SEXUAL MEDICINE REVIEWS REVIEW

Role of Androgens in Female Genitourinary Tissue Structure and Function: Implications in the Genitourinary Syndrome of Menopause

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ABSTRACT

Introduction: Genitourinary conditions in women increase in prevalence with age. Androgens are prerequisite hormones of estrogen biosynthesis, are produced in larger amounts than estrogens in women, and decrease throughout adulthood. However, research and treatment for genitourinary complaints have traditionally focused on estrogens to the exclusion of other potential hormonal influences.

Aim: To summarize and evaluate the evidence that androgens are important for maintaining genitourinary health in women and that lack of androgenic activity can contribute to the development of symptoms of the genitourinary syndrome of menopause.

Methods: The role of androgens in the pathophysiology, diagnosis, and treatment of genitourinary syndrome of menopause was discussed by an international and multidisciplinary panel during a consensus conference organized by the International Society for the Study of Women's Sexual Health. A subgroup further examined publications from the PubMed database, giving preference to clinical studies or to basic science studies in human tissues.

Main Outcome Measures: Expert opinion evaluating trophic and functional effects of androgens, their differences from estrogenic effects, and regulation of androgen and estrogen receptor expression in female genitourinary tissues.

Results: Androgen receptors have been detected throughout the genitourinary system using immunohistochemical, western blot, ligand binding, and gene expression analyses. Lower circulating testosterone and estradiol concentrations and various genitourinary conditions have been associated with differential expression of androgen and estrogen receptors. Supplementation of androgen and/or estrogen in postmenopausal women (local administration) or in ovariectomized animals (systemic administration) induces tissue-specific responses that include changes in androgen and estrogen receptor expression, cell growth, mucin production, collagen turnover, increased perfusion, and neurotransmitter synthesis.

Conclusion: Androgens contribute to the maintenance of genitourinary tissue structure and function. The effects of androgens can be distinct from those of estrogens or can complement estrogenic action. Androgen-mediated processes might be involved in the full or partial resolution of genitourinary syndrome of menopause symptoms in women. **Traish AM, Vignozzi L, Simon JA, et al. Role of Androgens in Female Genitourinary Tissue Structure and Function: Implications in the Genitourinary Syndrome of Menopause. Sex Med Rev 2018;X:XXX–XXX.**

Key Words: Testosterone; Dihydrotestosterone; Estradiol; Vulvovaginal Atrophy; Androgen Receptor; Estrogen Receptor

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INTRODUCTION

Menopause and aging are associated with decreased biosynthesis of sex steroid hormones resulting in structural and physiologic changes in the genitourinary tract, including, but not limited to, anatomic alterations in vulvar, clitoral, vestibular, urethral, vaginal, anterior vaginal wall, periurethral (prostate), and bladder tissues.[1–3] There can be thinning of the epithelium, decreased vascularity, altered smooth muscle structure, and decreased collagen and elastin content. Clinically, symptoms and physical examination findings can include thinning and resorption of the labia, retraction of the introitus with concomitant dyspareunia, genital dryness, loss of vaginal rugae, friable vaginal epithelium with signs of injury (petechiae) and/or inflammation, increased vaginal pH, protrusion and widening of the urethral meatus and urethral sensitivity, urinary frequency and urgency, recurrent urinary tract infections, and decreased lubrication, vaginal or vestibular discomfort, or pain with or without sexual activity.[4–7] Secondary to these symptoms, there also can be decreased libido and orgasmic response, significantly affecting sexual function. In symptomatic women, these changes can cause significant distress with decreased quality of life[8,9] and constitute part of the diagnosis for genitourinary syndrome of menopause (GSM).[1] GSM is chronic and progressive, increases in severity over time, and does not improve without treatment.[1,10]

Sex steroid hormones (androgens, estrogens, and progestins) play an important role in human reproductive and sexual function. In particular, androgens and estrogens are critical physiologic modulators during development and maintenance of genital tissue structure and function.[1,4–6,11] Despite the longstanding perspective that pathologic conditions affecting female genital and urinary tissues are due to estrogen deficiencies,[2] the biochemical and physiologic mechanisms regulating sex steroid action in the genitourinary system have not been fully investigated and the role of androgens is unrecognized or underappreciated. This review examines the evidence that androgens contribute to genitourinary health in women and that hormonal insufficiency of androgens and estrogens after menopause can lead to GSM.

METHODS

The International Society for the Study of Women's Sexual Health convened a multidisciplinary and international panel of 14 researchers and clinicians in obstetrics and gynecology, endocrinology, urogynecology, internal medicine, biochemistry, and physiology. During the 2-day conference, participants presented and discussed the current state of knowledge on the pathophysiology, diagnosis, and treatment of GSM and, in particular, the role of androgens in female genitourinary tissues. A working subgroup was designated to develop a scientific report to support a separate clinically focused consensus white paper. The writing team performed literature reviews using the US National Center for Biotechnology Information's PubMed database on (i) the effects of androgens in female genitourinary tissues; (ii) distinctions between androgenic and estrogenic effects; and (iii) regulation of androgen and estrogen receptors (AR and ER) in premenopausal and postmenopausal women, intact and ovariectomized animals, and animal models of human disease. Selection criteria were based on expert opinion and, whenever possible, preference was given to studies that included human tissues. There were no filters or restrictions on publication date. Because treatment recommendations and efficacy of therapies are not specifically discussed in this review, levels of evidence have not been included.

HORMONAL AND GENITOURINARY CHANGES IN POSTMENOPAUSAL WOMEN

During early childhood, plasma estradiol concentration generally remains below 13 pg/mL and gradually increases during prepuberty and through adolescence.[12] Plasma testosterone concentration in prepubertal girls is lower than 20 ng/dL.[12] During the reproductive years, mean plasma estradiol concentration varies throughout the menstrual cycle in women, ranging from 40 to 350 pg/mL, and mean total testosterone concentration is 35 ng/dL (range = 20–70 ng/dL).[13,14] In healthy premenopausal women, the vagina has a stratified squamous epithelium composed of 3 cell layers consisting of superficial and intermediate cells with few parabasal cells. Normal proliferation of the epithelium leads to the formation of moist and thick rugae on the mucosal surface of the vagina and glycogen released by exfoliated epithelial cells is hydrolyzed into glucose. Then, glucose is metabolized mainly by lactobacilli into lactic acid, creating an acidic environment (pH = 3.5–4.5) that discourages the growth of pathogenic bacteria and fungi.[6]

During menopause, mean estradiol concentration decreases to 13 pg/mL.[14] Although mean testosterone concentration is decreased slightly to a value of 25 ng/dL,[14] it is important to emphasize that androgen levels progressively decrease throughout adult life.[15,16] Thus, menopause itself is not associated with dramatic decreases in androgens but postmenopausal women can have significantly less endogenous androgens compared with younger women in their 20s and 30s. Interestingly, the perimenopausal state might be associated with a relatively stable serum concentration of free testosterone due to decreasing SHBG, caused in part by decreased estradiol.[17] The greatest decreases in SHBG levels have been reported to occur from 4 years before and up to 2 years after the final menstrual period.[17]

In the absence of sufficient levels of sex steroid hormones, the genitourinary organs essentially return to the structure and function more representative of prepuberty. Vulvar tissue can appear diminished, obliterated, or even fused, and irritation or erythema can be evident.[18] The introitus becomes narrow with a loss of hymenal remnants, the cervix can become flush with the vaginal vault, and pelvic organ prolapse is not uncommon.[18] The vagina can become shortened and narrowed; its surface can

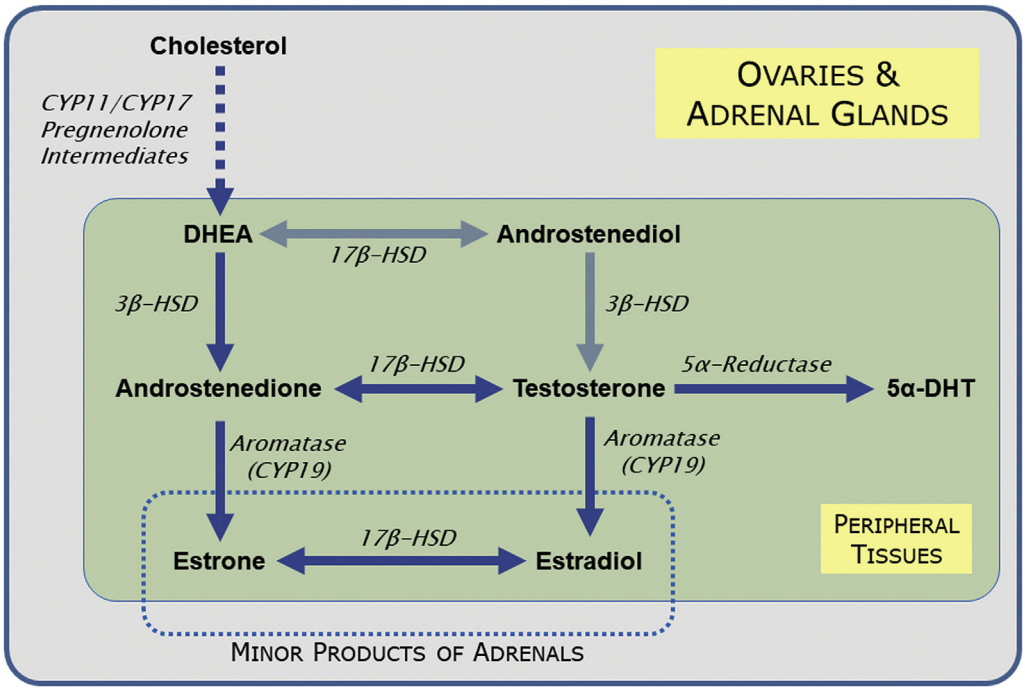

Figure 1. Synthesis of androgens. The major androgen secreted by the adrenals is DHEA, synthesized from cholesterol by the sequential action of CYP11 and CYP17 enzymes. Testosterone is synthesized from cholesterol in the ovaries and adrenals and from circulating DHEA in the peripheral tissues. Testosterone and androstenedione are the necessary precursors to the formation of estradiol and estrone. Estrogens are a minor product of the adrenal glands (dashed rectangle). Androstenedione also can be converted by aromatase to estrone and interconversion between estradiol and estrone is mediated by multiple isoforms of 17β-HSD. 5α-DHT is synthesized in target tissues from testosterone by the action of 5α-reductases. Major pathways of synthesis in humans are denoted by blue arrows and minor pathways are denoted by gray arrows. CYP = cytochrome P450; DHEA = dehydroepiandrosterone; DHT = dihydrotestosterone; HSD = hydroxysteroid dehydrogenase.

appear thinner, less elastic, and smoother (fewer rugae) with visible petechiae from intradermal or submucosal bleeding or inflammation. Urethral changes can include development of a urethral caruncle with urethral prolapse when the mucosa is circumferentially everted at the meatus. The loss of acidity in the vagina is associated with decreased resistance to non-native bacterial or fungal infection and there could be subsequent development of ascending bladder infections, overactive bladder, or recurrent urinary tract infections.[19]

ANDROGEN PRODUCTION AND SIGNALING

Androgens are 19-carbon steroid compounds that are synthesized from cholesterol (Figure 1). The 4 major androgens present in the systemic circulation of premenopausal women are dehydroepiandrosterone (DHEA; mostly as DHEA sulfate), androstenedione, testosterone, and 5α-dihydrotestosterone (5α-DHT). Androstenediol is a 5th androgen that is produced in lesser amounts. The synthesis of androgens in women takes place primarily in the ovaries and adrenal glands but can be synthesized in peripheral tissues. The ovaries secrete DHEA 1 to 2 mg/day and androstenedione 1 to 3.5 mg/day (peaking in peri-ovulatory phase) and the adrenal glands secrete DHEA sulfate 7 to 14 mg/day, DHEA 3 to 4 mg/day, and androstenedione 1 to 1.5 mg/day.[20] Total testosterone production has been estimated to be 0.1 to 0.4 mg/day, with the ovaries and adrenals synthesizing roughly equal amounts.[20,21] Similar amounts of estradiol (0.06–0.4 mg/day) are produced by the ovaries.[22] Thus, in healthy premenopausal women, production of androgens (DHEA, androstenedione, and testosterone) is significantly greater than that of estrogens.

It also is important to note that androgens are the necessary precursors for the biosynthesis of estrogens (Figure 1).[23–25] In the ovaries, adrenal gland, and peripheral tissues, DHEA and androstenedione can be converted to testosterone, which in turn can be converted to the more potent androgen 5α-DHT by the action of 5α-reductases or to estradiol by aromatase.[26] Aromatase also converts androstenedione to estrone, a weaker estrogen. A reversible reaction mediated by multiple isoforms of 17β-hydroxysteroid dehydrogenase can interconvert estrone and estradiol, but estrogens (18-carbon steroid compounds) are generally not converted back to androgens. In postmenopausal women, circulating DHEA and androstenedione are important precursors for the local synthesis of testosterone and estradiol in extragonadal tissues.[24,26]

The classic mechanism of androgen action through the intracellular AR has been well described in previous publications.[27,28] Briefly, binding of testosterone or 5α-DHT with the AR in target cells leads to receptor activation that involves conformational changes, including dissociation of heat shock proteins, receptor

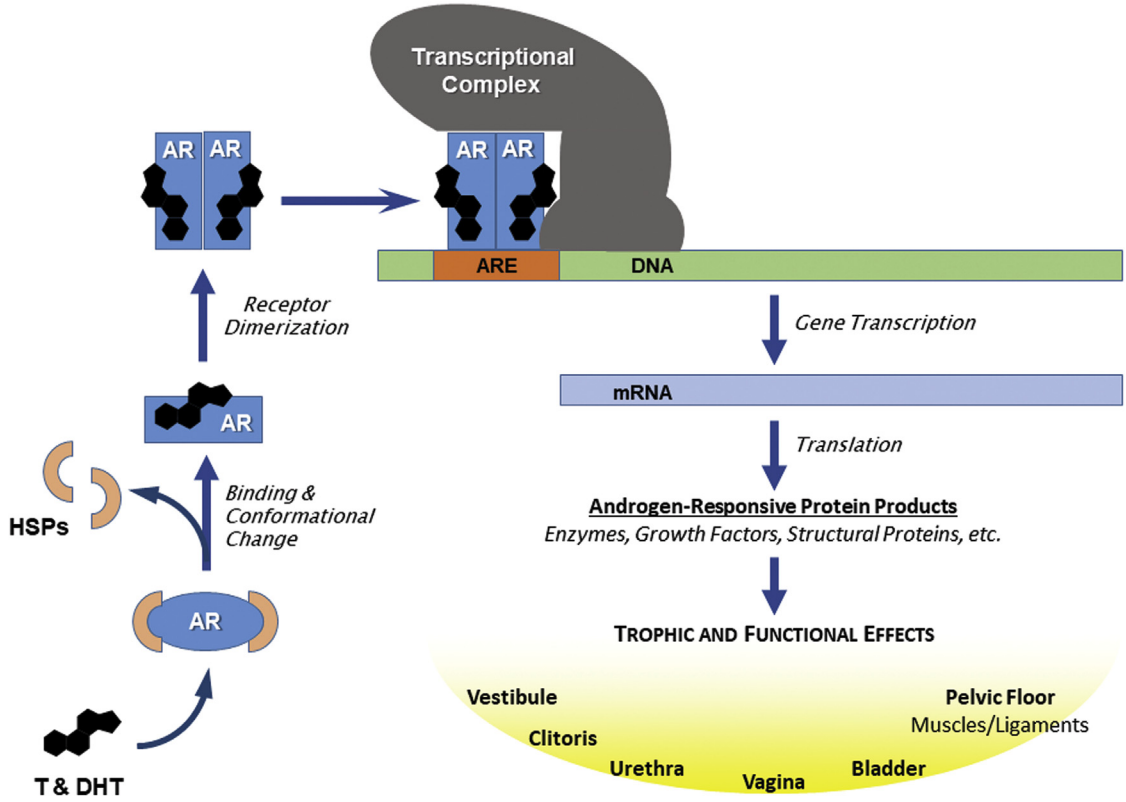

Figure 2. AR signaling. ARs are ligand-activated transcriptional regulators. Binding of T or 5α-DHT causes dissociation of HSPs, conformational change in the AR, and receptor dimerization. Ligand-bound receptor dimers bind to AREs on DNA and organize multiple transcriptional factors including RNA polymerase, coactivators and/or corepressors (transcriptional complex). Androgen-dependent protein products exert trophic effects on various genitourinary tissues. AR = androgen receptor; ARE = androgen-response element; DHT = dihydrotestosterone; HSPs = heat shock proteins; T = testosterone.

dimerization, and translocalization into the nucleus (Figure 2). This activated hormone-receptor complex binds to androgen response elements on the DNA with high affinity. Such specific binding to androgen response elements recruits transcriptional factors and co-activators or co-repressors, resulting in increased or decreased mRNA expression of specific androgen-responsive genes and subsequent changes in protein synthesis and cellular metabolism. Novel mechanisms involving non-genomic rapid signaling through cytoplasmic and plasma membrane ARs have been reported in neurons of the central nervous system, prostate cancer cells, and mouse Sertoli cells.[29–35] However, the significance of these signaling pathways in female genitourinary tissues is not clear and not discussed in this review.

EFFECTS OF ANDROGENS IN FEMALE GENITOURINARY TISSUES

Although the role of androgens in genitourinary tissues is based predominantly on data from animal studies, several lines of evidence from clinical studies and laboratory analyses of human tissues suggest that androgens also are important in human genitourinary physiology. Detection of ARs and ERs in genitourinary tissues and androgenic effects that are distinct from and contributory to estrogenic effects are discussed below.

Vagina

AR protein immunoreactivity and mRNA were detected throughout the human vagina (mucosa, submucosa, stroma, smooth muscle, and vascular endothelium).[36–38] Similar findings of positive AR immunoreactivity in the epithelium, lamina propria, blood vessels, and muscularis layer were reported in monkey vagina.[39] In addition, enzymes involved in androgen biosynthesis (17β-hydroxysteroid dehydrogenase and 5α-reductase) were expressed at high levels in the stratified squamous epithelium of monkey vagina and at lower levels in the muscularis layer and in the walls of blood vessels.[39]

ER is present in the epithelium and in stromal and muscle cells of the human vagina.[40,41] Interestingly, immunostaining for ER and AR was observed in epithelium, stroma, and smooth muscle of canine vagina and vulva but ER and AR staining intensity was generally higher in the stroma than in the epithelium.[42] Although direct evidence was not provided in this study, the investigators hypothesized that stromal-epithelial (paracrine) interactions might be important in mediating the effects of androgens and estrogens, particularly to the differentiation of vaginal epithelium.

It is well established that estrogen stimulates cell proliferation and thickening of the human vaginal epithelium.[43] More recent studies confirmed that epithelia throughout the lower urinary

tract of women exhibit increased proliferation in response to estrogen.[44] Less recognized is the observation that androgens also could independently regulate vaginal health.[45] Initial observations that vaginal atrophy was less prevalent in postmenopausal women with higher levels of androstenedione and testosterone[46,47] left open the possibility that testosterone could be converted to estradiol by aromatase. However, in a small study in women (N = 21) with breast cancer undergoing aromatase inhibitor therapy, intravaginal (vaginal mucosa) application of testosterone for 4 weeks was shown to ameliorate the signs and symptoms of vaginal atrophy (dyspareunia, vaginal dryness, vaginal pH, and epithelial maturation),[48] suggesting that androgens could have an independent effect on epithelial growth.

As noted earlier, circulating DHEA can be locally converted in genitourinary tissues to androgens and estrogens that bind AR and ER.[26] Thus, the effects of DHEA also could provide supportive evidence for androgens and estrogens in maintaining genitourinary tissue health. Indeed, in a randomized, double-blinded, placebo-controlled trial evaluating intravaginal DHEA for treating symptoms of GSM, daily application for 12 weeks in postmenopausal women improved vaginal pH, superficial and basal epithelial cell counts and relieved dyspareunia.[49] Currently, it remains unclear to what extent these clinical improvements can be attributed AR vs ER activation and it is generally assumed that androgen and estrogen metabolites of DHEA provide benefit. Limited evidence in cell lines and rodent models of diabetes, chemical carcinogenesis, and obesity suggests that DHEA also could act independently through specific membrane receptors or facilitate the activation of other receptor systems.[50–53] Further, a G-protein-coupled membrane receptor in bovine aortic endothelial cells that activates endothelial nitric oxide synthase has been reported.[54] Although a specific DHEA receptor has not been demonstrated in human tissues or cells, future research could uncover additional mechanisms of action of DHEA that go beyond the traditional view of this steroid as a prohormone.[55]

Vaginal perfusion also is regulated by androgens and estrogens in the context of baseline blood flow and during sexual arousal. Esterified estrogens increased baseline genital blood flow in postmenopausal women,[56] whereas testosterone or estradiol administration in ovariectomized rats restored vaginal blood flow responses to pelvic nerve stimulation.[57–59] Although specific mechanisms have yet to be characterized, testosterone and estradiol differentially regulate the expression of nitric oxide synthase and arginase,[60] key proteins regulating vaginal blood flow.[61,62] Interestingly, in a small double-blinded, randomized, placebo-controlled trial of 8 healthy women, vaginal vasocongestion and genital sensation in response to visual sexual stimulation was significantly increased 3 to 4.5 hours after sublingual administration of a single dose (0.5 mg) of testosterone undecanoate.[63] Although testosterone has been shown to have acute vasodilatory effects in other vascular beds,[64,65] the enhancement in vaginal response to visual sexual stimulation was significantly delayed in relation to peak plasma levels of testosterone that occurred 15 minutes after dosing and returned to baseline levels within 90 minutes. Conceivably, measurable increases in vaginal vasocongestion could require testosterone-induced upregulation of protein synthesis directly within the vagina and alterations in neuronal signaling pathways in the brain that process visual sexual stimuli.

In other functional studies in animals, androgens and estrogens were shown to regulate vaginal mucin production in epithelial cells[66–71] and to increase norepinephrine content or increase the density of adrenergic nerves in the vagina.[11,72–76] In more recent studies comparing androgenic and estrogenic effects, increased vaginal innervation was attributed to androgens but not to estrogens.[75,77] Direct comparisons between studies remain difficult because of different experimental paradigms (ovariectomy vs pregnancy vs specific hormone administration at different doses) and different methodologies of estimating neurotransmitter content or nerve fiber area or density in relation to tissue atrophy or growth. Nevertheless, although vaginal epithelial proliferation appears to be primarily an estrogenic effect, androgens do appear to have a role in regulating vaginal epithelial function (eg, mucin production). Whether maintenance of innervation requires a combination of androgens and estrogens or is exclusively an androgenic effect remains unclear. Interestingly, testosterone replacement in ovariectomized rabbits caused thickening of the submucosal lamina propria and muscularis layers and enhanced neurogenic and vasoactive intestinal peptide-induced relaxation of vaginal tissue strips, whereas estradiol replacement primarily stimulated epithelial thickening and did not improve neurogenic or vasoactive intestinal peptide-induced relaxation of the vaginal wall.[58] These observations support the perspective that vaginal health consists of more than epithelial health and the impact of androgens and estrogens should be evaluated in a more holistic manner with regard to overall structure and function.

Bladder, Urethra, Prostate

AR and ER have been detected in the epithelium and smooth muscle of the urethra and bladder in female rabbits[78] and specific receptor binding sites for estradiol and 5α-DHT were reported in the urinary tract of female baboons.[79] In ovariectomized female rats, the bladder exhibited changes in tissue histomorphology, growth factor expression, and contractile responses that were partly restored by estradiol or testosterone treatment.[80–82] Treatment with the combination of estradiol and testosterone was the most effective. In a separate study, ovariectomized rats treated with testosterone plus the aromatase inhibitor letrozole to inhibit the conversion of testosterone to estradiol normalized bladder relaxation responses to nitroprusside and increased the degree to which a phosphodiesterase type 5 inhibitor could potentiate nitroprusside-induced relaxation.[83] In female rabbits, ovariectomy produced urothelial hypoxia because of decreased blood flow[84] and estradiol was shown to improve bladder and urethral structure and function by increasing tissue vascularity.[85]

The responsiveness of bladder, urethral, and prostatic[86] tissue to sex steroid hormones in women is supported by several correlational studies. Although stress urinary incontinence and polycystic ovaries are not included within the spectrum of GSM, the following clinical studies are illustrative of trophic or functional effects of androgens and estrogens in the bladder, prostate, and urethra of women. Vascular resistance (inversely correlated with blood flow) was greater in the bladder neck of menopausal women than premenopausal women and systemic estrogen-progesterone hormone replacement therapy increased blood flow in menopausal women with urinary stress incontinence.[87] In addition, ultrasound assessment in normal-weight premenopausal women with and without polycystic ovary syndrome suggested a positive correlation between circulating testosterone levels and the volume of urethrovaginal tissue.[88] Although the full clinical significance remains unclear, in genitourinary tissue (vaginal punch biopsies near external urethral meatus) of women with and without stress urinary incontinence (36−88 years old), estradiol levels were positively correlated and testosterone levels were negatively correlated with markers of collagen turnover.[89] Insufficient amounts of collagen can lead to laxity or prolapse, whereas excess collagen accumulation can lead to tissue fibrosis. Thus, it stands to reason that significant decreases in estrogens and/or androgens or alterations in ER and/or AR expression can adversely affect the balance of collagen synthesis and degradation.

Clitoris, Labia, Vestibule

Positive immunostaining for AR and ER was demonstrated in epidermis and dermis of human labial tissue.[36,41] Compared with human vaginal epithelium, there was greater immunostaining for AR and less immunostaining for ER in labial skin.[36] Similarly, positive immunostaining for AR and ER has been reported in canine vulvar skin.[42] AR has been localized to the mucin-secreting vestibular (Bartholin) glands in humans[90] and ER has been detected in the vestibular glands of the cat.[91] However, aside from the well-known atrophic changes of the labia and vestibule in postmenopausal women, few studies have examined the potential clinical relevance of sex steroid hormones in these tissues.

In a small study examining skin from women with vulval lichen sclerosus (VLS; n = 7), the epidermal layer was significantly thinner but ER and AR immunostaining was similar to that of women without VLS (n = 8).[41] The main difference occurred in the subdermal fibromuscular layer, where ERα was the predominant receptor isoform in vulvar smooth muscle of healthy women but ERβ became predominant with concomitant disappearance of ERα in vulvar smooth muscle of women with VLS.[41] AR was only present in the subdermal vascular layer of healthy women and absent in the fibromuscular layer of the vulva of the 2 groups. Because the fibromuscular layers in women with VLS were negative for Ki-67, a cellular marker for proliferation, the investigators postulated that the appearance of ERβ could lead to other functional alterations not associated with cell growth (possibly collagen production), whereas ERα in healthy women without VLS could stimulate cell growth.[41]

In a separate study, AR was detected in the vulvar skin of 5 of 39 postmenopausal women with VLS and 4 of the women with positive staining for AR were asymptomatic.[92] However, the pathophysiology of lichen sclerosis remains unknown[93] and claims of normal function in this patient population should be interpreted cautiously. Thus, it remains unclear whether AR expression in vulvar skin confers any benefit with regard to symptoms experienced by women with VLS, especially because topical testosterone or 5α-DHT has been deemed ineffective in treating VLS symptoms.[94] With regard to the vestibule, administration of combined estradiol and testosterone implants in veal calves was associated with metaplasia and hyperplasia of the vestibular glands and this response was proposed as a marker for detecting supplemental administration of naturally occurring anabolic steroids.[95] Although the investigators assumed that this effect on the vestibular glands was an estrogenic effect, it remains unclear whether testosterone contributed to the transformational or proliferative effects.

The clitoris is well established as an androgen responsive organ during embryonic development but it also maintains androgen dependency in adulthood because it continues to express significant amounts of AR, although most tissues in the developing female genital tract cease to express AR after the 1st trimester of pregnancy.[96−98] Clitoral hypertrophy or clitoromegaly is considered one of the most sensitive markers for increased androgen production in women.[99] Yet, even in this androgen-sensitive organ, estrogen is functionally important. In healthy eumenorrheic premenopausal women, basal clitoral volume and blood flow varied during the menstrual cycle. In the absence of sexual arousal, estradiol levels were positively correlated with clitoral volume and negatively correlated with vascular resistance.[100] In ovariectomized female rats, treatment with testosterone was associated with increased endothelium-dependent vasodilation of the clitoral corpora cavernosa, a critical event for clitoral tumescence during sexual arousal.[101] This effect of testosterone was not due to conversion to estradiol because co-administration of testosterone and letrozole, an aromatase inhibitor, did not abolish the response. Ovariectomy also impaired clitoral vascular smooth muscle contractile signaling pathways involving RhoA and RhoA-associated protein kinase and this was reversed with estradiol treatment.[101] Thus, estradiol and testosterone are necessary for maintenance of clitoral tissue morphology and signaling pathways that regulate vascular responsiveness of the clitoris during sexual arousal.

Supporting Structures and Pelvic Floor

Similar to other skeletal muscle, ARs are highly prevalent throughout the pelvic floor musculature.[102] AR immunostaining was strongly detected in biopsies of levator ani muscle from premenopausal and postmenopausal women.[103] In a female rat model of stress urinary incontinence, a single dose of testosterone

increased myofiber area and prevented or ameliorated incontinent symptoms after early or delayed administration of testosterone.[104] In ovariectomized mice with atrophied pelvic muscles, treatment with selective AR modulators restored pelvic muscle mass and genes associated with muscle catabolism were inhibited.[105]

Whether ER participates in the regulation of pelvic muscle growth or function remains unclear because reports are inconsistent. ER was not detected in the levator ani muscle of women in some studies,[103,106] whereas others did report the presence of ER in the levator ani muscle of premenopausal and postmenopausal women.[107–109] Nevertheless, as discussed previously with periurethral tissue, ER does appear to have a role in regulating connective tissue and extracellular matrix. ER, along with AR, has been detected in the levator ani fascia of women undergoing surgery for gynecologic and urogynecologic conditions.[103] ER was increased in the levator ani fascia of symptomatic women compared with age-matched and asymptomatic women. In contrast, premenopausal women with pelvic organ prolapse had significantly lower ER levels in the cardinal ligament and uterosacral ligament.[110] ER also was decreased in periurethral fascia samples from menopausal women with stress urinary incontinence.[111] Whether these differences are due to the various tissues, anatomic locations, and/or conditions remains to be elucidated.

Somato-sensation

In general, women are more likely to experience chronic pain syndromes compared with men and this sex disparity has provided a rationale for investigating the role of sex steroid hormones in sensory perception and nociception. Symptoms and perception of pain are known to vary throughout the menstrual cycle in women diagnosed with chronic pelvic pain, painful bladder syndrome, and irritable bowel syndrome.[112] However, the effects of androgens and estrogens on attenuating or potentiating nociception are complex and not consistently predictable. Lower estradiol concentration was associated with weaker emotional control (reflective of central neuromodulation) over peripheral pain stimuli in healthy, normally cycling premenopausal women.[113] In a separate study of healthy premenopausal women assessing interindividual differences in pain perception using the McGill Pain Questionnaire, serum testosterone was positively correlated with an antinociceptive effect and serum estradiol was correlated with a pro-nociceptive effect.[114] In women with fibromyalgia not receiving hormone therapy (age range = 42–55 years), daily topical treatment with 1% testosterone gel 0.75 g improved self-reported symptoms related to this chronic pain condition (muscle pain, stiffness, and fatigue).[115] Although various experimental paradigms have attempted to discern the differences between central and peripheral effects, these types of clinical studies highlight the fact that the effects of androgens and estrogens on central mechanisms of pain perception are intimately associated with any potential peripheral effects on sensory nerves.

Immunohistochemical studies of vaginal tissue biopsies from postmenopausal women have demonstrated that total nerve density was highest in patients not receiving hormone therapy, moderate in those receiving systemic estrogen therapy, and lowest in those receiving intravaginal estrogen therapy.[116] This downregulation included parasympathetic, sympathetic, and sensory nerves. These findings also are supported by studies in female rats. Compared with rats in estrus, total nerve density was increased by 59% in vaginal tissue of ovariectomized rats and these effects persisted after correcting for differences in vaginal tissue volume.[117] When ovariectomized rats were treated with estradiol, the nerve density was decreased to levels that were similar to intact rats in estrus.[117] The investigators postulated that decreasing estrogen levels during menopause could increase sympathetic and sensory nerve density, leading to vasoconstriction, vaginal dryness, and pain.[117]

In functional studies, estrogen has been shown to increase the size of the sensory field for the pudendal nerve in female rats.[118] ERα protein and mRNA for ERα and ERβ have been detected in neurons of lumbosacral dorsal root ganglia[119] and could be important for growth and maintenance of sensory nerve pathways.[120] Estrogen also has been shown to enhance the response to opioids in rats and non-human primates,[121,122] although this is not a consistent finding.[123] Thus, data suggest that androgens and estrogens can modulate sensory nerve function and/or sensory perception. However, specific findings in genitourinary tissues are extremely limited and future research in this area would prove valuable to characterizing mechanisms of hypersensitivity and pain in GSM.

REGULATION OF AR AND ER EXPRESSION IN FEMALE GENITOURINARY TISSUES

Receptor protein expression is a critical mechanism regulating tissue responsiveness to hormones. Self-regulation and cross-regulation of AR and ER by androgens and estrogens can modulate the effects of sex steroid hormones in genitourinary tissues.

Regulation of AR by Testosterone

In vaginal tissue from women undergoing surgery for pelvic organ prolapse, AR protein and mRNA expression were significantly decreased in postmenopausal women compared with premenopausal women.[38] In a separate study examining tissue from women undergoing surgery for incontinence or pelvic organ prolapse, older age was correlated with decreased AR immunostaining in the vaginal epithelium.[37] Although plasma testosterone concentration was not available for all groups in these studies, the downregulation of AR expression is presumably further correlated with the well-documented decrease in androgens with advancing age.

In women treated with long-term administration of high-dose testosterone (Testoviron depot 100 mg intramuscularly every

7−10 days for ≥1 year) before female-to-male gender reassignment surgery, AR mRNA expression was significantly greater in vaginal tissue compared with that in postmenopausal women.[38] Similarly, ovariectomy of rats resulted in downregulation of AR expression, and testosterone replacement restored AR expression in the vagina, as assessed by ligand binding assays and western blot analyses.[59] Immunostaining studies also demonstrated increased AR density in the vaginal muscularis of ovariectomized rats supplemented with testosterone.[124] Thus, circulating testosterone is positively correlated with AR expression in the vagina. Whether a critical concentration of testosterone is required to maintain adequate levels of AR remains unknown.

Regulation of ER by Estrogen

In women undergoing surgery for urogynecologic conditions, ERα mRNA increased in the vagina of postmenopausal women and decreased after systemic estrogen-progestin replacement therapy, but not with intravaginal estrogen.[125] Although the exact types of hormone therapy were not specified, lower doses of estrogen were presumably used for intravaginal application compared with systemic administration. These findings are consistent with animal studies. Functional ER, assessed by immunostaining and binding of [^{3}H]estradiol, increased in vaginal tissue extracts of ovariectomized rats and decreased in ovariectomized rats supplemented with estradiol to physiologic levels.[57,124] However, ER remained elevated in vaginal tissue extracts of ovariectomized rats supplemented with sub-physiologic levels of estradiol.[57,110] In the same study, similar findings were obtained when ER was assessed by western blots.[57] In canine vagina, significant negative correlations were found between serum estradiol concentration and ERα immunostaining in epithelial and stromal cells, suggesting a negative feedback mechanism between estradiol and its receptor.[42] However, other investigators have not observed such reciprocal regulation between estradiol and ER. In a small cohort of patients undergoing surgery for vaginal prolapse, ERα immunostaining was significantly decreased in the vaginal mucosa but not in the vaginal stroma of postmenopausal women compared with premenopausal women.[126] ERβ was decreased in the stroma but not in the mucosa of postmenopausal women.[126] Although the significance of this differential regulation of ER subtypes remains unclear, this last study found an overall decrease in total ER in the vagina in postmenopausal women. It is unlikely that this inconsistency is due to differences in species or assessment technique because previous studies reported consistent findings among human, rat, and dog vaginal tissue using immunostaining, western blotting, and receptor binding assays.[42,57,124,125] Future studies should investigate potential differences between specific urogynecologic conditions and vaginal tissue (proximal vs distal vs periurethral).

Cross-Regulation of AR and ER

In addition to testosterone, 5α-DHT, and estradiol regulating their receptors, each hormone could cross-regulate the other receptor. In postmenopausal women receiving oral or transdermal estrogen replacement therapy, AR expression in the vaginal submucosa was significantly decreased compared with untreated postmenopausal women.[37] In ovariectomized rats, estradiol administration at physiologic levels decreased AR immunostaining in the vaginal epithelium and muscularis.[124] Although these changes in the rat vagina did not reach statistical significance (likely because of the large number of treatment groups compared), on average, AR immunostaining decreased 43% in the epithelium and 27% in the muscularis.[124] These findings suggest that estrogen could downregulate AR expression in the vagina.

As mentioned earlier, immunostaining for hormone receptors was performed in vaginal tissue from women undergoing long-term treatment of high-dose testosterone before gender reassignment surgery.[38,126] Testosterone treatment decreased the expression of ERα and ERβ in the stromal and epithelial layers compared with premenopausal women.[126] Further, testosterone administration induced profound morphologic changes to the vagina, manifested primarily as thinning of the vaginal epithelium and loss of glycogen content.[126] Unlike the effect of testosterone on AR expression, the effects of testosterone on ER have not been confirmed in animal studies. For example, in ovariectomized rats administered physiologic levels of testosterone, ER expression remained elevated at levels that were similar to ovariectomized rats treated with vehicle.[124] Thus, it remains unclear whether cross-regulation of ER by testosterone occurs in the vagina. Currently, the findings of ER downregulation by testosterone in women undergoing gender reassignment surgery should be interpreted cautiously until further studies can corroborate these data.

Receptor Polymorphisms

Aside from changes in expression of AR and ER proteins, hormone receptor polymorphisms could influence susceptibility to certain clinically significant conditions as hormone levels decrease. For example, in a small study of women taking combined hormonal contraceptives, those who developed vestibulodynia were more likely to have longer CAG repeats in the AR gene, a genetic characteristic associated with decreased responsiveness to androgens in target tissues.[127−130] Therefore, it can be speculated that the risk of developing vestibulodynia is due to lowered androgenic activity in the vulva. Another example is that the ERα genetic polymorphism rs2228480 G/A was significantly associated with pelvic organ prolapse in 2 separate studies.[131,132] Thus, genetic polymorphisms of receptors add another layer of complexity in understanding the molecular etiology of pathologic conditions.

SUMMARY AND CONCLUSIONS

Taken together, clinical and laboratory studies in human and animal tissues strongly suggest that decreasing androgens with advancing age and cessation of estrogen production during

menopause are important contributory factors in the development of the signs and symptoms of GSM. The actions of androgens can be distinct from those of estrogens and the effects of testosterone do not necessarily require its conversion to estradiol by aromatase. The most readily available clinical observations of androgenic trophic effects on the clitoris and estrogenic trophic effects on the labia and vaginal mucosa have likely resulted in an oversimplified understanding of the role of sex steroid hormones in genitourinary physiology and pathophysiology. The distinct regulation of AR and ER in different tissue layers of the vagina and the specific responses to androgen or estrogen administration is a prime example illustrating the perspective that a given sex steroid hormone cannot fully maintain tissue structure and function by itself. In addition, clinical assessments of genitourinary health (eg, parameters of epithelial health in the vagina, pain sensitivity, pallor, erythema, and dryness) are limited and do not necessarily reflect the health of the entire organ, especially when genitourinary system function is challenged by sexual activity.

Understanding the role of sex steroid hormones in genital and urinary tissues must go beyond the simple correlations with plasma levels of a given hormone. Downregulation or upregulation of receptors in target tissues could limit or enhance the action of a given concentration of androgens and estrogens in the circulation. Receptor subtypes with different signaling pathways and receptor polymorphisms that can alter interactions with other macromolecules add to the complexity of responses. Moreover, local synthesis of androgens and estrogens within genitourinary tissues can have physiologic effects that are not accurately reflected in assays of blood samples. A more complete understanding of the molecular mechanisms and individual actions associated with ARs and ERs could facilitate discovery of tissue-selective drugs that maximize beneficial effects and limit adverse effects of hormone therapy.

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