Testosterone improves verbal learning and memory in postmenopausal women: Results from a pilot study

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A B S T R A C T

Objective: To explore the effects of testosterone on cognitive performance in healthy postmenopausal women.

Study design: Open-label pilot study. Nine postmenopausal women on non-oral hormone replacement therapy, aged 47–60 years received transdermal testosterone spray for 26 weeks. A control group of 30 women provided normative data for comparison.

Main outcome measures: Scores from a computerized cognitive test battery performed pre- and post-treatment, at 0 and 26 weeks.

Results: There were no differences between treatment/normative groups in any parameter at baseline. At week 26 scores for the International Shopping list task including delayed recall (verbal learning and memory) and the continuous paired associate learning task (visual learning and memory) were significantly higher in the treatment group as compared to the normative group (p < 0.05). Significant improvements from baseline were observed for the International Shopping list delayed recall (verbal learning and memory) and Groton Maze recall tests (visual learning and memory) for the treatment group (both p < 0.05), after 26 weeks. There were no significant differences between baseline and week 26 in the normative group. In the regression analysis which modeled the score at week 26, and which included a bootstrapping approach, the beta coefficient for the treatment group was statistically significant when age and baseline score were taken into account for the International Shopping list task including delayed recall (both p < 0.02).

Conclusion: Testosterone improved cognitive performance in the domain of verbal learning and memory in a pilot study of healthy postmenopausal women and is worthy of further exploration in a randomized placebo controlled study.

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1. Introduction

The lifetime risk for dementia is higher in women [1–3]. It has been hypothesized that lower testosterone levels in women may contribute to the sex-difference in dementia prevalence [4]. Testosterone levels decline in women from their early adult years, reaching a nadir in the mid 60s [5], at which time dementia incidence rises [6]. Testosterone has been reported to have neuroprotective properties [7]. Higher endogenous testosterone levels in premenopausal women have been linked with better performance in tasks of spatial and mathematical ability [8], whilst in elderly women higher endogenous testosterone levels have been associated with superior performance on verbal fluency [9] and verbal memory tasks [10]. Mild cognitive impairment precedes the development of dementia by several years [11], and an interesting parallel is seen between the fall of androgen levels in women and the rise in incidence of mild cognitive decline and dementia that is yet to be explored in clinical studies. Whether exogenous testosterone can improve cognitive performance in postmenopausal women...
women is unknown. We therefore conducted this open label pilot study with the primary aim of investigating the effects of testosterone on cognitive function in healthy, non-depressed, cognitively unimpaired, estrogen replaced postmenopausal women. A control group of healthy women not on hormone replacement therapy (HRT) provided normative data for cognitive function testing over the same time span.

2. Methods

2.1. Subjects

Women were eligible for the study if they were aged 45–60 years and postmenopausal (age greater 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), stabilized for at least 8 weeks on standard parenteral HRT equivalent to at least 25 mcg 17β-estradiol by matrix patch or 1 g/day by transdermal gel, with non-oral progestogen treatment in those with an intact uterus.

A non-intervention group of thirty women, not using any systemic HRT were recruited to provide control data for cognitive testing at baseline and at 26 weeks. Participants were required to have no evidence of cognitive impairment or depression at baseline. Other exclusion criteria included a past history of neurological disorder including head injury, past recent significant psychiatric illness, significant systemic illness, use of psychoactive medications, recreational drug use or alcohol excess. The study was approved by Monash University Standing Committee on Ethics in Research Involving Humans and all subjects provided written informed consent.

3. Variables assessed in the participants

3.1. Cognitive function

Cognitive function was measured using the CogState computerized test battery which was specifically developed to minimize practice effects in the repeated testing of cognitive performance and use in clinical trials [12,13]. It has been demonstrated to have good acceptability, efficiency and stability for the repeated assessment of cognitive function [14]. Subjects are tested by being randomly presented different tasks within each cognitive domain, on each test occasion. The tasks included detection (psychomotor function/speed of processing), identification (visual attention/vigilance), one back (attention/working memory), International Shopping list learn and recall (verbal learning and memory), Groton Maze learning task and recall (executive function/spatial problem solving/visual learning and memory), and continuous paired associate learning (visual learning and memory). All women underwent 2 practice sessions prior to their baseline assessment and then provided data for assessments at week 0 (baseline), 12 and 26 weeks.

3.2. Biochemistry

Serum testosterone levels were determined at screening and week 26 in the treatment group. Total testosterone was measured by DPC radioimmunoassay (reference range 0.9–2.5 nmol/L; assay C.V.s of 14.6% and 8.0% at 0.36 and 2.6 nmol/L respectively), sex hormone binding globulin (SHBG) by DPC immunometric assay (reference range 27–109 nmol/L), and free testosterone was calculated using the Sodergard equation (reference range 13–39 pmol/L) [15].

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment group (n)</th>
<th>Normative group (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: (yrs), mean ± SD</td>
<td>55.4 ± 3.8</td>
<td>59.0 ± 6.3</td>
</tr>
<tr>
<td>Time since menopause: (yrs), mean ± SD</td>
<td>9.2 ± 6.8</td>
<td></td>
</tr>
<tr>
<td>Body mass index: (kg/m²), mean ± SD</td>
<td>26.5 ± 5.9</td>
<td></td>
</tr>
<tr>
<td>Previous gynecological surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hysterectomy alone</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Hysterectomy and bilateral oophorectomy</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Hormone therapy use</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Estradiol 25 mcg transdermal patch</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Estradiol 50 mcg transdermal patch ¹</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Estradiol transdermal 1% gel</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Estradiol 50 mcg/NETA</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>250 mcg transdermal patch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Completed primary school</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Completed secondary school</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Completed post-secondary school diploma/certificate</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Completed tertiary education or higher degree</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

SD = standard deviation, NETA = norethisterone acetate.

¹ One subject taking the estradiol 50 mcg patch also had a progesterone intrauterine device (Mirena) in situ.

3.3. Study treatment

All women in the treatment arm received daily treatment with a metered dose transdermal testosterone spray, which delivers 90 µl per spray at a concentration of 5% testosterone. The dose of testosterone delivered per spray is 4.55 mg, however the systemic absorption via the skin is reduced such that the remaining delivery of testosterone is equivalent to the daily production of testosterone in a young woman, i.e. approximately 300 mcg [16].

3.4. Statistical analysis

As the data distributions for the cognitive tests were not normal, medians and interquartile ranges were used to characterize performance in both groups. Means and standard deviations were used to report biochemical results. For each of the treatment and normative groups, cognitive test performance at week 26 was compared to baseline using the Wilcoxon signed ranks test. Similarly the Wilcoxon rank sum test was used to compare results for the treatment group and the normative group, at baseline and also at week 26. Linear regression modeling was performed for each measure of cognitive function with the outcome variable being the result at 26 weeks and the independent variables including treatment group, age and result at baseline. This allowed determination of between group differences at week 26 adjusted for age and any differences between the groups at baseline. To address outlying values, a bootstrapping approach with 5000 repetitions was used [17]. All statistical analysis was completed using the statistical program SPSS (SPSS Inc., IL, USA) and Stata 11.1.

4. Results

Nine women completed the treatment arm of the study. All were using non-oral systemic estrogen, and 2 women were also treated with non-oral progestins (Table 1). The non-intervention group of 30 women were slightly older, mean age of 59 (range 43–69 years) than the treatment group, mean age 55 years (range
Table 2  
Biochemistry results for treatment group, n=9.  

<table>
<thead>
<tr>
<th>Biochemistry</th>
<th>Baseline</th>
<th>Week 26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total testosterone (nmol/L)</td>
<td>0.8 ± 0.6</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>50.6 ± 30.0</td>
<td>56.0 ± 22.9</td>
</tr>
<tr>
<td>Calculated free testosterone (pmol/L)†</td>
<td>14.0 ± 12.9</td>
<td>40.4 ± 21.4</td>
</tr>
</tbody>
</table>

SD = standard deviation, SHBG = sex-hormone binding globulin.  
† Normal ranges for early reproductive aged women 0.9–2.5 nmol/L (total testosterone), 27–109 nmol/L (SHBG), 13–39 pmol/L (calculated free testosterone). To convert nmol/L to ng/dL, divide by 0.0347.  
† p < 0.01 for difference between weeks 0 and 26.

47–60 years), although this difference was not statistically significant (Table 1). A higher proportion of women in the treatment group had completed post-secondary level education. Testosterone levels increased significantly in all women in the treatment group indicating compliance with study medication (Table 2).

There were no differences between the treatment and the non-intervention group in any parameter at baseline. There were no significant differences between any baseline and week 26 scores in the non-intervention group. Significant improvements from baseline were observed for the International Shopping list delayed recall (verbal learning and memory) and Groton Maze recall tasks (visual learning and memory) for the treatment group (both p < 0.05), after 26 weeks (Table 3). At week 26 scores for the International Shopping list task including delayed recall (verbal learning and memory) and the continuous paired associate learning task (visual learning and memory) were significantly higher in the treatment group as compared to the normative group (p < 0.05, Table 3). In the regression analysis taking age into account and differences between groups at week 26, the beta coefficient for the treatment group was statistically significant when age and baseline were taken into account for the International Shopping list task including delayed recall (both p < 0.02) (Table 3).

5. Discussion

We have demonstrated improvements in verbal learning and memory after 26 weeks of transdermal testosterone therapy in a pilot study of cognitively unimpaired postmenopausal women on stable non oral estrogen therapy, compared with a non-intervention group of women over the same time period.

Our findings are in line with our earlier large open labeled study in which we observed a significant improvement in immediate and delayed verbal memory with transdermal testosterone in postmenopausal women on estrogen therapy using the California Verbal Learning Test (CVLT) [18]. Our earlier CVLT findings potentially reflected the well documented specific practice effect observed when normally functioning individuals are exposed to the same test materials within a brief time interval [19]. In the present study we have confirmed our earlier findings using the CogState battery, which has been shown to exhibit stable performance after subject familiarization [12].

Higher endogenous serum testosterone levels are associated with enhanced visuospatial skills and verbal fluency in men [20] and verbal fluency and memory in women [9,10]. Short term testosterone therapy has been shown to improve visuospatial ability and verbal fluency in older men [21] and in men with early AD [22]. In the present study we have observed improvements in the verbal learning and memory task of CogState (improvements in the International Shopping list task including delayed recall).

The decline in testosterone, and the adrenal pre-androgens, in women with age commences in the premenopausal years, such that levels in postmenopausal women are typically less than half of those seen in early reproductive-aged women [5]. There is biological plausibility that this decline in testosterone with age

Table 3  
Cognitive test results – treatment vs. normative group, baseline and week 26 and beta coefficient for treatment group variable in the regression analysis for week 26 result adjusted for age and baseline result.  

<table>
<thead>
<tr>
<th>CogState test</th>
<th>Cognitive domain</th>
<th>Baseline median (interquartile range)</th>
<th>Week 26 median (interquartile range)</th>
<th>Beta coefficient for treatment group (95% CI) and p-value in bootstrapping analysis††</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment group</td>
<td>Normative group</td>
<td>Treatment group</td>
<td>Normative group</td>
</tr>
<tr>
<td></td>
<td>(interquartile range)</td>
<td>(interquartile range)</td>
<td>(interquartile range)</td>
<td></td>
</tr>
<tr>
<td>International Shopping list†</td>
<td>Verbal learning and memory</td>
<td>29.0 (25.0–31.5)</td>
<td>27.0 (22.8–29.0)</td>
<td>30.0 (28.0–31.5) p = 0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0 (9.0–11.5)</td>
<td>9.5 (8.0–11.0)</td>
<td>12.0 (10.5–12.0) p = 0.015</td>
</tr>
<tr>
<td>Groton Maze learning task</td>
<td>Executive function/spatial problem solving</td>
<td>49.0 (38.5–55.0)</td>
<td>46.3 (39.6–50.3)</td>
<td>45.0 (31.3–54.5) p = 0.308</td>
</tr>
<tr>
<td>Identification</td>
<td>Visual learning and memory</td>
<td>6.0 (4.5–7.5)</td>
<td>5.0 (3.8–7.0)</td>
<td>6.0 (5.0–7.0) p = 0.007</td>
</tr>
<tr>
<td>Detection</td>
<td>Psychomotor function/speed of processing</td>
<td>2.5 (2.5–2.5)</td>
<td>2.5 (2.5–2.5)</td>
<td>2.5 (2.5–2.5) p = 0.307</td>
</tr>
<tr>
<td>Continuous paired associate learning</td>
<td>Visual learning and memory</td>
<td>15.0 (5.5–48.0)</td>
<td>29.0 (22.8–33.0)</td>
<td>11.0 (5.0–24.5) p = 0.007</td>
</tr>
<tr>
<td>One back†</td>
<td>Attention/working memory</td>
<td>2.9 (2.8–2.9)</td>
<td>2.9 (2.8–2.9)</td>
<td>2.9 (2.8–2.9) p = 0.007</td>
</tr>
</tbody>
</table>

As data were not normally distributed, medians and interquartile ranges are shown. CI = confidence interval.  
† p < 0.05 for difference between treatment and normative groups at baseline and at week 26 respectively, using the Wilcoxon rank sum test.  
†† A bootstrapping approach was utilized to analyse the effects of treatment between baseline, weeks 0 and week 26, comparing treatment and normative groups and taking age into account.  
† A higher score at week 26 compared to baseline represents an improvement in the cognitive domain measured; for unmarked tests a lower score at week 26 represents an improvement.  
†† p < 0.05 for difference between baseline and week 26 results in the treatment and normative groups respectively, using the Wilcoxon signed rank test.
may influence cognitive performance. Levels of testosterone in the human female brain during the reproductive years are several-fold greater than those of estradiol [23]. Within the brain testosterone exhibits neuroprotective effects including protection against oxidative stress, serum deprivation-induced apoptosis and soluble beta amyloid (Aβ) toxicity [7]. Although some of these effects of testosterone are blocked by aromatase inhibition and thus appear to be estrogen mediated [24], protection against Aβ toxicity appears to be androgen receptor (AR) mediated [7]. There is also evidence that endogenous androgens influence levels of Aβ possibly by an AR-dependent mechanism involving up-regulation of the Aβ catalyzing enzyme neprilysin [25]. In addition to having neuroprotective effects, testosterone has positive effects on endothelial function [26] and acts as a vasodilator [27], such that testosterone-induced vascular effects may have contributed to the changes we observed. The mechanism by which testosterone therapy may facilitate cognitive performance in postmenopausal women was not addressed in this study. The participant numbers were too small to explore the relationship between serum levels of testosterone and cognitive performance. Direct immunoassays such as the DPC assay for testosterone used in this study lack precision and accuracy at low levels of testosterone such as are seen in women and children, however the measurement of testosterone levels in the treatment group was used solely as an indicator of treatment compliance, and no analysis of cognitive test results in relation to testosterone levels was performed. As the cellular actions of testosterone involve intracrine metabolism via either 5α-reduction or aromatization [28] it is most likely that the downstream effects would not be reflected in absolute serum levels of testosterone.

We are aware of the potential issue of multiple comparisons in this study. However, the findings in relation to the International Shopping list tasks are unlikely to be due to chance. The difference seen in relation to the treatment group was not trivial in magnitude but represented a difference of about 10% of the baseline value in each case. Furthermore, the difference was highly statistically significant and the significant findings occurred in a cognitive domain in which we have seen an impact of testosterone previously [18].

The lack of improvement in other cognitive domains in this study may be due to testosterone improving online automatic processes involved in encoding, whereas all the other domains tested are utilizing strategic conscious higher order processes [29]. These processes are not so environmentally driven but rely more on innate abilities present from infancy [30].

Strengths of this study are the inclusion in the treatment group of healthy, non-depressed women less than 60 years of age using non-oral systemic estrogen. The study duration of 26 weeks also allowed the stabilization of testosterone levels, which may not have occurred with more short-term use. It is possible that the improvement in cognitive test scores was due to the effects of learning and the fact that subjects were more familiar with the test battery following repeat testing. However, we believe that the improvements in verbal learning and memory recorded with CogState were a treatment effect, as previous studies have shown performance on CogState does not improve with repeated testing [12], and no improvement was seen in women within the normative group. Limitations of the study include the small study sample in the treatment group and the absence of a placebo-controlled group for comparison. However, the inclusion of a normative group of 30 healthy non-depressed and cognitively intact women of the same age with testing at identical time points enabled us to determine whether practice effects were evident. The treatment group had a higher proportion of women who had completed post secondary level of education, and this may be a contributing factor to the positive effects of testosterone treatment on verbal learning and memory seen in this study. The treatment group were all taking hormone therapy, mostly estrogen only therapy, compared to no therapy in the normative group. This may also potentially explain the differences seen in cognitive testing between groups and suggest an independent effect of estrogen on cognitive performance. However the treatment group had been on stable hormone therapy for over 3 months prior to inclusion in the study, and the only change over the 6 months in the study was the addition of testosterone to the treatment group, compared to no change in the normative group, which makes it highly likely that any differences in cognitive performance between treatment and normative groups were due to testosterone treatment.

6. Conclusion

In this pilot study testosterone therapy improved cognitive performance in the areas of visual and verbal learning and memory in healthy postmenopausal women stabilized on estrogen, over 26 weeks. When the treatment group was compared to a non-intervention group of women the significant effect of testosterone treatment was on the cognitive domain of verbal learning and memory.

These findings are worthy of further investigation in a large randomized placebo controlled study.

Contributors


Competing interests

Dr. Paul Maruff is an employee of CogState Ltd., which provided the computerized testing. With respect to the use of testosterone, Professor Davis has received honoraria from Proctor & Gamble Pharmaceuticals, Acrux Australia and Organon, has been an investigator for Proctor & Gamble Pharmaceuticals, Acrux Australia and Organon, has received research support from BioSante, and unrestricted grant support from Proctor & Gamble Pharmaceuticals. Dr. Davison has received research support from Acrux Australia and Warner Chilcott Pharmaceuticals.

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Ethical approval

The study was approved by the Monash University Standing Committee on Ethics in Research Involving Humans.

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References