Testosterone Replacement Therapy in Older Male Subjective Memory Complainers: Double-Blind Randomized Crossover Placebo-Controlled Clinical Trial of Physiological Assessment and Sa...
Testosterone Replacement Therapy in Older Male Subjective Memory Complainants: Double-Blind Randomized Crossover Placebo-Controlled Clinical Trial of Physiological Assessment and Safety

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Abstract: Testosterone replacement therapy (TRT) has been investigated in older men as a preventative treatment against Alzheimer’s disease and dementia. However, previous studies have been contradictory. We assessed TRT physiological effects in 44 older men (aged 61 ± 7.7 years) with subjective memory complaints using a double blind, randomized, cross-over, placebo-controlled study. Participants were randomized into 2 groups, one group received transdermal testosterone (50 mg) daily for 24 weeks, followed by a 4 week wash-out period, then 24 weeks of placebo; the other group received the reverse treatment. Blood evaluation revealed significant increases in total testosterone, free (calculated) testosterone, dihydrotestosterone, and a decrease in luteinizing hormone levels (p<0.001) following TRT. Although there were significant increases in red blood cell counts, hemoglobin and prostate specific antigen levels following TRT, they remained within normal ranges. No significant differences in plasma amyloid beta, estradiol, sex hormone binding globulin, insulin levels, body fat percentage, or body mass index were detected. This is the first carefully controlled study that has investigated the influence of TRT in Indonesian men on blood biomarkers linked to dementia risk. Our study suggests TRT is safe and well-tolerated in this Indonesian cohort, yet longitudinal studies with larger cohorts are needed to assess TRT further, and to establish whether TRT reduces dementia risk.

Keywords: Aging, Alzheimer’s disease, dementia, testosterone, luteinizing hormone, plasma.

1. INTRODUCTION

Hormonal changes due to the dysregulation of the hypotalmic pituitary gonadal (HPG) axis during aging have been associated with cognitive impairment, the risk of developing dementia and the pathogenesis of Alzheimer’s disease (AD). Longitudinal studies indicate that men normally experience a gradual loss of testosterone as they age [1-3], and neuropathological evidence suggests that testosterone levels in the brain also decrease with age [4]. However, men with neuropathological diagnoses of AD or mild cognitive impairment (MCI) have lower testosterone levels compared to healthy men of a similar age [4]. The gradual decline in testosterone can manifest itself in many ways, including sexual dysfunction, diminished physical stamina, and loss of muscle and bone mass. Low testosterone has also been associated with systolic hypertension, the development of abdominal visceral fat mass, insulin resistance, high levels of cholesterol, LDL and triglycerides, low HDL levels, as well as impaired quality of life due to symptoms such as depression and cognitive impairment [5-8]. The age-related decline in testosterone levels usually starts in the fifth decade [9], with men aged 70 and older having the lowest levels. Estimates for the prevalence of men with low testosterone levels range from 1 in 200 [10], 1 in 20 [11], to 1 in 4 [12], depending on the cut-off point, the population age, ethnicity and health factors. Men with testosterone levels significantly lower than normal may benefit from carefully monitored testosterone replacement therapy (TRT).

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In vivo and in vitro studies have demonstrated that testosterone may be beneficial in the prevention and treatment of AD, at least in part by reducing brain beta amyloid (Aβ) levels. Testosterone treatment in castrated guinea pigs has been found to reduce cerebral and CSF Aβ levels [13]. Testosterone has also been shown to decrease Aβ levels in primary neuronal cultures [14]. In clinical studies, Gandy et al. [15] recorded an elevation in plasma Aβ levels in men being treated with testosterone suppression drugs for prostate cancer, and low testosterone levels have been associated with increased plasma Aβ levels in men [16]. Furthermore, testosterone supplementation in men with AD or MCI has been shown to improve spatial memory and cognition [17, 18]. Although there have been some inconsistencies in study results, most studies suggest that TRT may prevent or delay AD [19].

Some other studies have linked testosterone treatment to adverse outcomes such as increased risks of atherosclerosis, gynecomastia and in particular, prostate cancer [20-22]. However, the prostate cancer link has been strongly contested. Testosterone is now believed not to increase the risk when only raised to high physiological levels, even in people already at high risk of developing prostate cancer [23, 24]. TRT that has resulted in unfavourable outcomes has usually involved people deliberately trying to achieve supra-physiological levels to improve athletic performance, for example [25]. Such results do not apply to patients trying to maintain testosterone at physiological levels.

Almost universally, a testosterone-deficient state in older men is permanent, and as a result, TRT may be for life. Therefore, testosterone treatments need to be carefully designed and extensively tested in properly controlled clinical trials. Many TRT studies have already been undertaken [17-19]. However, there is a lack of suitable longitudinal clinical studies of aging men that have investigated comprehensively the long-term effects of testosterone treatment, including effects on the cardiovascular and immune systems, body metabolism, and the brain, and no such studies have been carried out in an Indonesian population. In this double-blind, randomized, cross-over, placebo-controlled study, we have investigated the effect of testosterone treatment in a group of elderly male subjective memory complainers (SMC), who had testosterone levels at or below what is considered to be a low normal level. The blood biomarkers included total testosterone and its derivative dihydrotestosterone (DHT), SHBG, estradiol, luteinizing hormone (LH) and insulin. Free testosterone levels were calculated, lipids profiles were obtained, plasma Aβ levels were measured, and general hematologic measures were also monitored. Potential unwanted side-effects of the treatment were monitored: we assessed markers of cardiovascular disease, and monitored prostate specific antigen (PSA) levels to assess prostate cancer risk, as well as any signs of gynecomastia.

2. MATERIALS AND METHODS

2.1. Participants

This study was undertaken at the Siloam Hospital in Lippo Karawaci, Tangerang, Indonesia. Human 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, prior to study commencement. The participants were recruited from areas surrounding Jakarta and Tangerang, Indonesia, including patients from Siloam Hospital, Lippo Karawaci, Indonesia and the nursing homes in those areas.

The following eligibility criteria were used: the male participants needed to 1) be 50 years of age or older, and have 2) a memory complaint, 3) testosterone levels between 300-600 ng/dL (~10.4 - 20.8 nmol/L), 4) normal levels of PSA, 5) blood pressure within normal limits, between 120/80 mmHg to 90/60 mmHg 6) no signs of diabetes mellitus, 7) normal liver and kidney enzyme function, and 8) they should not have suffered from major head injury. The diagnosis of subjective memory loss (complaint of memory loss but not meeting the criteria for dementia) was reached after a full clinical evaluation including a review of medical and drug history (for example, whether a participant had had any medication such as donepezil or rivastigmine) and a physical examination. Participants were partly selected based on their Mini Mental State Examination (MMSE) assessment: a score of 24 or above determined eligibility. The final decision for inclusion was determined jointly by a neurologist, a neuropsychologist, and the researchers in charge of the project. All participants in this study had at least 6 years of education. Written informed consent was obtained from all participants.

Fifty male subjective memory complainers met the eligibility criteria, and 44 of these completed the study. They were recruited and assigned to one of the two parallel groups; placebo (n=22) or T (n=22) treatment from 2nd September 2006 to 20th October 2007. Calculation of the sample size based on the types of superiority design (randomized crossover trial) and measures of continuous outcomes. For allocation of the participants, a blocked randomization 1:1 ratio was used to ensure each arm of the study was balanced. Block randomization was by a random numbers-table prepared by an investigator with no clinical involvement in the trial. After the research nurse had obtained the patient’s consent, she telephoned a contact who was independent of the recruitment process for allocation of the participants. The study design was such that participants received testosterone treatment or placebo for 24 weeks, followed by a wash-out period of 4 weeks. Following this step, the participant groups were “crossed over”, such that the placebo group received testosterone and vice versa for a further 24 weeks. For all participants, 11 clinic visits to Siloam Hospital, Lippo Karawaci (Indonesia) were required during the study period. The participants and the investigators were blinded to treatment assignment until study completion (Fig. 1).

2.2. Procedures

During the first (baseline) visit, participant blood pressure, height, weight, body fat percentage and body mass index (BMI) were measured and blood samples taken. Blood (± 45 ml) was collected using serum separating tubes (SST), that include a gel and blood clot activator), EDTA collection tubes, and heparin collection tubes. Blood in SST collection
Analysis of blood samples provided testosterone, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), luteinizing hormone (LH), estradiol, prostate-specific antigen (PSA), insulin and amyloid-β (Aβ) levels, and provided blood lipid profiles.

Blood samples were taken every 8 weeks of the 24-week cross-over period, showing the weeks that blood samples were taken. Every 4 weeks for 0-28 weeks, and every 8 weeks of the 24-week cross-over period, blood samples were taken. Analysis of blood samples provided testosterone, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), luteinizing hormone (LH), estradiol, prostate-specific antigen (PSA), insulin and amyloid-β (Aβ) levels, and provided blood lipid profiles.

For the testosterone treatment group (n=22), testosterone (50 mg in the form of Andromen\textsuperscript{a} 5\% FORTE cream obtained from Lawley Pharmaceuticals, Perth, Western Australia) was applied daily to the scrotum, thus using a transdermal route (topically), for 24 weeks. For the placebo group (n=22) the same amount of the cream base [dl-\(\alpha\)-tocopherol acetate (vitamin E), without the testosterone] was applied with the same frequency and in the same manner.

At each of the six remaining clinic visits i.e. at 4, 8, 12, 16, 20 and 24 weeks during the first treatment period, blood was collected as described for the baseline measurement. During the 4 week washout period, the participants were given an equivalent amount of cream base lacking testosterone and vitamin E, to be applied to the scrotum. The placebo and testosterone treatment groups of the study were then crossed-over for the second 24-week treatment period. During this period, the participants were examined every eight weeks (one bleed at the start of the period (end of washout), followed by bleeds at weeks 8, 16 and 24 of the cross-over period); and blood samples were analysed as described previously.

2.3. Blood Biomarker Measurement

Serum samples (n=535) were analysed for total testosterone (TT) and DHT using an isotope dilution LC-MS/MS method at the Anzac Research Institute, NSW. Other serum samples were analysed for LH, estradiol, PSA, SHBG and insulin in PathWest Laboratory, Perth, WA. Lipid profiles were obtained and albumin levels measured using heparin-treated plasma samples. Serum-free testosterone (calculated free testosterone: CFT) was calculated using TT, SHBG and albumin levels following a standard formula \[26\]. Plasma Aβ40 and Aβ42 levels were measured using an ELISA kit \[27\]. Changes of all of these blood biomarker measurements are the primary outcomes of the study, compared to baseline.

2.4. Statistical Analysis

Statistical analysis was performed using the Statistical Package of Social Sciences (SPSS version 21, SPSS Inc, Chicago, USA). The mixed model ANOVA analysis consisted of fixed effects for treatment (to compare testosterone with placebo treatment), treatment period (testosterone or placebo), and sequence (testosterone period first or placebo period first), irrespective of the baseline. Carry-over effects were tested from differences from baseline in the first period (week 0) to the end of the washout period (week 24-28), which is the baseline time-point for the second period, based on APOE\textsubscript{ε}4 genotype, on each arm of the study as well as on the entire data set. When directly comparing two groups based on the presence of APOE\textsubscript{ε}4 genotype, two tailed independent t-tests were performed. All analyses were two-tailed and the alpha level was set at 0.05.

3. RESULTS

Forty-four of the initial 50 male participants completed both treatment periods. The other 6 men did not complete the treatment due to personal reasons (n=5), and moving interstate (n=1), as shown in the participant flowchart (Fig. 2). After categorizing the men according to APOE\textsubscript{ε}4 status \[32\] APOE\textsubscript{ε}4- (72\%) and 12 APOE \textsubscript{ε}4+ (27\%), the characteristics of the 44 SMC elderly men were as reported in Table 1. There were no significant differences between \textsubscript{ε}4+ and \textsubscript{ε}4- participants in terms of age or education. The treatment suppliers were provided with treatments labeled “A” and “B” to give to the participants, which eventually were revealed to be A = testosterone treatment, and B = placebo after the end of all treatment. For ease of understanding, testosterone and placebo treatment periods are referred to as “T” and “P” respectively, in the figures/tables of this paper.

Overall, at week 0, there were no significant differences between the APOE\textsubscript{ε}4+ and \textsubscript{ε}4- subjects in any of the blood biomarkers measured. There were also no significant sequence effects, period effects or carry-over effects for any variable following the wash-out period (week 28) of either treatment sequence. As a result, all further analyses were carried out after pooling all testosterone treatment results and all placebo results, regardless of original order of treatment (T→P or P→T). This improved statistical power, and allowed us to focus on comparing placebo with testosterone treatments.

3.1. Hormones

From the graphs in Fig. (3) and Table 2 it is clear that testosterone levels were significantly higher following
testosterone compared to placebo treatment (F=41.471, p<0.001). At four weeks post-testosterone treatment (end of washout period), testosterone was back at baseline levels, which indicated no carry-over effects. The results for DHT and calculated free testosterone levels were similar to total testosterone level results, with 4-fold and 2-fold increases respectively (F=151.793, p<0.001; F=37.875, p<0.001 Table 2 and Fig. 3b). LH levels significantly decreased following testosterone treatment (F=43.466, p<0.001, Table 1), yet rose back to pre-treatment levels by the end of the washout period in the T→P treatment group (Fig. 3c). In contrast, estradiol levels did not show significant changes following testosterone treatment (F=4.677, p=0.37). No significant changes in insulin levels were observed (F=1.32, p=0.261).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (Mean SD)</th>
<th>Years of Education (Mean SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment T→P</td>
<td>22</td>
<td>59.2 (7.18)</td>
<td>14.0 (2.84)</td>
</tr>
<tr>
<td>APOE ε4-</td>
<td>14</td>
<td>59 (5.82)</td>
<td>13.8 (3.31)</td>
</tr>
<tr>
<td>APOE ε4+</td>
<td>8</td>
<td>59.6 (9.58)</td>
<td>14.2 (1.91)</td>
</tr>
<tr>
<td>Treatment P→T</td>
<td>22</td>
<td>62.9 (8.22)</td>
<td>13.8 (3.39)</td>
</tr>
<tr>
<td>APOE ε4-</td>
<td>18</td>
<td>62.7 (7.31)</td>
<td>13.5 (3.62)</td>
</tr>
<tr>
<td>APOE ε4+</td>
<td>4</td>
<td>63.8 (13)</td>
<td>15 (2)</td>
</tr>
</tbody>
</table>

T = testosterone, P = placebo. Results shown as means (SD).

3.2. Plasma Aβ in Response to Testosterone Treatment

Overall, there were no significant changes in plasma Aβ levels, both Aβ40 and Aβ42 (F= 2.273, p=0.136; F=0.47, p=0.498 as shown in Table 2). Furthermore, based on the individual data (data not shown), most participants had no plasma Aβ40 and Aβ42 changes in response to testosterone treatment (24 plasma Aβ40 non-responsive (54.5%) and 29 plasma Aβ42 non-responsive participants (65.9%)). Whereas the rest of the participants had either a 10%-30% increase or decrease of their plasma Aβ compared to the baseline.

3.3. Lipid Profiles, Hematology and Cardiovascular Risk

There were no significant differences observed in total cholesterol (F=3.31, p=0.081), HDL (F=3.623, p=0.065), or LDL (F=0.159, p=0.693) levels following treatment with either testosterone or placebo (Table 1). Cholesterol/HDL and LDL/HDL ratios were also calculated to monitor atherosclerosis risk, and both did not show any significant changes following testosterone treatment (F=1.14, p=0.294; F=2.418, p=0.132, respectively). In contrast, RBC counts and hemoglobin levels both increased following testosterone treatment (F=43.317, p<0.001; F=41.634, p<0.001, Table 2).

3.4. High PSA Levels and Gynecomastia as Potential Side-Effects of TRT

Significant increases in PSA levels were observed following testosterone treatment compared to placebo.
The risk of developing gynecomastia was estimated by measuring the estradiol/testosterone ratio, and testosterone treatment caused no change to this ratio (F=0.31, p=0.974 Table 2).

3.5. Physical Measures

There were no significant differences following testosterone and placebo treatments in body fat percentage (F=0.088, p=0.77) and body mass index (BMI) (F=0.51, p=0.481 Table 2).

4. DISCUSSION

This study investigated the effect of topical transdermal testosterone treatment in a group of Indonesian male subjective memory complainers (SMC). A selection of blood biomarkers was investigated, including plasma Aβ. In addition, the study monitored possible risks of testosterone treatment such as the potentially increased risks of developing prostate cancer, cardiovascular disease, and gynecomastia.

All participants in this 12 month double-blind, randomized, and placebo-controlled trial underwent both...
Table 2. Combined blood biomarker results (n=44) from the two treatment groups.

<table>
<thead>
<tr>
<th>Blood Biomarkers</th>
<th>Baseline</th>
<th>Testosterone</th>
<th>Placebo</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (nmol/L)*</td>
<td>17.4</td>
<td>27.5 ± 1.22</td>
<td>16.6 ± 1.22</td>
<td>*** p&lt;0.001</td>
</tr>
<tr>
<td>DHT (nmol/L)*</td>
<td>1.87</td>
<td>8.96 ± 1.66</td>
<td>1.73 ± 0.42</td>
<td>*** p&lt;0.001</td>
</tr>
<tr>
<td>Calculated free testosterone</td>
<td>363</td>
<td>662 ± 36.4</td>
<td>362 ± 36.4</td>
<td>*** p&lt;0.001</td>
</tr>
<tr>
<td>Estradiol (pmol/L)</td>
<td>82.3</td>
<td>89.1 ± 4.28</td>
<td>78.6 ± 4.28</td>
<td>p= 0.37</td>
</tr>
<tr>
<td>Estradiol/Testosterone</td>
<td>0.005</td>
<td>0.003 ± 0.05</td>
<td>0.004 ± 0.05</td>
<td>p= 0.974</td>
</tr>
<tr>
<td>LH (U/L)*</td>
<td>4.83</td>
<td>2.77 ± 0.26</td>
<td>5.17 ± 0.26</td>
<td>*** p&lt;0.001</td>
</tr>
<tr>
<td>Abeta 40 (pg/mL)</td>
<td>137</td>
<td>137 ± 2.94</td>
<td>143 ± 2.94</td>
<td>p= 0.136</td>
</tr>
<tr>
<td>Abeta 42 (pg/mL)</td>
<td>31.6</td>
<td>31.2 ± 0.69</td>
<td>31.8 ± 0.67</td>
<td>p= 0.498</td>
</tr>
<tr>
<td>Abeta 42 : Abeta 40</td>
<td>0.22</td>
<td>0.21 ± 0.01</td>
<td>0.18 ± 0.02</td>
<td>p=0.084</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.77</td>
<td>4.65 ± 0.087</td>
<td>4.8 ± 0.087</td>
<td>p= 0.081</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.17</td>
<td>1.12 ± 0.02</td>
<td>1.17 ± 0.02</td>
<td>p= 0.065</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>3.01</td>
<td>2.98 ± 0.076</td>
<td>3.01 ± 0.076</td>
<td>p= 0.693</td>
</tr>
<tr>
<td>Total cholesterol/HDL</td>
<td>4.09</td>
<td>4.25 ± 0.08</td>
<td>4.16 ± 0.08</td>
<td>p= 0.294</td>
</tr>
<tr>
<td>LDL / HDL</td>
<td>2.58</td>
<td>2.72 ± 0.07</td>
<td>2.61 ± 0.07</td>
<td>p= 0.132</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>7.29</td>
<td>7.42 ± 0.32</td>
<td>7.87 ± 0.32</td>
<td>p= 0.261</td>
</tr>
<tr>
<td>PSA (µg/L)*</td>
<td>1.32</td>
<td>1.41 ± 0.07</td>
<td>1.19 ± 0.07</td>
<td>** p=0.014</td>
</tr>
<tr>
<td>Red blood cells (x10⁶/µL)</td>
<td>4.88</td>
<td>5.2 ± 0.04</td>
<td>4.81 ± 0.04</td>
<td>*** p&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)*</td>
<td>14.4</td>
<td>15.2 ± 0.11</td>
<td>14.2 ± 0.11</td>
<td>*** p&lt;0.001</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>36.0</td>
<td>34.5 ± 0.56</td>
<td>34.3 ± 0.56</td>
<td>p= 0.843</td>
</tr>
<tr>
<td>% body fat</td>
<td>22.5</td>
<td>25.4 ± 0.29</td>
<td>25.4 ± 0.29</td>
<td>p= 0.77</td>
</tr>
<tr>
<td>Body mass index</td>
<td>25.5</td>
<td>22.8 ± 0.4</td>
<td>22.5 ± 0.4</td>
<td>p= 0.481</td>
</tr>
</tbody>
</table>

Data are expressed as mean and SEM of the average of readings from the blood samples taken over the 6 months from both treatment groups (T→P and P→T). Baseline levels are the week 0 (starting baseline) and week 28 (end of washout period) readings. *Levels on these measures were significantly different post-testosterone vs post-placebo.

24 weeks of testosterone and 24 weeks of placebo treatments. Thus all participants served as their own “controls”, and the study reduced the problems of patient-to-patient variability. Another strength of the study came from the fact that both potential orders of treatment were tested – testosterone followed by placebo, as well as the reverse. From the participant point of view, the role was the same all year – all stages required a cream to be applied daily.

4.1. Hormones

When measuring testosterone levels, the assay for total plasma testosterone levels is the test of choice as it is one of the most widely available as well as the gold standard of the testosterone assays. Total testosterone levels were also used to monitor the efficacy of testosterone treatment. Total plasma testosterone represents testosterone that is tightly bound to SHBG (44%) as well as that weakly associated with albumin (54%), with the remaining 2% free (and able to pass the blood brain barrier) [28]. With aging, total testosterone levels decrease on average, approximately 110 ng/dL (~3.8 nmol/L) per decade [29]. An early morning plasma testosterone level of less than 300 ng/dL (~ 10.4 nmol/L) indicates that a patient should be considered testosterone-deficient [30]. However, the threshold of testosterone levels for various symptoms can vary widely, and individual variation also occurs. The total testosterone levels of the study participants were slightly above the borderline for testosterone-deficient men, as they ranged between 10-20 nmol/L. This study aimed to raise serum testosterone levels to that seen in the mid-normal range, which for these Indonesian elderly men is 20-35 nmol/L, based on the laboratory reference range using immunoassays.

Since day-to-day variations in testosterone levels may be considerable, blood samples were taken at 4 week intervals in weeks 4-24, and at 8 week intervals in weeks 28-52 and averages for treatment periods were calculated. From the results (Fig. 3a), it is clear testosterone levels were significantly higher during testosterone treatments, irrespective of treatment order (T→P or P→T). However, individual increases in testosterone levels varied somewhat (data not shown). This may have been due to the possibility that the amounts of testosterone cream applied may have varied from one individual to another, although the testosterone cream application instructions had been well demonstrated and documented in the T/P tubes. In addition, androgen receptor polymorphisms (CAG repeats) conferring different individual sensitivity to testosterone may also affect testosterone treatment efficiency. The calculated free testosterone result showed a similar outcome.
Testosterone’s mechanisms of action on target organs may be mediated directly via testosterone or indirectly via a major metabolic product, dihydrotestosterone (DHT). The testosterone cream resulted in elevated serum DHT levels, with no carry-over effect, most likely due to high serum levels of 5-alpha reductase. Much of the testosterone must have been converted to DHT, which can bind to androgen receptors. The results agree with those of Cunningham et al. [31] and other studies [32] where it has also been found that DHT is elevated following testosterone treatment using a transdermal cream.

Another metabolic product of testosterone is estradiol. Testosterone’s conversion to estradiol via cytochrome P450 aromatase in men, unlike women, helps to maintain or modulate levels of serum estradiol as men age [9, 33, 34]. Thus, in the presence of aromatase, testosterone can exert its effects on cell metabolism by influencing estrogen receptors as well as androgen receptors [35]. Aging has been linked to enhanced peripheral aromatization of androgens as well as increased estradiol levels. Higher estradiol levels promote increased adiposity or increased body fat, as well as an increase in SHBG levels [30]. In our patients, there was a small (non-significant) rise in estradiol levels observed during testosterone treatment (Table 2). The lack of change in estradiol levels may partly explain why there were no significant changes observed in body fat or SHBG levels. Estradiol levels were still in the mid- to lower-normal range, which is 10-82 pg/mL in fertile Asian men [36].

It was clear that testosterone treatment had a significant inhibitory effect on LH levels in plasma (Table 2; Fig. 3c), and that once the participants stopped TRT, LH levels rose back to baseline levels. This indicated no carry-over or long-term effects, and that the normal feedback loops and enzymatic conversions of testosterone were occurring. In men, both testosterone and DHT exert their effects on LH mainly at the hypothalamic level, by decreasing the frequency of LH pulses, whilst estradiol reduces the amplitude of LH at the pituitary level [37]. Among testosterone-deficient men, LH has been shown to be suppressed down to levels in the eugonadal range following injectable and implantable testosterone [38, 39].

4.2. Effect of Testosterone Treatment on Plasma Aβ Levels

Ideally, CSF Aβ40 or Aβ42 levels would be investigated to determine testosterone effects and future risk of AD, as CSF levels of these biomarkers are considered much more useful than plasma Aβ levels when assessing AD risk [40, 41]. However, CSF sampling is not likely to be adopted for routine population screening, mostly due to cost and discomfort to patients, thus we have concentrated on blood screening. In this study, there were no significant changes in plasma Aβ40 or Aβ42 levels following testosterone treatment.

Many factors influence the measurement as well as the levels of Aβ40 or Aβ42 levels. For example: most of the Aβ in plasma is bound to triglyceride-rich lipoproteins, soluble low-density lipoprotein receptor-related protein-1 and possibly albumin, leaving little free Aβ [42-44]. Levels of albumin and lipoproteins and the amounts of Aβ bound to them are influenced by many factors, and thus measured Aβ levels may also be affected by such confounding factors [45]. Plasma Aβ is also rapidly broken down or removed by peripheral tissues, particularly the liver [46].

Recent clinical trials have shown that the diagnostic utility of plasma Aβ levels in relation to risk of AD have been limited due to the lack of correlation between plasma, CSF Aβ, and PET amyloid plaque measurement [47-49]. There are several reasons that can explain these findings. Firstly, Aβ in the plasma and blood can be found not only in the brain, but also in almost all peripheral cells that metabolize APP [50, 51], that travel through the blood-brain barrier penetration which is limited. Thus plasma Aβ might only be partially reflect altered APP metabolism in the brain. Secondly, plasma Aβ was found to be independent to that in the CSF of both dementia and healthy people [47]. Thirdly, like CSF, plasma Aβ has been found to have a circadian pattern that is inversely correlated with age but is unaffected by amyloid deposition [52].

Even though plasma Aβ is not a significant diagnostic AD biomarker, but it can be an important prognostic biomarker. Several studies have demonstrated that plasma levels of Aβ40 and Aβ42, when combined with a panel of other peripheral biomarkers, can help in the assessment of AD risk. However, Aβ changes are not substantial, and not all studies agree. Individual variation may be influenced by the confounding factors mentioned above [45], as well as genetic differences such as APOE allele status, stage of AD neuropathology (if present) and the many health conditions that can afflict the elderly as well as associated medications. Nevertheless, animal and cell culture studies support the concept that lower testosterone levels are associated with higher Aβ levels [13, 14]. Furthermore, in clinical studies, low levels of testosterone due to androgen blockade therapy (flutamide and leuprolide) have been associated with increased plasma Aβ levels in men [16], and serum total testosterone and SHBG have been found to correlate inversely with plasma Aβ40 levels in older men with memory loss or dementia [53]. The authors of these studies suggested that subclinical androgen deficiency enhances the expression of AD-related peptides. One study which did not find an inverse relationship between testosterone levels and Aβ levels is a study which found that testosterone treatment raised plasma Aβ40 levels, in an animal model of hypogonadism [13].

Studies have also not been conclusive concerning the relationship between pre-MCI plasma Aβ and the risk of AD or dementia. For example, individuals with plasma Aβ40 levels above the median baseline level for the group in one particular study had an increased risk of developing dementia [54]; however, another study found low plasma Aβ40 levels can predict incident AD in elderly men [55]. Yet another study found that plasma Aβ40 and Aβ42 levels increase with age and are strongly correlated with each other, and that plasma Aβ40 and Aβ42 levels are elevated in some patients before and during the early stages of AD but decline thereafter [56]. A recent review has found that low plasma Aβ42:Aβ40 ratios are the best predictors of AD and dementia development [57]. Interestingly, an increase in plasma Aβ oligomers, known to be important in AD pathogenesis, has been associated with decreased MMSE.
and AMT (Abbreviated Mental Test) scores [58]. In our study however, plasma Aβ42:Aβ40 ratios did not change though it should be recognized that a longer follow up on these subjects would be needed to reach a definitive conclusion.

The lack of conclusion in all these studies may be partly attributable to small cohort sizes, as well as differences between the studies, for example in the ethnic origins of the populations studied, the ages of study participants, the follow-up periods used, plasma Aβ assays, and the cognitive and physical health status of participants at the start of the studies [59]. The standardization of procedures will be essential to improve study comparisons [59, 60]. Plasma Aβ peptides or oligomer levels may eventually be part of a blood biomarker panel used to determine AD risk or AD status [40].

4.3. Lipid Profiles and Hematology

Studies of the effects of testosterone on lipid metabolism have produced conflicting results. As in this study, some have found no significant effects on LDL levels, testosterone has also been found to result in a mild decrease in HDL levels [61-63]. However, some have demonstrated an inverse correlation between testosterone and LDL levels [64-67]. The significance of a small HDL level decrease is still unknown. It is known, however, that serum HDL levels are lower in men than in premenopausal women [68], and a key enzyme responsible for HDL clearance, hepatic endothelial triglyceride lipase, has been found to have greater activity in men, and to be stimulated by androgens and suppressed by estrogens [69]. Overall, the effects of testosterone on lipid profiles remain uncertain.

Testosterone has long been known to stimulate red blood stem cell numbers [70]. Several TRT studies have reported significant increases in hemoglobin and hematocrit levels [29, 71-73], with greater increases in RBC in older men than in young men [71]. Our results also show that TRT enhances RBC and hemoglobin levels. However, it has been found that testosterone-treated men are nearly four times as likely to have hematocrits of >50% compared to placebo-treated men [74]. Therefore, TRT should not be administered to men with baseline RBC or hematocrits of 50% or greater without appropriate evaluation and treatment of erythrocytosis [75], since TRT may worsen pre-existing erythrocytosis. Although it had been postulated that testosterone stimulates erythropoiesis by modulating erythropoietin and stem cell proliferation, recent data suggests testosterone increases red cell mass by inhibiting hepcidin [71]. The normal hemoglobin range in men is 14-18 ng/dL [75], and although significant increases in hemoglobin were observed following TRT, the levels seen in our results were still in the safe healthy range.

4.4. PSA, Cardiovascular Disease, and Gynecomastia as Potential Risks of TRT

In the past, there have been major concerns regarding testosterone therapy promoting the growth of metastatic prostate cancer. However, it has now been established that there is no evidence for an increased risk of prostate cancer [24, 76]. The view now is that testosterone (at physiological levels) does not promote prostate cancer, but can exacerbate existing cancer [77, 78] and should not be administered to men with already high PSA levels [75]. In fact, one study has found that an increased risk of prostate cancer was associated with low plasma testosterone [20]. Most studies have shown no significant increases in PSA or prostate volume following TRT in testosterone-deficient men [79]. Although PSA levels did increase following TRT in our study, levels still remained within the normal range of 0-4 µg/L (American Cancer Society). Similarly, a study by Khera et al. [80] showed a small increase in PSA levels following 12 months of testosterone treatment. According to the American Cancer Society, a PSA level of 4 - 10 µg/L indicates a 25% increased risk of prostate cancer, and a level over 10 µg/L increases the risk by 50% or more. A number of open-label trials have also reported that testosterone treatment results in very low rates of prostate cancer [81, 82].

Cross-sectional studies indicate an inverse relationship between cardiovascular disease (CVD) incidence and/or severity, and endogenous testosterone levels, irrespective of age, when considering men with low testosterone levels [83]. For example, in hypogonadal men, testosterone treatment increases coronary blood flow and reduces signs of myocardial ischemia in CVD [21, 84, 85]. However, testosterone treatment has been contra-indicated in some studies of people with hypertension, diabetes and hyperlipidemia, thus maintenance of testosterone levels within a normal range is recommended, and the monitoring of CVD indicators is advisable [84]. To this end, total cholesterol:HDL ratios and LDL:HDL ratios were monitored in our study. The total cholesterol:HDL ratio should be kept below 5:1, ideally below 3.5:1. In our study, no significant changes in total cholesterol:HDL ratios were found, and the ratios remained below 5:1. Also, no significant changes to HDL levels or LDL:HDL ratios were detected, which suggests that the testosterone treatment in this study did not increase CVD risk.

An increased production or action of estradiol compared to testosterone will result in gynecomastia: benign enlargement of the male breast; a side-effect occasionally associated with TRT. In our studies, the ratio of testosterone to estradiol was calculated to monitor the risk of gynecomastia. No significant changes were observed, reflecting the fact that testosterone increases were greater than estradiol increases, and implying that gynecomastia risk was minimal.

Studies with larger numbers of participants are needed to get a clearer picture of any differences in all of the blood parameters measured, particularly plasma Aβ40 and lipid profiles. Ideally, CSF Aβ40 and Aβ42 levels should be measured, as these reflect AD neuropathology more directly. However, sampling CSF is uncomfortable and costly. If plasma Aβ40 or Aβ42 levels are found to correlate with AD risk, then plasma would clearly be preferred for screening. Another possible problem is the reliance on daily TRT self-administration, some men may have forgotten to apply the cream daily, and/or the dosage may have been incorrect. However the blood testosterone levels suggest strongly that these problems did not occur.
Truly testosterone-deficient men who have no contraindications to TRT may benefit more from such treatment. Lower testosterone levels are associated with increased risks of osteoporosis, metabolic syndrome, type 2 diabetes mellitus and mortality. Nevertheless, it is still uncertain whether a reduced testosterone level causes ill-health or whether it is a marker of pre-existing condition or disease, as some illnesses can lower testosterone levels.

CONCLUSION

This study has shown that 6 months of transdermal TRT in Indonesian elderly men with SMC is well-tolerated, and results in a significant increase in testosterone and its metabolic products, which in turn suppress LH levels. The TRT-induced trend towards decreasing plasma Aβ40 levels warrants investigation using a larger cohort, to establish more conclusively the benefits of TRT in elderly men with SMC with low testosterone level.

TRIAL REGISTRATION

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

LIST OF ABBREVIATIONS

Aβ = Beta Amyloid
AD = Alzheimer’s Disease
CFT = Calculated Free Testosterone
DHT = Dihydrotestosterone
LDL = Low Density Lipoprotein
LH = Luteinizing Hormone
MCI = Mild Cognitive Impairment
P = Placebo
PSA = Prostate Specific Antigen
RBC = Red Blood Cells
SMC = Subjective Memory Complainers
SHBG = Sex Hormone Binding Globulin
TRT = Testosterone Replacement Therapy
T = Testosterone

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

We acknowledge the McCusker Alzheimer’s Disease Research Foundation, Perth Western Australia and Siloam Hospitals Lippo Village, Tangerang Indonesia for generously funding this study. We are indebted to the Indonesian participants and research volunteers for making this study possible.

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Testosterone Replacement Therapy in Older Men

CNS & Neurological Disorders - Drug Targets, 2015, Vol. 14, No. 4

Received: August 11, 2014
Revised: January 9, 2015
Accepted: January 20, 2015


