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Effects of aromatase inhibition on sexual function and well-being in postmenopausal women treated with testosterone: a randomized, placebo-controlled trial

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Abstract

Objective: The extent to which aromatization of testosterone (T) to estradiol is required for the observed effects of testosterone therapy on sexual function and well-being are not known. Therefore, the authors investigated the effects of aromatase enzyme inhibition on sexual function, well-being, and mood in estrogen- and T-replete postmenopausal women in a double-blind, randomized, placebo-controlled study.

Design: Postmenopausal women using transdermal estrogen therapy for at least 8 weeks and reporting low sexual satisfaction (score <42 for the Sabbatsberg Sexual Self-rating Scale [SSS]) with a total T value of less than 1.2 nmol/L were treated with 400 μL of a 0.5% T gel (total dose 2 mg) and were randomly assigned to receive treatment with either 2.5 mg/day of letrozole or an identical placebo tablet. Women were assessed at baseline (week −2) and at 0, 4, 8, and 16 weeks. Sexual function was assessed with the SSS, well-being was assessed with the Psychological General Well-being Index, and mood was assessed with the Beck Depression Inventory at 0 and 16 weeks. Eighty-one women were screened, 76 were randomly assigned to a treatment group, and 30 in each group completed the study. Because this was a mechanistic study, only the 60 women who completed the study per protocol were included in the final analysis.

Results: Total T and calculated free T increased from baseline in both groups, with no difference between groups. At 16 weeks, estradiol, sex hormone-binding globulin, fasting lipids, lipoprotein(a), and C-reactive protein did not differ from baseline or between groups. Significant increases in total Sabbatsberg Sexual Self-rating Scale scores, total Psychological General Well-being Index scores, and a reduction in Beck Depression Inventory scores from baseline to 16 weeks was seen for both treatment groups, with no effect of treatment allocation. No adverse treatment effects were reported.

Conclusions: Increases in total and free T in the physiologic range in postmenopausal women were associated with improved sexual satisfaction, well-being, and mood. In this study, aromatase inhibition did not influence any of these outcomes. Short-term transdermal T therapy did not modify fasting lipids, lipoprotein(a), or C-reactive protein.

Key Words: Testosterone – Postmenopausal androgen therapy – Androgen action.

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Testosterone seems to play a major role in stimulating sexual motivational behaviors, maintaining optimal levels of sexual desire, and possibly contributing to sexual gratification.[1-3] Improvements in sexual function, mood, and well-being have been demonstrated in both premenopausal and postmenopausal women treated with testosterone.[4-12] Acutely, compared with placebo, testosterone therapy also results in increased vaginal vasocongestion, with increased subjective arousal in young eugonadal women.[13]

Transdermal testosterone replacement in surgically menopausal women significantly improves the Psychological General Well-being (PGWB) Index[14] score over placebo, with the greatest change being in improved general well-being and less depressed mood.[5,15] We have reported similar improvements in the total and all subscales of the PGWB Index with testosterone administration in premenopausal women.[4]

Whether the above effects are caused by testosterone acting directly via the androgen receptor or as a precursor for extragonadal estrogen biosynthesis and then via the estrogen receptor (ER) is not known. Testosterone may also act by decreasing sex hormone binding globulin (SHBG) and increasing free circulating levels of multiple sex steroids. There are both androgen receptors and ERs in the healthy female brain,[16] and high concentrations of estradiol (E_2) and testosterone have been demonstrated in the human female hypothalamus and preoptic area, the concentrations of testosterone being 10-fold greater than those of E_2.[17] These regions correlate with regions of high aromatase activity in animals.[18] ERα knockout (KO) mice and aromatase KO mice, despite high circulating androgen levels, exhibit almost no sexual interest,[19,20] whereas ERβKO mice exhibit normal reproductive behavior.[19] In birds, visual exposure to sexual intercourse evokes increased aromatization of testosterone to E_2 in the preoptic area.[21] Therefore, findings from animal models imply that testosterone influences sexual behavior in the brain via conversion to E_2, which then acts through ERα.

To explore whether aromatization is required to achieve the reported sexual and mood effects of testosterone in postmenopausal women, we evaluated the effects of aromatase enzyme inhibition in estrogen-replete postmenopausal women, free of vasomotor symptoms but presenting with low libido, treated with concurrent transdermal testosterone in a double-blind, placebo-controlled, randomized study. To maximize the opportunity to explore the mechanism of testosterone action, we monitored circulating levels during the study and adjusted doses to minimize the likelihood that testosterone levels did not exceed those considered physiologic for young women. Therefore, a placebo-placebo group was not included because this would have resulted in unblinding. Because all women were treated with testosterone, this study also provided the opportunity to examine the short-term effects of transdermal testosterone on established markers of cardiovascular disease risk.

METHODS

Study population

The study involved postmenopausal women (aged older than 55 years or bilateral oophorectomy or >12 months amenorrhea and FSH >20 IU/L, or treated for climacteric symptoms with postmenopausal estrogen therapy for >12 months) with the following characteristics: a body mass index between 18 and 34 kg/m^2, diminished sexuality [Sabbatsberg Sexual Self-rating Scale (SSS)[22] score less than 42 out of a possible 84[23]], no evidence of severe clinical depression on the Beck Depression Inventory (BDI) (score <28),[24] and an early morning serum total testosterone of less than 1.2 nmol/L. Participants were required to have been stabilized for at least 8 weeks on standard transdermal estrogen therapy equivalent at least to 50 μg of 17β-E_2 by matrix patch or 1 mg/day by transdermal gel to eliminate any effect of estrogen therapy on SHBG, with appropriate cyclical or continuous-combined progestin for those with an intact uterus and to be free of vasomotor symptoms and vaginal dryness.

All women were in general good health on history and physical examination and had a normal cervical smear within the past year. Women who had relationship problems, poor feelings for their partner, or dyspareunia were excluded, as were women receiving pharmacotherapy for depression, those taking medication known to interfere with normal sexual function (such as β-blockers and α-blockers), and those who had received tibolone in the past 4 weeks, transdermal testosterone in the last 2 weeks, oral androgen therapy in the previous 3 months, or testosterone injections or implants in the previous 12 months. Women with a history of acne or hirsutism, estrogen-related cancer, confirmed thromboembolic disease, a previous cerebrovascular accident, uncontrolled hypertension (blood pressure >160/95 mm Hg), unstable cardiovascular disease (a myocardial infarction or coronary or peripheral angioplasty within preceding 3 months), genital bleeding of unknown cause, alcohol intake greater than 30 g/day, insulin-dependent diabetes mellitus or unstable

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non–insulin-dependent diabetes mellitus, homozygous familial hypercholesterolemia, or abnormal liver function test results or who were taking medications known to interfere with sex steroid metabolism, a neurologic disorder that would affect cognition, including head injury, epilepsy, history of seizures, or intellectual disability, were also excluded. The use of thyroid hormone was acceptable if the dose was expected to remain stable throughout the study.

Participants were recruited from the Jean Hailes Medical Centre, Melbourne, Australia, and from the general population via television and radio announcements over a 12-month period. The protocol was approved by the Human Research and Ethics Committee, Southern Health (Clayton, Victoria, Australia), and all participants gave written informed consent.

Study design

The study was a randomized, placebo-controlled trial with a 16-week treatment period. All participants continued their transdermal E_2 therapy and in addition received testosterone in the form of 400 μL of a 0.5% testosterone gel (total dose 2 mg) administered to a skin surface area of approximately 60 cm^2 (the size of the average female palm) dispensed from a canister that releases 200 μL per depression, packaged and supplied by Cellergy Pty, Ltd. Women were randomly assigned to receive either the aromatase inhibitor, letrozole, 2.5 mg orally once daily (dose selected on the ability to inhibit in vivo aromatization by 98%[25]), or an identical placebo tablet, one tablet daily. Randomization sequences were generated centrally at Novartis (Basel, Switzerland), and Novartis also supplied and packaged the active and placebo tablets. Women were seen at baseline (week −2) and at 0, 4, 8, and 16 weeks to review therapy and document any adverse events. Women were also reviewed at 4 weeks to have blood drawn for measurement of free testosterone. If the value of the free testosterone, measured by the direct radioimmunoassay, was found to exceed 12.5 pmol/L (above the upper limit of normal provided by the manufacturers of the assay kit for a young woman), the dose was reduced to 200 μL/day and blood was drawn again 2 weeks later to determine the new level achieved. This was to prevent excessive testosterone exposure, both to protect participants and to limit excessive dosing overriding physiologic systems.

Serum hormone measurements

Serum total testosterone, SHBG, E_2, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, lipoprotein(a) [Lp(a)], and highly sensitive C-reactive protein (hsCRP) were measured at 0 and 16 weeks. Thyroid-stimulating hormone, prolactin, and follicle-stimulating hormone were measured at screening. Hormone analyses were performed by Mayne Health Dorevitch Pathology (Melbourne, Australia).

Total testosterone was measured by a validated direct radioimmunoassay method (Biosource Europe S.A., Belgium)[26] using antibody-coated tubes and iodine-labeled testosterone tracer. Mean between-batch coefficients of variation (CVs) were 12.8%, 9.7%, 8.8%, and 7.1% at 0.17, 0.61, 1.77, and 11.5 nmol/L (n = 100), respectively; mean within-batch CVs were 10.5%, 5.3%, 4.2%, and 4.7% at the same concentrations (n = 20). SHBG was measured by a solid-phase, two-site chemiluminescent enzyme immunometric assay using the Immulite 2000 automated analyzer (Diagnostic Products Corporation, Los Angeles, CA). Intra-assay and interassay CVs for SHBG are 6.5% and 8.7%, respectively. During the conduct of the study, free testosterone was measured using a direct assay (Diagnostic Products Corporation) for the purpose of adjusting the testosterone dose. The interassay CVs for this assay were 23.2%, 15.2%, 8.8%, and 9.3% at concentrations of 3.4, 9.7, 33.0, and 77.0 pmol/L, respectively. Calculated free testosterone (using the Sodergard equation[27]) was determined for reporting purposes but was not available to the investigators during the study.

Total cholesterol was determined by the CHOD-PAP method, and triglycerides were determined by the GPO-PAP method using a Boehringer Mannheim Systems Hitachi 747 machine. HDL cholesterol was also measured by an enzymatic colorimetric test on a Boehringer Mannheim/Hitachi 747 machine. Lp(a) was determined by a rate nephelometry method with a kit from Beckman Image Immunochemistry Systems (Beckman Instruments, Inc.). LDL cholesterol was calculated according to Friedewald et al.[28]

Measurement of hsCRP was by a particle-enhanced immunoturbidimetric assay performed on the Hitachi 917 analyzer. The assay range is 0.1 to 20 mg/L, intra-assay CVs are 1.34% at 0.55 mg/L and 0.28 at 12.36 mg/L, interassay CVs are 5.7% at 0.52 mg/L and 2.51% at 10.98 mg/L, and the detection limit is 0.03 mg/L.

Evaluation of well-being, mood, and sexual function

Overall general well-being was assessed with the PGWB Index, a validated 22-item multiple-choice questionnaire.[14] It includes subscales for anxiety, depressed mood, positive well-being, self-confidence,

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general health, and vitality. The composite score ranges from 0 (most negative affective experience) to 110 (most positive affective experience).

Depressed mood was assessed by the BDI, a widely used, validated questionnaire that contains 13 variables to assess mood and depression.[24] Possible scores ranged from 0 (minimal depression) to 63 (severe depression).

The SSS[22] is a multiple-choice questionnaire containing seven aspects of sexuality (sexual interest, sexual activity, satisfaction of sexual life, experience of sexual pleasure, sexual fantasy, orgasm capacity, and sexual relevancy). We have considerable experience with using this questionnaire.[4,11] Possible composite scores range from 0 (low sexuality) to 84 (high sexuality). Each of the three questionnaires was self-administered at screening, at baseline, and at each study visit.

Evaluation of safety

At each visit, hirsutism was evaluated using the Ferriman and Gallwey score[29] by a physician blinded to treatment allocation. Adverse events were recorded. Measurement of lipids, Lp(a), and hsCRP were performed as described.

Statistics

Sample Size

Calculation of the sample size was based on the assumptions that the baseline total score for the PGWB Index, the changes expected with treatment, and the within-group SD for the PGWB Index in postmenopausal women would be similar to those seen in the premenopausal women recently studied.[4] With these assumptions, a sample size of 30 women per group would be required to have an 80% power of detecting an effect size of at least 0.75 ($\alpha = 0.05$). Anticipating a dropout rate of 33%, we decided to recruit 40 women per group (total of 80 women) to the study.

Statistical analyses

Because this was designed as a mechanistic study, only women who provided evaluable data at baseline and 16 weeks were included in the analysis.

All primary and secondary outcomes are continuous variables, although they are not all normally distributed. Where the distribution of the results approximates that of a normal distribution, results are presented as mean (SD), and differences between means are compared using *t* tests. Where the results do not approximate a normal distribution, the results are presented as medians, and the results are compared using non-parametric tests. Unpaired tests were used to assess the characteristics of the group of women excluded from the study compared with those who remained in the analysis, baseline differences between the intervention and control groups, differences between groups at 16 weeks, and change in results between week 0 and week 16 in the two treatment groups. Paired *t* tests were used to assess change from baseline to week 16 in the two treatment groups combined. The contribution of baseline value to the value of week 16 for the outcomes of SSS, BDI, and PGWB Index was assessed using linear regression, with the value at week 16 as the dependent variable and the value at week 0 and group allocation (letrozole or placebo) being the dependent variables. A *P* value of 0.05 was considered statistically significant, and all tests were two-tailed.

When biochemical results were reported as being below the level of detection, these results were ascribed the value of the level of detection. Where a considerable number of results were affected, such as for serum E_2, where 12 of the 60 results at baseline were reported as less than 100 pmol/L, results were compared using ranks rather than means.

RESULTS

Eighty-one women were screened, and 76 were randomly assigned to groups. Reasons for exclusion included total testosterone greater than 1.2 nmol/L (n = 2), increased BDI score (n = 1), detection of a breast lump (n = 1), and failure to attend follow-up (n = 1). Sixty women completed the study according to protocol, with equal numbers in each group. Reasons for discontinuation are listed in Table 1. No significant differences were found for women who did and did not complete the study in relation to any of the parameters measured at screening and baseline. Characteristics of those who completed the study are listed in Table 2. There were no differences in biochemical measures at baseline between the two groups (Table 3). Per protocol reduction in testosterone dose based on increased free testosterone, measured by the direct assay, was made at week 4 in two women in the letrozole group.

Effects on circulating hormone levels and biochemical parameters

The results of biochemical measures are summarized in Table 3. There were no statistically significant differences between treatment groups at week 16 for any biochemical parameter.

The calculated free testosterone values were less than the 90th centile value for young women in 80% of the

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TABLE 1. *Reasons for study discontinuation*

Reason for discontinuation	Letrozole + testosterone	Placebo + testosterone
Decided to stop	2	2
Fatigue	1	0
Feeling "low"/depression	1	1
Insomnia, mood swings, and hot flushes	2	2
Rash	1	0
Insomnia/mood swings and rash	1	
Postcoital bleeding		1
Uncontactable		1
Ineligible, randomized in error		1
Total	8	8

participants at week 16. Of the women who had values above 38.64 pmol/L at week 16, 7 were in the letrozole group and 5 were in the placebo group. Furthermore, 30% of women had free testosterone values below the 10th centile for young women at week 16.

There was no difference between the two groups for the change in total testosterone from week 0 to week 16 [letrozole group, mean ± SD, 1.54 ± 2.61 nmol/L, and placebo group, 1.38 ± 2.02 nmol/L; mean difference 0.16 nmol/L (95% CI, −1.04 to 1.37 nmol/L)] or for the change in calculated free testosterone from week 0 to week 16 [letrozole group, mean ± SD, 23.88 ± 39.51 nmol/L, and placebo group, 18.34 ± 24.65 nmol/L; mean difference 5.54 nmol/L (95% CI, −11.48 to 22.56 nmol/L)].

Because the use of letrozole was not associated with any biochemical differences between the treatment groups, to examine the effects of transdermal testosterone therapy on each of the biochemical end points, the two treatment groups were combined and considered as one group. Between week 0 and week 16, there were statistically significant increases in the mean levels of total and calculated free testosterone. Testosterone therapy was not associated with any statistically significant changes in SHBG, E_2, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, Lp(a), or hsCRP (Table 3).

Sexual function, general well-being, and mood

The treatment groups were comparable in terms of baseline values for sexual function (SSS), mood (BDI), and well-being (PGWB Index) (Table 4). There was no difference between groups at week 16 for SSS, BDI, or PGWB Index.

We again looked at the effects of testosterone treatment in the two groups combined. There was a highly significant increase in the mean scores for the SSS and the PGWB Index and a highly significant decrease in the mean scores for the BDI between weeks 0 and 16. In the regression models for each outcome, the baseline value for the PGWB Index and BDI made a statistically significant contribution ($P < 0.001$) to the value of the PGWB Index and the BDI at week 16. The value of SSS at baseline did not make a statistically significant contribution to the value for SSS at week 16. Group allocation did not contribute significantly to the determination of the value at week 16 for any of these parameters.

TABLE 2. *Screening characteristics of women who completed the study*

Participant characteristic	Letrozole + testosterone, mean (SD) (n = 30)	Placebo + testosterone, mean (SD) (n = 30)	Mean difference (95% CI) between groups or *P* value
Age (y)	53.8 (7.2)	54.2 (5.7) 30	−0.4 (−3.7 to 2.9)
Married (n)	27	25	$P = 0.71$[a]
Sexual preference	All heterosexual	All heterosexual	$P = 1.0$[a]
Sexually active (n)	29 active	28 active	$P = 1.0$[a]
Menopause status	9 natural 21 surgical	9 natural 21 surgical	$P = 1.0$[b]
Estradiol use (n)			
Transdermal patch	24	20	[c]
Gel	3	6	
Implant	2	3	
Intranasal	1	1	
Progestin	2	3	
Weight (kg)	72.3 (10.2) 30	73.5 (10.7) 30	−1.2 (−6.6 to 4.2)
Height (cm)	164.5 (4.8) 30	163.8 (5.8) 30	0.7 (−2.1 to 3.4)
BMI (kg/m^2)	26.8 (3.7) 30	27.4 (3.5) 30	−0.6 (−2.5 to 1.2)
Systolic blood pressure (mm Hg)	130.8 (16.8) 30	138.0 (13.7) 30	−7.2 (−15.1 to 0.7)
Diastolic blood pressure (mm Hg)	80.7 (9.1) 30	82.8 (7.3) 30	−2.1 (−6.3 to 2.1)
Pulse rate (beats/min)	69.7 (7.1) 29	67.9 (5.7) 30	1.8 (−1.6 to 5.2)

BMI, body mass index.
[a]Fisher's exact test.
[b]Yates' corrected $\chi 2$.
[c]For descriptive purposes only.

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TABLE 3. *Biochemical variables at baseline and week 16 for the two treatment groups*

Biochemical variable (normal range)	Letrozole + testosterone, mean (SD) (n = 30)	Placebo + testosterone, mean (SD) (n = 30)	Mean difference (95% CI) between groups	Mean difference from week 0 for combined treatment groups (n), *P* value
Total testosterone (nmol/L) (0.86-2.47)[a]				
Week 0	0.62 (0.45)	0.67 (0.55)	−0.05 (−0.3 to 0.2)	1.46 (60), $P < 0.001$
Week 16	2.17 (2.7)	2.06 (2.13)	0.11 (−1.15 to 1.37)	
Free testosterone[b](pmol/L) (12.91-38.64)[a]				
Week 0	8.40 (5.11)	9.75 (8.5)	−1.35 (−4.97 to 2.27)	21.1 (60), $P < 0.001$
Week 16	32.28 (40.68)	28.09 (25.89)	4.19 (−13.4 to 21.8)	
SHBG testosterone (nmol/L) (27-109)				
Week 0	52.4 (21.7)	58.2 (38.0)	−5.8 (−21.8 to 10.2)	0.6 (60), $P = 0.84$
Week 16	49.6 (20.1)	59.7 (42.1)	−10.1 (−27.2 to 6.8)	
Estradiol (pmol/L) (early follicular <575)				
Week 0	Median 215	Median 235	Difference between medians = 20, $P = 0.876$[c]	Median change = 0
Week 16	Median 199	Median 195	Difference between medians = 4, $P = 0.73$[c]	$P = 0.83$[d]
Total cholesterol (mmol/L) (<5.5)				
Week 0	5.7 (1.1)	5.4 (0.7)	0.3 (−0.2 to 0.8)	0.0 (59), $P = 0.80$
Week 16	5.5 (1.0)	5.5 (0.8)	0.0 (−0.44 to 0.46)	
LDL cholesterol (mmol/L) (3.4)				
Week 0	3.4 (1.0)[e]	3.2 (0.7)	0.2 (−0.2 to 0.7)	0.01 (58), $P = 0.82$
Week 16	3.3 (0.9)[e]	3.2 (0.7)	0.1 (−0.34 to 0.50)	
HDL cholesterol (mmol/L) (0.9-2.2)				
Week 0	1.7 (0.4)	1.7 (0.4)	0.0 (−0.2 to 0.2)	0.03 (59), $P = 0.19$
Week 16	1.6 (0.5)[e]	1.7 (0.4)	−0.1 (−0.35 to 0.10)	
Triglyceride (mmol/L) (0.5-2.0)				
Week 0	1.5 (1.0)	1.2 (0.5)	0.3 (−0.1 to 0.7)	−0.1 (59), $P = 0.39$
Week 16	1.4 (0.8)[e]	1.3 (0.7)	0.1 (−0.30 to 0.45)	
Lipoprotein(a) (mg/L) (<270)				
Week 0	Median 83.5	Median 76.0	Difference between medians = 7.5, $P = 0.77$	
Week 16	Median 100.5	Median 84.5	Difference between medians = 15.5, $P = 0.95$	Median change = 0.0, $P = 0.42$[d]
hsCRP (mmol/L) (<3.0)				
Week 0	Median 2.0	Median 1.4	Difference between medians = 0.6, $P = 0.10$[c]	Median change = −0.05
Week 16	Median 2.3	Median 1.8	Difference between medians = 0.5, $P = 0.10$[c]	$P = 0.46$[d]

SHBG, sex hormone-binding globulin; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hsCRP, highly sensitive C-reactive protein.
[a]10th to 90th centile values for women aged 18 to 24 years[30]; to convert nmol/L to ng/dL or pmol/L to pg/dL, divide by 0.0347.
[b]Calculated using the Sodergard equation.
[c]Wilcoxon rank sum test.
[d]Wilcoxon signed-rank test.
[e]n = 29.

The changes in SSS domain scores for the two treatment groups are shown in Table 5.

The change in the mean score for the domain of sexual activity was significantly greater in the placebo group than the letrozole group ($P = 0.05$), but a statistically significant difference was not seen for any other sexual domain.

There were no significant differences between treatment groups seen for changes in any of the domains of the PGWB Index.

Safety

Application of the transdermal gel was well tolerated, although 2 women (1 in each group) reported hair growth at the site of application, and one woman reported itchiness at the application site.

Hirsutism scores out of a total of 44 changed by up to 2 points in 10 women (a decrease in 4 women and an increase in 6 women), which we considered within observational error. One woman treated with testosterone plus letrozole had an increase of 4 points at week

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TABLE 4. *Effects of therapy on sexual function, well-being, and mood*

	Letrozole + testosterone, mean (SD) (n = 30)	Placebo + testosterone, mean (SD) (n = 30)	Mean difference (95% CI) between groups	Mean difference from week 0 for both treatment groups (n = 60), *P* value for difference from week 0
Sabbatsberg Sexual Self-rating Scale, total score				
Week 0	21.8 (9.6)	23.7 (9.1)	−1.9 (−6.8 to 2.9)	
Week 16	33.3 (17.5)	37.9 (14.4)	−4.6 (−12.8 to 3.7)	12.8, $P < 0.001$
Psychological General Well-being Index, total score				
Week 0	69.9 (17.5)	70.2 (15.4)	−0.3 (−8.8 to 8.2)	
Week 16	78.16 (14.89)	79.13 (15.91)	−0.97 (−8.93 to 7.0)	8.6, $P < 0.001$
Beck Depression Inventory, total score				
Week 0	11.3 (7.7)	10.3 (7.4)	1.0 (−2.9 to 4.9)	
Week 16	7.0 (6.3)	6.0 (6.0)	1.0 (−2.16 to 4.16)	−4.3, $P < 0.001$

16, achieving a final score of 8 of 44. No woman had a score higher than 8 at week 16.

One woman treated with testosterone plus letrozole and 2 women treated with testosterone reported acne. No serious adverse event occurred during the study.

DISCUSSION

The aim of this study was to investigate whether aromatization plays a significant role in the therapeutic action of testosterone in women, as suggested by animal models. Significant and equivalent increases in the mean sexual function and well-being scores and a decrease in the mean BDI scores was seen in both the letrozole and placebo groups, indicating that inhibiting the aromatization of testosterone to E_2 does not impair the effects of testosterone on sexual function and mood in estrogenized postmenopausal women.

All women who participated were postmenopausal at entry into the study and were considered estrogen replete, being free of vasomotor symptoms and vaginal dryness on their ongoing transdermal oestrogen therapy. All received transdermal testosterone and achieved increased circulating total and free testosterone levels with this therapy.

The study design did not include a placebo-placebo treatment group because it was a mechanistic study, not an efficacy study. Furthermore, the inclusion of a placebo-placebo group would have meant that we could not monitor serum levels and include the opportunity for dose adjustment without unblinding

TABLE 5. *Change between baseline and week 16 in Sabbatsberg Sexual Self-rating Scale domains, Psychological General Well-being Index domains, and Beck Depression Inventory*

	Testosterone + letrozole	Testosterone + placebo	Mean difference	95% CI for mean difference
Sabbatsberg Sexual Self-rating Scale domains				
Sexual interest	1.77 (3.23)	2.27 (2.01)	0.5	−0.89 to 1.89
Sexual activity	1.10 (2.96)	2.53 (2.32)	1.43	0.06 to 2.81[a]
Satisfaction of sexual life	1.90 (3.62)	2.47 (2.70)	0.57	−1.08 to 2.21
Sexual pleasure	1.93 (3.29)	1.90 (2.45)	−0.03	−1.53 to 1.47
Sexual fantasy	1.80 (3.68)	2.23 (2.37)	0.43	−1.17 to 2.03
Orgasm	1.97 (3.65)	2.03 (2.12)	0.06	−1.48 to 1.61
Importance of sex	1.03 (2.55)	0.70 (2.18)	−0.33	−1.56 to 0.89
Total score	11.50 (20.91)	14.13 (13.40)	2.63	−6.45 to 11.71
Psychological General Well-being Index domains				
Anxiety	1.23 (5.37)	1.00 (4.65)	−0.23	−2.83 to 2.36
Depressed mood	0.77 (2.76)	1.20 (2.64)	0.43	−0.96 to 1.83
Positive well-being	1.83 (3.36)	1.90 (3.84)	0.07	−1.80 to 1.93
Self-control	1.07 (2.50)	0.90 (2.54)	−0.17	−1.47 to 1.14
General health	1.30 (2.31)	1.33 (2.02)	0.03	−1.09 to 1.15
Vitality	2.03 (3.68)	2.57 (4.30)	0.53	−1.53 to 2.60
Total score	8.23 (16.62)	8.90 (14.78)	0.67	−7.46 to 8.80
Beck Depression Inventory	−4.26 (6.50)	−4.26 (5.69)	0.00	−3.16 to 3.16

[a]Difference statistically significant at the 5% level.

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the study. Not only did monitoring protect participants, but limiting excessive exposure was also intended to minimize the possibility of achieving supraphysiologic testosterone levels that would override the normal physiology we wished to study.

The mean and range of baseline total and calculated free testosterone levels were consistent with mean levels we have recently reported for Australian women aged 55 to 64 years.[30] Furthermore, the mean levels achieved in total and free testosterone were within the range reported for young healthy women[30] at week 16. However, 30% of women had low values and 80% had higher values at this time point despite attempted titration at week 4. This may reflect variations in timing of application of the final dose or possibly differences in transdermal absorption of the gel. Mean SHBG levels did not change during the study period. Therefore, we believe we achieved a sound mechanistic model in which to study the effects of aromatase enzyme inhibition.

There were no significant differences between letrozole and placebo treatment groups for any total score or domain score other than the sexual activity domain of the SSS. As an isolated finding and in the setting of multiple statistical comparisons, this is of questionable significance and is very likely to be a chance finding.

The improvements in sexual function and well-being over baseline were clinically significant and equal in magnitude to increases previously reported by us and others with exogenous testosterone therapy.[4,5,15] Such substantial increases in these parameters are highly unlikely to be attributable to a placebo effect and have not been seen with placebo therapy in our studies.[4,31] Although Shifren et al reported a significant placebo effect on sexual function, this did not apply to the PGWB Index in the same study.[5] Therefore, we do not believe the lack of an effect of aromatase inhibition in our study is simply due to a placebo effect of testosterone in both treatment groups. The baseline scores for the BDI and PGWB Index made highly significant contributions to each of these measures at week 16. That this did not hold true for the assessment of sexual function suggests that the determination of sexual function is complex, and its assessment remains problematic.

Letrozole is a nonsteroidal aromatase inhibitor. Clinical tracer studies in postmenopausal women show that letrozole inhibits peripheral aromatase by more than 98% and suppresses blood and urinary estrogen levels by more than 95% after 2 weeks of treatment.[25] Despite the possible contribution of a placebo effect, we conclude that near-complete blockade of aromatization of exogenous and endogenous testosterone does not impair improvements in sexual function and well-being with testosterone therapy. Therefore, our findings would support further evaluation of the use of poorly aromatizable androgens such as methyltestosterone and nonsteroidal selective androgen receptor modulators[32] for the treatment of low libido in postmenopausal women.

There is considerable concern regarding potential adverse cardiovascular effects of testosterone in postmenopausal women. In this study, increase of testosterone levels into the high normal range for young women with testosterone gel therapy was not associated with any changes in lipoprotein lipids or SHBG. In contrast, methyltestosterone therapy significantly reduces HDL cholesterol, triglycerides, and SHBG.[33,34] Furthermore, over the 4-month treatment period, we demonstrated no adverse effects of transdermal testosterone therapy on circulating hsCRP and Lp(a), which are each considered independent risk markers for cardiovascular disease.[35,36] Although these findings are of interest, it is important to note that this study was of short duration and was not powered to detect small changes in these parameters.

In summary, aromatase inhibition did not seem to negate the favorable effect of testosterone therapy on sexual function and well-being. Short-term restoration of total and free testosterone to the physiologic range for young, healthy women did not adversely affect fasting lipids, Lp(a), or hsCRP.

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