari A, Liu J, Fekih M, Qublan HS, Amorim MM, Bujold E 2011 Role of fetal sex in the outcome of antenatal glucocorticoid treatment to prevent respiratory distress syndrome: systematic review and meta-analysis. *J Obstet Gynaecol Can* 33:216–226
 265. Northway Jr WH, Rosan RC, Porter DY 1967 Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. *N Engl J Med* 276:357–368
 266. Fanaroff AA, Hack M, Walsh MC 2003 The NICHD neonatal research network: changes in practice and outcomes during the first 15 years. *Semin Perinatol* 27:281–287
 267. Bhandari A, Bhandari V 2009 Pitfalls, problems, and progress in bronchopulmonary dysplasia. *Pediatrics* 123:1562–1573
 268. Popova AP, Bozyk PD, Bentley JK, Linn MJ, Goldsmith AM, Schumacher RE, Weiner GM, Filbrun AG, Hershenson MB 2010 Isolation of tracheal aspirate mesenchymal stromal cells predicts bronchopulmonary dysplasia. *Pediatrics* 126:e1127–e1133
 269. Gortner L, Misselwitz B, Milligan D, Zeitlin J, Kollée L, Boerch K, Agostino R, Van Reempts P, Chabernaud JL, Bréart G, Papiernik E, Jarreau PH, Carrapato M, Gadziowski J, Draper E 2011 Rates of bronchopulmonary dysplasia in very preterm neonates in Europe: results from the MOSAIC cohort. *Neonatology* 99:112–117
 270. Trotter A, Maier L, Kron M, Pohlandt F 2007 Effect of oestradiol and progesterone replacement on bronchopulmonary dysplasia in extremely preterm infants. *Arch Dis Child Fetal Neonatal Ed* 92:F94–F98
 271. Thome UH, Bischoff A, Maier L, Pohlandt F, Trotter A 2006 Amiloride-sensitive nasal potential difference is not changed by estradiol and progesterone replacement but relates to BPD or death in a randomized trial on preterm infants. *Pediatr Res* 60:619–623
 272. McCurnin DC, Pierce RA, Willis BC, Chang LY, Yoder BA, Yuhanna IS, Ballard PL, Clyman RI, Waleh N, Maniscalco W, Crapo JD, Grubb PH, Shaul PW 2009 Postnatal estradiol up-regulates lung nitric oxide synthases and improves lung function in bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 179:492–500
 273. Gurwitz D, Corey M, Francis PW, Crozier D, Levison H 1979 Perspectives in cystic fibrosis. *Pediatr Clin North Am* 26:603–615
 274. Davis PB 1999 The gender gap in cystic fibrosis survival. *J Gend Specif Med* 2:47–51
 275. Rosenfeld M, Davis R, FitzSimmons S, Pepe M, Ramsey B 1997 Gender gap in cystic fibrosis mortality. *Am J Epidemiol* 145:794–803
 276. Demko CA, Byard PJ, Davis PB 1995 Gender differences in cystic fibrosis: *Pseudomonas aeruginosa* infection. *J Clin Epidemiol* 48:1041–1049
 277. Johannesson M, Lúdvíksdóttir D, Janson C 2000 Lung function changes in relation to menstrual cycle in females with cystic fibrosis. *Respir Med* 94:1043–1046
 278. Coakley RD, Sun H, Clunes LA, Rasmussen JE, Stackhouse JR, Okada SF, Fricks I, Young SL, Tarran R 2008 17β -Estradiol inhibits Ca^{2+} -dependent homeostasis of airway surface liquid volume in human cystic fibrosis airway epithelia. *J Clin Invest* 118:4025–4035
 279. Ghayee HK, Auchus RJ 2007 Basic concepts and recent developments in human steroid hormone biosynthesis. *Rev Endocr Metab Disord* 8:289–300
 280. Payne AH, Hales DB 2004 Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. *Endocr Rev* 25:947–970
 281. Luu-The V, Labrie F 2010 The intracrine sex steroid biosynthesis pathways. *Prog Brain Res* 181:177–192
 282. Labrie F, Simard J, Luu-The V, Trudel C, Martel C, Labrie C, Zhao HF, Rheume E, Couet J, Breton N 1991 Expression of 3β -hydroxysteroid dehydrogenase/ Δ delta 5- Δ 4 isomerase (3β -HSD) and 17β -hydroxysteroid dehydrogenase (17β -HSD) in adipose tissue. *Int J Obes* 15(Suppl 2):91–99
 283. Labrie F 2003 Extragonadal synthesis of sex steroids: intracrinology. *Ann Endocrinol (Paris)* 64:95–107
 284. Elmlinger MW, Kühnel W, Ranke MB 2002 Reference ranges for serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), prolactin, progesterone, sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), cortisol and ferritin in neonates, children and young adults. *Clin Chem Lab Med* 40:1151–1160
 285. Cummings SR, Browner WS, Bauer D, Stone K, Ensrud K, Jamal S, Ettinger B 1998 Endogenous hormones and the risk of hip and vertebral fractures among older women. Study of Osteoporotic Fractures Research Group. *N Engl J Med* 339:733–738
 286. Lippe BM, LaFranchi SH, Lavin N, Parlow A, Coyotupa J, Kaplan SA 1974 Serum 17α -hydroxyprogesterone, progesterone, estradiol, and testosterone in the diagnosis and management of congenital adrenal hyperplasia. *J Pediatr* 85:782–787
 287. Bennett NC, Gardiner RA, Hooper JD, Johnson DW, Gobe GC 2010 Molecular cell biology of androgen receptor signalling. *Int J Biochem Cell Biol* 42:813–827
 288. Lamont KR, Tindall DJ 2010 Androgen regulation of gene expression. *Adv Cancer Res* 107:137–162
 289. Foradori CD, Weiser MJ, Handa RJ 2008 Non-genomic actions of androgens. *Front Neuroendocrinol* 29:169–181
 290. Edwards DP 2005 Regulation of signal transduction pathways by estrogen and progesterone. *Annu Rev Physiol* 67:335–376
 291. Simoncini T, Genazzani AR 2003 Non-genomic actions of sex steroid hormones. *Eur J Endocrinol* 148:281–292
 292. Miller VM, Duckles SP 2008 Vascular actions of estrogens: functional implications. *Pharmacol Rev* 60:210–241
 293. Papadopoulou N, Papakonstanti EA, Kallergi G, Alevizopoulos K, Stournaras C 2009 Membrane androgen receptor activation in prostate and breast tumor cells: molecular signaling and clinical impact. *IUBMB Life* 61:56–61
 294. Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Ström A, Treuter E, Warner M,

- Gustafsson JA** 2007 Estrogen receptors: how do they signal and what are their targets. *Physiol Rev* 87:905–931
295. **Gilliver SC** 2010 Sex steroids as inflammatory regulators. *J Steroid Biochem Mol Biol* 120:105–115
296. **Beato M, Klug J** 2000 Steroid hormone receptors: an update. *Hum Reprod Update* 6:225–236
297. **McKenna NJ, O'Malley BW** 2002 Minireview: nuclear receptor coactivators—an update. *Endocrinology* 143:2461–2465
298. **Zhang Z, Teng CT** 2000 Estrogen receptor-related receptor α 1 interacts with coactivator and constitutively activates the estrogen response elements of the human lactoferrin gene. *J Biol Chem* 275:20837–20846
299. **Hall JM, McDonnell DP** 1999 The estrogen receptor β -isoform (ER β) of the human estrogen receptor modulates ER α transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. *Endocrinology* 140:5566–5578
300. **Giangrande PH, McDonnell DP** 1999 The A and B isoforms of the human progesterone receptor: two functionally different transcription factors encoded by a single gene. *Recent Prog Horm Res* 54:291–313; discussion 313–314
301. **Lee YF, Shyr CR, Thin TH, Lin WJ, Chang C** 1999 Convergence of two repressors through heterodimer formation of androgen receptor and testicular orphan receptor-4: a unique signaling pathway in the steroid receptor superfamily. *Proc Natl Acad Sci USA* 96:14724–14729
302. **Zhou ZX, Wong CI, Sar M, Wilson EM** 1994 The androgen receptor: an overview. *Recent Prog Horm Res* 49:249–274
303. **Filardo EJ, Quinn JA, Frackelton AR Jr, Bland KI** 2002 Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. *Mol Endocrinol* 16:70–84
304. **Thomas P, Pang Y, Filardo EJ, Dong J** 2005 Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology* 146:624–632
305. **Le Mellay V, Grosse B, Lieberherr M** 1997 Phospholipase C β and membrane action of calcitriol and estradiol. *J Biol Chem* 272:11902–11907
306. **Grosse B, Kachkache M, Le Mellay V, Lieberherr M** 2000 Membrane signalling and progesterone in female and male osteoblasts. I. Involvement of intracellular Ca(2+), inositol trisphosphate, and diacylglycerol, but not cAMP. *J Cell Biochem* 79:334–345
307. **Benten WP, Lieberherr M, Stamm O, Wrehlke C, Guo Z, Wunderlich F** 1999 Testosterone signaling through internalizable surface receptors in androgen receptor-free macrophages. *Mol Biol Cell* 10:3113–3123
308. **Lieberherr M, Grosse B** 1994 Androgens increase intracellular calcium concentration and inositol 1,4,5-trisphosphate and diacylglycerol formation via a pertussis toxin-sensitive G-protein. *J Biol Chem* 269:7217–7223
309. **Valverde MA, Rojas P, Amigo J, Cosmelli D, Orio P, Bahamonde MI, Mann GE, Vergara C, Latorre R** 1999 Acute activation of Maxi-K channels (hSlo) by estradiol binding to the β subunit. *Science* 285:1929–1931
310. **Lambert JJ, Belelli D, Peden DR, Vardy AW, Peters JA** 2003 Neurosteroid modulation of GABAA receptors. *Prog Neurobiol* 71:67–80
311. **Frye CA, Gardiner SG** 1996 Progestins can have a membrane-mediated action in rat midbrain for facilitation of sexual receptivity. *Horm Behav* 30:682–691
312. **White MM, Zamudio S, Stevens T, Tyler R, Lindendorf J, Leslie K, Moore LG** 1995 Estrogen, progesterone, and vascular reactivity: potential cellular mechanisms. *Endocr Rev* 16:739–751
313. **Harrison DA, Carr DW, Meizel S** 2000 Involvement of protein kinase A and A kinase anchoring protein in the progesterone-initiated human sperm acrosome reaction. *Biol Reprod* 62:811–820
314. **Nazareth LV, Weigel NL** 1996 Activation of the human androgen receptor through a protein kinase A signaling pathway. *J Biol Chem* 271:19900–19907
315. **Bagowski CP, Myers JW, Ferrell Jr JE** 2001 The classical progesterone receptor associates with p42 MAPK and is involved in phosphatidylinositol 3-kinase signaling in *Xenopus* oocytes. *J Biol Chem* 276:37708–37714
316. **Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, Masushige S, Gotoh Y, Nishida E, Kawashima H, Metzger D, Chambon P** 1995 Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. *Science* 270:1491–1494
317. **Endoh H, Sasaki H, Maruyama K, Takeyama K, Waga I, Shimizu T, Kato S, Kawashima H** 1997 Rapid activation of MAP kinase by estrogen in the bone cell line. *Biochem Biophys Res Commun* 235:99–102
318. **Pearson G, Barry P, Timmins C, Stickley J, Hocking M** 2001 Changes in the profile of paediatric intensive care associated with centralisation. *Intensive Care Med* 27:1670–1673
319. **Migliaccio A, Piccolo D, Castoria G, Di Domenico M, Bilancio A, Lombardi M, Gong W, Beato M, Auricchio F** 1998 Activation of the Src/p21ras/Erk pathway by progesterone receptor via cross-talk with estrogen receptor. *EMBO J* 17:2008–2018
320. **Simoncini T, Genazzani AR, Liao JK** 2002 Nongenomic mechanisms of endothelial nitric oxide synthase activation by the selective estrogen receptor modulator raloxifene. *Circulation* 105:1368–1373
321. **Kousteni S, Bellido T, Plotkin LI, O'Brien CA, Bodenner DL, Han L, Han K, DiGregorio GB, Katzenellenbogen JA, Katzenellenbogen BS, Roberson PK, Weinstein RS, Jilka RL, Manolagas SC** 2001 Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. *Cell* 104:719–730
322. **Migliaccio A, Castoria G, Di Domenico M, de Falco A, Bilancio A, Lombardi M, Barone MV, Ametrano D, Zanini MS, Abbondanza C, Auricchio F** 2000 Steroid-induced androgen receptor-oestradiol receptor β -Src complex triggers prostate cancer cell proliferation. *EMBO J* 19:5406–5417
323. **Heinlein CA, Chang C** 2002 Androgen receptor (AR) coregulators: an overview. *Endocr Rev* 23:175–200
324. **Thakur MK, Paramanik V** 2009 Role of steroid hormone coregulators in health and disease. *Horm Res* 71:194–200
325. **Tremblay GB, Giguère V** 2002 Coregulators of estrogen receptor action. *Crit Rev Eukaryot Gene Expr* 12:1–22
326. **Smith CL, O'Malley BW** 2004 Coregulator function: a key

- to understanding tissue specificity of selective receptor modulators. *Endocr Rev* 25:45–71
327. Shirasaki H, Watanabe K, Kanaizumi E, Konno N, Sato J, Narita S, Himi T 2004 Expression and localization of steroid receptors in human nasal mucosa. *Acta Otolaryngol* 124:958–963
 328. Tulchinsky D, Hobel CJ, Yeager E, Marshall JR 1972 Plasma estrone, estradiol, estriol, progesterone, and 17-hydroxyprogesterone in human pregnancy. I. Normal pregnancy. *Am J Obstet Gynecol* 112:1095–1100
 329. Haeggström A, Ostberg B, Stjerna P, Graf P, Hallén H 2000 Nasal mucosal swelling and reactivity during a menstrual cycle. *ORL J Otorhinolaryngol Relat Spec* 62:39–42
 330. Paulsson B, Gredmark T, Burian P, Bende M 1997 Nasal mucosal congestion during the menstrual cycle. *J Laryngol Otol* 111:337–339
 331. Ellegård E, Karlsson G 1994 Nasal congestion during the menstrual cycle. *Clin Otolaryngol Allied Sci* 19:400–403
 332. Philpott CM, Wild DC, Wolstensholme CR, Murty GE 2008 The presence of ovarian hormone receptors in the nasal mucosa and their relationship to nasal symptoms. *Rhinology* 46:221–225
 333. Levin ER 2001 Cell localization, physiology, and non-genomic actions of estrogen receptors. *J Appl Physiol* 91:1860–1867
 334. Taylor M 1961 An experimental study of the influence of the endocrine system on the nasal respiratory mucosa. *J Laryngol Otol* 75:972–977
 335. Zondek B, Bromberg YM 1947 Mechanism of endocrine allergy; interpretation of principal phenomena observed in hypersensitivity to the endogenous hormones. *Acta Med Orient* 6:1–8
 336. Pelikan Z 1978 Possible immediate hypersensitivity reaction of the nasal mucosa to oral contraceptives. *Ann Allergy* 40:211–219
 337. Mortimer H, Wright RP, Collip JB 1936 The effect of the administration of oestrogenic hormones on the nasal mucosa of the monkey (*Macaca mulatta*). *Can Med Assoc J* 35:503–513
 338. Topozada H, Michaels L, Topozada M, El-Ghazzawi I, Talaat M, Elwany S 1982 The human respiratory nasal mucosa in pregnancy. An electron microscopic and histochemical study. *J Laryngol Otol* 96:613–626
 339. Konno A, Terada N, Okamoto Y 1986 Effects of female hormones on the muscarinic and α 1-adrenergic receptors of the nasal mucosa. An experimental study in guinea pigs. *ORL J Otorhinolaryngol Relat Spec* 48:45–51
 340. Hamano N, Terada N, Maesako K, Ikeda T, Fukuda S, Wakita J, Yamashita T, Konno A 1998 Expression of histamine receptors in nasal epithelial cells and endothelial cells—the effects of sex hormones. *Int Arch Allergy Immunol* 115:220–227
 341. Kirsch EA, Yuhanna IS, Chen Z, German Z, Sherman TS, Shaul PW 1999 Estrogen acutely stimulates endothelial nitric oxide synthase in H441 human airway epithelial cells. *Am J Respir Cell Mol Biol* 20:658–666
 342. Ivanova MM, Mazhawidza W, Dougherty SM, Klinge CM 2010 Sex differences in estrogen receptor subcellular location and activity in lung adenocarcinoma cells. *Am J Respir Cell Mol Biol* 42:320–330
 343. Townsend EA, Meuchel LW, Thompson MA, Pabelick CM, Prakash YS 22 September 2011 Estrogen increases nitric oxide production in human bronchial epithelium. *J Pharmacol Exp Ther* doi:10.1124/jpet.111.184416
 344. Sud N, Wiseman DA, Black SM 2010 Caveolin 1 is required for the activation of endothelial nitric oxide synthase in response to 17 β -estradiol. *Mol Endocrinol* 24:1637–1649
 345. Hamad AM, Clayton A, Islam B, Knox AJ 2003 Guanylyl cyclases, nitric oxide, natriuretic peptides, and airway smooth muscle function. *Am J Physiol Lung Cell Mol Physiol* 285:L973–L983
 346. Folkerts G, Nijkamp FP 2006 Nitric oxide in asthma therapy. *Curr Pharm Des* 12:3221–3232
 347. Batra J, Pratap Singh T, Mabalirajan U, Sinha A, Prasad R, Ghosh B 2007 Association of inducible nitric oxide synthase with asthma severity, total serum immunoglobulin E and blood eosinophil levels. *Thorax* 62:16–22
 348. Morris NH, Sooranna SR, Steer PJ, Warren JB 1996 The effect of the menstrual cycle on exhaled nitric oxide and urinary nitrate concentration. *Eur J Clin Invest* 26:481–484
 349. Jilma B, Kastner J, Mensik C, Vondrovec B, Hildebrandt J, Krejcy K, Wagner OF, Eichler HG 1996 Sex differences in concentrations of exhaled nitric oxide and plasma nitrate. *Life Sci* 58:469–476
 350. Vihko P, Herrala A, Härkönen P, Isomaa V, Kaija H, Kurkela R, Pulkka A 2006 Control of cell proliferation by steroids: the role of 17HSDs. *Mol Cell Endocrinol* 248:141–148
 351. Folkerd EJ, Dowsett M 2010 Influence of sex hormones on cancer progression. *J Clin Oncol* 28:4038–4044
 352. Dammann CE, Ramadurai SM, McCants DD, Pham LD, Nielsen HC 2000 Androgen regulation of signaling pathways in late fetal mouse lung development. *Endocrinology* 141:2923–2929
 353. Provost PR, Simard M, Tremblay Y 2004 A link between lung androgen metabolism and the emergence of mature epithelial type II cells. *Am J Respir Crit Care Med* 170:296–305
 354. Nielsen HC 1985 Androgen receptors influence the production of pulmonary surfactant in the testicular feminization mouse fetus. *J Clin Invest* 76:177–181
 355. Ishibashi H, Suzuki T, Suzuki S, Niikawa H, Lu L, Miki Y, Moriya T, Hayashi S, Handa M, Kondo T, Sasano H 2005 Progesterone receptor in non-small cell lung cancer—a potent prognostic factor and possible target for endocrine therapy. *Cancer Res* 65:6450–6458
 356. Márquez-Garbán DC, Chen HW, Fishbein MC, Goodglick L, Pietras RJ 2007 Estrogen receptor signaling pathways in human non-small cell lung cancer. *Steroids* 72:135–143
 357. Mikkonen L, Pihlajamaa P, Sahu B, Zhang FP, Jänne OA 2010 Androgen receptor and androgen-dependent gene expression in lung. *Mol Cell Endocrinol* 317:14–24
 358. Márquez-Garbán DC, Chen HW, Goodglick L, Fishbein MC, Pietras RJ 2009 Targeting aromatase and estrogen signaling in human non-small cell lung cancer. *Ann NY Acad Sci* 1155:194–205
 359. Plante J, Simard M, Rantakari P, Côté M, Provost PR, Poutanen M, Tremblay Y 2009 Epithelial cells are the major site of hydroxysteroid (17 β) dehydrogenase 2 and an-

- drogen receptor expression in fetal mouse lungs during the period overlapping the surge of surfactant. *J Steroid Biochem Mol Biol* 117:139–145
360. Ino T, Aviado DM 1971 Cardiopulmonary effects of progestational agents in emphysematous rats. *Chest* 59:659–666
361. Ito H, Aviado DM 1968 Prevention of pulmonary emphysema in rats by progesterone. *J Pharmacol Exp Ther* 161:197–204
362. Giles RE, Finkel MP, Williams JC, Winbury MM 1974 The therapeutic effect of progesterone in papain-induced emphysema. *Proc Soc Exp Biol Med* 147:489–493
363. Giles RE, Williams JC, Finkel MP, Winbury MM 1973 Progesterone antagonism of papain emphysema: role of sex, estrogens and serum antitrypsin. *Proc Soc Exp Biol Med* 144:487–491
364. Tyler JM 1960 The effect of progesterone on the respiration of patients with emphysema and hypercapnia. *J Clin Invest* 39:34–41
365. Hershberger PA, Vasquez AC, Kanterewicz B, Land S, Siegfried JM, Nichols M 2005 Regulation of endogenous gene expression in human non-small cell lung cancer cells by estrogen receptor ligands. *Cancer Res* 65:1598–1605
366. Márquez DC, Lee J, Lin T, Pietras RJ 2001 Epidermal growth factor receptor and tyrosine phosphorylation of estrogen receptor. *Endocrine* 16:73–81
367. Stabile LP, Lyker JS, Gubish CT, Zhang W, Grandis JR, Siegfried JM 2005 Combined targeting of the estrogen receptor and the epidermal growth factor receptor in non-small cell lung cancer shows enhanced antiproliferative effects. *Cancer Res* 65:1459–1470
368. Kawai H, Ishii A, Washiya K, Konno T, Kon H, Yamaya C, Ono I, Ogawa J 2005 Combined overexpression of EGFR and estrogen receptor α correlates with a poor outcome in lung cancer. *Anticancer Res* 25:4693–4698
369. Weinberg OK, Marquez-Garban DC, Fishbein MC, Goodglick L, Garban HJ, Dubinett SM, Pietras RJ 2005 Aromatase inhibitors in human lung cancer therapy. *Cancer Res* 65:11287–11291
370. Mah V, Seligson DB, Li A, Márquez DC, Wistuba II, Elshimali Y, Fishbein MC, Chia D, Pietras RJ, Goodglick L 2007 Aromatase expression predicts survival in women with early-stage non small cell lung cancer. *Cancer Res* 67:10484–10490
371. Su JM, Hsu HK, Chang H, Lin SL, Chang HC, Huang MS, Tseng HH 1996 Expression of estrogen and progesterone receptors in non-small-cell lung cancer: immunohistochemical study. *Anticancer Res* 16:3803–3806
372. Nishizawa Y, Yamasaki M, Katayama H, Amakata Y, Fushiki S, Nishizawa Y 2007 Establishment of a progesterone-sensitive cell line from human lung cancer. *Oncol Rep* 18:685–690
373. Verstuyf A, Sobis H, Vandeputte M 1989 Morphological and immunological characteristics of a rat choriocarcinoma. *Int J Cancer* 44:879–884
374. Maasberg M, Rotsch M, Jaques G, Enderle-Schmidt U, Weehle R, Havemann K 1989 Androgen receptors, androgen-dependent proliferation, and 5 α -reductase activity of small-cell lung cancer cell lines. *Int J Cancer* 43:685–691
375. Recchia AG, Musti AM, Lanzino M, Panno ML, Turano E, Zumpano R, Belfiore A, Andò S, Maggiolini M 2009 A cross-talk between the androgen receptor and the epidermal growth factor receptor leads to p38MAPK-dependent activation of mTOR and cyclin D1 expression in prostate and lung cancer cells. *Int J Biochem Cell Biol* 41:603–614
376. Kellermann G, Shaw CR, Luyten-Kellerman M 1973 Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. *N Engl J Med* 289:934–937
377. Bullard MK, Bir N, Kwan R, Cureton E, Knudson P, Harken A 2010 Women rule. *Surgery* 147:134–137
378. Wang M, Crisostomo PR, Markel T, Wang Y, Lillemoec KD, Meldrum DR 2008 Estrogen receptor β mediates acute myocardial protection following ischemia. *Surgery* 144:233–238
379. Choudhry MA, Schwacha MG, Hubbard WJ, Kerby JD, Rue LW, Bland KI, Chaudry IH 2005 Gender differences in acute response to trauma-hemorrhage. *Shock* 24(Suppl 1):101–106
380. Raju R, Chaudry IH 2008 Sex steroids/receptor antagonist: their use as adjuncts after trauma-hemorrhage for improving immune/cardiovascular responses and for decreasing mortality from subsequent sepsis. *Anesth Analg* 107:159–166
381. Ananthakrishnan P, Cohen DB, Xu DZ, Lu Q, Feketeova E, Deitch EA 2005 Sex hormones modulate distant organ injury in both a trauma/hemorrhagic shock model and a burn model. *Surgery* 137:56–65
382. Caruso JM, Deitch EA, Xu DZ, Lu Q, Dayal SD 2003 Gut injury and gut-induced lung injury after trauma hemorrhagic shock is gender and estrus cycle specific in the rat. *J Trauma* 55:531–539
383. Caruso JM, Xu DZ, Lu Q, Dayal SD, Deitch EA 2001 The female gender protects against pulmonary injury after trauma hemorrhagic shock. *Surg Infect (Larchmt)* 2:231–240
384. Speyer CL, Rancilio NJ, McClintock SD, Crawford JD, Gao H, Sarma JV, Ward PA 2005 Regulatory effects of estrogen on acute lung inflammation in mice. *Am J Physiol Cell Physiol* 288:C881–C890
385. Tesfaigzi Y, Rudolph K, Fischer MJ, Conn CA 2001 Bcl-2 mediates sex-specific differences in recovery of mice from LPS-induced signs of sickness independent of IL-6. *J Appl Physiol* 91:2182–2189
386. Yu HP, Hsieh YC, Suzuki T, Shimizu T, Choudhry MA, Schwacha MG, Chaudry IH 2006 Salutary effects of estrogen receptor- β agonist on lung injury after trauma-hemorrhage. *Am J Physiol Lung Cell Mol Physiol* 290:L1004–L1009
387. Ebina M, Takahashi T, Chiba T, Motomiya M 1993 Cellular hypertrophy and hyperplasia of airway smooth muscles underlying bronchial asthma. A 3-D morphometric study. *Am Rev Respir Dis* 148:720–726
388. Camp PG, Appleton J, Reid WD 2000 Quality of life after pulmonary rehabilitation: assessing change using quantitative and qualitative methods. *Phys Ther* 80:986–995
389. Degano B, Mourlanette P, Valmary S, Pontier S, Prévost MC, Escamilla R 2003 Differential effects of low and high-dose estradiol on airway reactivity in ovariectomized rats. *Respir Physiol Neurobiol* 138:265–274
390. Kouloumenta V, Hatziefthimiou A, Paraskeva E, Gourgoulanis K, Molyvdas PA 2007 Sexual dimorphism

- in airway responsiveness to sex hormones in rabbits. *Am J Physiol Lung Cell Mol Physiol* 293:L516
391. Pang JJ, Xu XB, Li HF, Zhang XY, Zheng TZ, Qu SY 2002 Inhibition of β -estradiol on trachea smooth muscle contraction in vitro and in vivo. *Acta Pharmacol Sin* 23:273–277
 392. Townsend EA, Thompson MA, Pabelick CM, Prakash YS 2010 Rapid effects of estrogen on intracellular Ca²⁺ regulation in human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 298:L521–L530
 393. Prakash YS, Thompson MA, Vaa B, Matadin I, Peterson TE, He T, Pabelick CM 2007 Caveolins and intracellular calcium regulation in human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 293:L1118–L1126
 394. Ay B, Prakash YS, Pabelick CM, Sieck GC 2004 Store-operated Ca²⁺ entry in porcine airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 286:L909–L917
 395. Jia S, Zhang X, He DZ, Segal M, Berro A, Gerson TG, Wang Z, Casale TB 3 June 2011 Expression and function of a novel variant of estrogen receptor-ER- α 36 in mouse airway. *Am J Respir Cell Mol Biol* doi: 10.1165/rcmb.2010–0268OC
 396. Perusquía M, Hernández R, Montaña LM, Villalón CM, Campos MG 1997 Inhibitory effect of sex steroids on guinea-pig airway smooth muscle contractions. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 118:5–10
 397. Amrani Y, Syed F, Huang C, Li K, Liu V, Jain D, Keslacy S, Sims MW, Baidouri H, Cooper PR, Zhao H, Siddiqui S, Brightling CE, Griswold D, Li L, Panettieri Jr RA 2010 Expression and activation of the oxytocin receptor in airway smooth muscle cells: regulation by TNF α and IL-13. *Respir Res* 11:104
 398. Grazzini E, Guillon G, Mouillac B, Zingg HH 1998 Inhibition of oxytocin receptor function by direct binding of progesterone. *Nature* 392:509–512
 399. Kouloumenta V, Hatziefthimiou A, Paraskeva E, Gourgoulis K, Molyvdas PA 2006 Non-genomic effect of testosterone on airway smooth muscle. *Br J Pharmacol* 149:1083–1091
 400. Bordallo J, de Boto MJ, Meana C, Velasco L, Bordallo C, Suárez L, Cantabrana B, Sánchez M 2008 Modulatory role of endogenous androgens on airway smooth muscle tone in isolated guinea-pig and bovine trachea: involvement of β 2-adrenoceptors, the polyamine system and external calcium. *Eur J Pharmacol* 601:154–162
 401. Rodríguez D, Keller AC, Faquim-Mauro EL, de Macedo MS, Cunha FQ, Lefort J, Vargaftig BB, Russo M 2003 Bacterial lipopolysaccharide signaling through Toll-like receptor 4 suppresses asthma-like responses via nitric oxide synthase 2 activity. *J Immunol* 171:1001–1008
 402. Stewart AG, Fernandes D, Tomlinson PR 1995 The effect of glucocorticoids on proliferation of human cultured airway smooth muscle. *Br J Pharmacol* 116:3219–3226
 403. Dashtaki R, Whorton AR, Murphy TM, Chitano P, Reed W, Kennedy TP 1998 Dehydroepiandrosterone and analogs inhibit DNA binding of AP-1 and airway smooth muscle proliferation. *J Pharmacol Exp Ther* 285:876–883
 404. Stamatou R, Paraskeva E, Papagianni M, Molyvdas PA, Hatziefthimiou A 2011 The mitogenic effect of testosterone and 17 β -estradiol on airway smooth muscle cells. *Steroids* 76:400–408
 405. Kawai K, Akaza H 2003 Bleomycin-induced pulmonary toxicity in chemotherapy for testicular cancer. *Expert Opin Drug Saf* 2:587–596
 406. Voltz JW, Card JW, Carey MA, Degraff LM, Ferguson CD, Flake GP, Bonner JC, Korach KS, Zeldin DC 2008 Male sex hormones exacerbate lung function impairment after bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol* 39:45–52
 407. Tofovic SP, Zhang X, Jackson EK, Zhu H, Petrussevska G 2009 2-Methoxyestradiol attenuates bleomycin-induced pulmonary hypertension and fibrosis in estrogen-deficient rats. *Vascul Pharmacol* 51:190–197
 408. Kondo H, Kasuga H, Noumura T 1983 Effects of various steroids on in vitro lifespan and cell growth of human fetal lung fibroblasts (WI-38). *Mech Ageing Dev* 21:335–344
 409. Flores-Delgado G, Bringas P, Buckley S, Anderson KD, Warburton D 2001 Nongenomic estrogen action in human lung myofibroblasts. *Biochem Biophys Res Commun* 283:661–667
 410. Milam JE, Keshamouni VG, Phan SH, Hu B, Gangireddy SR, Hogaboam CM, Standiford TJ, Thannickal VJ, Reddy RC 2008 PPAR- γ agonists inhibit profibrotic phenotypes in human lung fibroblasts and bleomycin-induced pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 294:L891–L901
 411. Smith AM, Bennett RT, Jones TH, Cowen ME, Channer KS, Jones RD 2008 Characterization of the vasodilatory action of testosterone in the human pulmonary circulation. *Vasc Health Risk Manag* 4:1459–1466
 412. Rabinovitch M, Gamble WJ, Miettinen OS, Reid L 1981 Age and sex influence on pulmonary hypertension of chronic hypoxia and on recovery. *Am J Physiol* 240:H62–H72
 413. McMurtry IF, Frith CH, Will DH 1973 Cardiopulmonary responses of male and female swine to simulated high altitude. *J Appl Physiol* 35:459–462
 414. Resta TC, Kanagy NL, Walker BR 2001 Estradiol-induced attenuation of pulmonary hypertension is not associated with altered eNOS expression. *Am J Physiol Lung Cell Mol Physiol* 280:L88–L97
 415. Lahm T, Patel KM, Crisostomo PR, Markel TA, Wang M, Herring C, Meldrum DR 2007 Endogenous estrogen attenuates pulmonary artery vasoreactivity and acute hypoxic pulmonary vasoconstriction: the effects of sex and menstrual cycle. *Am J Physiol Endocrinol Metab* 293:E865–E871
 416. Wetzel RC, Zacur HA, Sylvester JT 1984 Effect of puberty and estradiol on hypoxic vasomotor response in isolated sheep lungs. *J Appl Physiol* 56:1199–1203
 417. Kiyatake K, Kakusaka I, Kasahara Y, Qi TM, Suzuki A, Nakano K, Kaneko N, Kitada M, Kuriyama T 1994 [Relationship between the converting ability of liver microsomes and monocrotaline-induced pulmonary hypertension in male, female and castrated male rats]. *Nihon Kyobu Shikkan Gakkai Zasshi* 32:125–129
 418. Farhat MY, Chen MF, Bhatti T, Iqbal A, Cathapermal S, Ramwell PW 1993 Protection by oestradiol against the development of cardiovascular changes associated with monocrotaline pulmonary hypertension in rats. *Br J Pharmacol* 110:719–723
 419. Ahn BH, Park HK, Cho HG, Lee HA, Lee YM, Yang EK,

- Lee WJ 2003 Estrogen and enalapril attenuate the development of right ventricular hypertrophy induced by monocrotaline in ovariectomized rats. *J Korean Med Sci* 18:641–648
420. **Tofovic S, Rafikova O** 2009 Preventive and therapeutic effects of 2-methoxyestradiol, but not estradiol, in severe occlusive pulmonary arterial hypertension in female rats. *Am J Respir Crit Care Med* 179:A1802
421. **Dempsey Y, Nilsen M, Loughlin L, MacLean MR** 2009 The effects of gender on the development of pulmonary arterial hypertension in mice over-expressing the serotonin transporter. *Am J Respir Crit Care Med* 179:A1809
422. **Bolego C, Cignarella A, Sanvito P, Pelosi V, Pellegatta F, Puglisi L, Pinna C** 2005 The acute estrogenic dilation of rat aorta is mediated solely by selective estrogen receptor- α agonists and is abolished by estrogen deprivation. *J Pharmacol Exp Ther* 313:1203–1208
423. **Douglas G, Cruz MN, Poston L, Gustafsson JA, Kublickiene K** 2008 Functional characterization and sex differences in small mesenteric arteries of the estrogen receptor- β knockout mouse. *Am J Physiol Regul Integr Comp Physiol* 294:R112–R120
424. **Tostes RC, Nigro D, Fortes ZB, Carvalho MH** 2003 Effects of estrogen on the vascular system. *Braz J Med Biol Res* 36:1143–1158
425. **Akarasreenont P, Techatraisak K, Thaworn A, Chotewuttakorn S** 2000 The induction of cyclooxygenase-2 by 17 β -estradiol in endothelial cells is mediated through protein kinase C. *Inflamm Res* 49:460–465
426. **Prakash YS, Togaibayeva AA, Kannan MS, Miller VM, Fitzpatrick LA, Sieck GC** 1999 Estrogen increases Ca²⁺ efflux from female porcine coronary arterial smooth muscle. *Am J Physiol* 276:H926–H934
427. **Kitazawa T, Hamada E, Kitazawa K, Gaznabi AK** 1997 Non-genomic mechanism of 17 β -oestradiol-induced inhibition of contraction in mammalian vascular smooth muscle. *J Physiol* 499:497–511
428. **Nakajima T, Kitazawa T, Hamada E, Hazama H, Omata M, Kurachi Y** 1995 17 β -Estradiol inhibits the voltage-dependent L-type Ca²⁺ currents in aortic smooth muscle cells. *Eur J Pharmacol* 294:625–635
429. **Shimokawa H, Takeshita A** 2005 Rho-kinase is an important therapeutic target in cardiovascular medicine. *Arterioscler Thromb Vasc Biol* 25:1767–1775
430. **Abe K, Shimokawa H, Morikawa K, Uwatoku T, Oi K, Matsumoto Y, Hattori T, Nakashima Y, Kaibuchi K, Sueishi K, Takeshit A** 2004 Long-term treatment with a Rho-kinase inhibitor improves monocrotaline-induced fatal pulmonary hypertension in rats. *Circ Res* 94:385–393
431. **Nagaoka T, Fagan KA, Gebb SA, Morris KG, Suzuki T, Shimokawa H, McMurtry IF, Oka M** 2005 Inhaled Rho kinase inhibitors are potent and selective vasodilators in rat pulmonary hypertension. *Am J Respir Crit Care Med* 171:494–499
432. **Abe K, Morikawa K, Hizume T, Uwatoku T, Oi K, Seto M, Ikegaki I, Asano T, Kaibuchi K, Shimokawa H** 2005 Prostaglandin does not inhibit rho-kinase: an implication for the treatment of pulmonary hypertension. *J Cardiovasc Pharmacol* 45:120–124
433. **Barberis MC, Veronese S, Bauer D, De Juli E, Harari S** 1995 Immunocytochemical detection of progesterone receptors. A study in a patient with primary pulmonary hypertension. *Chest* 107:869–872
434. **English KM, Jones RD, Jones TH, Morice AH, Channer KS** 2001 Gender differences in the vasomotor effects of different steroid hormones in rat pulmonary and coronary arteries. *Horm Metab Res* 33:645–652
435. **Li HF, Zheng TZ, Li W, Qu SY, Zhang CL** 2001 Effect of progesterone on the contractile response of isolated pulmonary artery in rabbits. *Can J Physiol Pharmacol* 79:545–550
436. **Tofovic PS, Zhang X, Petrusevska G** 2009 Progesterone inhibits vascular remodeling and attenuates monocrotaline-induced pulmonary hypertension in estrogen-deficient rats. *Prilozi* 30:25–44
437. **Vázquez F, Rodríguez-Manzanique JC, Lydon JP, Edwards DP, O'Malley BW, Iruela-Arispe ML** 1999 Progesterone regulates proliferation of endothelial cells. *J Biol Chem* 274:2185–2192
438. **Morey AK, Pedram A, Razandi M, Prins BA, Hu RM, Biesiada E, Levin ER** 1997 Estrogen and progesterone inhibit vascular smooth muscle proliferation. *Endocrinology* 138:3330–3339
439. **Lee WS, Liu CW, Juan SH, Liang YC, Ho PY, Lee YH** 2003 Molecular mechanism of progesterone-induced antiproliferation in rat aortic smooth muscle cells. *Endocrinology* 144:2785–2790
440. **Pribylova NN, Goloshchapov OA, Parygina EI** 1984 [Significance of progesterone in the pathogenesis and treatment of pulmonary hypertension and cardiopulmonary failure]. *Ter Arkh* 56:94–97
441. **Jones RD, English KM, Pugh PJ, Morice AH, Jones TH, Channer KS** 2002 Pulmonary vasodilatory action of testosterone: evidence of a calcium antagonistic action. *J Cardiovasc Pharmacol* 39:814–823
442. **Rowell KO, Hall J, Pugh PJ, Jones TH, Channer KS, Jones RD** 2009 Testosterone acts as an efficacious vasodilator in isolated human pulmonary arteries and veins: evidence for a biphasic effect at physiological and supra-physiological concentrations. *J Endocrinol Invest* 32:718–723
443. **Pugh PJ, Jones TH, Channer KS** 2003 Acute haemodynamic effects of testosterone in men with chronic heart failure. *Eur Heart J* 24:909–915
444. **Bonnet S, Dumas-de-La-Roque E, Bégueret H, Marthan R, Fayon M, Dos Santos P, Savineau JP, Baulieu EE** 2003 Dehydroepiandrosterone (DHEA) prevents and reverses chronic hypoxic pulmonary hypertension. *Proc Natl Acad Sci USA* 100:9488–9493
445. **Farrukh IS, Peng W, Orlinska U, Hoidal JR** 1998 Effect of dehydroepiandrosterone on hypoxic pulmonary vasoconstriction: a Ca(2+)-activated K(+)-channel opener. *Am J Physiol* 274:L186–L195
446. **Oka M, Karoor V, Homma N, Nagaoka T, Sakao E, Golembeski SM, Limbird J, Imamura M, Gebb SA, Fagan KA, McMurtry IF** 2007 Dehydroepiandrosterone upregulates soluble guanylate cyclase and inhibits hypoxic pulmonary hypertension. *Cardiovasc Res* 74:377–387
447. **Liu D, Iruthayanathan M, Homan LL, Wang Y, Yang L, Wang Y, Dillon JS** 2008 Dehydroepiandrosterone stimulates endothelial proliferation and angiogenesis through extracellular signal-regulated kinase 1/2-mediated mechanisms. *Endocrinology* 149:889–898

448. Edwards LJ, Constantinescu CS 2004 A prospective study of conditions associated with multiple sclerosis in a cohort of 658 consecutive outpatients attending a multiple sclerosis clinic. *Mult Scler* 10:575–581
449. Provenzano G, Donato G, Brai G, Rinaldi F 2002 Prevalence of allergic respiratory diseases in patients with RA. *Ann Rheum Dis* 61:281
450. Rudwaleit M, Andermann B, Alten R, Sörensen H, Listing J, Zink A, Sieper J, Braun J 2002 Atopic disorders in ankylosing spondylitis and rheumatoid arthritis. *Ann Rheum Dis* 61:968–974
451. Bergamaschi R, Villani S, Crabbio M, Ponzio M, Romani A, Verri A, Bargiggia V, Cosi V 2009 Inverse relationship between multiple sclerosis and allergic respiratory diseases. *Neurol Sci* 30:115–118
452. Tremlett HL, Evans J, Wiles CM, Luscombe DK 2002 Asthma and multiple sclerosis: an inverse association in a case-control general practice population. *QJM* 95:753–756
453. Straub RH 2007 The complex role of estrogens in inflammation. *Endocr Rev* 28:521–574
454. Pierdominici M, Maselli A, Colasanti T, Giammarioli AM, Delunardo F, Vacirca D, Sanchez M, Giovannetti A, Malorni W, Ortona E 2010 Estrogen receptor profiles in human peripheral blood lymphocytes. *Immunol Lett* 132:79–85
455. Soldan SS, Alvarez Retuerto AI, Sicotte NL, Voskuhl RR 2003 Immune modulation in multiple sclerosis patients treated with the pregnancy hormone estriol. *J Immunol* 171:6267–6274
456. Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J, Pacifici R 2000 Estrogen deficiency induces bone loss by enhancing T-cell production of TNF- α . *J Clin Invest* 106:1229–1237
457. Lambert KC, Curran EM, Judy BM, Milligan GN, Lubahn DB, Estes DM 2005 Estrogen receptor α (ER α) deficiency in macrophages results in increased stimulation of CD4+ T cells while 17 β -estradiol acts through ER α to increase IL-4 and GATA-3 expression in CD4+ T cells independent of antigen presentation. *J Immunol* 175:5716–5723
458. Butts CL, Shukair SA, Duncan KM, Harris CW, Belyavskaya E, Sternberg EM 2007 Evaluation of steroid hormone receptor protein expression in intact cells using flow cytometry. *Nucl Recept Signal* 5:e007
459. Chiu L, Nishimura M, Ishii Y, Nieda M, Maeshima M, Takedani Y, Shibata Y, Tadokoro K, Juji T 1996 Enhancement of the expression of progesterone receptor on progesterone-treated lymphocytes after immunotherapy in unexplained recurrent spontaneous abortion. *Am J Reprod Immunol* 35:552–557
460. Mansour I, Reznikoff-Etievant MF, Netter A 1994 No evidence for the expression of the progesterone receptor on peripheral blood lymphocytes during pregnancy. *Hum Reprod* 9:1546–1549
461. Schust DJ, Anderson DJ, Hill JA 1996 Progesterone-induced immunosuppression is not mediated through the progesterone receptor. *Hum Reprod* 11:980–985
462. Miyaura H, Iwata M 2002 Direct and indirect inhibition of Th1 development by progesterone and glucocorticoids. *J Immunol* 168:1087–1094
463. Huck B, Steck T, Habersack M, Dietl J, Kämmerer U 2005 Pregnancy associated hormones modulate the cytokine production but not the phenotype of PBMC-derived human dendritic cells. *Eur J Obstet Gynecol Reprod Biol* 122:85–94
464. Hardy DB, Janowski BA, Corey DR, Mendelson CR 2006 Progesterone receptor plays a major antiinflammatory role in human myometrial cells by antagonism of nuclear factor- κ B activation of cyclooxygenase 2 expression. *Mol Endocrinol* 20:2724–2733
465. Huber SA, Kupperman J, Newell MK 1999 Hormonal regulation of CD4(+) T-cell responses in coxsackievirus B3-induced myocarditis in mice. *J Virol* 73:4689–4695
466. Namazi MR 2009 The Th1-promoting effects of dehydroepiandrosterone can provide an explanation for the stronger Th1-immune response of women. *Iran J Allergy Asthma Immunol* 8:65–69
467. Suzuki T, Suzuki N, Daynes RA, Engleman EG 1991 Dehydroepiandrosterone enhances IL2 production and cytotoxic effector function of human T cells. *Clin Immunol Immunopathol* 61:202–211
468. Araghi-Niknam M, Zhang Z, Jiang S, Call O, Eskelson CD, Watson RR 1997 Cytokine dysregulation and increased oxidation is prevented by dehydroepiandrosterone in mice infected with murine leukemia retrovirus. *Proc Soc Exp Biol Med* 216:386–391
469. Yu CK, Liu YH, Chen CL 2002 Dehydroepiandrosterone attenuates allergic airway inflammation in *Dermatophagoides farinae*-sensitized mice. *J Microbiol Immunol Infect* 35:199–202
470. Choi IS, Cui Y, Koh YA, Lee HC, Cho YB, Won YH 2008 Effects of dehydroepiandrosterone on Th2 cytokine production in peripheral blood mononuclear cells from asthmatics. *Korean J Intern Med* 23:176–181
471. Melgert BN, Oriss TB, Qi Z, Dixon-McCarthy B, Geerlings M, Hylkema MN, Ray A 2010 Macrophages: regulators of sex differences in asthma? *Am J Respir Cell Mol Biol* 42:595–603
472. Paharkova-Vatchkova V, Maldonado R, Kovats S 2004 Estrogen preferentially promotes the differentiation of CD11c+ CD11b(intermediate) dendritic cells from bone marrow precursors. *J Immunol* 172:1426–1436
473. Canning MO, Grotenhuis K, de Wit HJ, Drexhage HA 2000 Opposing effects of dehydroepiandrosterone and dexamethasone on the generation of monocyte-derived dendritic cells. *Eur J Endocrinol* 143:687–695
474. Riffo-Vasquez Y, Ligeiro de Oliveira AP, Page CP, Spina D, Tavares-de-Lima W 2007 Role of sex hormones in allergic inflammation in mice. *Clin Exp Allergy* 37:459–470
475. Klebanoff SJ 1977 Estrogen binding by leukocytes during phagocytosis. *J Exp Med* 145:983–998
476. Katayama ML, Federico MH, Brentani RR, Brentani MM 1998 Eosinophil accumulation in rat uterus following estradiol administration is modulated by laminin and its integrin receptors. *Cell Adhes Commun* 5:409–424
477. Mayo JC, Sáinz RM, Antolín I, Uría H, Menéndez-Peláez A, Rodríguez C 1997 Androgen-dependent mast cell degranulation in the Harderian gland of female Syrian hamsters: in vivo and organ culture evidence. *Anat Embryol (Berl)* 196:133–140
478. Belot MP, Abdennebi-Najar L, Gaudin F, Lieberherr M, Godot V, Taïeb J, Emilie D, Machelon V 2007 Progester-

- one reduces the migration of mast cells toward the chemokine stromal cell-derived factor-1/CXCL12 with an accompanying decrease in CXCR4 receptors. *Am J Physiol Endocrinol Metab* 292:E1410–E1417
479. Vasiadi M, Kempuraj D, Boucher W, Kalogeromitros D, Theoharides TC 2006 Progesterone inhibits mast cell secretion. *Int J Immunopathol Pharmacol* 19:787–794
480. Gritz ER, Nielsen IR, Brooks LA 1996 Smoking cessation and gender: the influence of physiological, psychological, and behavioral factors. *J Am Med Womens Assoc* 51:35–42
481. Dijkstra A, Howard TD, Vonk JM, Ampleford EJ, Lange LA, Bleecker ER, Meyers DA, Postma DS 2006 Estrogen receptor 1 polymorphisms are associated with airway hyperresponsiveness and lung function decline, particularly in female subjects with asthma. *J Allergy Clin Immunol* 117:604–611
482. Oberholzer A, Keel M, Zellweger R, Steckholzer U, Trentz O, Ertel W 2000 Incidence of septic complications and multiple organ failure in severely injured patients is sex specific. *J Trauma* 48:932–937
483. Angele MK, Schwacha MG, Ayala A, Chaudry IH 2000 Effect of gender and sex hormones on immune responses following shock. *Shock* 14:81–90
484. Kher A, Wang M, Tsai BM, Pitcher JM, Greenbaum ES, Nagy RD, Patel KM, Wairiuko GM, Markel TA, Meldrum DR 2005 Sex differences in the myocardial inflammatory response to acute injury. *Shock* 23:1–10
485. Deitch EA, Livingston DH, Lavery RF, Monaghan SF, Bongu A, Machiedo GW 2007 Hormonally active women tolerate shock-trauma better than do men: a prospective study of over 4000 trauma patients. *Ann Surg* 246:447–453; discussion 453–445
486. Offner PJ, Moore EE, Biffl WL 1999 Male gender is a risk factor for major infections after surgery. *Arch Surg* 134:935–938; discussion 938–940
487. Ashcroft GS, Greenwell-Wild T, Horan MA, Wahl SM, Ferguson MW 1999 Topical estrogen accelerates cutaneous wound healing in aged humans associated with an altered inflammatory response. *Am J Pathol* 155:1137–1146
488. Kovacs EJ, Messingham KA, Gregory MS 2002 Estrogen regulation of immune responses after injury. *Mol Cell Endocrinol* 193:129–135
489. Jarrar D, Wang P, Knöferl MW, Kuebler JF, Cioffi WG, Bland KI, Chaudry IH 2000 Insight into the mechanism by which estradiol improves organ functions after trauma-hemorrhage. *Surgery* 128:246–252
490. Yu HP, Chaudry IH 2009 The role of estrogen and receptor agonists in maintaining organ function after trauma-hemorrhage. *Shock* 31:227–237
491. Martin TR, Castile RG, Fredberg JJ, Wohl ME, Mead J 1987 Airway size is related to sex but not lung size in normal adults. *J Appl Physiol* 63:2042–2047
492. Wang X, Dockery DW, Wypij D, Gold DR, Speizer FE, Ware JH, Ferris Jr BG 1993 Pulmonary function growth velocity in children 6 to 18 years of age. *Am Rev Respir Dis* 148:1502–1508
493. Harms CA 2006 Does gender affect pulmonary function and exercise capacity? *Respir Physiol Neurobiol* 151:124–131
494. Pride NB 2005 Ageing and changes in lung mechanics. *Eur Respir J* 26:563–565
495. Bertelsen RJ, Instanes C, Granum B, Lødrup Carlsen KC, Hetland G, Carlsen KH, Mowinckel P, Løvik M 2010 Gender differences in indoor allergen exposure and association with current rhinitis. *Clin Exp Allergy* 40:1388–1397
496. Loyd JE, Butler MG, Foroud TM, Conneally PM, Phillips 3rd JA, Newman JH 1995 Genetic anticipation and abnormal gender ratio at birth in familial primary pulmonary hypertension. *Am J Respir Crit Care Med* 152:93–97
497. 2010 Putting gender on the agenda. *Nature* 465:665