The Effects of Testosterone Supplementation on Cognitive Functioning in Older Men

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Abstract: Reduction in testosterone levels in men during aging is associated with cognitive decline and risk of dementia. Animal studies have shown benefits for testosterone supplementation in improving cognition and reducing Alzheimer's disease pathology. In a randomized, placebo-controlled, crossover study of men with subjective memory complaint and low testosterone levels, we investigated whether testosterone treatment significantly improved performance on various measures of cognitive functioning. Forty-four men were administered a battery of neuropsychological tests to establish the baseline prior to being randomly divided into two groups. The first group (Group A) received 24 weeks of testosterone treatment (T treatment) followed by 4 weeks washout, and then 24 weeks of placebo (P); the second group (Group B) received the same treatments, in reverse order (Placebo, washout, and then T treatment). In group A (TèP), compared to baseline, there was a modest (1 point) but significant improvement in general cognitive functioning as measured by the Mini Mental State Examination (MMSE) following testosterone treatment. This improvement from baseline was sustained following the washout period and crossover to placebo treatment. Similar Mini Mental State Examination (MMSE) scores were observed when comparing testosterone treatment with placebo. In group B (PèT) a significant increase was observed from baseline following testosterone treatment and a trend towards an increase when compared to placebo treatment. Improvements in baseline depression scores (assessed by Geriatric Depression Scale) were observed following testosterone/placebo treatment in both groups, and no difference was observed when comparing testosterone with placebo treatment. Our findings indicate a modest improvement on global cognition with testosterone treatment. Larger clinical trials with a longer follow- up and with the inclusion of blood and brain imaging markers are now needed to conclusively determine the significance of testosterone treatment.

1. INTRODUCTION

Aging is associated with a gradual decline in sex hormone levels in men, together with a deterioration in general health, mood and cognitive abilities. In epidemiological studies of healthy older men, higher testosterone concentrations have been associated with better global cognition, executive functions, and verbal memory [1]. Most recently, testosterone levels have been associated with an increase in the brain amyloid load, a major Alzheimer's disease (AD) pathological hallmark, in men at risk of AD [2].

The potential benefits of testosterone replacement therapy for the safety and physiological assessment has been demonstrated in men with low testosterone levels and who have subjective memory complaints at risk of cognitive decline [3]. Even elderly men who are above 65 years old with late onset hypogonadism benefit as much as the younger men in terms of safety profile and increasing testosterone levels [4]. With respect to cognition, although cell culture and animal studies have provided support for testosterone in having potential therapeutic benefits in AD

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[5]. there have been relatively few studies that have trialed testosterone supplementation in men with AD or mild cognitive impairments (MCI) and none have evaluated testosterone treatment in individuals that are pre-symptomatic but at a greater risk of cognitive decline. These studies have shown mixed outcomes with improvements in certain cognitive domains [6], to no improvement [7, 8]. A number of factors may account for the discrepancies in the outcomes of these trials, including differences in type, mode and dosage of treatment. Furthermore, modifying roles for genetic factors such as the presence of the AD risk factor, apolipoprotein E (*APOE ε4*) allele, may also influence outcomes of trials and the benefits of testosterone on cognition or AD like pathology [9]. In addition, testosterone supplementation as potential prevention and/or therapeutics prevention of AD should start at the earliest phase of the disease when primary prevention may provide maximal benefit. In the current study, the efficacy of testosterone treatment on cognitive function and depression was assessed during a six-month, double blind, cross-over treatment trial in older men with subjective memory complaints and low testosterone levels.

2. MATERIALS AND METHODS

2.1. Participants

This study was undertaken at the Siloam Hospital in Lippo Karawaci, Tangerang, Indonesia. Ethics approval was obtained from the Independent Human Ethics Committee, Faculty of Medicine, University of Indonesia as well as from Edith Cowan University, Western Australia. Informed consent was obtained from all participants prior to the study. Participants were recruited from all areas surrounding Jakarta and Tangerang, Indonesia. The eligibility criteria included: 1) Males aged ≥50 yrs old; 2) Presenting with subjective memory complaints; 3) Testosterone levels of 300- 600 ng/dL (~10.4-20.8 nmol/L); 4) Normal prostate-specific antigen (PSA) levels; 5) Normal blood pressure of 120/80 mmHg to 90/60 mmHg; 6) No diagnosis of diabetes mellitus; 7) Normal liver and kidney enzyme function; 8) No previous history of severe head injury and stroke ; 9) No significant history of alcohol abuse ; 10) Normal cognitive function, established using a score ≥24 on the Mini Mental State Examination (MMSE); and 11) ≥6 years of education. A clinical panel consisting of a neurologist and a neuropsychologist reviewed each volunteer's cognitive and pathology blood results prior to recruitment into the study.

Fig. (**1**) depicts the participant recruitment and randomization process and outlines number of participants recruited from the initial screen and drop-outs during this double blind, randomized study. A total of 44 men were assigned to one of the two parallel, crossover groups, starting with placebo (n=22) or testosterone (n=22) treatment and transferred to testosterone and placebo after a 4 week wash-out period. Assignment was random using stratification for memory performance at baseline. Each participant had a total of 11 clinic visits within the 52-week study period. Intervention included applying a testosterone cream, namely 50 mg of AndroForte® 5% manufactured by Lawley Pharmaceuticals, Perth, Australia, applied daily to the scrotum *via* transdermal route for a period of 24 weeks. This cream contains dl-α- tocopherol acetate (vitamin E) and almond oil formulated to protect testosterone from oxidation. It also contains cetomacrogol 1000, cetostearyl alcohol, butylated hydroxytoluene, anhydrous citric acid, triethanolamine, carbomer 940, B & J Phenonip® and purified water. The placebo included dl-alpha tocopherol acetate (vitamin E) only [without the active ingredient: testosterone] and was applied in a similar manner to the testosterone cream. Dose and site of application were evaluated in a separate small pilot study as presented in supplementary information.

2.2. Clinical and Neuropsychological Measures

Neuropsychological measurements were performed at four different time points: week 0 (baseline), week 24 (1st treatment period), week 28 (washout period) and week 52 (2nd treatment or cross-over period), while blood biomarker measurements and apolipoprotein (APOE) genotyping were performed as described previously [3]. Global cognitive function was assessed using the Mini Mental State Examination (MMSE) [10]. Verbal memory was assessed using Rey Auditory Verbal Learning Test (RAVLT) [11]. Comparable, parallel Indonesian versions of the RAVLT were administered at each time point to limit the practice and learning effects. A standardized Indonesian translation of Geriatric Depression Scale (GDS-30) with a cut off score of ≥11 was used to measure depressive symptoms [12]. Physicians, participants, and investigators were blind to the treatment conditions.

2.3. Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 21, SPSS Inc, Chicago, IL). The Kolmogorov-Smirnov and Levene's tests for the MMSE, RAVLT, and GDS indicated normally distributed data. To analyse each arm of the study individually (testosterone to placebo "T→P" and placebo to testosterone "P→T") and overall, a mixed model ANOVA analysis was used, consisted of fixed effects for treatment (to compare testosterone and placebo treatments), treatment period (testosterone or placebo), and sequence (testosterone treatment first or placebo treatment first). Carry-over effects were investigated by looking for differences between baseline readings and readings at week 28 after the washout period (weeks 24-28), was considered as the baseline reading for the second treatment period. To analyse the results of each treatment arms independently, one-way repeated measures ANOVA was performed.

3. RESULTS

A total of 44 elderly men (32 *APOE ε4* non carriers, 72% and 12 *APOE ε4* carriers) were randomized. Participants in both T→P & P→T groups were comparable on age and education (Age : T→P 59.2 ± 7.2, P→T 62.9 ± 8.2 ; Education: T→ P 13.9 ± 2.8, P→T 13.7 ± 3.3). There were no significant differences in age or education between the *APOE ε4* carriers and non-*APOE ε4* carriers.

Testosterone supplementation significantly increased the serum levels of T and its metabolite DHT and reduced the
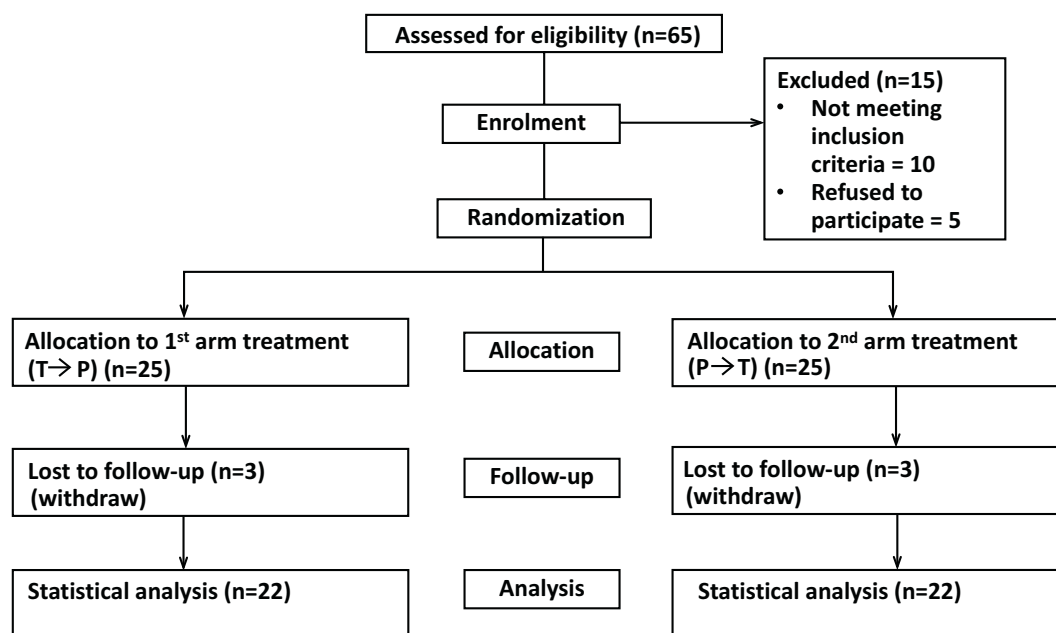
Fig. (1). An outline of participant's recruitment and randomization process [3].

LH levels, when compared to baseline, placebo administration or the washout period prior to crossover treatment. Estradiol levels were similar following all treatment periods (Tables **1** and **2**). The results confirm that the treatment regime was effective at increasing serum testosterone and lowering LH levels.

3.1. Effects of Testosterone on Measures of Cognitive Functioning and Depression

Compared to baseline MMSE scores, participants in the testosterone then placebo (T→P) the treatment arm (Group A) showed a modest but significant improvement with testosterone supplementation (Table **1**). The MMSE scores of participants from the T→P arm remained at approximately the same level for 52 weeks despite the fact that testosterone treatment had been stopped at week 24 and replaced with placebo at week 28 following the washout period. In the P→T arm (started with placebo and transferred to testosterone after the washout period, Group B) the MMSE scores were only increased when they started the testosterone application (Table **2**). In both treatment arms, no significant changes in MMSE scores were observed from baseline following placebo treatment. In both groups, MMSE scores were similar when comparing testosterone treatment with placebo treatment. Also, no significant differences were observed in verbal memory across treatment groups as measured by immediate and delayed recall on the RAVLT (Tables **1** and **2**).

Depression scores significantly decreased following testosterone treatment and the observed improvement maintained during the washout and following crossover to the administration of placebo compared to baseline in the T→P treatment (Group A) (Table **1**). Interestingly, the placebo group showed a trend towards improvement in their follow up depression scores compared to the baseline in the P→T treatment (Group B) (Table **2**). When compared to

placebo, testosterone treatment significantly decreased depression scores in the T→P treatment, but not the P→T treatment (Tables **1** and **2**).

4. DISCUSSION

Our findings showed that significant improvements in baseline MMSE scores occurred after testosterone treatment in both arms of the study compared to baseline, consistent with previous studies assessing testosterone in AD and MCI subjects. In addition, when testosterone was administered first, we saw an improvement in MMSE score at the end of the washout and placebo phases. This finding suggests that there may be carry-over effect of testosterone treatment. These potentially lasting, beneficial effects of testosterone on global cognition are in line with previous studies that indicated changes in cognitive capacity following hormonal manipulation do not return to baseline levels but are preserved or continue to change in the same direction after cessation of hormone treatment [7, 13]. This finding may partially be a by-product of baseline hormonal state because it has been indicated that baseline hormonal state may affect ones response with carry-over effects [14]. A comparison between placebo and testosterone treatment in group B (commenced with placebo treatment), revealed no significant differences (although a trend towards an increase was noted, p=0.087). It is not clear why no differences were observed in comparing placebo and testosterone. One potential contributing factor maybe the similar ingredient that made up the base of the cream. Both creams contained low doses of dl-α-tocopherol acetate (Vitamin E) and almond oil to allow greater systemic absorption of testosterone. Vitamin E has shown to alter cognition in AD and MCI patients at ~2000 IU/day when consumed orally from 6 months to 4 years [15, 16]. In our study the cream contained less than 5% of the dose of Vitamin E needed for clinical efficacy (~111IU/day) and was administered topically, thus it is

Table 1.   **Cognitive scores and serum testosterone, DHT and estradiol for men that were administered Testosterone then Placebo (Group A, n=22, Mean±SD).**

	Baseline (Week 0)	**Testosterone (Week 24)**	**Wash Out**	**Placebo (Week 52)**
MMSE[1]	27.3±1.7	28.3±1.5*	28.0±1.2*	28.2±1.3*
RAVLT[2]				
Immediate Recall[3]	44.3±6.9	46.9±7.8	47.9±7.9	47.0±10.1
Delayed Recall	8.4±2.2	8.9±1.9	9.5±2.5	9.6±2.6
GDS[4]	7.1±5.5	4.5±3.3*#	3.5±3.1*	3.2±2.8*
Testosterone (nmol/L)	16.5±4.4	26.7±12.1*#	17.3±4.4	15.3±4.1
DHT (nmol/L)	1.84±0.9	9.1±4.9*#	1.8±1.7	1.7±0.8
Estradiol (pmol/L)	81.2±22.7	85.6±33.8	83.1±7.9	92.4±18.6
LH (U/L)	4.2±2.8	2.1±1.2*#	4.2±2.4	4.8±3.3

[1]Mini Mental State Examination; [2]Rey Auditory Verbal Learning Test; [3]RAVLT Learning Trial 1-5 total score; [4]Geriatric Depression Scale. *$p<0.05$, values significantly different compared to baseline; #$p<0.05$, values significantly different compared to placebo.

Table 2.   **Cognitive scores and serum testosterone, DHT and estradiol for men that were administered Placebo then Testosterone (Group B, n=22, Mean±SD).**

	Baseline (Week 0)	**Placebo (Week 24)**	**Wash Out**	**Testosterone (Week 52)**
MMSE[1]	27.05±1.64	27.82±1.3	27.77±1.5	28.14±1.8*
RAVLT[2]				
Immediate Recall[3]	42.4±9	44.1±8.2	47.4±8.6	46.7±10.2
Delayed Recall	8.5±3	8.1±3.2	9.8±2.8	9.7±3.5
GDS[4]	6.4±5.6	4.9±3.9	4.9±4.5	4.5±4*
Testosterone (nmol/L)	17.9±6.3	16.6±4.6	16.8±5.9	24.5±13.8*#
DHT (nmol/L)	2.8±0.8	1.7±0.9	1.7±1.6	8.4±5.7*#
Estradiol (pmol/L)	85.8±26.4	88.4±29.6	84.8±29.3	91.3±40.1
LH (U/L)	4.7±2.6	5.6±3.4	4.6±1.9	3.1±2.6* #

[1]Mini Mental State Examination; [2]Rey Auditory Verbal Learning Test; [3]RAVLT Learning Trial 1-5 total score; [4]Geriatric Depression Scale. *$p<0.05$, values significantly different from baseline; #$p<0.05$, values significantly different from placebo.

unlikely that enough Vitamin E was systemically absorbed to have cognitive enhancing effects.

There are, however, conflicting findings on the effects of testosterone on global cognition with studies showing no evidence of cognitive enhancing properties with testosterone. A randomized double blind placebo controlled study by Haren *et al*., 2005, showed no increase in MMSE scores in 76 men following 12 months of oral testosterone undecanoate or placebo, after taking into the account of the baseline hormone and MMSE levels [17]. Another study by Kenny *et al*., 2004 also showed no significant changes in global cognitive performance with 12 weeks of intramuscular testosterone treatment in older men with early cognitive decline and low testosterone levels [18].

Despite relatively modest improvements in baseline global cognition with testosterone, our study showed no beneficial effects on episodic verbal memory. This finding is in accordance with those from another study that did not show a consistent improvement across all cognitive domains in relation to higher levels of testosterone [19]. While some studies have found an association between testosterone and

verbal memory [20, 21], other studies have not reported similar results [22, 23]. Moreover, the improvement on verbal memory was found to last only for a short term, as shown by Cherrier *et al*., 2015 that this improvement was observed at 3 months and was not sustained at 6 months of treatment [20]. It has been suggested that inconsistencies in these studies could be explained by the concept of changes in the optimal testosterone range and the possibility of a curvilinear association between testosterone and cognitive performance [19, 21]. For example, Cherrier *et al*. (2007) reported that men with moderate increases in serum testosterone and its metabolites showed significant improvements in verbal and spatial memory, but that these effects were not observed in men with larger or negligible to low increases in testosterone concentration. In addition, men who had higher levels of both testosterone and E2 subsequent to testosterone treatment showed better performance on tests of verbal memory compared to pre-treatment scores and compared to placebo treatment [21]. The E2 levels reached in our study (following transdermal testosterone administration) may not have been sufficient to improve memory. The peak serum levels of E2 in our study

were in the lower one-third of the normal range for men. We believe our mode of application of testosterone on the scrotum explains the above finding with testosterone being converted to DHT and thus not being susceptible to aromatization which is usually observed in older men [24].

Our study shows that, albeit modestly, testosterone can increase baseline global cognition following 24 weeks but taken together with the above mentioned studies, it also highlights the need for further validation studies. These may involve a longer follow-up periods, reversing treatment to placebo after the crossover completion or an open label study following completion. As highlighted in a study of 500 elderly men that lower testosterone levels at baseline was associated with a higher risk of cognitive decline at a 2 year follow up [25]. Thus follow-up studies with larger participants and longer treatment durations are required to determine the efficacy of testosterone in preventing or slowing down the progression of cognitive decline.

Depression symptoms can contribute to future cognitive decline in older adults [26, 27]. Studies of testosterone treatment in relation to depression levels have produced conflicting results. It is likely that the relationship is complex in nature and involves interaction between genetic, environmental and lifestyle factors. Some studies have shown no association between endogenous testosterone levels and depressive symptoms [28] and may not be beneficial for patients who have reached a clinical depression state or are highly depressed [29]. However, a significant improvement in depression following testosterone treatment has been previously reported, with one study observing the strongest effect in men with the lowest baseline total testosterone [30]. It was shown that men with sub-threshold levels of depression treated with testosterone had greater reductions in symptoms of depression than placebo treated men [31]. Furthermore, a recent study by Cherrier *et al*. (2015) reported that men with MCI and low testosterone levels had decreased depression symptoms following 6 month transdermal testosterone gel treatment [20]. In the present study, compared to baseline, depression levels decreased following testosterone treatment in both treatment arms. In T➔P treatment (Group A), GDS scores were reduced following administering testosterone, in the wash-out period and following administering placebo. This could be partially explained by the lasting benefits of testosterone (similar to the effects on global cognition). However, further reduction was observed when placebo at 52 weeks was compared to testosterone at 24 weeks. In addition, in the P➔T treatment (Group B), although not significant, there was a trend towards a reduction when placebo was administered with a further reduction following testosterone treatment. Overall, these results may suggest a placebo effect on reducing GDS scores. As discussed above the low dose of Vitamin E within the creams is not likely to confer benefits over the treatment period. Other factors may contribute such as being active in the trial and engaging with researchers on a regular basis.

It is also difficult to determine whether there is a placebo effect in interpreting this result when the course of development of AD is unknown in this cohort [32]. The physician-patient relationship which resulted in the expectations and motivations of the patient thus probably also has modifying changes on the placebo response [33], and where there is no untreated (control) group to define the true placebo effect [33, 34].

CONCLUSION

Some limitations of the present study should be acknowledged. Although larger than some of the previous studies, this study was relatively underpowered with small sample sizes (n=44) to conclusively determine testosterone treatment on cognition. It is also possible that our memory measure, the RAVLT, was not able to detect small or subtle changes in cognitively intact participants even though we used parallel forms to avoid the practice effect and the RAVLT has proven to appropriately evaluate episodic verbal memory [11]. Despite these limitations, this is the first double blind randomized crossover placebo-controlled study that evaluated the effect of short term testosterone treatment on cognitive functioning in older men with low to low-normal testosterone levels and who have a subjective memory complaint. When combined with genetic risk factors, brain amyloid imaging and other AD related biomarkers, subjective memory complainers are at higher risk of clinically defined cognitive decline than non-memory complainers. We have also previously shown that in addition to APOEε4, changes in hormone levels (LH) is strongly correlated with greater brain amyloid load in subjective memory complainers [2], providing further support that this is an appropriate cohort to include in such a trial. Data from our current study indicated that testosterone supplementation lowered LH, investigating the impact on the association between LH and brain amyloid load in subjective memory complainers would be further warranted. Our findings of a modest improvement from baseline in global cognition are promising but require comprehensive larger longer term studies to investigate the efficacy of testosterone treatment on cognitive and clinical measures with the inclusion of blood and brain imaging markers.

TRIAL REGISTRATION

The Australian New Zealand Clinical Trials Registry (ANZCTR). Trial ID ACTRN12614000277640.

LIST OF ABBREVIATIONS

AD	=	Alzheimer's Disease
DHT	=	Dihydrotestosterone
GDS	=	Geriatric Depression Scale
LH	=	Luteinizing Hormone
MCI	=	Mild Cognitive Impairment
MMSE	=	Mini Mental State Examination
P	=	Placebo
PSA	=	Prostate Specific Antigen
RAVLT	=	Rey Auditory Verbal Learning Test
T	=	Testosterone

CONFLICT OF INTEREST

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

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