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Role of androgens in women's sexual dysfunction

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Abstract

Objective: Although suspected, androgen deficit in women with sexual dysfunction has never been established. Given that serum testosterone levels are of limited value, we sought to compare total androgen activity in women with and without hypoactive sexual desire disorder (HSDD). Intracellular production in target tissues is the major source of testosterone in older women and can now be measured. Androgen metabolites, specifically androsterone glucuronide (ADT-G), reflect intracellular and ovarian sources of testosterone. Thus, we predicted significantly lowered levels of metabolites in women with sexual dysfunction.

Methods: A detailed assessment of the sexual function of women without depression, without serious relationship discord, or receiving medications affecting sexual function included 121 women with HSDD and 124 sexually healthy community controls. Sexual function was assessed using structured interviews, validated questionnaires, and steroid analysis–mass spectrometry levels of ADT-G, testosterone, and precursor hormones.

Results: No group differences in serum levels of testosterone or ADT-G were found. Significantly lower levels of two precursor hormones, dehydroepiandrosterone sulfate and androstene-3β,17β-diol, were found in women with sexual dysfunction ($P = 0.006$ and $P = 0.020$, respectively). The variability of metabolite and precursor levels was substantial for all women.

Conclusions: Significantly lower levels of the two precursor steroids dehydroepiandrosterone sulfate and androstene-3β,17β-diol but not the major androgen metabolite ADT-G were found in women with HSDD. Although the significance of the former awaits further study, androgen deficiency in women with HSDD was not confirmed. Given the unknown long-term effects of testosterone supplementation, women receiving testosterone therapy should be informed that a deficit of testosterone activity in women with HSDD has not been identified.

Key Words: Androgens – Women's sexual desire – Sexual dysfunction.

The complaint of low sexual desire is highly prevalent,[1] leading to widespread off-label prescription of testosterone using locally compounded formulations or adapting formulations approved for men.[2] Women's sexual function is complex, but androgen activity has long been thought to modulate women's sexual desire and response. Modest benefit has been demonstrated in randomized controlled trials (RCTs) of supplemental transdermal testosterone to estrogen-replete[3-7] and estrogen-deficient[8] postmenopausal women reporting low sexual desire and inconsistent ability to be sexually satisfied. This has led to the approval of such therapy in some countries. Concerns remain about the long-term safety of testosterone,[2] but there is a more fundamental issue: unlike traditional endocrine therapy for a clearly identified deficit, testosterone lack has not been demonstrated among women with sexual complaints. Serum levels of testosterone have not been shown to correlate with women's sexual function.[9-11] In contrast, the consistent finding is a strong association between women's sexual function and mental health.[12-15]

One major confounding factor in exploring possible androgen influence has been the inability to measure testosterone produced in peripheral cells from the precursor hormone dehydroepiandrosterone (DHEA) of predominantly adrenal origin. Although lessening by some two thirds between the fourth and seventh decades,[16] DHEA production continues, thereby providing postmenopausal women with both estrogen and testosterone made in the peripheral target tissues possessing the required enzymes to transform DHEA into androgens

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and/or estrogens at various rates in a cell-specific manner by the process of intracrinology.[17] Intracrinology is the production of hormones within a cell to exert their activity in that same cell with minimal release of active hormones into the circulation.[16,18,19]

The secretion of DHEA after menopause protects many older women from the consequences of sex steroid deficiency (eg, vaginal atrophy). In peripheral cells possessing the required steroidogenic enzymes, DHEA can be converted to androstenedione (4-dione) and testosterone to potentially aromatize into estrogens. Measuring intracrine testosterone production has proved challenging as very little spills into the serum. Mass spectrometry (liquid chromatography/tandem mass spectrometry [LC/MS/MS]) methods have now become established means of measuring total androgen production (Fig. 1).[19] The small amount of testosterone derived from the ovaries delivered to cells via the bloodstream plus the larger amount made intracellularly from DHEA in target tissues are all ultimately metabolized, predominantly (some 93%)[19] to androsterone glucuronide (ADT-G), with small amounts of androstane-3α,17β-diol-3-glucuronide (3α-diol-3G) and androstane-3α,17β-diol-17-glucuronide (3α-diol-17G).[18,20] These more water-soluble metabolites, which reflect the total systemic androgen pool, diffuse into the bloodstream and can be accurately measured.[18] To date, there has been minimal study of these metabolites in women with known sexual function status.

Aside from their inability to reflect intracrine androgens, radioimmunoassays of serum testosterone do not possess the required specificity and sensitivity to measure the low levels in women.[21-23] Doing so with good laboratory practice (GLP)–validated mass spectrometry is the preferred method[24]: this has not yet been used in epidemiological studies on sexual function.

In view of these new findings on endocrine physiology in women, to identify a scientific basis for testosterone treatment in women and to assist any subsequent targeting of RCTs of testosterone therapy, we investigated the relationship between total androgen activity, as measured by the major androgenic metabolites, and women's sexual function. We measured the serum levels of androgen metabolites along with the precursor hormones DHEA, dehydroepiandrosterone sulfate (DHEAS), 4-dione, and androstene-3β,17β-diol (5-diol), as well as testosterone, dihydrotestosterone (DHT), estrone (E_1), estradiol (E_2), and E_1 sulfate (E_1S), in carefully evaluated nondepressed

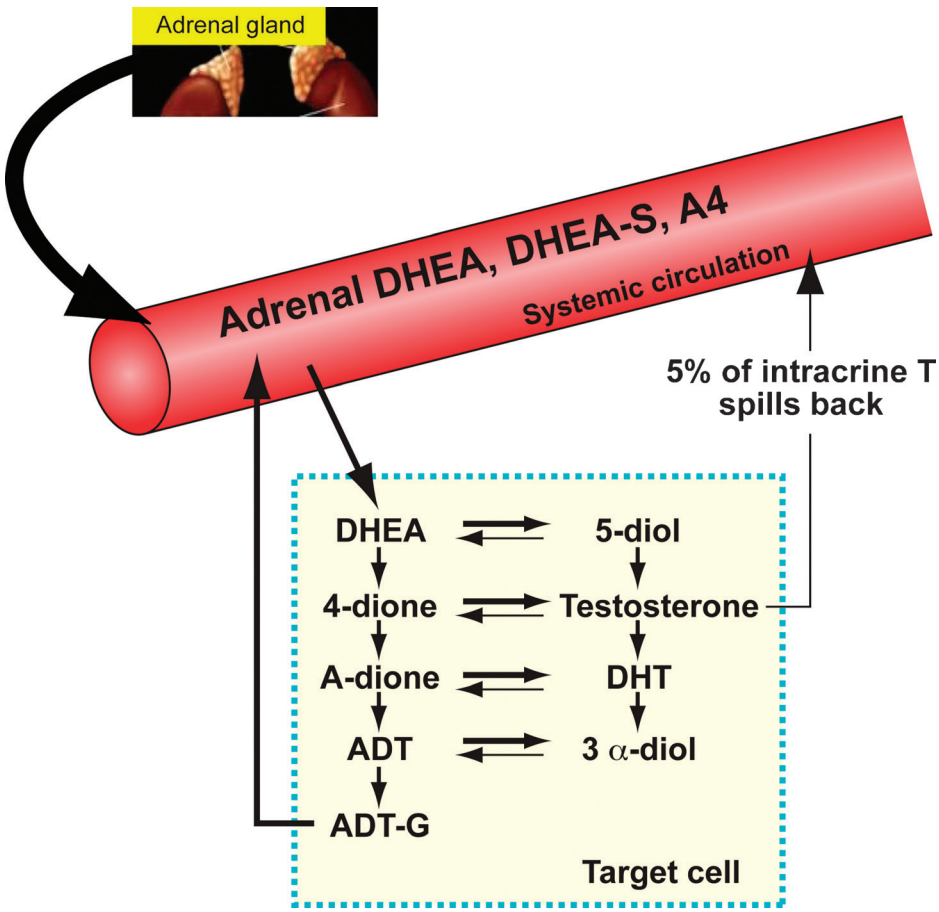

FIG. 1. Intracrine production of testosterone in target cell from circulating precursor steroids, most notably adrenal DHEA. Other precursor hormones include 5-diol and 4-dione, as well as DHEAS. Only some 10% of intracrine testosterone spills into the circulation. Intracrine and ovarian testosterone is further metabolized, mainly to ADT-G, which diffuses into the circulation and can be measured. DHEA, dehydroepiandrosterone; 5-diol, androstene-3β,17β-diol; 4-dione, androstenedione; DHEAS, dehydroepiandrosterone sulfate; ADT-G, androsterone glucuronide; DHT, dihydrotestosterone.

women with and without hypoactive sexual desire disorder (HSDD). Our primary hypothesis was to predict significantly lower levels of serum ADT-G, but not serum testosterone, in women with sexual dysfunction. If low levels of ADT-G are associated with sexual dysfunction, testosterone therapy can be administered in keeping with the concept of an endocrine deficiency state, thereby reducing the number of women receiving supraphysiological supplementation.

RCTs of testosterone patches have been criticized for showing only modest benefit.[25] Moreover, there was no targeting of women with low androgen status, and the participants may not have been sexually dysfunctional.[26] Many would argue that the ability of the recruited women to experience two to three sexually satisfying events per month at baseline is hardly reflective of a sexual disorder and, in fact, is often a desirable outcome for those seeking treatment. However, many women engage sexually without desire but do not have a satisfactory outcome on any occasion. To address the current dilemma over what constitutes a sexual disorder[27-30] and to target more severely impaired women, we further categorized the women with HSDD by distinguishing between those who reported distress from lack of sexual fantasies and absence of sexual desire at the outset of sexual engagement (D1) from women who, in addition, were consistently unable to become aroused and trigger desire during any sexual experiences (D2). Both groups of women reported distress about sexual infrequency and met the criteria for HSDD according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision*.[31] Women in D2 also met a diagnosis of sexual desire/interest disorder as per definitions recommended by the American Urology Association Foundation.[26] The American Urology Association Foundation also separated the subgroup of women who retain subjective excitement but have specifically lost genital responsivity (genital sexual arousal disorder), forming group D3. A secondary hypothesis was that there would be significantly lower ADT-G levels in D2 women compared with controls, given the wider scope of their sexual difficulties. Hypothesis 3 was that ADT-G levels in D1 and D3 women would be only marginally less than in controls, with both groups having significantly higher levels than D2 women.

METHODS

Participants

From January 2005 to March 2009, women with generalized sexual desire and arousal disorders (groups D1, D2, and D3) acquired after the age of 35 years diagnosed at a Canadian sexual medicine center were eligible to participate. Women without sexual dysfunction were recruited from community advertisements. Their healthy sexual function was confirmed by a structured telephone interview and completion of questionnaires. The interview focused on sexual desire, motivation, arousal/excitement, genital arousal/wetness, genital sexual sensitivity, orgasm, pleasure, and pain. Difficulties in any of these areas excluded women from the control group.

Exclusion criteria for both groups were clinical depression, as assessed by interview and completion of the Beck Depression Inventory[32] using a cut-off score of 18; body mass index less than 18.5 kg/m^2 or greater than 29.9 kg/m^2; current use of sex hormones or medications known to affect sexual function, including antidepressants; medical conditions known to potentially inhibit sexual function, including bilateral oophorectomy; situational sexual dysfunction (ie, the dysfunction is partner or context specific); dyspareunia; substance abuse; cigarette smoking; severe relationship discord; or lack of English fluency. Lack of a current partner did not preclude inclusion. Menopausal status was not specified in the recruitment nor adjusted for in the statistical analyses.

Power calculations were based on our pilot data collected from 13 women with sexual dysfunction and 25 age-matched women without sexual dysfunction, who all satisfied the criteria for this study (n = 38 total). Given that there are no available data on the androgen metabolite levels in women with and without sexual dysfunction, these pilot data on 38 women provide the best available data from which to estimate an effect size. Power calculations were based on group difference, in ng/mL, for ADT-G because it accounts for 93% of the obligatory metabolites of androgen elimination.[19] The mean blood value of ADT-G for women in the clinical group for this variable was 20.9 (SD, 24.2) and for women in the control group was 30.1 (SD, 24.8), a group difference that we expected to be clinically meaningful. With a study designed to have at least 80% power of detecting a true difference of 9 ng/mL in the mean ADT-G blood values of the clinical and control groups, a two-sided test based on Student's t statistic, and with an α of 0.05, we required at least 117 women in each of the control and HSDD groups.

Measures of sexual function, mood, and relationship satisfaction

Sexual function, relationship satisfaction, and mood were measured with (1) the Sexual Interest and Desire Inventory (SIDI),[33] (2) the Detailed Assessment of Sexual Arousal,[34] (3) the Dyadic Adjustment Scale,[35] and (4) the Positive and Negative Affect Scale.[36] These questionnaires were identified only by number and were completed in private.

TABLE 1. *Demographic characteristics of the control and sexual dysfunction subgroups*

	Control group (D0)	HSDD alone (D1)	HSDD + SDID (D2)	GSAD (D3)
Number of participants	124	58	52	11
Age, mean (SD), y	48.3 (8.6)	52.5 (7.1)	50.8 (7.8)	56.5 (8.2)
BMI, mean (SD), kg/m^2[a]	25.5 (3.5)	25.5 (3.8)	24.9 (5.8)	25.5 (4.3)
SIDI, mean (SD)[a]	38.2 (8.8)	19.1 (8.8)	13.1 (7.3)	28.9 (10.8)

HSDD, hypoactive sexual desire disorder; SDID, sexual desire/interest disorder; GSAD, genital sexual arousal disorder; BMI, body mass index; SIDI, Sexual Interest and Desire Inventory.
[a]Missing values—BMI: D0 = 3, D1 = 8, D2 = 3, D3 = 2; SIDI: D0 = 1, D1 = 1, D2 = 2, D3 = 2.

Outcome measures: serum steroid analyses

Steroid measurements were performed at the Laboratory of Molecular Endocrinology and Oncology under GLP-validated methodology. Extraction and analysis of conjugated and nonconjugated steroids were performed as previously described.[19,37] Serum levels of DHEA, 5-diol, 4-dione, testosterone, DHT, E_1, and E_2 were analyzed by gas chromatography-mass spectrometry, whereas ADT-G, 3α-diol-3G, 3α-diol-17G, DHEAS, and E_1S were analyzed by LC/MS/MS using TurboIonSpray.[19,37-39] Regarding indices of validity, in each assay of the three different methods used for steroid quantitation, at least six nonzero calibration standard samples were prepared in duplicate from the same stock solutions, and three quality control samples prepared from the same batch at low and medium levels of each steroid were included. For the calibration standards, at least one of the two must have an accuracy between 85% and 115% of each nominal concentration (80%-120% for the lower limit of quantitation). To accept an assay, two thirds of the total calibration standards must meet these acceptance criteria. For the quality control samples, at least two of the three quality control samples analyzed at each level must have an accuracy between

TABLE 2. *Outcome measures in the control and sexual dysfunction groups*

Outcome measure	Control group (n = 124)	Sexual dysfunction group (n = 121)	P^a without age adjustment	P^b with age adjustment
ADT-G, ng/mL			0.11	0.33
Mean (SD)	24.65 (17.27)	21.09 (17.46)		
Min, Max	2.42, 109.38	0.36, 103.00		
5%, 95% percentile	7.52, 53.88	5.35, 56.79		
Testosterone, ng/mL			0.071	0.19
Mean (SD)	0.21 (0.10)	0.19 (0.11)		
Min, Max	0.02, 0.54	0.03, 0.68		
5%, 95% percentile	0.08, 0.40	0.06, 0.37		
DHEA, ng/mL			0.005	0.065
Mean (SD)	3.61 (2.60)	2.81 (1.67)		
Min, Max	0.56, 19.52	0.07, 8.36		
5%, 95% percentile	0.88, 8.05	0.77, 5.62		
DHEAS, μg/mL			<0.001	0.006
Mean (SD)	1.08 (0.68)	0.79 (0.46)		
Min, Max	0.11, 3.65	0.02, 2.29		
5%, 95% percentile	0.35, 2.50	0.18, 1.63		
5-diol, pg/mL			0.001	0.02
Mean (SD)	403.81 (220.51)	318.61 (185.76)		
Min, Max	42.56, 1,196.16	31.26, 911.03		
5%, 95% percentile	104.84, 799.31	68.83, 642.01		
4-dione, ng/mL			0.005	0.29
Mean (SD)	0.77 (0.42)	0.63 (0.36)		
Min, Max	0.15, 2.42	0.04, 1.71		
5%, 95% percentile	0.25, 1.50	0.18, 1.30		
DHT, pg/mL			0.017	0.35
Mean (SD)	71.25 (42.47)	59.03 (36.59)		
Min, Max	13.07, 290.50	5.00, 167.44		
5%, 95% percentile	21.36, 142.24	13.55, 130.75		
3α-diol-3G, ng/mL			0.98	0.81
Mean (SD)	0.97 (0.66)	0.97 (0.86)		
Min, Max	0.10, 3.42	0.10, 5.67		
5%, 95% percentile	0.10, 2.23	0.10, 2.65		
3α-diol-17G, ng/mL			0.13	0.31
Mean (SD)	0.77 (0.68)	0.65 (0.64)		
Min, Max	0.10, 3.51	0.10, 3.14		
5%, 95% percentile	0.10, 2.09	0.10, 2.16		
E_1, pg/mL			0.010	0.19
Mean (SD)	54.12 (48.70)	39.15 (41.15)		
Min, Max	4.00, 319.25	1.99, 241.57		
5%, 95% percentile	8.69, 143.07	6.93, 113.26		
E_2, pg/mL			0.021	0.36
Mean (SD)	77.83 (84.29)	52.34 (87.32)		
Min, Max	0.73, 429.39	0.23, 466.36		
5%, 95% percentile	1.48, 238.76	1.03, 238.39		
E_1S, ng/mL			0.002	0.051
Mean (SD)	1.03 (1.28)	0.60 (0.85)		
Min, Max	0.04, 8.43	0.03, 4.93		
5%, 95% percentile	0.08, 3.91	0.05, 2.17		

ADT-G, androsterone glucuronide; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; 5-diol, androstene-3β, 17β-diol; 4-dione, androstenedione; DHT, dihydrotestosterone; 3α-diol-3G, androstane-3α,17β-diol-3-glucuronide; 3α-diol-17G, androstane-3α,17β-diol-17-glucuronide; E_1, estrone; E_2, estradiol; E_1S, E_1 sulfate; ANCOVA, analysis of covariance.

[a]Student's *t* test comparing the control and sexual dysfunction groups.

[b]ANCOVA comparing the control and sexual dysfunction groups.

85% and 115% of the nominal concentration (80%-120% for the low quality control sample). In addition, three samples of a reference standard were injected at the beginning and three samples were injected at the end of the sequence run to test the validity of the LC/MS/MS or gas chromatography-mass spectrometry system. The coefficient of variation for each analyte response of these six injections must be less than 10%.

Regarding reproducibility, the intra-assay and interassay coefficients of variation obtained during the validation of the three different assays performed under GLP guidelines with six replicates in each steroid assay are indicated in articles published in 2006[19] and 2009.[40]

Procedures

Sexual dysfunction diagnoses for D1, D2, and D3 resulted from a 2-hour semistructured clinical interview of both partners (together and individually): their experiences of sexual desire, motivation, arousal/excitement, genital arousal/wetness, genital sexual sensitivity, orgasm, pleasure, and pain were addressed in detail. Five investigators carried out the interviews, with more

TABLE 3. *Outcome measures in the sexual dysfunction subgroups*

Outcome measure	HSDD alone (n = 58)	HSDD + SDID (n = 52)	GSAD (n = 11)	P^a without age adjustment	P^b with age adjustment
ADT-G, ng/mL				0.26	0.62
Mean (SD)	21.22 (17.50)	22.19 (18.63)	15.20 (9.99)		
Min, Max	3.18, 103.00	1.54, 80.41	0.36, 33.04		
5%, 95% percentile	5.45, 47.21	6.08, 59.76	1.92, 29.48		
Testosterone, ng/mL				0.24	0.34
Mean (SD)	0.19 (0.12)	0.18 (0.08)	0.22 (0.12)		
Min, Max	0.03, 0.68	0.04, 0.37	0.03, 0.42		
5%, 95% percentile	0.05, 0.41	0.08, 0.31	0.08, 0.41		
DHEA, ng/mL				0.038	0.18
Mean (SD)	2.91 (1.91)	2.66 (1.13)	2.94 (2.40)		
Min, Max	0.23, 8.36	0.78, 5.42	0.07, 7.71		
5%, 95% percentile	0.69, 5.82	1.09, 4.67	0.36, 7.09		
DHEAS, μg/mL				0.002	0.028
Mean (SD)	0.81 (0.48)	0.75 (0.41)	0.81 (0.60)		
Min, Max	0.10, 2.29	0.15, 1.71	0.02, 1.84		
5%, 95% percentile	0.20, 1.49	0.25, 1.54	0.10, 1.68		
5-diol, pg/mL				0.008	0.047
Mean (SD)	341.26 (223.89)	294.03 (121.13)	315.40 (217.67)		
Min, Max	31.26, 911.03	82.59, 534.50	50.00, 744.45		
5%, 95% percentile	58.76, 795.17	119.55, 511.68	50.00, 642.13		
4-dione, ng/mL				0.021	0.67
Mean (SD)	0.64 (0.37)	0.65 (0.35)	0.48 (0.35)		
Min, Max	0.13, 1.57	0.20, 1.71	0.04, 1.24		
5%, 95% percentile	0.17, 1.33	0.26, 1.33	0.11, 1.12		
DHT, pg/mL				0.049	0.68
Mean (SD)	60.04 (37.54)	61.43 (37.35)	42.43 (24.16)		
Min, Max	5.00, 167.44	12.72, 160.82	5.33, 83.47		
5%, 95% percentile	12.76, 119.49	19.29, 131.95	9.44, 73.97		
3α-diol-3G, ng/mL				0.94	0.96
Mean (SD)	0.97 (0.93)	1.00 (0.83)	0.84 (0.66)		
Min, Max	0.10, 5.67	0.10, 2.91	0.10, 1.75		
5%, 95% percentile	0.10, 2.34	0.10, 2.80	0.10, 1.69		
3α-diol-17G, ng/mL				0.47	0.77
Mean (SD)	0.66 (0.52)	0.66 (0.76)	0.55 (0.58)		
Min, Max	0.10, 2.64	0.10, 3.14	0.10, 1.65		
5%, 95% percentile	0.10, 1.40	0.10, 2.30	0.10, 1.56		
E_1, pg/mL				0.023	0.26
Mean (SD)	44.16 (45.30)	37.78 (39.29)	19.27 (12.73)		
Min, Max	1.99, 199.64	7.84, 241.57	3.56, 43.04		
5%, 95% percentile	4.43, 133.41	8.85, 92.23	3.78, 38.70		
E_2, pg/mL				0.025	0.22
Mean (SD)	64.46 (99.33)	47.82 (79.15)	9.76 (18.91)		
Min, Max	0.23, 466.36	1.53, 349.07	1.03, 65.55		
5%, 95% percentile	0.84, 248.67	1.86, 214.16	1.33, 40.35		
E_1S, ng/mL				0.012	0.14
Mean (SD)	0.71 (0.93)	0.55 (0.83)	0.25 (0.23)		
Min, Max	0.03, 4.93	0.05, 4.78	0.06, 0.65		
5%, 95% percentile	0.04, 2.26	0.08, 1.70	0.07, 0.63		

HSDD, hypoactive sexual desire disorder; SDID, sexual desire/interest disorder; GSAD, genital sexual arousal disorder; ADT-G, androsterone glucuronide; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; 5-diol, androstene-3β,17β-diol; 4-dione, androstenedione; DHT, dihydrotestosterone; 3α-diol-3G, androstane-3α,17β-diol-3-glucuronide; 3α-diol-17G, androstane-3α,17β-diol-17-glucuronide; E_1, estrone; E_2, estradiol; E_1S, E_1 sulfate; ANOVA, analysis of variance; ANCOVA, analysis of covariance.

[a]ANOVA comparing the control and three sexual dysfunction subgroups.

[b]ANCOVA comparing the control and three sexual dysfunction subgroups.

than 40% of the interviews taking place with two clinicians. Interclinician reliability was established at the beginning of the study through a workshop led by the principal investigator, where all methodologies were explained and clinician ratings to case examples were discussed. The women with HSDD also completed the same battery of questionnaires as the control women, and this was arranged through a study coordinator who was not involved in interviews with the clinical group. Early morning (menstrual cycle days 8-10 if relevant), 5-mL samples of serum were taken from all women, packaged on dry ice, and sent for analysis. Approval for the study was obtained from the university research ethics board, and all women provided informed consent.

Statistical analysis

Differences between groups on continuous variables were initially assessed using Student's *t* test and analysis of variance. Analysis of covariance (ANCOVA) provided corresponding comparisons after adjustment for age. An expanded model including interactions between group and age provided a diagnostic assessment of the ANCOVA common slope assumption. When appropriate, pairwise comparisons of the sexual dysfunction subgroups with the controls were conducted subsequent to analysis of variance and ANCOVA. Analyses other than the comparisons of serum testosterone and ADT-G in the clinical and control groups were considered secondary, so no formal adjustments were made for multiple testing. All

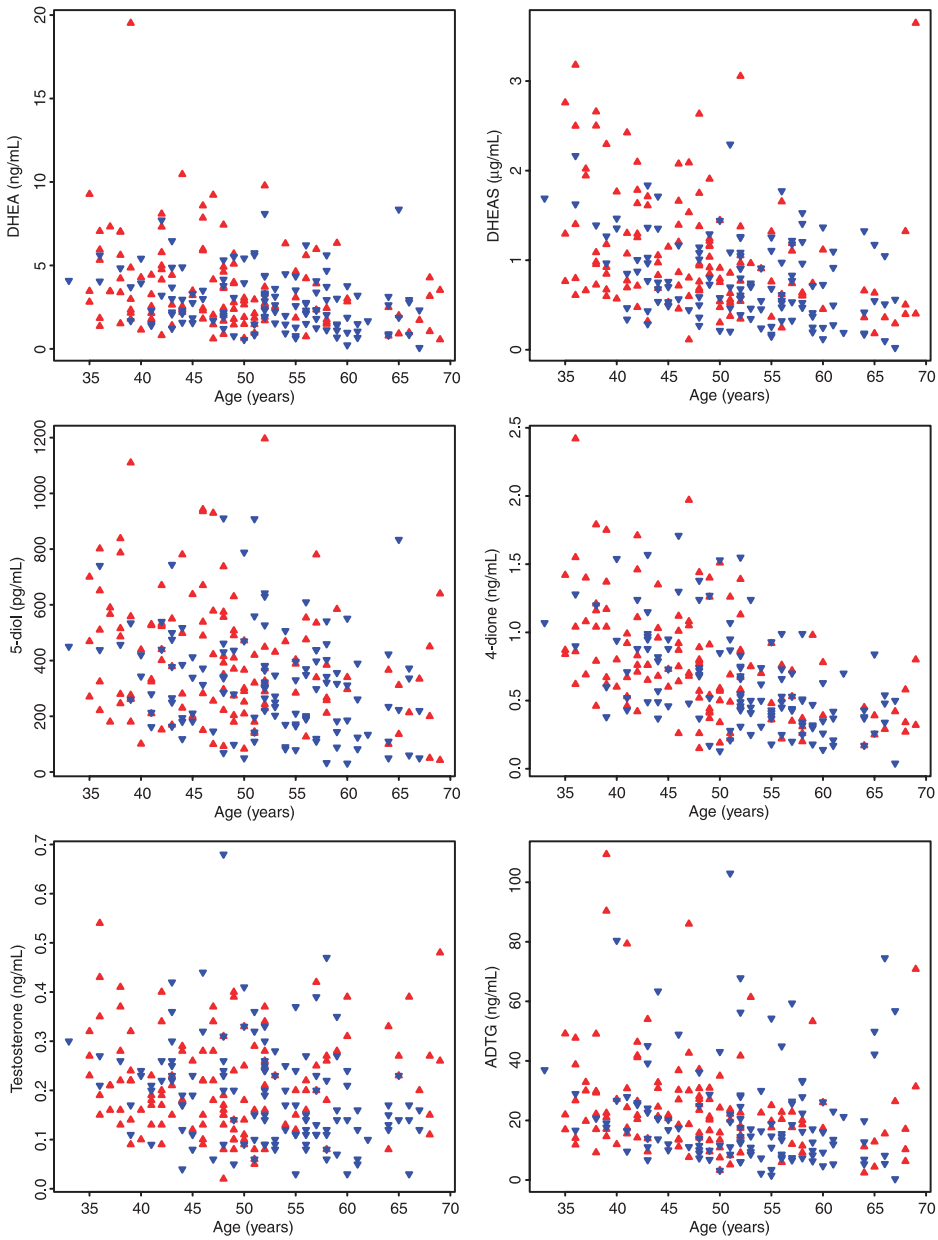

FIG. 2. Serum levels of DHEA, DHEAS, 5-diol, 4-dione, TESTO, and ADT-G plotted versus age (y). Values for sexually dysfunctional women are in blue and those for control women are in red. DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; 5-diol, androstene-3β,17β-diol; 4-dione, androstenedione; TESTO, testosterone; ADT-G, androsterone glucuronide.

statistical analyses were carried out using the free and open source R environment for statistical computing and graphics.

The funding body approved our design and protocol for the study, as well as our proposed data collection, management, and analysis procedures.

RESULTS

A total of 134 women fulfilling inclusion and exclusion criteria were diagnosed with sexual dysfunction, and 121 followed through with steroid measurements—58 with HSDD alone (D1), 52 with HSDD plus sexual desire/interest disorder (D2), and 11 with genital sexual arousal disorder (D3). A total of 140 community control women were screened, and steroid levels were obtained from all of the 124 women fulfilling study criteria.

Demographic characteristics are presented in Table 1. The clinical group was older, on average, than the control group ($P < 0.001$), as were each of the sexual dysfunction subgroups. No group differences on body mass index were noted ($P = 0.83$).

Comparing SIDI scores, the sexual dysfunction group had significantly lower SIDI scores, on average, than the control group ($P < 0.001$). In addition, the mean levels in the control group were significantly higher than in each of the sexual dysfunction subgroups ($P < 0.002$), with scores in D2 being the lowest.

Table 2 shows the serum levels of DHEA, DHEAS, 5-diol, and 4-dione to be 28% ($P = 0.005$), 37% ($P < 0.001$), 27% ($P = 0.001$), and 22% ($P = 0.005$) higher in the control group than in the dysfunctional group. Given that the women with sexual dysfunction were older, and sex steroids are known to decrease with age,[16] age-adjusted analyses were conducted. Preliminary diagnostic assessment provided no indication that the relationships with age differed among groups for these outcome measures ($P > 0.38$ in all cases). When the ANCOVA correction was made for the difference in age between the two groups, the differences decreased, but those for DHEAS ($P = 0.006$) and 5-diol ($P = 0.020$) remained significant, whereas the difference for DHEA was nearly significant at $P = 0.065$ (Table 2). Subgroup comparisons (with ANCOVA) produced the same conclusion of statistically significant differences for DHEAS ($P = 0.028$) and 5-diol ($P = 0.047$) but no significant group difference for DHEA ($P = 0.18$) or 4-dione ($P = 0.67$; Table 3). Table 3 indicates that D2 had the lowest values for DHEAS and 5-diol.

Figure 2 shows the wide range of individual serum steroid concentrations in parallel with increasing number of enzymatic steps. In the full sample of women, the negative correlation between ADT-G and age was highly significant (Pearson's $r = -0.18$, $P = 0.004$; 95% CI, -0.30 to -0.06); the correlation between testosterone and age was weaker ($r = -0.14$, $P = 0.023$; 95% CI, -0.27 to -0.02). The weak correlation for 3α-diol-3G ($r = -0.07$) was not significant, and that for 3α-diol-17G was significant but low ($r = -0.15$, $P = 0.022$). Correlations for the remaining individual steroids, although modest in magnitude (between -0.28 and -0.53), were all negative and highly significant ($P < 0.001$).

There was no statistically significant difference in age-adjusted serum testosterone ($P = 0.19$) or DHT ($P = 0.35$) levels between the control and overall sexual dysfunction group. The ANCOVA of subgroups (Table 3) produced the same finding of no statistically significant differences for testosterone ($P = 0.34$) or DHT ($P = 0.68$).

After age adjustment, for androgen metabolites, serum ADT-G was not statistically significantly higher in the control group ($P = 0.33$). Subgroup comparisons also showed no statistically significant difference for ADT-G ($P = 0.62$), 3α-diol-17G ($P = 0.77$), or 3α-diol-3G ($P = 0.96$).

After age adjustment, there were no significant subgroup and control group differences for E_1 ($P = 0.26$), E_2 ($P = 0.22$), or E_1S ($P = 0.14$).

DISCUSSION

Contrary to our primary hypothesis, our study did not show significantly lower levels of ADT-G in women with sexual dysfunction. In addition, contrary to our second hypothesis, we did not demonstrate lower levels of ADT-G in women in D2[26] who had arguably a more severe form of sexual dysfunction. Interestingly, we did find, for the first time, decreased serum levels of main precursors of testosterone and estrogens in women with sexual dysfunction. Significantly lower levels of 5-diol and DHEAS and marginally significantly lower levels of DHEA were found in women with sexual dysfunction. The overall subgroup analysis was significant, and analysis of Table 3 shows that D2 had the lowest values for DHEAS and 5-diol. Decreases accounted for solely by age were controlled for in all analyses.

After entry into the individual cells in peripheral tissues, these steroid precursors are converted into active androgens and/or estrogens in a cell-specific fashion according to the steroidogenic pathways shown in Fig. 1.[17,18] Declining precursor hormone production may not be symptomatic if the enzymes involved, including 3β-hydroxysteroid dehyrogenases 17β-hydroxysteroid dehyrogenase and 17,20-lyase, are sufficiently active to compensate for the substrate deficit. The global activity of these steroidogenic enzymes and the availability of the precursors are reflected by measurement of androgen metabolites.[18,19] However, our study did not show significantly lower levels of ADT-G in women with sexual dysfunction. The only evidence of lower ADT-G in the group with sexual dysfunction was that a larger proportion of women with sexual dysfunction had ADT-G values in the lowest quartile of serum ADT-G (data not shown).

Attempting to understand the apparent paradox of lower precursor hormones but not lower androgen metabolites, we must stress that there was marked variability in serum DHEA, DHEAS, 5-diol, and 4-dione levels in all women. With this marked variability in substrate levels, their further modification via multiple enzyme steps produces even more variability of metabolite values (Fig. 2). This is despite our a priori power calculation and recruitment of a sufficient number of women

to adequately power the study. Of note, in all women, the variability in ADT-G levels was substantial, the SD being almost as large as the group mean, making it difficult to detect statistically significant group differences. Correlations between serum DHEA and ADT-G, 3α-diol-3G, and 3α-diol-17G have been measured at 0.65, 0.52, and 0.47, respectively, in postmenopausal women aged 55 to 65 years, thus illustrating the individual variability in the efficacy of transformation of DHEA into androgens between different women.[19] In this study, comparable correlations were 0.58, 0.55, and 0.49 in controls and 0.48, 0.44, and 0.41 in the sexual dysfunction group. Identifying any differences in ADT-G levels between functional and dysfunctional women may require extremely large sample sizes, particularly when it is acknowledged that psychosexual factors could contribute further to the variance.

To reconcile our results with those of recent RCTs of transdermal testosterone, which led to limited approval of such therapy, it is necessary to recall the sexual function status of the women recruited to those trials. Compared with our clinical group, they would seem to be relatively sexually healthy. At baseline, the women in the RCTs reported two to three sexually satisfying events per month, and when such details were given, this represented some 50% of their total sexual experiences.[8] Not recruited to the trials were women who are willing to be sexual for reasons other than desire[41] but are consistently unable to have a satisfactory sexual response, that is, similar to our women in D2. Also excluded were women with HSDD to curtail sexual frequency to less than two to three times a month—the typical situation for most of our participants. Thus, it remains unclear if women with a more extensive pattern to their sexual disorder would benefit from testosterone therapy.

Our finding of increased DHEA in the control women, as well as previous research giving some support to reduction of DHEA[10] in sexually dysfunctional women, necessitates considering that DHEA is acting via an alternative pathway (nonandrogen), perhaps activating γ-aminobutyric acid and/or *N*-methyl-D-aspartate receptors, to potentially improve mood[42] and thereby improve sexual function.[12-15] Even when women with clinical depression are excluded (as in our study), research suggests that most women seeking help for low desire have more depressed and anxious thoughts and lower self-esteem than do controls.[43] Current use of antidepressants (an exclusion factor) necessitated excluding some 80% of women referred to our clinic for low desire, hence the extended recruitment period. There is limited study of DHEA replacement for adrenal insufficiency. Due perhaps to their low power, previous RCTs of DHEA replacement have not consistently shown significant improvements in sexual function.[44]

The negative correlation between ADT-G and age was highly significant. A weaker negative correlation between testosterone and age was marginally significant, as expected from previous data[19,45] and consistent with age-related declines in intracrine production of androgens. While acknowledging that neither measurement of intracrine testosterone nor the use of mass spectrometry measurement of serum testosterone was used, previous epidemiological studies suggest that natural menopause per se has little effect on testosterone production.[24] In keeping with their older ages, compared with controls, more of our sexual dysfunction group was postmenopausal.

Testosterone levels did not significantly differ between the groups, in agreement with past research using assays other than mass spectrometry. The low contribution of ovarian testosterone to sexual function is in keeping with the lack of sexual deficit after bilateral salpingo-oophorectomy for benign disease in perimenopausal women reported recently in three different prospective studies.[46-48] A recent large epidemiological survey confirmed increased distress about low sexual desire in women with past bilateral salpingo-oophorectomy but no increased prevalence of low desire per se.[15]

A number of issues remain to be explored. The question of DHEA acting directly on γ-aminobutyric acid receptors merits further evaluation. It is also possible that more robust DHEA levels indicate a healthy hypothalamic-pituitary-adrenal response: childhood abuse/neglect has been associated with adult cortisol dysregulation[49] as well as with adult sexual dysfunction.[50] Future study might usefully include diurnal cortisol levels and cortisol awakening responses in women with and without dysfunction. Alternatively, androgens might not be relevant: although complete androgen insensitivity syndrome may not represent a clear model for testosterone action in women, as these women are genetically male, it is of note that their normal desire and response are documented.[51] Another possibility is that, perhaps, only a minority of women are sensitive to low levels of androgens, with psychosexual factors being more important for the majority. Further complexities include variability in androgen receptor sensitivity and, perhaps most importantly, in the production of sex steroids within the brain.[52] These are also important areas for future research.

Past research has shown minimal correlation between levels of precursor hormones and sexual function.[10,14,53] However, past studies did not include comparable detail of assessment of sexual function nor exclusion of depression or medications known to reduce sexual function (most notably antidepressants). Our Medline search for studies of sexual function and androgen metabolites published up to November 2009 identified only one study: metabolites did not correlate with sexual function subsequent to breast cancer treatment.[54]

The external validity of our study is limited because of the necessary exclusion of women with depression or antidepressant use, making our sample not representative of the majority of women seeking treatment for low sexual desire.[43] Given the known complexity of women's sexual dysfunctions and the robust correlation between psychosocial factors and women's sexual function, very large sample sizes may be needed to confirm or refute any correlations with androgen metabolites. In addition, the aforementioned marked variability in substrate levels and their further modification via multiple enzyme steps similarly suggests that identifying important differences in metabolites between functional and dysfunctional women may

require an extremely large sample size. There was a significant group difference in age, despite our wish to have similarly aged groups. This was due to concurrent recruitment of women to the clinical and control groups. Although age matching was not possible, we statistically controlled for age in all relevant analyses. Although five different treating clinicians assessed both participants and controls, the telephone interviews of community volunteers were all done by the study coordinator, who is an experienced sexual health clinician. Strengths include the exclusive use of mass spectrometry assays for the measurement of intracrine as well as gonadal testosterone. In addition, the assessment of sexual function was comprehensive, and we focused both on women with HSDD alone and on women with HSDD and inability to respond to sexual stimulation to allow a satisfying sexual experience.

CONCLUSIONS

In conclusion, we did not find reduced androgen activity in women with HSDD or in women with HSDD plus concomitant inability to experience a satisfying sexual response. Women receiving testosterone supplementation should be advised that an androgen deficit has not been demonstrated in dysfunctional women. Two million prescriptions for non-compounded formulations were written for women in 2006 to 2007 by US physicians.[55] Although the present data do suggest a role for the sex steroids derived from adrenal precursors in the modulation of women's sexual function, clarifying this role awaits further study. Intriguingly, the link could be past (neglect/ abuse) or present (mood) psychological factors. For current clinical practice, targeting women for testosterone therapy for sexual dysfunction remains a challenge.

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