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# Testosterone and Cognition in Elderly Men: A Single Testosterone Injection Blocks the Practice Effect in Verbal Fluency, but Has No Effect on Spatial or Verbal Memory

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**Background:** *The relevance of the age-associated decline in testosterone for cognition in elderly men is still poorly understood. One hypothesis is that testosterone enhances spatial abilities, while it might impair verbal skills.*

**Methods:** *Thirty elderly men received a single testosterone (250 mg testosterone enanthate) or placebo injection. Cognitive performance was tested before and 5 days after treatment using spatial as well as verbal tests.*

**Results:** *Five days after injection, testosterone and estradiol levels were still in the supraphysiologic range. In the verbal fluency task, the placebo group, but not the testosterone group, showed a practice effect. Therefore, the testosterone group performed significantly worse than the placebo group after treatment. No effects of testosterone were observed in the other verbal and spatial tasks.*

**Conclusions:** *The present finding, that testosterone blocks the practice effect in verbal fluency, partly supports the general idea that sex steroids modulate performance in tests with known gender differences. Moreover it demonstrates that these effects can occur rapidly. However, beneficial effects on spatial cognition or memory might need more time to develop and/or might only occur when a less pronounced testosterone increase is induced.* Biol Psychiatry 2000;47: 650–654 © 2000 Society of Biological Psychiatry

**Key Words:** Aging, cognition, humans, sex steroids, gender differences, replacement

## Introduction

In aging men, there is a decrease in total and free (unbound) testosterone levels (Vermeulen 1991). However, the relevance of this for cognitive impairments and other age-associated psychiatric problems is still poorly understood (Sternbach 1998). Estradiol replacement in women has resulted in conflicting findings (see for review: Haskell et al 1997; Rice et al 1997). At least studies by Sherwin et al suggest that estradiol specifically enhances verbal memory (Phillips and Sherwin 1992; Sherwin and Tulandi 1996), and studies from our laboratory indicate that this effect is already detectable after a short treatment period (Wolf et al 1999). With respect to the effects of testosterone replacement in elderly men, Janowsky et al (1994) reported an increase in spatial cognition (block design subtest of the WAIS-R) after transdermal testosterone treatment, whereas another study, which did not measure spatial cognition, failed to find effects on several other cognitive tests (Sih et al 1997). Recently, Cherrier et al (1998) reported preliminary evidence that testosterone treatment increases spatial as well as verbal memory. The authors suggested that the effects on verbal memory might have been caused by the treatment induced estradiol rise. The study by Janowsky et al (1994), as well as correlational observations in young women and men (reviewed in Kimura and Hampson 1994), clinical endocrine syndromes (reviewed in Hampson 1995), and transsexuals (Van Goozen et al 1995) seem to support the hypothesis that sex steroids especially influence cognitive domains where gender differences are apparent. Moreover, sex steroids might have enhancing as well as impairing properties depending on the domain tested, at least in women (Hampson 1995; Kimura and Hampson 1994). Testosterone may enhance spatial abilities and impair verbal skills, while the opposite seems to be the case for estradiol. However, whether similar effects occur in response to hormone replacement in elderly men is not established yet.

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At least the preliminary data by Cherrier et al (1998) suggest a testosterone and/or estradiol effect on memory functions rather than specific effects on spatial or verbal skills with known gender differences.

Given the lack of experimental studies in elderly men, the present study investigated the effects of a single testosterone depot injection on cognition tested 5 days later, at a time when blood levels of testosterone are still high. With this design, activation effects of this steroid and its metabolites could be tested.

## Methods and Materials

Thirty healthy elderly men, who were recruited by newspaper advertisements, participated in the experiment. They underwent a comprehensive medical examination for past or current health problems including an ultrasonic screening for prostate abnormalities. Subjects with psychiatric, endocrine, cardiovascular, other chronic diseases, or those medicated with psychoactive drugs or glucocorticoids, were excluded from participation. Seventeen subjects (age:  $68.7 \pm 1.9$  years; body mass index (BMI):  $25.5 \pm 0.6$  kg/m<sup>2</sup>; years of formal education:  $10.4 \pm 0.9$ ) received testosterone, 13 subjects (age:  $67.1 \pm 1.8$  years; BMI:  $25.2 \pm 0.7$  kg/m<sup>2</sup>, years of formal education:  $13.6 \pm 1.3$ ) received placebo. The two groups did not differ significantly in these demographic variables (all  $p > .05$ ). In order to compare the hormone levels with a young control group, blood samples were also obtained from 23 young subjects (age:  $25.5 \pm 0.5$  years; BMI:  $22.8 \pm 0.4$  kg/m<sup>2</sup>). The study was approved by the Ethics Committee of the University of Trier (Germany), and all subjects gave written informed consent.

### Procedure

The study design was placebo controlled and double blind with testosterone or placebo treatment. At baseline and 5 days after injection, subjects underwent a cognitive test battery (see "Cognitive Tests"). Subjects received the injection 2 to 4 days after the baseline tests. Cognitive testing was performed in the early afternoon (between 2:30 and 4 PM).

### Hormonal Treatment

Subjects received testosterone (250 mg testosterone enanthate; Rotexmedica GmbH Trittau, Germany) or placebo (saline) injection (IM). The used testosterone dose results in a strong testosterone rise in the first 24 hours after injection. Thereafter, the levels gradually decline and reach baseline levels approximately after 9 days. For the clinical treatment of hypogonadal men, the injection is usually repeated every 3 weeks (information from the drug manufacturer).

### Biochemical Analyses

After both cognitive test sessions, blood samples were drawn in order to measure free testosterone, estradiol (RIAs, Biermann,

Bad Nauheim, Germany), and total testosterone (RIA, IBL, Hamburg, Germany).

### Cognitive Tests

Parallel versions of the following cognitive tests were used in a counterbalanced fashion.

**VERBAL FLUENCY.** For 1 min subjects had to generate as many words as possible to a given first letter. Thereafter, a second letter was introduced with another 1 min test period. In general, women outscore men in this test (Horn 1983).

**SPATIAL MEMORY.** Subjects were asked to memorize a route marked in a city map within 2 min. Immediate as well as delayed recall (after the mental rotation task, approximately 10 min later) was assessed by asking the subject to draw the learned route onto an unmarked map. The number of correctly chosen roads (maximum 31) was used as test score. In general, men outscore women in this test (Baeumler 1974).

**VERBAL MEMORY.** Six word-pairs were read to the subject (one word per sec). Immediate as well as delayed recall (after the delayed spatial memory recall, ~10 min later) was tested by presenting the first word of each pair as a cue. If the subject could not recall the correct word, the whole pair was read to him again. In both recall condition, each pair was tested twice. In general, no gender differences are observed in this task (Oswald and Fleischmann 1994).

**STROOP.** For each card, the time needed to read or name the items was assessed and the difference between cards 3 and 2 was used as interference score. In general, no gender differences are observed with respect to this interference score (Oswald and Fleischmann 1994).

**MENTAL ROTATION.** Five copies of a specific letter or number were presented on a piece of paper. Each item was rotated to different degrees from the normal horizontal position. In addition, one item in each line was flipped horizontally. This item had to be recognized and crossed out by the subject. The subject was given 2 min to complete as many items as possible. No normative data on the presence or absence of gender differences are provided in the test handbook (Horn 1983).

### Statistical Analyses

Endocrine as well as cognitive data were analyzed with ANOVAs. For all computed ANOVA models post hoc testing was done using Newman Keuls post hoc test. For all analyses, the significance level was  $\alpha = .05$ . All results shown are the mean  $\pm$  standard error of mean (sem).

## Results

The elderly men had significantly lower levels of total testosterone, free testosterone, and estradiol at baseline.

Table 1. Hormone Levels in Elderly Subjects Receiving Testosterone or Placebo Treatment and in Young Control Subjects (Mean  $\pm$  SEM)

Hormone	Group				Young control subjects
	Placebo		Testosterone		
	Before treatment	After treatment	Before treatment	After treatment	No treatment
Free testosterone (pg/mL)	12.6 $\pm$ 1.2 <sup>a</sup>	10.9 $\pm$ 0.9	13.7 $\pm$ 1.3 <sup>a</sup>	68.7 $\pm$ 15.0 <sup>b</sup>	21.0 $\pm$ 1.5
Total testosterone (ng/mL)	3.37 $\pm$ 0.3 <sup>a</sup>	3.04 $\pm$ 0.2	3.77 $\pm$ 0.3 <sup>a</sup>	16.5 $\pm$ 5.5 <sup>b</sup>	4.98 $\pm$ 0.3
Estradiol (pg/mL)	26.5 $\pm$ 3.1 <sup>a</sup>	26.1 $\pm$ 3.3	30.7 $\pm$ 3.6 <sup>a</sup>	55.4 $\pm$ 5.1 <sup>b</sup>	45.8 $\pm$ 2.8

<sup>a</sup>*p* < .05 compared to young control subjects.<sup>b</sup>*p* < .05 compared to placebo group and to young control subjects.

Testosterone treatment led to a significant increase in all three hormones, which were higher compared to baseline, as well as higher than those from the placebo and the young control group, respectively (Table 1).

The results of the cognitive tests are presented in Table 2. A significant group by treatment interaction was observed in the verbal fluency task [ $F(1,28) = 4.5$ ;  $p < .05$ ]. Post hoc testing revealed that both groups did not differ from each other at baseline, however, the placebo group was significantly better than the testosterone group after treatment ( $p < .05$ ). While the placebo group showed an increase in performance at the second test day (a practice effect;  $p < .05$ ), the testosterone group did not ( $p = .24$ ). The group main effect was not significant [ $F(1,28) = 0.7$ ;  $p = .40$ ], but the treatment (time) main effect was [ $F(1,28) = 20.1$ ;  $p < .05$ ].

A practice effect was also evident in the Stroop [ $F$

(1,28) = 15.7;  $p < .05$ ], as well as in the mental rotation task [ $F(1,28) = 13.4$ ,  $p < .05$ ]. In both tasks, however, this effect occurred in both groups (treatment main effect but no treatment by group interaction). Moreover, the placebo group was significantly better than the testosterone group in the mental rotation task at baseline and after treatment [ $F(1,28) = 7.7$ ;  $p < .05$ ], demonstrating baseline differences between the two groups in this test. Identical results were obtained for all ANOVAs described using years of formal education as a covariate in an ANCOVA model.

## Discussion

A single testosterone injection resulting in supraphysiologic hormone levels had no beneficial effects on the five cognitive tests used in this study. It blocked the practice

Table 2. Cognitive Performance in Elderly Subjects Receiving Testosterone or Placebo Treatment (Mean  $\pm$  SEM)

Test	Treatment groups				Group by treatment interaction
	Placebo		Testosterone		
	Before treatment	After treatment	Before treatment	After treatment	
Verbal fluency (number of words produced)	28.5 $\pm$ 2.1	34.9 $\pm$ 2.1 <sup>a</sup>	28.1 $\pm$ 2.0	30.4 $\pm$ 2.3	$p < .05$
Spatial memory immediate recall (number of correct decisions)	17.2 $\pm$ 2.0	20.1 $\pm$ 1.9	14.9 $\pm$ 1.0	15.8 $\pm$ 1.4	n.s.
Spatial memory delayed recall (number of correct decisions)	17.1 $\pm$ 1.9	19.1 $\pm$ 1.8	15.1 $\pm$ 1.1	14.8 $\pm$ 1.4	n.s.
Verbal memory immediate recall (number of recalled pairs)	5.1 $\pm$ 0.9	5.7 $\pm$ 0.9	3.5 $\pm$ 0.7	4.0 $\pm$ 0.8	n.s.
Verbal memory delayed recall (number of recalled pairs)	6.9 $\pm$ 1.0	6.8 $\pm$ 1.0	5.1 $\pm$ 0.8	4.6 $\pm$ 0.9	n.s.
Stroop (interference score: card 3 minus card 2 in seconds)	18.9 $\pm$ 2.0	16.4 $\pm$ 1.0	24.9 $\pm$ 3.7	20.3 $\pm$ 2.8	n.s.
Mental rotation (number of correctly detected flipped items)	18.2 $\pm$ 1.6	20.9 $\pm$ 0.9	13.8 $\pm$ 1.2	15.9 $\pm$ 1.3	n.s.

<sup>a</sup>*p* < .05 compared to testosterone as well as compared to baseline performance; all n.s. are  $p > .20$ .

effect in verbal fluency, however, which is typically observed in this task (e.g., Wolf et al 1999) and which occurred in the placebo group. Since testosterone treatment did not influence the practice effect in the Stroop and the mental rotation task, the conclusion seems justified that the effect was specific to verbal fluency and is not the result of a more global effect on procedural learning. Such a negative effect on verbal fluency was not observed by Sih et al (1997) and Cherrier et al (1998), while Janowsky et al (1994) did not measure verbal fluency. Moreover, treatment with the adrenal androgen dehydroepiandrosterone does not impair verbal fluency (Wolf et al 1997, 1998). The current finding supports the hypothesis that male sex steroids can impair performance in tests where women typically outperform men. The present results are in line with a report in female-to-male transsexuals, where the authors observed a decrease in verbal fluency after testosterone treatment (Van Goozen et al 1995). The present study demonstrates that cognitive effects of sex steroids can occur rapidly, which corresponds with other replacement studies from this laboratory (Wolf et al 1998, 1999) as well as with observational studies in younger humans (Kimura and Hampson 1994) and experimental studies in rodents, respectively (McEwen et al 1997).

Several reasons for the absence of beneficial effects on memory or mental rotation have to be considered. First, the mental rotation task used in our study differs substantially from those used by other researchers (e.g., Janowsky et al 1994; Kimura and Hampson 1994; Van Goozen et al 1997) since it uses letters and numbers instead of geometric objects. Gender differences are most reliably observed in the mental rotation of geometric figures (like the one developed by Vandenberg and Kuse 1978). If such a task had been used in the present study, the results might have been different.

Second, the relationship between testosterone levels and spatial cognition is still discussed as a controversy. Some studies in younger subjects reported a linear relationship between the two (e.g., Christiansen and Knusmann 1987), while other researchers observed a curvilinear relationship when they included men and women into the analysis (Gouchie and Kimura 1991; Moffat and Hampson 1996). The latter studies support Nyborg's theory (Nyborg 1983) that improvements in cognitive performance might only emerge in an optimal (medium) hormone range. This points to the possibility that the testosterone increases induced in the present study might have been too high, since the elderly subjects in the testosterone group had levels 3 times as high as the young control group. Indeed, the only study which reported an enhancement in spatial skills after testosterone treatment tested the subjects directly after application of a new patch, at a time when testosterone levels probably were only slightly elevated

and estradiol levels were actually reduced (Janowsky et al 1994). The authors of this experiment suggested that the reduction in estradiol levels might have been the reason for the enhancement in spatial cognition. In our study, as well as in the two other studies which also used testosterone injections rather than patch treatment, estradiol levels rose (as expected) after testosterone treatment (Cherrier et al 1998; Shi et al 1997). This might in part explain the different results obtained. The fact, that testosterone treatment normally increases estradiol levels complicates illumination of the individual contributions of these sex steroids to observed effects. Especially since it is not known if the two hormones have independent effects on cognition, or may act in an antagonistic or synergistic fashion.

Third, the short treatment duration in the present experiment might be responsible for the failure to observe beneficial effects on spatial cognition or memory. The two studies that observed cognition enhancing effects of testosterone treated their subjects for 3 weeks (Cherrier et al 1998) or 3 months (Janowsky et al 1994). A third study, failed to find beneficial effects in a 12-month trial (Sih et al 1997).

In sum, a single testosterone injection in healthy elderly men blocks the practice effect in verbal fluency without affecting other cognitive domains. Whether this effect is transient or would also be detectable after long-term testosterone treatment remains to be shown in future studies. Beneficial effects on spatial cognition or memory might need more time to develop and/or might only occur when a less pronounced testosterone increase is induced.

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