Androgen Actions in the Female – They Don't Just Make a Man out of You

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Dr. Hammes is interested in ovarian physiology and the role that sex steroids such as androgen play in normal and pathologic follicular development. In addition, Dr. Hammes is interested in transcription-independent, or nongenomic, signaling by steroids.

Introduction

The importance of androgens such as testosterone and dihydrotestosterone (DHT) in mediating normal male sexual development and fertility is without question. In contrast, the role and significance of androgens in regulating normal female physiology is not well understood at this time. The goals of this Internal Medicine Grand Rounds are to review and critique some of the existing literature with regard to three important issues concerning androgens and female physiology: 1) How do androgens effect normal female physiology? 2) Do we need to replace androgens in women later in life? 3) Is too much androgen a bad thing in women? Androgen effects on the cardiovascular system, bones, brain, and ovary will be addressed, with a final discussion on some of the mechanisms that androgens may utilize to regulate female fertility.

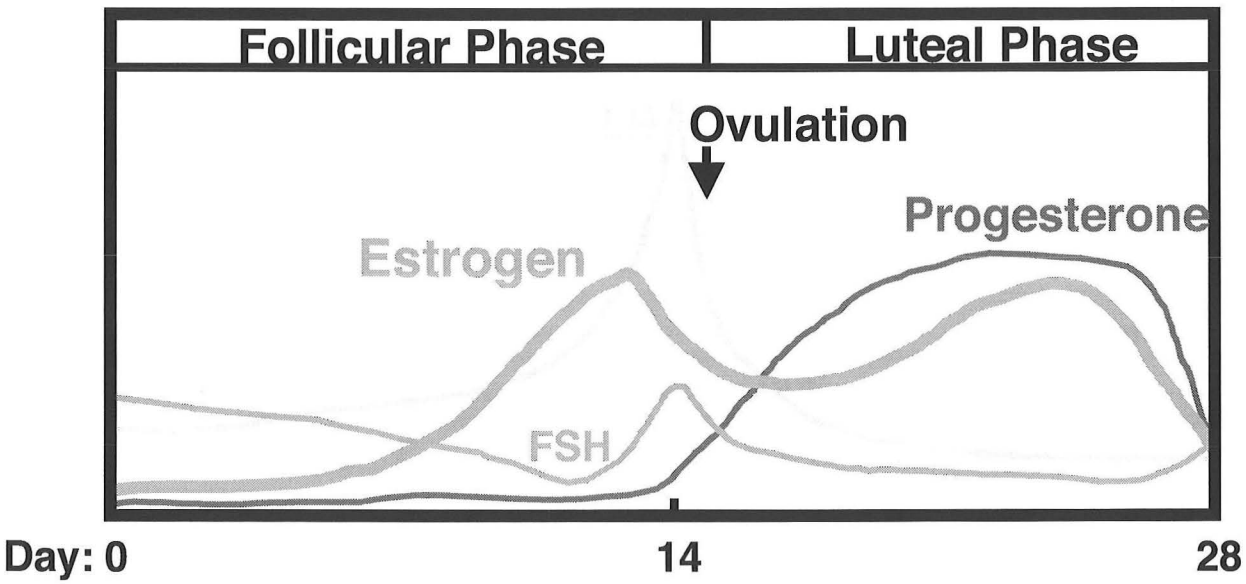

Figure 1. Hormonal changes during the menstrual cycle. Williams Endocrinology.

Androgen production in women

Precise regulation of pituitary and ovarian hormone secretion is crucial for normal fertility in women. Most graphic representations of hormone levels during the menstrual cycle focus on the pituitary peptide hormones LH and FSH and the ovarian steroid hormones estrogen and progesterone (Figure 1). In contrast, very little is written about production of one of the major estrogen precursors: testosterone. Notably, serum testosterone levels in women are actually quite high when compared to estradiol (Table 1). Testosterone concentrations range from 200 –750 pg/mL, while estradiol levels vary from 20 – 60 pg/mL to >200 pg/mL in the follicular and luteal phases, respectively (Wilson et al., 1998). In addition, testosterone production increases with estradiol during the mid-cycle ovulatory surge, suggesting that androgens might be playing some role in regulating ovulation (Mushayandebvu et al., 1996).

The presence of a surge in testosterone production prior to ovulation implies that testosterone synthesis occurs in the ovaries. Testosterone levels in women drop by 50% after oophrectomy, however, suggesting that serum testosterone is actually derived equally from the ovary and from androgen precursors secreted by the adrenal glands (Leder et al., 2002). Interestingly,

Hormone	Basal	Ovulatory Surge
Estradiol	20 - 60 pg/mL	> 200 pg/mL
Testosterone		
Total	200 - 450 pg/mL	500 - 750 pg/mL
Free	1 - 3 pg/mL	3 - 5 pg/mL
Bioavailable	16 - 127 pg/mL	

Table 1. Serum estradiol and testosterone levels during the menstrual cycle. Williams Endocrinology; Mushayandebvu et al., Fertility and Sterility

serum testosterone concentrations are reduced by only 30% in women undergoing physiologic menopause, indicating that post-menopausal ovaries may still be capable of producing significant amounts of androgens. These differences in post-menopausal reductions of serum testosterone levels further imply that studies examining the effects of androgen replacement in women with post-surgical menopause may not apply to those undergoing natural menopause (Guzick and Hoeger, 2000; Khosla and Bilezikian, 2003).

Androgen actions on the heart

Androgen effects on the cardiovascular system are not well understood. Intriguingly, there appears to be a gender-specific relationship between serum androgen levels and the metabolic syndrome, which consists of increased visceral obesity, insulin resistance, low HDL, high LDL, high triglycerides, and an increased risk of coronary artery disease. In men, HYPOandrogenemia is associated with the metabolic syndrome. Several epidemiologic studies have shown an inverse association between free testosterone levels and coronary artery disease (Wu and Von Eckardstein, 2003). Whether the low androgen levels are actually causing the metabolic syndrome remains unclear, however, as androgen deprivation in castrated men and male to female transgender patients does not seem to change their risk of CAD, nor does excess use of anabolic steroids in athletes (Eckardstein and Wu, 2003).

In contrast to men, women with HYPERandrogenemia have an increased risk of metabolic syndrome and CAD. For example, women with polycystic ovarian syndrome (PCOS) and accompanying hyperandrogenemia have an approximately 7-fold increased risk of metabolic syndrome (Dunaif, 1997; Lobo and Carmina, 2000). Again, this increased risk my be due to factors other than simply hyperandrogenemia, however, as female to male transgender patients and women taking anabolic steroids do not appear to have significantly increased risks of metabolic syndrome (Eckardstein and Wu, 2003).

Some studies have suggested that androgens might increase the risk of cardiovascular disease due to their negative effects on serum lipids. While estrogen replacement in post-menopausal women has been shown to lower LDL and raise HDL, the addition of oral methyltestosterone to the hormone replacement regimen seems to abolish these effects, leading to lower HDL and higher LDL levels (Dunaif, 1997; Lobo and Carmina, 2000; Raisz et al., 1996; Watts et al., 1995). Interestingly, women in a similar hormone replacement study using trans-dermal rather than oral testosterone had no statistically significant changes in their lipid levels when compared to taking estrogen alone (Shifren et al., 2000), suggesting that the negative effects of testosterone on lipid levels may occur only when testosterone in taken orally.

In summary: 1) The effects of androgens on normal female cardiovascular physiology are still not known. 2) Androgen replacement for postmenopausal women will probably not significantly improve the risk of CAD. 3) Excess endogenous androgen production in women (e.g. PCOS) appears to be associated with an increased risk of CAD; however, use of exogenous androgens, especially when taken by injection or trans-dermally, is not.

Androgen actions on bones

Androgens are generally felt to be important for the normal development of bones; however, their specific roles in regulating bone growth and bone mineral density (BMD) are still controversial. Women with excess androgen production and PCOS appear to have higher BMDs than age-matched controls (Khosla and Bilezikian, 2003; Zborowski et al., 2000), suggesting the androgens may be capable of promoting bone growth and density. This difference loses significance when weight and BMI are taken into consideration (Good et al., 1999), however, indicating that androgens alone may not be responsible for the higher BMDs in patients with PCOS. Interestingly, genetic males with testicular feminization due to mutations in their androgen receptors (ARs) often have low BMDs (Marcus et al., 2000), suggesting that androgen actions via the AR might

be playing an important role in attaining and/or maintaining normal BMD. In addition, mice in which the gene encoding the AR has been deleted have decreased bone volume (Yeh et al., 2002), implying that androgens might play a role in regulating bone size.

In support of androgen actions being important for the regulation of bone density, several studies comparing the effects of estrogen versus estrogen plus androgen replacement on BMD in post-menopausal women have shown that the addition of androgens leads to improvement in bone mineral density (Figure 2) (Davis et al., 1995; Lobo et al., 2003; Raisz et al., 1996; Watts et al., 1995). One possible target for androgen action in these patients might be the osteoblast, as testosterone appears to attenuate estrogen-mediated decreases in bone formation based on measurements of osteocalcin, and bone specific alkaline phosphastase (Raisz et al., 1996). Other studies instead argue that both androgens and estrogens are acting similarly in osteoblasts to attenuate apoptosis (Kousteni et al., 2001), although whether osteoblast apoptosis is playing a significant physiologic role in regulating BMD is still controversial.

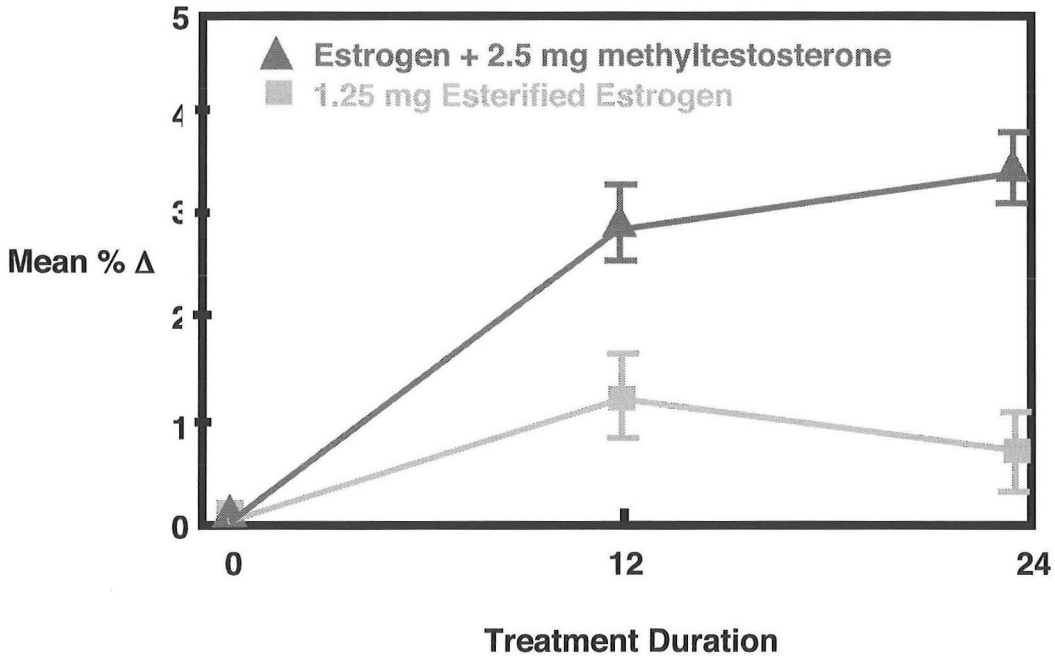

Figure 2. Addition of testosterone to estradiol improves BMD. Watts, Obst & Gyn

In summary: 1) Androgens may be playing an important role in maintaining normal bone size and BMD. 2) Androgen replacement in postmenopausal women appears to improve BMD and bone volume; however, whether it will significantly improve BMD and fracture when compared to other modalities such as bis-phosphonates is still not known. 3) Excess endogenous androgen production does not appear to adversely effect bones, but no good studies examining excess exogenous androgen use have been published.

Androgen actions in the brain

Many of the strong advocates of androgen replacement in postmenopausal women point to their potential positive effects on mood, sexual desire and function, and feelings of well-being in postmenopausal women (Davis, 1999; Davis and Burger, 2003; Guzick and Hoeger, 2000). Indeed, several studies have shown that the addition of oral testosterone to estrogen

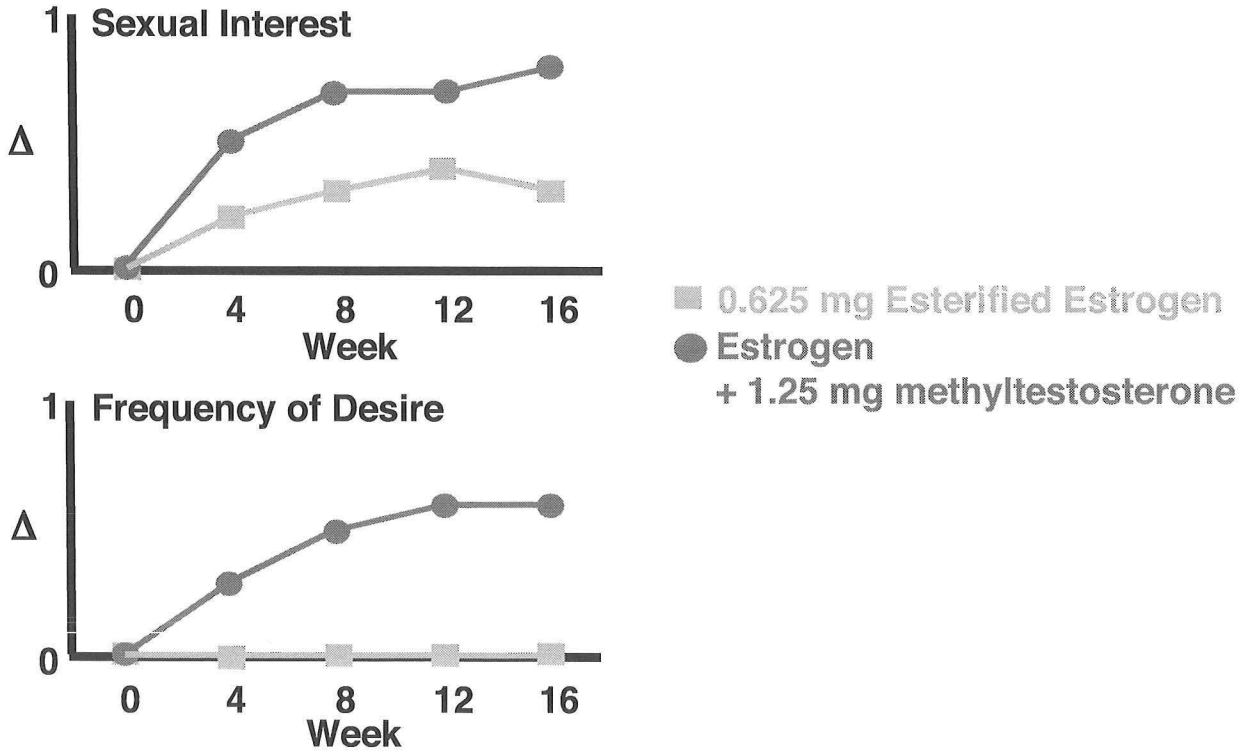

Figure 3. Androgen replacement improves sexual desire. Lobo et. al., Fertility and Sterility

replacement in postmenopausal women improves all of these parameters (Figure 3). These studies are hindered in that 1) many are done in women with post-surgical menopause, who generally have lower testosterone levels than women who have undergone physiologic menopause; 2) many of the studies used women who were actively seeking therapy, as they were having problems on

DIMENSION	BASE LINE	PLACEBO	150 μg OF TESTOSTERONE PER DAY	300 μg OF TESTOSTERONE PER DAY
Composite score	52±27	72±38	74±37	81±37†
Thoughts–desire	48±31	67±40	72±40	77±40
Arousal	58±31	80±40	73±40	84±40
Frequency of sexual activity	41±31	53±41	58±40	64±40‡
Receptivity–initiation	68±33	89±39	86±39	92±39
Pleasure–orgasm	48±42	65±53	70±52	80±52‡
Relationship satisfaction	73±33	82±32	86±32	87±32
Problems affecting sexual function	116±48	108±49	97±49	98±49

Table 2. Placebo and androgen replacement improve sexual function. Shifren et. al., NEJM

estrogen replacement alone; 3) many of the women had side-effects associated with androgen usage, including worsening lipids, hirsutism, and acne. In order to address some, but not all, of these issues, Shifren et. al. performed a blinded cross-over study using women with post-surgical menopause who were already on estrogen replacement but were sexually unsatisfied (Shifren et al., 2000). Women were treated for 12 weeks with placebo, 150 micrograms trans-dermal testosterone, and 300 micrograms of trans-dermal testosterone. These patients demonstrated marked improvements in scores of sexual desire and function, as well as feelings of well-being (Table 2). However, these scores improved similarly in patients taking the placebo patches, suggesting a large placebo effect. In addition, androgen levels were raised to the high-end of normal or above, which would likely eventually lead to androgen mediated side-effects such

as acne and hirsutism (12 weeks may have been too short to detect these changes).

In summary: 1) Although androgens play an important role in male sexuality and gender identity (Wilson, 1999), their contribution to female sexuality is still unclear. 2) Studies examining the effects of androgen replacement on sexuality and feeling of well-being in postmenopausal women show that they improve these parameters; however, the placebo effect is very strong in these individuals, making interpretation of the results difficult. Still, androgen replacement may prove beneficial for a select group of patients. 3) Excess serum androgens from exogenous sources do not appear to negatively influence sexuality and feelings of well-being in women, though it has not been carefully examined.

Androgen actions in the ovary

As mentioned, excess ovarian androgen production is clearly linked to abnormal ovarian growth, as exemplified by the polycystic ovarian syndrome. Whether the excess androgens are playing a role in promoting the ovarian pathology in these patients is controversial, however, as PCOS patients often have confounding factors that may also affect ovarian growth, including insulin resistance and abnormal gonadotropin secretion (Azziz, 2003; Dunaif, 1997; Dunaif, 2003; Lobo, 2003; Lobo and Carmina, 2000). In contrast, individuals with elevated serum androgens due to congenital adrenal hyperplasia (Speiser, 2001), aromatase deficiency (Ito et al., 1993), or exogenous androgen use (Pache et al., 1991), have an increased incidence of polycystic ovaries in the absence of these additional components, implying that the high circulating androgens may indeed be regulating abnormal follicular growth in the ovary. Interestingly, the AR antagonist flutamide improves infertility in some women with PCOS (De Leo et al., 1998; Rittmaster, 1999), consistent with androgen signaling through the AR playing a significant role in the infertility associated with polycystic ovaries (De Leo et al., 1998; Rittmaster, 1999).

If elevated androgens are mediating abnormal follicular growth, could physiologic androgen concentrations be important for normal follicular growth and fertility? A recent study of female mice lacking ARs suggests that this may indeed be true (Yeh et al., 2002; Yeh et al., 2003). These mice had pronounced reproductive defects, with significantly reduced pregnancy rates and litter sizes. In addition, histologic analysis of the ovaries revealed abnormally small follicles containing immature oocytes.

A possible mechanism of androgen action in the ovary

Together, the studies in humans and mice indicate that androgens may play an important role in ovarian function. Our laboratory has been interested in studying androgen actions in the ovary, and has recently focused on the actions of androgens in promoting mouse oocyte maturation. The maturation of an oocyte refers to its meiotic stage. "Immature" oocytes are arrested in prophase of meiosis I. Just before ovulation, oocytes are induced to reenter the cell cycle, finally resting in metaphase II. "Mature" oocytes are then competent for ovulation and subsequent fertilization, after which meiosis is completed. Interestingly, we found that mouse, as well as amphibian, oocyte maturation was mediated in a transcription-independent fashion (nongenomic) by testosterone via interactions with the AR (Hammes, 2003; Lutz et al., 2003). Further, we discovered that the compound R1881, which is a potent promoter or AR-mediated transcription, was a very poor inducer of transcription-independent oocyte maturation. This result suggests that it should be possible to isolate other compounds that specifically modulate genomic versus nongenomic signaling, and that these substances could then be used to specifically alter oocyte maturation *in vivo*. Since previous work by others has demonstrated that communication between oocytes and surrounding follicular cells is essential for normal follicular growth and development (Eppig et al., 2002; Matzuk et al., 2002), drugs that specifically modulate oocyte maturation might therefore prove useful in treating patients with infertility due to PCOS or other hyper-androgenic states.

We propose a model of oocyte maturation whereby constitutive inhibitory signals within the ovary (I) hold meiosis in prophase I (Figure. 4). Prior to ovulation, gonadotropins promote follicular growth and production of sex steroids (T = testosterone; E = estradiol). Only one or a few dominant follicles might be sufficiently stimulated by gonadotropins to produce enough steroid to overcome the inhibitory signals and allow meiosis and subsequent ovulation to progress. In contrast over-stimulation of oocytes in women with hyperandrogenemia might lead to unregulated growth of many follicles, resulting in the polycystic ovarian phenotype. Blockade of this over-stimulation with flutamide, aromatase inhibitors, or even R1881 might attenuate this unregulated growth, thus allowing normal oocyte maturation, follicular growth, and ovulation to occur.

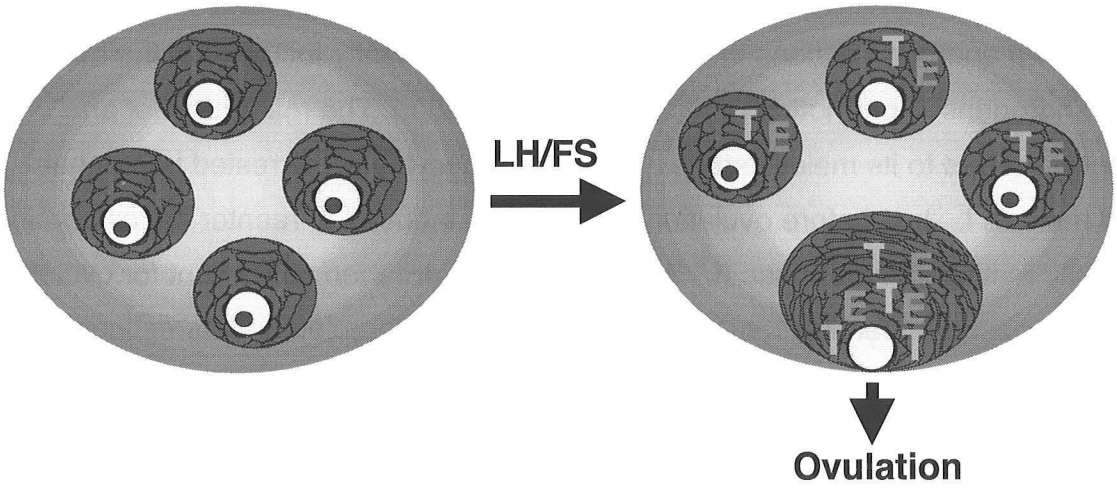

Figure 4. Release of inhibition model for normal oocyte maturation and ovulation. Ovarian follicles produce inhibitor "I" that holds oocyte in meiotic arrest. Gonadotropins stimulate follicular growth and steroid production, resulting in oocyte maturation and ovulation in a dominant follicle.

In summary, 1) Androgens likely play an important role in regulating normal ovarian physiology. 2) Androgen replacement later in life will probably not benefit the ovary. 3) Excess androgens are clearly detrimental to normal ovarian function and fertility.

Conclusions

The roles of androgens in normal and pathologic female physiology are just beginning to be elucidated. With the generation of a female AR knockout mouse model, perhaps some of the controversies concerning androgen actions in the female will be resolved. As for androgen replacement in postmenopausal or androgen-deficient women, large prospective placebo-controlled clinical trials still need to be performed in order to determine whether true long-term benefits exist.

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