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PERSONAL PERSPECTIVE

A sex-specific dose-response curve for testosterone: could excessive testosterone limit sexual interaction in women?

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Abstract

Testosterone treatment increases sexual desire and well-being in women with hypoactive sexual desire disorder; however, many studies have shown only modest benefits limited to moderate doses. Unlike men, available data indicate women show a bell-shaped dose-response curve for testosterone, wherein a threshold dosage of testosterone leads to desirable sexual function effects, but exceeding this threshold results in a lack of further positive sexual effects or may have a negative impact. Emotional and physical side-effects of excess testosterone, including aggression and virilization, may counteract the modest benefits on sexual interaction, providing a possible explanation for a threshold dose of testosterone in women. In this commentary, we will review and critically analyze data supporting a curvilinear dose-response relationship between testosterone treatment and sexual activity in women with low libido, and also explore possible explanations for this observed relationship. Understanding optimal dosing of testosterone unique to women may bring us one step closer to overcoming regulatory barriers in treating female sexual dysfunction.

Key Words: Dose-response curve – Female aggression – Female sexual dysfunction – Hypoactive sexual desire disorder – Testosterone – Virilization.

In women, testosterone, produced by the ovaries and adrenal glands, declines with age, oophorectomy, and certain medical conditions.[1,2] Decreasing testosterone concentrations may contribute to low libido and hypoactive sexual desire disorder (HSDD), although evidence for a direct relationship between measured androgen levels and low sexual desire is lacking.[3,4] Difficulties in defining and measuring low testosterone in women have made the diagnosis of decreased sexual desire challenging, likely related to limitations of available testosterone assays and complexity of female sexual behavior.[2] Randomized studies have shown that testosterone formulations both with and without estrogen increase sexual desire and well-being in premenopausal, perimenopausal, naturally postmenopausal, and surgically menopausal women with low libido.[5-20] These findings have led to the development of testosterone therapy for women; however, no testosterone products have been approved by the US Food and Drug Administration (FDA) for the treatment of postmenopausal HSDD to date. In comparison, over 30 testosterone products have been US FDA-approved for men to treat androgen deficiency.[21] Although political and regulatory barriers are often cited, could difficulties in the approval process be related to limitations in our understanding of the testosterone dose-response curve specific to women?

Unlike the linear testosterone dose-response curve in men, data indicate the possibility of a curvilinear or bell-shaped

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relationship between dosage of testosterone therapy and sexual outcomes in both premenopausal and postmenopausal women. In postmenopausal women with HSDD, doses of testosterone around 300 μg/d significantly increase sexual desire, well-being, and frequency of satisfying sexual episodes. In multicenter, randomized trials, doses lower than 300 μg/d did not lead to increased sexual outcomes.[15,20] Interestingly, women receiving the highest testosterone dose of 450 μg/d also did not show a significant increase in sexual desire or frequency of satisfying sexual activity compared with those receiving 300 μg/d.[15] A similar dose-response relationship was found in premenopausal women treated with 56, 90, and 180 μL/d of testosterone (50 μg of testosterone per μL), with significant sexual outcomes seen only in the moderate-dose group.[20]

If there is a curvilinear relationship between testosterone and sexual function in females, as indicated by these data, what are the possible explanations for this? The physical and emotional side-effects of testosterone are well-documented, predominately virilization (hirsutism, alopecia, acne, seborrhea) and psychological side-effects (aggression, anxiety, depression).[22] These factors may temper positive effects of testosterone on sexual function by altering psychosexual aspects in the woman, or even affecting her attractiveness to a partner.[23] This commentary will explore the possibility that increasing concentrations of testosterone in women increase sexual function to certain point until androgenic side-effects potentially limit sexual function, resulting in a bell-shaped, rather than a linear dose-response relationship.

SEX-SPECIFIC TESTOSTERONE DOSE-RESPONSE: AN OBSERVATION

As men age, there is a natural decrease in testosterone levels.[24] Testosterone replacement therapy (TRT) is associated with improvements in sexual symptoms, and may improve morbidity in men with low testosterone.[25,26] Despite the potential benefits of TRT for men with hypogonadism, this treatment has been met with controversy over its long-term safety, especially in regard to risk of prostate cancer. However, a recent meta-analysis suggests that TRT is effective and generally safe for men with normal prostate-specific antigen (PSA) levels.[27] Contraindications to TRT include polycythemia, prostate cancer, poorly controlled congestive heart failure, and a history of breast cancer.[25,27]

To understand the dose-response relationship for testosterone in men and women, a Medline search was conducted using the keywords ''testosterone'' and ''dose-response'' or ''dose-response relationship'' and ''sexual behavior,'' producing 22 results. References were reviewed for additional related resources. In older men with hypogonadism, studies suggest that there is a testosterone dose-response relationship, in which changes in overall sexual function differ depending upon the dose of testosterone.[28] A number of studies have shown that testosterone supplementation in men results in increased libido and improvement in erections; however, the use of only one to two testosterone doses in these studies has limited understanding of a potential dose-response curve for TRT.[24] Gray et al[28] conducted a randomized study investigating the effects of five different doses of testosterone enanthate (25, 50, 125, 300, and 600 mg) weekly for 20 weeks on sexual function, mood, and visuospatial cognition in 60 healthy, older men between the ages of 60 to 75 years. Concurrently, these men were receiving a long-acting gonadotropin-releasing hormone agonist to suppress endogenous testosterone. Increases in overall sexual function scores, waking erections, and libido in men who were already sexually active were seen with increasing doses of testosterone (Fig. 1). Interestingly, there was no significant change in intercourse or masturbation frequency (sexual events) in the men treated with testosterone in this study, although their relationship status was not reported. Log-free testosterone levels during treatment significantly correlated with changes in overall sexual function ($P = 0.001$), waking erections ($P = 0.040$), spontaneous erections ($P = 0.047$), and libido ($P = 0.047$) in a linear fashion.[28]

Although the dose-response curve for testosterone in men appears to be linear, this may not be the case in women. Whereas data are limited for a testosterone dose-response curve in women, there are two relatively large, multicenter studies that indicate a curvilinear relationship between testosterone and sexual outcomes in women with low sexual desire.[15,20] Braunstein et al[15] conducted a 24-week randomized double-blind trial involving 318 postmenopausal women with HSDD who had undergone bilateral salpingo-oophorectomy and hysterectomy, and who were receiving a stable dose of estrogen. These women were in monogamous relationships with sexually functional men, but developed HSDD after oophorectomy. The women were assigned to one of three testosterone doses or placebo. The participants received a 150, 300, or 450 μg/d testosterone patch, in addition to continuing their established dose of estrogen (50% >0.625 mg/d conjugated equine estrogens and 50% <0.625 mg/d conjugated equine estrogens). The

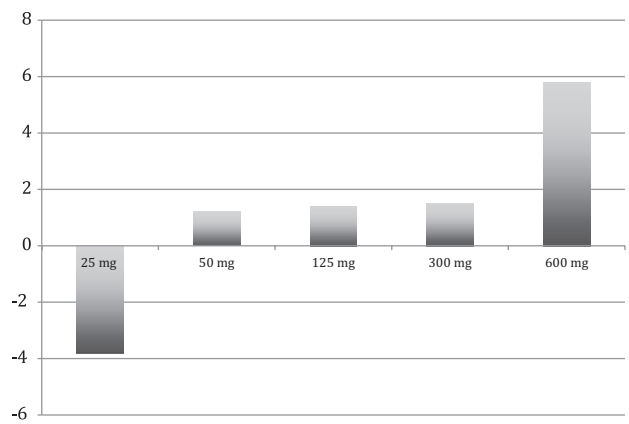

FIG. 1. Difference in libido score from baseline in sexually active males by testosterone. In a two-way analysis of variance (ANOVA), in men a significant interaction between reported sexual activity and testosterone dose was found ($P = 0.009$).[28]

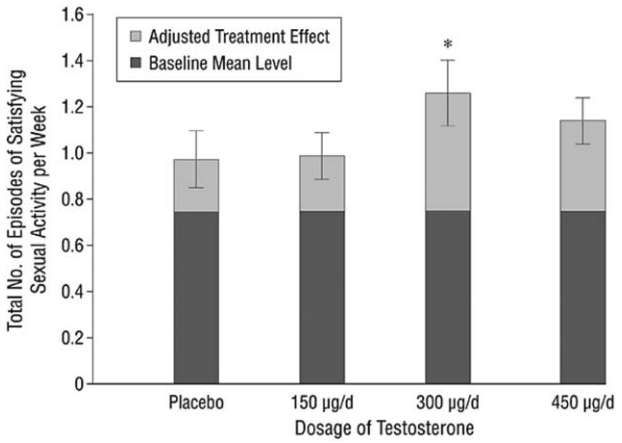

FIG. 2. Frequency of sexual activity at 24 weeks of treatment. Asterisk indicates *P* less than 0.05 versus placebo. Limit lines indicate standard error of the mean. Reprinted from Braunstein et al[15] with permission of the publisher. © 2005 American Medical Association.

primary outcomes were change in sexual desire and frequency of satisfying sexual activity (events).[15]

Women who received the lower-dose testosterone of 150 μg/d did not demonstrate significant changes in sexual desire or frequency of satisfying sexual activity; however, women taking 300 μg/d dosing did experience significant increases from baseline compared with those on placebo. Sexual desire increased 67% from baseline compared with a 48% increase from baseline for those taking placebo ($P = 0.05$). The frequency of satisfying sexual activity in the 300 μg/d group increased 79% from baseline compared with 43% for those on placebo ($P = 0.049$). Interestingly, women receiving the highest testosterone dose of 450 μg/d did not show a significant increase in sexual desire or frequency of satisfying sexual activity compared with those receiving 300 μg/d. The change for both measures (although numerically higher than the 300 μg/d) was not significantly different from either the placebo group or the group receiving 300 μg/d (Fig. 2). When measuring the domains (desire, pleasure, arousal, responsiveness, self-image, orgasm, and concerns) of the Profile of Female Sexual Function (PFSF) questionnaire, desire and arousal were significantly higher for only the 300 μg/d group, relative to the change for those receiving placebo ($P < 0.05$) (Fig. 3). Serum testosterone levels were not measured in this study. Adverse events included acne, breast pain, headache, and hirsutism, which were found to be similar between treatment groups. Forty-seven women withdrew from the study due to adverse events, although the authors describe no trend related to treatment group.[15]

These findings are consistent with a previous study indicating increasing doses of transdermal testosterone increase sexual function, although higher doses above 300 μg/d were not evaluated.[10] In this placebo-controlled, multicenter trial, 75 surgically postmenopausal women with impaired sexual function were randomized to placebo or treatment with either 150 μg/d or 300 μg/d of transdermal testosterone for 12 weeks. Scores for sexual function increased from 52% at baseline to 72% for placebo, 75% for treatment with 150 μg/d testosterone and 81% with 300 μg/d testosterone, with dose-dependent increases in frequency of sexual fantasy, masturbation, and sexual intercourse. Frequency of sexual activity and pleasure orgasm was significantly higher for women receiving the 300 μg/d dose of testosterone. Mean serum concentrations of testosterone increased with treatment dose, with levels of free and total testosterone in the high-normal or exceeding normal ranges during treatment with the 300 μg/d dose. Adverse events of hirsutism and acne did not increase significantly during treatment; however, the mean facial-depilation rate did increase with the 300 μg/d testosterone treatment.[10] The placebo effect tends to be strong in studies of both postmenopausal and premenopausal women with impaired sexual function. Although some authors indicate that the placebo effect is stronger in younger surgically

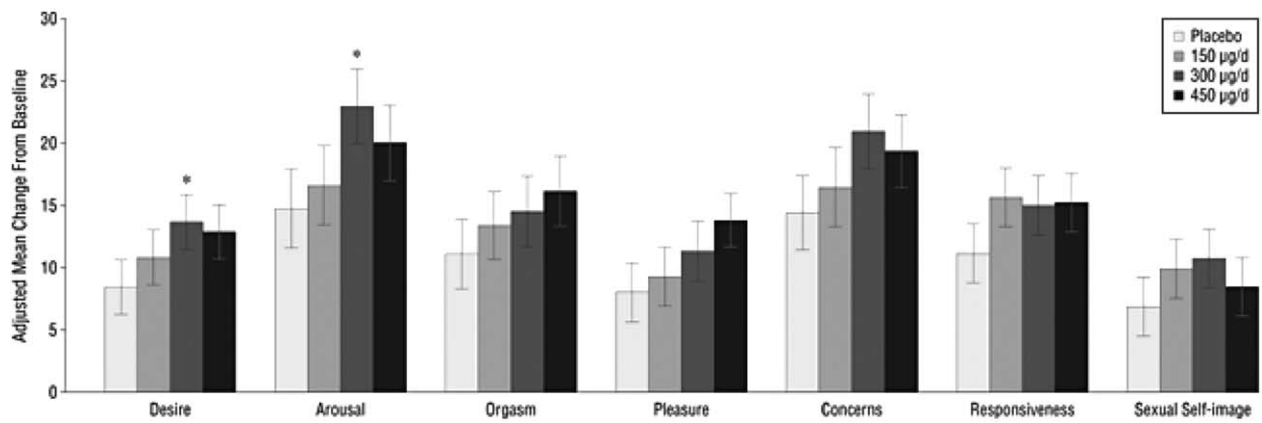

FIG. 3. Profile of female sexual function sexuality domain change from baseline at 24 weeks by treatment and dosage. Limit lines indicate standard error of the mean; asterisks, *P* less than 0.05 compared with placebo. Reprinted from Braunstein et al[15] with permission of the publisher. © 2005 American Medical Association.

postmenopausal women, there are a number of potential confounders including time since oophorectomy and hysterectomy, length of time in a stable relationship, and age of the male partner. In addition, sequence of exposure to study conditions could lead to carry over effect, which may have potentiated the placebo effect in younger participants.

Davis et al[18] conducted a multicenter, double-blind, placebo-controlled trial to determine efficacy and safety of testosterone treatment for postmenopausal women with HSDD not receiving estrogen. Women received 150 or 300 μg/d testosterone patch. At 24 weeks, participants experienced a significant increase of 2.1 satisfying sexual episodes/4 wks compared with 0.7 episodes in those receiving placebo ($P < 0.001$).[18] These findings are supported by previous studies of surgically postmenopausal women treated with 300 μg/d testosterone patch, where increases from baseline ranged from 1.56 to 2.1 sexually satisfying episodes/4 wks versus 0.73 to 0.98 episodes for placebo, which were found to be significant.[16,17] The higher 450 μg/d dose was not evaluated in any of these studies.

To determine a dose-response relationship, Huang et al[19] randomized 71 menopausal women with low testosterone levels (total testosterone <31ng/dL or free testosterone <3.5 pg/mL) to weekly intramuscular testosterone injections of 3, 6.25, 12.5, or 25 mg, in addition to standardized transdermal estradiol (E_2) treatment for 24 weeks. The researchers did not specifically recruit women with HSDD or sexual dysfunction in this study. Increases in sexual function scores measured by the Brief Index of Sexual Functioning for Women (BISF-W), and also sexual thoughts, desire, and frequency of sexual activity were found to be significant in only the 25-mg group compared with placebo. Changes in sexual activity scores were significantly related to increases in free testosterone concentrations, with the 25-mg group exhibiting supraphysiologic testosterone levels. Sexual encounters in the 25-mg testosterone group increased by an average of 2.7 per week. Hirsutism scores did increase significantly in the 12.5 and 25-mg dose groups compared with placebo. It is important to note that this population, which did not present with HSDD or other forms of sexual dysfunction, did not exhibit increased sexual activity at physiologic testosterone dosage levels. As the authors discuss, it is possible that lower testosterone doses or levels could have improved sexual function in women with HSDD.[19] However, studies have shown that low serum androgen levels do not necessarily correlate with low self-reported sexual desire and satisfaction.[4]

The possibility of a nonlinear dose-response curve has also been suggested in premenopausal women with low libido. Davis et al[20] conducted a study of premenopausal women with low circulating testosterone levels (≤1.1 pg/mL) and reporting low sexual satisfaction. This trial included 261 women ranging in age from 35 to 46 years with regular menstrual cycles who engaged in at least one sexual event per 28 days, but exhibited a low sexuality score on the Sabbatsberg Sexual Self-Rating Scale with no evidence of severe clinical depression. The participants also reported a decrease or decline in satisfaction with sexual activity. In this double-blind, placebo-controlled trial, participants were randomized to three different doses of testosterone or placebo. Participants administered variations of the 56 and 90 μL (50 μg of testosterone per μL) metered-dose spray: one 56 μL spray, one 90 μL spray, or two 90 μL sprays. The highest dose was anticipated to increase mean serum free testosterone levels to about 75% of normal range for premenopausal women. The primary outcome was mean number of self-reported satisfying sexual events (SSEs) per 4 weeks after 16 weeks of treatment. Women who received the 90-μL dose of testosterone spray reported 2.48 SSEs versus 1.7 events for placebo, with a mean increase of 0.8 SSEs per month with treatment. The frequency of SSEs did not differ from placebo for either the lower or the higher testosterone dose groups (Fig. 4).[20]

Women in all three testosterone groups showed a significant increase in serum testosterone concentrations compared with placebo at weeks 8 and 16, with a higher increase in the highest testosterone dose group. However, no relationship was found between number of SSEs and free testosterone levels. Thirty-five per cent of women in the highest dosage group had supraphysiologic free testosterone levels. The most frequent adverse events related to treatment included hypertrichosis, headache, nausea, acne, and dysmenorrhea, leading to eight women withdrawing from the study. Hypertrichosis was found to be dose-related, and incidence of acne increased slightly after active treatment.[20]

There are a number of limitations to these studies, and comparing studies is difficult due to differences in methodology, testosterone preparations, and lack of statistical analysis for dose-response relationships.[22] However, these findings at least raise the possibility of a bell-shaped dose-response curve for testosterone in women with low sexual desire. In a proposed bell-shaped dose-response model, a threshold dosage of testosterone leads to desirable sexual effects, but exceeding this threshold results in a lack of further

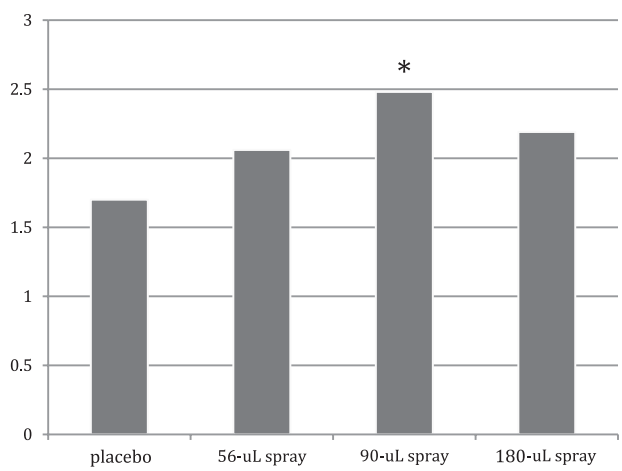

FIG. 4. Mean sexually satisfying events per month by testosterone dose.[20] *$P = 0.04$ versus placebo.

positive sexual effects or possibly negative effects. Even though it is significant, studies suggest that the impact of testosterone treatment has been modest, at least on the ''downstream'' endpoint of sexual desire, namely satisfying sexual events. With such a modest effect, there are a number of subtle factors that could interfere with these perceived benefits.

This leads to an intriguing question: Could perceived negative side-effects of excess testosterone in women, namely aggression and virilization, impact sexual activity and function? There are no human studies that specifically evaluate how known side-effects of excess testosterone in females impact sexual activity. To better understand the complex relationship between testosterone and female sexual function, the authors will first review animal studies elucidating how testosterone affects sexual activity in the brain, then discuss the relationship between testosterone and female aggression, and finally explore the effects of virilization on sexual interaction.

TESTOSTERONE AND ESTROGEN IN THE BRAIN CONTROL OF SEXUAL INTERACTION IN ANIMAL MODELS

Sexual response and arousal have been associated with multiple areas of the brain stem and cerebral cortex, with the hypothalamus playing a pivotal role.[29,30] Studies have shown that lesions of the hypothalamic preoptic area in rodents cause negative effects on copulation, whereas electrical stimulation of this area of the brain facilitates copulation in male rodents.[31,32] In female rodents, neurons of the hypothalamic ventromedial nucleus are associated with lordosis posturing and induction of sexual receptivity.[33] The regions of the brain that have been identified as important areas for controlling sexual function in males and females express androgen receptors (ARs), estrogen receptors (ERs), and aromatase, which is critical for the conversion of testosterone to estrogen. High levels of aromatase activity have been found in the hypothalamus of animals, and also humans, highlighting the important role of sex steroids in this region of the brain. In male rodents, hypothalamic levels of aromatase are higher compared with female rodents[34]; however, in humans, the levels of aromatase have been found to be the same.[35]

The expression of steroid receptors is complex, with some cells in the brain expressing both ARs and ERs.[36] In rodents, androgens increase the expression of both AR and ER levels, whereas estrogens down-regulate their own expression and have negligible effects on AR levels.[37] This intricate expression pattern is made more complex by the conversion of testosterone to estrogen by aromatase. In male rats, the effects of testosterone conversion to estrogen are critical for the male sexual differentiation of the central nervous system.[38] These complex relationships make understanding the precise mechanisms of the effects of exogenous testosterone and estrogen on libido and other functions, such as mood and cognition, difficult to determine. Ongoing studies in animal models and humans are attempting to elucidate the direct and indirect effects of these steroids in the brain.

The complex interaction of testosterone and estrogen has been demonstrated in mouse models that were able to separate the activities in genetically manipulated mice. Research on mice has clearly demonstrated that estrogen receptor-α (ER-α) gene disruption greatly modifies female sexual response. Female ER-α knockout (ERαKO) mice showed a lack of lordosis behavior when exposed to stud male mice, which did not change after administration of progesterone.[39] Even more interesting, gonadally intact ERαKO females not only lacked sexual receptivity, but were vigorously attacked by the males, suggesting that in gonadally intact ERαKO females, male-type characteristics were present and acting through testosterone and the ARs. Further research showed that estrogen-treated gonadectomized ERαKO females were not attacked by stud males, despite their lack of sexual responsiveness. These results suggest that elevated levels of testosterone found in gonadally intact ERαKO females may affect pheromone production, leading to male-type characteristics, which has been shown in other studies.[40] Supporting this theory, the excess serum androgen found in these gonadally intact ERαKO female mice caused the preputial (clitoral) glands of these mice to be highly stimulated compared with wild-type females.[39]

TESTOSTERONE AND AGGRESSION

Aggression likely has different evolutionary roles for males versus females.[41] In males, aggression plays a role in obtaining resources and intrasexual competition, which may compensate for an inferior physical strength. For females, aggression provides protection of offspring from predators or other environmental threats. On the contrary, for males and females, aggression may also have negative consequences, as it may lead to behaviors that can cause injury or death. Many studies have positively correlated testosterone levels with aggression in animals and men[42,43]; however, findings in women are less clear. In one meta-analysis, the tests used to evaluate aggression were confounded by an increase in testosterone by the experimental test being used to evaluate aggression.[44] In 2001, a large meta-analysis[45] was conducted based on 45 independent studies. This meta-analysis provided support for an overall weak positive correlation ($r = 0.14$) between testosterone and aggression.[45]

In 2009, Yu and Shi[46] conducted a study where they examined the saliva of 20 aggressive and 20 nonaggressive students who had been matched for sex, age, and pubertal development. Children were determined to be aggressive based on the child behavior checklist (CBCL), and also reports by parents and other people who were well acquainted with the children. The salivary testosterone levels were 22.20 ± 14.50 and 19.54 ± 12.52 pg/mL in aggressive male and female students, respectively. These levels were significantly higher ($P < 0.05$) than those found in nonaggressive male and female students, which were 13.20 ± 6.85 and 5.24 ± 3.03 pg/mL, respectively. This study also found that salivary cortisol levels were significantly lower in aggressive males and females than in the nonaggressive groups, but

reached significance only in a comparison of males ($P < 0.05$). Interestingly, when CBCL scores were compared with testosterone levels, there was a significant association with higher testosterone levels and CBCL scores ($P < 0.05$) in the females, but not the males. However, there was a significant correlation between lower cortisol levels in boys and CBCL scores, suggesting lower cortisol levels may be a better predictor of aggression in boys than elevated testosterone levels.[46] These findings highlight potential sex differences in the role of testosterone and cortisol on aggression, and also the difficulties in drawing precise conclusions given the complex endocrine environment found in humans.

To examine the potential for aggression/hostility in women taking testosterone, it may be relevant to examine the effects of exogenous testosterone in women. Estratest (1.25 mg esterified estrogens, 2.5 mg methyltestosterone; Reid-Provident Laboratories, Inc, later aquired by Solvay Pharmaceuticals, Inc) was the first estrogen-testosterone combination oral therapy marketed in the United States (1965) for postmenopausal symptoms, and later, another formulation—Estratest HS (0.625 mg esterified estrogens, 1.25 mg methyltestosterone)—was marketed in 1975; however, neither product received US FDA approval.[47] Estratest was marketed before the more rigid US FDA rules for approval were in place, requiring large clinical trials to confirm its efficacy. Smaller trials showed that methyltestoterone independently contributed to the efficacy of Estratest, but the trials were not large enough to satisfy US FDA criteria.[13,48] Due to controversy surrounding US FDA approval, the prescription medication was discontinued by the innovator company in March of 2009; however, so-called ''generic'' versions such as esterified estrogens/methyltestosterone (formerly marketed at Syntest by Breckenridge Pharmaceutical, Inc) and Covaryx (Centrix Pharmaceuticals, Inc) are still being marketed.

In a summary of the safety surveillance data of Estratest and Estratest HS conducted from 1989 to 2002, hostility was noted as the third most frequently reported event within the ''body as a whole'' body system, occurring in 1.3% of the participants.[23] Although a Medline search did not reveal any published case studies describing aggressive behavior in women taking Estratest, anecdotal information can be found in online message boards, which provide interesting insight on patients' personal experiences with Estratest. On the popular online patient medical resource WebMD, patients can write user reviews for specific medications. In regard to Estratest, one patient writes, ''My libido increased way too much so I cut down to 1/2 pill a day. I also noticed that I felt more aggressive and angry. The one thing it did for me was to relieve hot flashes very well, especially when used in combo (combination) with Vivelle dot patch. However, my depression/mood worsened, have had bad headaches and worst of all, gained 15 lbs in 1 month and was still going up so I decided to stop and try something else.''[49] On an online support group for women after hysterectomy, called Hystersisters, one woman writes, ''These are the reasons why I am going off testosterone: I have been aggressive, combative, argumentative, obsessive, irritable, sharp tongued, high strung, rude, and short tempered. My EEMT (Estratest) was raised from 1/2 strength to full strength, 10 days after my surgery, due to night sweats. This is when the scale tipped.''[50] Another patient on a ''Testosterone and Libido'' message board asks, ''I know the current story is that testosterone cream boosts libido, but have any of you actually found this to be true? I used a 0.2% cream briefly and it only made me mean and aggressive. My GYN (gynecologist) said that he has had other ladies tell him the same thing.''[51] Although self-reported statements from individual patients on message boards have clear limitations from a research standpoint including bias and lack of generalizability, these insights may provide a starting point for future research on this topic.

Is it possible that elevated testosterone may lead to aggressive sexual fantasies or behaviors that decrease sexual attraction and prevent sexual encounters in women? Animal models are often useful in exploring the effect of medication on specific behavior. To investigate the effects of anabolic androgenic steroids (AASs) on female sexual behavior, Barreto-Estrada et al[52] employed a mouse model to quantify the sexual response of anabolic androgen-exposed (AAS-exposed) female mice when paired with female or male partners. Female mice were administered 17a-methyltestosterone (7.5 mg/kg) for 17 days, and on days 15 to 17, they were allowed to mate with males or proestrus female mice. This study found that males displayed a significant decrease in the frequency of mounts to androgen-exposed females when compared with control females (Fig. 5), and androgen-treated females attempted to mount female mice, but not male mice. Also interestingly, androgen-exposed females demonstrated significant increases in the number of fights, escapes, and rejections to males (Fig. 5).[52] Similar results on the effects of androgens in female mice were seen in a previous study.[53] The significance of these findings with respect to the effect high exogenous testosterone may have on the sexual behaviors of women await further investigation. One could hypothesize, based on findings in animal models, that women exposed to

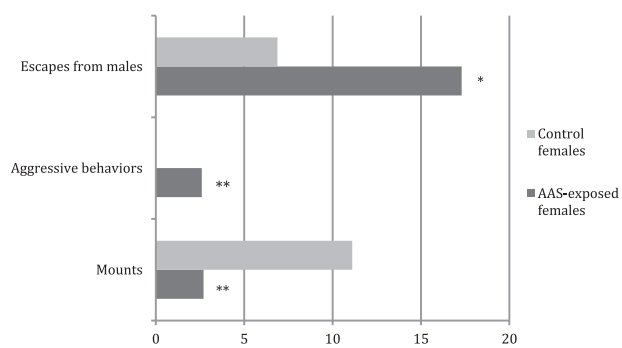

FIG. 5. Number of activities for anabolic androgenic steroid exposed (AAS-exposed) female rats versus control female rats.[52] $^{*}P \leq 0.05$. $^{**}P \leq 0.005$.

high levels of testosterone may exhibit sexual behaviors that differ from women with normal testosterone levels.

EFFECTS OF VIRILIZATION ON SEXUAL INTERACTION

Virilizing side-effects of high testosterone levels may also affect sexual encounters. Virilizing effects (deepening of the voice, hirsutism, acne, alopecia, clitoromegaly, and menstrual irregularities) were noted in 20% of women taking esterified estrogens plus methyltestosterone (Estratest; Solvay Pharmaceuticals, Inc). The most commonly reported virilizing adverse events from the safety surveillance data for Estratest were alopecia (8.8%), acne (5.6%), and hirsutism (4.5%).[23] The association between methyltestosterone and masculinization was first described in the early 1990s.[54] Urman et al[54] conducted a retrospective study of nine postmenopausal women using a long-term injectable combination androgen-estrogen (testosterone enanthate benzilic acid hydrozone-estradiol dienanthate/estradiol benzoate), finding all nine postmenopausal women reported virilizing side-effects. Hirsutism was the major complaint in almost all of the participants. Serum testosterone levels were elevated in eight of the women who had available measurements. The elevation in testosterone ranged from 5.7 to 14.9 nmol/L, compared with a range of 0.35 to 2.43 nmol/L found in postmenopausal women not receiving testosterone, and approximately 19 nmol/L in men 45 to 59 years of age.[54]

Although the link between testosterone and virilization of females is well demonstrated, do these physical effects impact sexual interaction? Exploring sexual function in women with hyperandrogenic conditions causing virilization, specifically women with congenital adrenal hyperplasia (CAH) and polycystic ovary syndrome (PCOS), may provide insight. CAH due to 21-hydroxylase deficiency results in decreased synthesis of cortisol and increased adrenocorticotropic hormone (ACTH) secretion, leading to increased production in androgens. Studies investigating sexual function in women with classical CAH do show decreased FSFI scores including decreased sexual desire; however, these patients are often receiving treatment with hydrocortisone and have undergone reconstructive vulvovaginal surgery.[55] These findings are more likely attributed to abnormal genital sensitivity and vaginal stenosis rather than hyperandrogenism. A recent study examined sexual activity in untreated women with nonclassic CAH compared with age-matched controls. Women with nonclassic CAH had higher total and free testosterone, androstenedione, and dehydroepiandrosterone sulfate (DHEAS) levels, and also higher hirsutism scores compared with controls. Women with CAH had lower total FSFI scores. Specifically, scores for sexual arousal, lubrication, satisfaction, and dyspareunia were significantly lower compared with controls; however, scores for sexual desire and orgasm were similar to healthy age-matched women. Sexual desire and orgasm scores correlated with testosterone levels and hirsutism scores. Depression screening scores were also found to be higher in women with nonclassic CAH, correlating with testosterone levels and hirsutism.[56] The authors note the contradiction of these findings with interventional testosterone studies. However, it is possible that decreased sexual function at supraphysiologic levels of testosterone seen in women with CAH represent the downsloping portion of a curvilinear testosterone dose-response curve, as described previously.

Adamopoulos et al[57] evaluated sexual activity in 51 women with hirsutism associated with increased levels of androgen compared with 52 controls. Forty-four of the hirsute women met the diagnostic criteria for polycystic ovary syndrome (PCOS). At baseline, bound testosterone was found to be elevated in 87% of the hirsute women. This group was further subdivided into married women, single women with partners, and single women without partners. The study group was matched with a reference group of women with no hirsutism. Sexual activity was measured by a sexual questionnaire, which assessed frequency of sexual intercourse, masturbation, physical arousal, sexual feeling, thoughts and fantasies, and sexual problems. Outcomes were measured in hirsute women before, during, and after two cycles of treatment with cyproterone acetate (50 mg/d), an antiandrogen, combined with ethinyl estradiol (50 μg/d at 2 and 6-month intervals).[52]

Based on radioimmunoassay measurement, unbound testosterone levels were higher in the hirsute women than in the control group (2.18 vs 1.7%; $P < 0.001$). When comparing all hirsute women with control women, those with hirsutism had a significantly lower coital frequency, but a higher masturbation frequency than women in the control group ($P < 0.001$) (Fig. 6). When analyzed by groups, the subgroup of single women with partners was the only subgroup that showed statistically significant differences for coital and masturbation frequency compared with controls. When analyzed by matching age between groups, only a decrease in coital frequency reached significance compared with the age-matched controls.[57]

Treatment with the androgen antagonist, cyproterone acetate, resulted in a significant decline in free testosterone levels (2.18 vs 1.8%; $P < 0.05$) and in the mean hirsutism index after 6 months. Results showed there were no significant

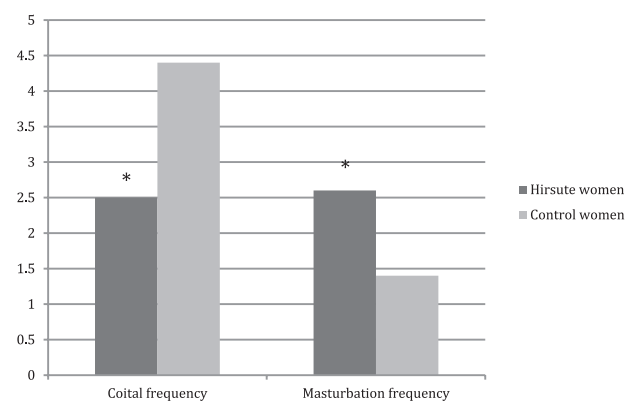

FIG. 6. Coital and masturbation frequency in hirsute versus control women.[57] *$P < 0.001$.

changes in coital or masturbation frequency in any group of women after treatment with cyproterone and estradiol, although there was a numerical increase in coital frequency and decrease in masturbation frequency in single women with a partner.[57]

Comparing these results with other studies on women with PCOS, there appears to be some correlation with higher testosterone levels and degree of sexual dysfunction.[58,59] In a study of 92 women with PCOS compared with 82 controls, Stoval et al[58] reported that women with PCOS had significantly lower orgasm/completion scores compared with the women in the control group ($P < 0.001$). However, when analyzing the subdomains, only increased body mass index correlated significantly with a decrease in the orgasm/completion subdomain in women with PCOS.[58] In another study of women with PCOS, desire and arousal were the most negatively affected domains of sexual function.[54] Overall, these results may suggest that higher testosterone levels (within normal range) or hirsutism in women with PCOS may have a negative effect on a woman's sexual activity. In women with PCOS, obesity and issues with body image may affect sexual desire and function; however, studies have shown a minimal or no association between weight and sexual function in women with PCOS.[60] This is a complex issue that needs further investigation to clarify the factors that may be at play with respect to both physiologic and possible psychologic factors affecting these outcomes.

CONCLUSIONS

Many trials have shown the benefits of testosterone therapy for women with low testosterone levels and/or HSDD. Evidence of a bell-shaped dose-response curve for effects of testosterone on various aspects of sexual function in women is intriguing. Efforts to elucidate a dose-response relationship for testosterone in women with low sexual function have been limited by differences in methodology, including variable study populations, instruments used to measure sexual function, testosterone preparations, and dosing. Difficulties in measuring testosterone concentrations in women, and also the lack of correlation between serum testosterone levels and sexual activity pose challenges in standardizing studies and determining the nature of the dose-response relationship for testosterone in women, and specifically in women with decreased sexual desire. Finding the correct dose of testosterone based on individual needs may obviate overmedication and limit negative side-effects, such as mood changes and hirsutism, which have the potential to decrease sexual activity. In the animal models, androgen-exposed female rats were not only mounted less by male rats, but when sexually approached, exhibited aggressive and avoidant behavior. There are no studies in humans or nonhuman primates that suggest such an extreme reaction to high testosterone, but there is the possibility of unknown neuropsychological effects that may alter sexual function in humans. Additional dose-response relationship studies are crucial to further elucidate a potential bell-shaped testosterone curve for women, with a more detailed analysis of the reasons for higher masturbation rates and lower sexual intercourse rates found in some studies. The absence of a clearly linear dose-response relationship for testosterone on sexual function, particularly sexual activity, in women, may represent an additional barrier to regulatory approval. As such, testosterone dosing that can ''hit the sweet spot'' of sexual activity in women may be difficult to determine. As we forge ahead in discovering the benefits of testosterone on sexual function in women, further research would be helpful in determining how increased levels and doses of testosterone affect sexual interactions.

REFERENCES

1. Davis SR, Wahlin-Jacobsen S. Testosterone in women: the clinical significance. *Lancet Diabetes Endocrinol* 2015;3:980-992.
2. Wierman ME, Arlt W, Basson R, et al. Androgen therapy in women: a reappraisal: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2014;99:3489-3510.
3. West S, D'Aloisio AA, Agans RP, Kalsbeek WD, Borisov NN, Thorp JM. Prevalence of low sexual desire and hypoactive sexual desire disorder in a nationally representative sample of US women. *Arch Intern Med* 2008;168:1441-1449.
4. Davis SR, Davison SL, Donath S, Bell RJ. Circulating androgen levels and self-reported sexual function in women. *JAMA* 2005;294:91-96.
5. Sherwin BB, Gelfand MM, Brender W. Androgen enhances sexual motivation in females: a prospective, crossover study of sex steroid administration in the surgical menopause. *Psychosom Med* 1985;47:339-351.
6. Sherwin BB, Gelfand MM. Sex steroids and affect in the surgical menopause: a double-blind, cross-over study. *Psychoneuroendocrinol* 1985;10:325-335.
7. Meyers LS, Dixen J, Morrissette D, Carmichael M, Davidson JM. Effects of estrogen, androgen, and progestin on sexual psychophysiology and behavior in postmenopausal women. *J Clin Endocrinol Metab* 1990;70:1124-1131.
8. Davis SR, McCloud P, Strauss BJ, Burger H. Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality. *Maturitas* 1995;21:227-236.
9. Sarrel P, Dobay B, Wiita B. Estrogen and estrogen–androgen replacement in postmenopausal women dissatisfied with estrogen-only therapy. *J Reprod Med* 1998;43:847-856.
10. Shifren JL, Braunstein GD, Simon JA, et al. Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. *N Engl J of Med* 2000;343:682-688.
11. Dobs AS, Nguyen T, Pace C, Roberts CP. Differential effects of oral estrogen versus oral estrogen-androgen replacement therapy on body composition in postmenopausal women. *J Clin Endocrinol Metab* 2002;87:1509-1516.
12. Floter A, Nathorst-Boos J, Carlstrom K, von Schoultz B. Addition of testosterone to estrogen replacement therapy in oophorectomized women: effects on sexuality and well-being. *Climacteric* 2002;5:357-365.
13. Lobo RA, Rosen RC, Yang HM, Block B, Van Der Hoop RG. Comparative effects of oral esterified estrogens with and without methyltestosterone on endocrine profiles and dimensions of sexual function in postmenopausal women with hypoactive sexual desire. *Fertil Steril* 2003;79:1341-1352.
14. Goldstat R, Briganti E, Tran J, Wolfe R, Davis S. Transdermal testosterone improves well-being, mood and sexual function in premenopausal women. *Menopause* 2003;10:390-398.
15. Braunstein GD, Sundwall DA, Katz M, et al. Safety and efficacy of a testosterone patch for the treatment of hypoactive sexual desire disorder in surgically menopausal women: a randomized, placebo-controlled trial. *Arch Intern Med* 2005;165:1582-1589.
16. Buster JE, Kingsberg SA, Aguirre O, et al. Testosterone patch for low sexual desire in surgically menopausal women: a randomized trial. *Obstet Gynecol* 2005;105:944-952.
17. Simon J, Braunstein G, Nachtigall L, et al. Testosterone Patch increases sexual activity and desire in surgically menopausal women with hypoactive sexual desire disorder. *J Clin Endocrinol Metab* 2005;90:5226-5233.
18. Davis SR, Moreau M, Kroll R, et al. Testosterone for low libido in postmenopausal women not taking estrogen. *N Engl J Med* 2008;359:2005-2017.

19. Huang G, Basaria S, Travison TG, et al. Testosterone dose-response relationships in hysterectomized women with and without oophorectomy: effects on sexual function, body composition, muscle performance and physical function in a randomized trial. *Menopause* 2014;21:612-623.
20. Davis S, Papalia MA, Norman RJ, et al. Safety and efficacy of a testosterone metered-dose transdermal spray for treating decreased sexual satisfaction in premenopausal women: a randomized trial. *Ann Int Med* 2008;148:569-577.
21. United States Food and Drug Administration. FDA Approved Drug Products: Testosterone. Available at: https://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm. Accessed October 21, 2016.
22. Somboonporn W, Bell RJ, Davis SR. Testosterone for peri and postmenopausal women. *Cochrane Library* 2009; Available at: http://www.hormonebalance.org/images/documents/Somboonporn%2005%20Testosterone%20peri%20and%20post%20men%20women%20Cochrane.pdf. Accessed December 19, 2016.
23. Phillips EH, Ryan S, Ferrari R, Green C. Estratest and Estratest HS (esterified estrogens and methyltestosterone) therapy: a summary of safety surveillance data, January 1989 to August 2002. *Clin Ther* 2003;25:3027-3043.
24. Wu FC, Tajar A, Beynon JM, et al. Identification of late-onset hypogonadism in middle-aged and elderly men. *N Engl J Med* 2010;363:123-135.
25. Hassan J, Barkin J. Testosterone deficiency syndrome: benefits, risks, and realities associated with testosterone replacement therapy. *Can J Urol* 2016;23(suppl 1):20-30.
26. Yeap BB, Alfonso H, Chubb SA, et al. In older men an optimal plasma testosterone is associated with reduced all-cause mortality and higher dihydrotestosterone with reduced ischemic heart disease mortality, while estradiol levels do not predict mortality. *J Clin Endocrinol Metab* 2014;99:E9-E18.
27. Guo C, Gu W, Liu M, et al. Efficacy and safety of testosterone replacement therapy in men with hypogonadism: a meta-analysis study of placebo-controlled trials. *Exp Ther Med* 2016;11:853-863.
28. Gray PB, Singh AB, Woodhouse LJ, et al. Dose-dependent effects of testosterone on sexual function, mood, and visuospatial cognition in older men. *J Clin Endocrinol Metab* 2005;90:3838-3846.
29. Karama S, Lecours AR, Leroux JM, et al. Areas of brain activation in males and females during viewing of erotic film excerpts. *Hum Brain Mapp* 2002;16:1-13.
30. Stoléru S, Fonteille V, Cornélis C, Joyal C, Moulier V. Functional neuroimaging studies of sexual arousal and orgasm in healthy men and women: a review and meta-analysis. *Neurosci Biobehav Rev* 2012;36:1481-1509.
31. Larsson K, Heimer L. Mating behavior of male rats after lesions in the preoptic area. *Nature* 1964;202:413-414.
32. Twiggs DG, Popolow HB, Gerall AA. Medial preoptic lesions and male sexual behavior: age and environmental interactions. *Science* 1978; 200:1414-1415.
33. Pfaff DW, Sakuma Y. Deficit in the lordosis reflex of female rats caused by lesions in the ventromedial nucleus of the hypothalamus. *J Physiol* 1979;288:203-210.
34. Roselli CE, Ellinwood WE, Resko JA. Regulation of brain aromatase activity in rats. *Endocrinology* 1984;114:192-200.
35. Biegon A, Kim SW, Alexoff DL, et al. Unique distribution of aromatase in the human brain: in vivo studies with PET and [N-methyl-11C]vorozole. *Synapse* 2010;64:801-807.
36. Simerly RB. Distribution and regulation of steroid hormone receptor gene expression in the central nervous system. In: Seil FJ, ed. *Advances in Neurology*. New York: Raven Press; 1993:207-226.
37. Lisciotto CA, Morrell JI. Circulating gonadal steroid hormones regulate estrogen receptor mRNA in the male rat forebrain. *Brain Res Mol Brain Res* 1993;20:79-90.
38. Balthazart J. Steroid control and sexual differentiation of brain aromatase. *J Steroid Biochem Mol Biol* 1997;61:323-339.
39. Ogawa S, Eng V, Taylor J, Lubahn DB, Korach KS, Pfaff DW. Roles of estrogen receptor-α gene expression in reproduction-related behaviors in female mice. *Endocrinology* 1998;139:5070-5081.
40. Lee C, Ingersoll DW. Pheromonal influence on aggressive behavior. In: Svare BB, ed. *Hormones and Aggressive Behavior*. New York: Plenum Press; 1981:373-392.
41. Craig IW, Halton KE. Genetics of human aggressive behaviour. *Hum Genet* 2009;126:101-113.
42. Rada RT, Kellner R, Winslow WW. Plasma testosterone and aggressive behavior. *Psychosomatics* 1976;17:138-142.
43. Mazur A, Booth A. Testosterone and dominance in men. *Behav Brain Sci* 1998;21:353-363.
44. Archer J. The influence of testosterone on human aggression. *Br J Psychol* 1991;82:1-28.
45. Book AS, Starzyk KB, Quinsey VL. The relationship between testosterone and aggression: a meta-analysis. *Aggression and Violent Behavior* 2001;6:579-599.
46. Yu YZ, Shi JX. Relationship between levels of testosterone and cortisol in saliva and aggressive behaviors of adolescents. *Biomed Environ Sci* 2009;22:44-49.
47. National Women's Health Network. Available at: http://www.fda.gov/ohrms/dockets/dockets/06p0346/06p-0346-cp00001-01-vol1.pdf. Accessed November 4, 2016.
48. Simon J, Klaiber E, Wiita B, Bowen A, Yang HM. Differential effects of estrogen-androgen and estrogen-only therapy on vasomotor symptoms, gonadotropin secretion, and endogenous androgen bioavailability in postmenopausal women. *Menopause* 1999;6:138-146.
49. Web MD User Review for Estratest Oral. Available at: http://www.webmd.com/drugs/drugreview-1226+Estratest+Oral.aspx?drugid=1226&drugname=Estratest+Oral. Accessed July 21, 2016.
50. Hystersisters. Message Board on Hystersisters: Woman-to-woman Hysterectomy Support. Available at: http://www.hystersisters.com/vb2/showthread.php?p=3114086. Accessed July 21, 2016.
51. Garden Web. Message Board on Testosterone and Libido. Available at: http://ths.gardenweb.com/forums/load/menopause/msg0314452818178.html. Accessed July 21, 2016.
52. Barreto-Estrada JL, Parrilla-Carrero J, Carlos J. Exposure to an anabolic steroid changes female mice's sexual responses according to sex partner. *J Sex Med* 2007;4:878-885.
53. Bronson FH. Effects of prolonged exposure to anabolic steroids on the behavior of male and female mice. *Pharmacol Biochem Behav* 1996;53:329-334.
54. Urman B, Pride SM, Yuen BH. Elevated serum testosterone, hirsutism, and virilism associated with combined androgen-estrogen hormone replacement therapy. *Obstet Gynecol* 1991;77:595-598.
55. Gastaud F, Bouvattier C, Duranteau L, et al. Impaired sexual and reproductive outcomes in women with classical forms of congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 2007;92:1391-1396.
56. Krysiak R, Drosdzol-Cop A, Skrzypulec-Plinta V, Okopien B. Sexual function and depressive symptoms in young women with nonclassic congenital adrenal hyperplasia. *J Sex Med* 2016;13:34-39.
57. Adamopoulos DA, Kampyli S, Georgiacodis F, Kapolla N, Abrahamian-Michalakis A. Effects of antiandrogen-estrogen treatment on sexual and endocrine parameters in hirsute women. *Arch Sex Behav* 1988;17:421-429.
58. Stovall DW, Scriver JL, Clayton AH, Williams CD, Pastore LM. Sexual function in women with polycystic ovary syndrome. *J Sex Med* 2012;9:224-230.
59. Bazarganipour F, Ziaei S, Montazeri A, Foroozanfard F, Kazemnejad A, Faghihzadeh S. Health-related quality of life in patients with polycystic ovary syndrome (PCOS): a model-based study of predictive factors. *J Sex Med* 2014;11:1023-1032.
60. Benetti-Pinto CL, Ferreira SR, Antunes A Jr, Yela DA. The influence of body weight on sexual function and quality of life in women with polycystic ovary syndrome. *Arch Gynecol Obstet* 2015;291:451.

