Sex steroid hormones and cognitive functioning in healthy, older men

Rose H. Matousek and Barbara B. Sherwin*

Department of Psychology, McGill University, 1205 Dr. Penfield Avenue, Montreal, Quebec, Canada H3A 1B1

Abstract

The precise impact of age-related changes in hormone levels on cognition in men is still unclear due to differing study designs and contradictory findings. This study was undertaken to examine the relationship between endogenous sex hormone levels and cognitive functioning in healthy older men using a comprehensive battery of neuropsychological tests and measurement of serum sex hormone levels. Verbal learning and memory, visual-motor processing, spatial abilities, working memory and attention, and levels of testosterone and estradiol were evaluated in 54 healthy older men. Regression analyses revealed significant curvilinear associations between working memory function and both free and bioavailable testosterone levels, suggesting that an optimal hormone level may exist for maximal performance on tasks of executive/frontal lobe functioning. However, no other relationships were evident between either estradiol or testosterone levels and any of the other cognitive functions evaluated. Hormone assays performed at the end of the study revealed that a considerable portion of the healthy elderly men in our sample met criteria for hypogonadism and suggests that their low hormone levels may have mitigated against discovering other significant hormone–cognition relationships.

Keywords

Steroid hormones; Testosterone; Estradiol; Cognition; Memory; Aging; Men; Correlational

Introduction

In men, approximately 50–60% of circulating testosterone is tightly bound to sex hormone-binding globulin (SHBG), a hepatic protein, while approximately 40–50% is more loosely bound to albumin (Kaufman and Vermeulen, 2005). Consequently, only about 1–2% of circulating testosterone in men is free or unbound in serum (Kaufman and Vermeulen, 2005). The relevance of these steroid dynamics lies in the fact that the portion of testosterone bound to SHBG is not available to the tissues for biological activity but the portion of testosterone bound to albumin and the portion that is unbound are available to exert biological activity (Manni et al., 1985; Pardridge, 1981; Pardridge and Mietus, 1979; Vermeulen et al., 1969). Therefore, the most meaningful estimate of the amount of

*Corresponding author. Fax: +1 514 398 4896. barbara.sherwin@mcgill.ca (B.B. Sherwin).

testosterone available for biological activity in men is determined by the assay of bioavailable testosterone (i.e., the free and the albumin-bound fractions).

With increasing age, declines in total, free, and bioavailable testosterone levels occur in healthy males (Feldman et al., 2002; Gray et al., 1991; Harman et al., 2001; Morley et al., 1997; Zmuda et al., 1997). Several large cross-sectional and longitudinal studies that have investigated sex steroid hormone levels in elderly men found that free and bioavailable testosterone levels decline by approximately 1–2% annually from age 40 onwards (Feldman et al., 2002; Gray et al., 1991), while the incidence of hypogonadism (total testosterone levels lower than 11.3 nmol/l or 325 ng/dl) rises from roughly 20% in 60–69 year old men to approximately 50% in those over 80 years of age (Harman et al., 2001). Moreover, since 80% of estradiol in males comes from the peripheral conversion of testosterone (Braunstein, 1994; Vermeulen et al., 2002), a natural consequence of the gradual age-related decreases in testosterone levels in aging men is a concomitant decline in total and bioavailable estradiol levels with increasing age (Ferrini and Barrett-Connor, 1998).

The gradual decrease in testosterone and estradiol levels in aging men has numerous physiological and behavioral effects, most notably, decreases in muscle mass, bone mass, immune function, libido, and mood (for review, see Kaufman and Vermeulen, 2005). There is also evidence that declining sex steroid hormone levels are implicated in age-related changes in cognitive function, such that verbal and spatial abilities are most affected. Specifically, testosterone may enhance spatial abilities in healthy men. Correlational studies found that higher endogenous levels of testosterone were positively associated with scores on tests of spatial abilities in both younger and older men (Christiansen and Knussmann, 1987; Silverman et al., 1999; Thilers et al., 2006; van Strien et al., 2009). Randomized controlled trials have also found a beneficial effect of exogenous testosterone on spatial abilities in both healthy older men (Cherrier et al., 2001, 2007; Janowsky et al., 1994) and in men with mild cognitive impairment (Cherrier et al., 2005a). However, numerous studies failed to demonstrate a positive relationship between endogenous testosterone levels and spatial cognition in young (Halari et al., 2005; McKeever and Deyo, 1990; Moffat and Hampson, 1996; Shute et al., 1983; Yonker et al., 2006) and in older men (Fonda et al., 2005; Perry et al., 2001; Yonker et al., 2006), or have failed to find an enhancement of spatial abilities following testosterone treatment in older men (Sih et al., 1997; Wolf et al., 2000). These inconsistencies suggest that the relationship between testosterone and spatial cognition may be complex. Moreover, the diversity in experimental designs and in methodologies used to investigate the relationship between the sex steroid hormones and aspects of cognition in men (correlational vs. experimental), as well as differences in subject populations (young vs. old, hypogonadal vs. eugonadal) and the neuropsychological measures used, have likely contributed to the lack of consistency between studies, making it difficult to draw definitive conclusions.

In addition to the partial support for the positive relationship between testosterone and spatial cognition, there is reason to believe that estradiol has a positive influence on performance on tasks of verbal memory in men, just as it does in women (Sherwin and Henry, 2008). For example, in randomized controlled trials, older men who had higher levels of both testosterone and estradiol subsequent to testosterone treatment exhibited better

performance on tests of verbal memory compared to their own pretreatment scores (Cherrier et al., 2001, 2005b, 2007) and compared to men treated with placebo (Cherrier et al., 2001, 2005b). Moreover, when testosterone plus an aromatase inhibitor or testosterone-alone were randomly administered to older men, improvements in verbal memory were evident only in the group treated with testosterone-alone (Cherrier et al., 2005b), suggesting that estradiol, aromatized from testosterone, was responsible for the enhancement of verbal memory that occurred in these testosterone-treated older men. Despite these findings, however, others have failed to demonstrate a positive relationship between estradiol levels and verbal memory in men (Carlson and Sherwin, 2000; Hogervorst et al., 2004; Janowsky et al., 1994; Kampen and Sherwin, 1996; Muller et al., 2005). Moreover, significant positive associations between estradiol levels and performance on tests of visual memory (Kampen and Sherwin, 1996), spatial span (Hoger-vorst et al., 2004), and global cognitive functioning (Senanarong et al., 2002) have also been found in men, suggesting that estradiol may affect numerous cognitive functions in addition to its potential influence on verbal memory.

Finally, few studies have investigated the relationship between testosterone and working memory, a frontal lobe function, in older men. In a double-blind randomized-controlled trial, working memory performance improved pre- to post-treatment in older men given 4-weeks of testosterone supplementation whereas no change occurred in those who received placebo (Janowsky et al., 2000). However, Cherrier et al. (2005b) failed to find changes in working memory in healthy older men treated with testosterone compared to an age-matched control group who were not taking testosterone. Given that the prefrontal cortex is among the first brain regions to show signs of atrophy with age (Raz et al., 1997; Salat et al., 1999; Raz, 2000, 2004; Anderton, 2002; Shan et al., 2005), and that areas in the prefrontal cortex are involved in working memory functions (see review by Owen, 1997), more studies evaluating the effects of testosterone on frontal lobe-mediated working memory function in aging men are needed to further elucidate this relationship.

The present study was undertaken to examine possible relationships between endogenous sex hormone levels and cognitive functioning in healthy older men using a comprehensive battery of neuropsychological tests and measurement of serum sex hormone levels. The rationale for choosing specific cognitive tests was that they either demonstrated sex differences or their scores correlated with circulating levels of the sex steroid hormones in men or women in previous studies. It was hypothesized that testosterone levels would be positively associated with performance on tasks of visual-motor processing and spatial abilities (e.g., mental rotation task, paper folding task, water level test, block design task, digit symbol) and working memory in healthy, older men. Second, it was predicted that estradiol levels would be positively related to performance on tasks of verbal memory (e.g., verbal memory/paragraph recall, verbal paired-associates test) in older men.

Methods

The study was approved by the Institutional Review Board, Faculty of Medicine, McGill University, Montreal, Canada. Written informed consent was obtained from all participants.

Participants

Sixty-six healthy male volunteers were recruited through advertisements posted in local newspapers. Potential participants were screened over the telephone to ensure that they had no current medical or psychiatric disorders and were not taking any medications that could potentially interfere with cognitive functioning, including psychotropic medications (mood stabilizers, antipsychotics, antidepressants, anxiolytics, anticonvulsants, stimulants or sedatives). Men who were taking medications that could potentially influence their sex hormone levels (i.e., leuprolide acetate, goserelin acetate, or finasteride) were also excluded from the study. Additional exclusion criteria included a history of stroke, transient ischemic attack, myocardial infarction, and psychiatric illness (e.g., bipolar disorder, major depressive disorder, generalized anxiety disorder, schizophrenia), smoking 10 cigarettes or more a day for a period of 20 years or equivalent, intake of four alcoholic drinks or more a day for 3 years, history of head injury that resulted in loss of consciousness, or the suspected or confirmed presence of mild cognitive impairment or other neurological disorders (e.g., Alzheimer's disease, Parkinson's disease). Of the 66 men who answered the advertisement, 2 participants were excluded because they had a history of a heart attack, 1 had a history of a transient ischemic attack, 1 had an history of a viral central nervous system illness, 1 had uncontrolled diabetes, 2 were excluded because they were currently using psychotropic medications, and 2 for the current use of finasteride. Two participants with a score of 14 or higher on the Beck Depression Inventory were excluded and, with their permission, were referred for psychiatric services to their local community health clinic. Additionally, one participant with a score of 21 on the Mini-Mental State Examination was excluded and, with his permission, was referred to his family physician for follow-up. Therefore, the final sample consisted of 54 men ranging in age from 61 to 77 years (mean=68.55±4.39 years) with a mean of 15.15 (±3.34) years of formal education (Table 1).

Test battery

Demographics questionnaire—Potential participants were screened over the telephone using a brief questionnaire to assess their general medical history and health habits including drinking and smoking. Sociodemographic information such as age and level of education was also collected as these factors are independent predictors of cognitive performance (Evans et al., 1997).

Screening measures

Depression measures: The Beck Depression Inventory (BDI) (Beck and Steer, 1993) is a 21-item self-report inventory designed to measure the extent and nature of depressive symptoms during the previous two weeks. A higher score is indicative of a higher level of depression. Scores between 0 and 13 are considered to be within the normal range.

The Geriatric Depression Scale (GDS): The Geriatric Depression Scale (GDS) (Yesavage et al., 1983) is a 30-item inventory designed to measure the extent and nature of depressive symptoms on the day of administration. A higher score is indicative of a higher level of depression. Participants with a score of 12 or higher on the GDS were considered to be depressed.

General cognitive and mental status: The Mini-Mental State Examination (MMSE) (Folstein et al., 1975) assesses global cognitive functioning. A score between 24 and 30 is considered to be within the normal range (Folstein et al., 1975). Participants with a score of 23 or lower on the MMSE were excluded from the study.

Neuropsychological test battery

Spatial ability

Mental rotation test: The adaptation (Vandenberg and Kuse, 1978) of the three-dimensional mental rotation test (Shepard and Metzler, 1971) was used. This test measures one's capacity for mental manipulation and rotation of geometric shapes. Participants are presented with an image of a three-dimensional geometric object and asked to determine what the object would look like if it had been rotated in space and were required to choose two correct responses from the four possibilities presented for each item. Participants were presented with 12 such items and were given 8 min to complete as many of them as possible. For a correct response, both choices for an item had to be correct. One point was awarded for each correct response.

Water-Level Test: The Water-Level Test (Piaget and Inhelder, 1956) is a measure of spatial perception. Participants were presented with six different images of empty bottles that were tilted at various angles. They were asked to imagine where the liquid would be in that bottle if it was half full of liquid, and were asked to mark the appropriate water level for each tilted bottle. The number of degrees between the participant's response and the correct response was tabulated. The smaller the discrepancy between the participant's response and the correct response, the lower is the score on this task and, thus, the better the performance. A discrepancy of 5° or less was considered a correct response, for a maximum score of six.

Paper Folding Test: The Paper Folding Test (Ekstrom et al., 1976) is a measure of mental manipulation. Participants were presented with graphical representations of a piece of paper being folded several times before a hole was punched through it. They were then asked to imagine the placement of the holes if the paper was unfolded. For each item, participants were presented with five possibilities from which to choose. Ten items were presented within a time limit of 3 min. One point was awarded for each correct response, for a maximum score of 10.

Block Design: The Block Design subtest of the WAIS-III (Wechsler, 1997) is a measure of spatial/visuoconstructive skill known to be sensitive to T levels. Participants were presented with a series of designs and were asked to reconstruct each image as quickly as they could with the blocks provided. The test consists of nine images with three images per difficulty level (i.e., easy, moderate, and hard). Participants were given 60 or 120 s to reconstruct each image, depending on the difficulty level. Points were allocated based on time to completion of the task, with time bonuses offered for faster completion times. If a participant was unable to reconstruct three consecutive images, the task was discontinued.

Verbal ability

Verbal memory/paragraph recall: The Logical Memory subtest of the Wechsler Memory Scale-Revised (Wechsler, 1987) measures verbal recall. Participants were presented with a story consisting of five sentences and were asked to recall the paragraph verbatim immediately following presentation and again, following a 45-min delay during which other tests were being administered. Scores on the immediate and delayed portions of the test were then computed using the Wechsler (1987) guidelines.

Verbal Paired-Associates: The Verbal Paired-Associates is a subtest from the Wechsler Memory Scale-Revised (Wechsler, 1987). Participants were read a list of 10 word pairs presented in random order several times. Immediately following each presentation and, again, following a 45-min delay, participants were cued with the first word in the pair and were asked to recall the accompanying paired word. One point was awarded for each related paired associate recalled (i.e., easy task) and two points for each unrelated paired associate recalled (i.e., hard task).

Visuomotor scanning

Digit Symbol: The Digit Symbol subtest of the WAIS-III (Wechsler, 1981) is a measure of visuomotor scanning and processing speed in which females have been shown to excel (Mann et al., 1990). Participants are presented with a row of numbers at the top of the page, ranging from 1 to 9, each of which had a symbol directly below it. Below, participants are presented with a series of randomly devised numbers and are required to match each number with its corresponding symbol. They are given 2 min to complete as many symbols as possible. One point is allocated for each correct response.

Working memory

Letter-Number Sequencing: The Letter-Number Sequencing task (Wechsler, 1981) involves the oral presentation of a string of numbers and letters which participants are asked to order, starting with the numbers in ascending order, followed by the letters in alphabetical order. One point is awarded for each correct response generated for a maximum score of 21 points. The task is discontinued when a participant receives a score of zero on all three strings of letters and numbers in a given span size.

Hormone assays

Total testosterone, total estradiol, albumin, and SHBG were assayed following the conclusion of the study by the Endocrine Research Laboratory, Hotel Dieu Hospital, Montreal, Canada. T levels were assayed using the DSL Radioimmunoassay kit (Diagnostic Systems Laboratories, Webster, TX). The intra-assay (within-run) coefficient of variation (C.V.) for this assay varied between 7.8% and 9.6% and the inter-assay C.V. varied between 8.4% and 9.1% for total testosterone in the ranges from 2.4 to 68.4 nmol/l. This assay has a sensitivity of 0.28 nmol/l. Estradiol levels were assayed using the ADVIA Centaur Estradiol-6 assay kit (Bayer Corporation, USA). The intra-assay C.V. was 9.7% for estradiol in the range from 36.8 to 461.6 pmol/l. The minimum sensitivity/detection limit of estradiol was 36.7 pmol/l. Plasma SHBG levels were measured using the DPC Immunoradiometric

assay kit (Diagnostic Product Corporation, Los Angeles, CA). The intra-assay C.V. for this assay ranged from 2.8% to 5.3% and the inter-assay C.V. ranged from 7.9% to 8.5%. Sensitivity of this assay is 0.04 nmol/l. The percentages of undetectable levels were 0.0% for testosterone, estradiol and SHBG. Plasma albumin levels were measured using the ADVIA 1650 Albumin assay kit (Bayer Corporation). Bioavailable testosterone was calculated using the Södergård equation (Sodergard et al., 1982) using constants from Rosner (1997).

Procedure

Fifty-four men who met the selection criteria voluntarily agreed to participate in the study and signed a consent form that had been approved by the Institutional Review Board, Faculty of Medicine, McGill University, Montreal, Canada. Participants were tested individually in the Human Psychoneuroendocrinology Laboratory at McGill University. A blood sample was obtained from each participant by a registered nurse or blood technician at the beginning of each test session. Samples were centrifuged immediately to separate the serum which was then stored at -50°C until the end of the study. All test sessions were scheduled between 9 and 11 AM to control for the circadian rhythm in testosterone levels (Bremner et al., 1983). Following the drawing of the blood sample, a general demographics questionnaire was completed and then the battery of neuropsychological tests was administered. The test session lasted approximately 2.5 h. Participants were remunerated \$15 for their transportation expenses. Anonymity and confidentiality were maintained by assigning each participant a number used to identify their questionnaires and blood samples.

Statistical analyses

All statistical analyses were performed using the SPSS version 11.0 program. All raw scores were transformed into z -scores to facilitate comparison across tasks. A Principal Components Analysis (PCA) was performed to cluster related variables into components that could represent a specific cognitive ability (not shown). Based on the PCA, the z -scores of highly loading variables were averaged to form a composite score. Hierarchical regressions, controlling for the effects of age and education, were then used to examine the associations between the sex steroid hormone levels and scores on tests of cognitive function. The presence of curvilinear relationships was tested with the addition of quadratic terms to the regression models. Thus, each cognitive measure (variable or component) was first regressed on age and education levels in Block 1, followed by the linear term for the hormonal variable of interest in Block 2 and finally on the quadratic term for the hormonal variable of interest in Block 3. To reduce the influence of outliers, subjects with values greater than 3 SD from the mean on any outcome measure were excluded from analyses involving that variable; three such cases existed.

Results

Sample characteristics

Demographic characteristics of the participants are presented in Table 1. The men were, on average, 68.6 years of age, middle-class and well-educated, with a mean of 15 years of formal education. Mean total testosterone, free testosterone, bioavailable testosterone, total estradiol, albumin and SHBG levels for the entire sample of men are presented in Table 2.

According to the norms provided by the laboratory that performed the assays, mean total testosterone, free testosterone, bioavailable testosterone, and total estradiol levels fell within the low normal range for men in this age group, and 50% of the sample met criteria for hypogonadism (bioavailable testosterone levels below 3.8 nmol/l). However, none of the participants had values at or near the detection limit, with the lowest individual total testosterone value in our sample being 5.66 nmol/l. Total testosterone, free testosterone, and bioavailable testosterone levels were moderately and significantly correlated with one another (total and free testosterone, $r=.510$, $p<.001$; total and bioavailable testosterone, $r=.504$, $p<.001$). Age was negatively associated with lower free testosterone levels ($r=-.343$, $p<.001$) and with bioavailable testosterone levels ($r=-.343$, $p<.001$), and positively associated with higher SHBG levels ($r=.270$, $p<.001$).

Neuropsychological testing

Table 3 contains the mean scores and standard deviations for the psychological and cognitive test measures of the entire sample of healthy, older men.

Spatial abilities—Spatial abilities were tested using the Mental Rotation Test, the Paper Folding Test, the Water Level Test, and the Block Design Test. Scores on these tests were significantly correlated, with correlation coefficients ranging between 0.3 and 0.7. Hierarchical linear regression coefficients for the association of circulating estradiol and testosterone levels and spatial abilities were not significant.

Verbal memory—Verbal memory performance was tested using the immediate and delayed test scores from both the Logical Memory and the Verbal Paired Associates Tests. Scores on these tests were significantly correlated, with correlation coefficients ranging between 0.5 and 0.8. Hierarchical linear regression coefficients for the association of circulating estradiol and testosterone levels and verbal memory performance were not significant.

Working memory—Working memory function was evaluated using the Letter–Number Sequencing Test. Results of the hierarchical linear regression analyses, in which age and education were covariates, revealed significant quadratic relationships between working memory performance and both free testosterone, $R^2=.183$, adjusted $R^2=.116$, $F(4, 53)=2.738$ ($p=.039$), and bioavailable testosterone, $R^2=.183$, adjusted $R^2=.117$, $F(4, 53)=2.752$ ($p=.038$), respectively. Hierarchical regression results for free testosterone and working memory appear in Table 4. Regression results for bioavailable testosterone levels and working memory were similar (not shown).

Visuomotor processing speed—Visuomotor processing speed was evaluated using the Digit Symbol Test. Hierarchical linear regression coefficients for the association of circulating estradiol and testosterone levels and visuomotor processing speed were not significant.

Discussion

The present study was undertaken to examine the relationship between endogenous sex hormone levels and cognitive functioning in a sample of 54 healthy elderly men. Contrary to our first hypothesis, regression analyses failed to reveal significant associations between either total, free or bioavailable testosterone levels and performance on tasks of spatial abilities (as measured by a composite score). These results are consistent with those of several other studies that failed to find a relationship between testosterone levels and spatial abilities in men (Bhasin et al., 2001; Fonda et al., 2005; Halari et al., 2005; Perry et al., 2001). In particular, our results accord with those from a large correlational study in which there was no association between either total or free testosterone levels and performance on tests of spatial abilities in a sample of 981 men whose mean age was 63 years (Fonda et al., 2005).

However, our findings are inconsistent with those of other studies that found either positive linear relationships (Christiansen and Knussmann, 1987; Gordon and Lee, 1986; Hooven et al., 2004; Moffat et al., 2002; Silverman et al., 1999; Thilers et al., 2006; van Strien et al., 2009) or quadratic/curvilinear relationships (Gouchie and Kimura, 1991; Moffat and Hampson, 1996; O'Connor et al., 2001) between endogenous testosterone levels and spatial abilities in men. For example, in a large population-based study conducted in 1107 men aged 35–90 (mean age=62±14 years), higher free testosterone levels were associated with better performance on the Block Design subtest of the WAIS (Thilers et al., 2006). Similarly, a prospective longitudinal study conducted in 400 men aged 50–91 years of age (mean age=64±9 years) followed for over 10 years reported a significant positive relationship between free testosterone levels and performance on the Card Rotations Test, a task measuring mental rotation abilities (Moffat et al., 2002). Thus, in these two studies with significantly larger sample sizes than the present study, a different pattern of results emerged, with significant positive relationships between endogenous free testosterone levels and spatial abilities. Indeed, the main strengths of these two studies were their large sample sizes and wide age ranges, which may have allowed them to capture more variability in testosterone levels than occurred in our study of older men with a more restricted age range. It is also possible that differences in data analytic methods (composite scores vs. individual test scores), in hormone assay methods, and the use of different cognitive measures may also have accounted for the inconsistent findings.

Other studies found quadratic, or curvilinear, relationships between endogenous testosterone levels and scores on tests of spatial abilities (Gouchie and Kimura, 1991; Moffat and Hampson, 1996; O'Connor et al., 2001; Shute et al., 1983). However, all four studies sampled young, college-age participants and three of the four (Gouchie and Kimura, 1991; Moffat and Hampson, 1996; Shute et al., 1983) tested both women and men and analyzed their data together so that it is difficult to interpret these findings. However, a study that evaluated the effects of different doses of exogenous testosterone on cognition in 57 healthy, eugonadal older men aged 50–90 years provided more definitive evidence of a curvilinear relationship between testosterone levels and spatial abilities in men (Cherrier et al., 2007). Significant improvements occurred in verbal and in spatial memory scores in men whose testosterone levels were elevated into the normal to high-normal range of serum values

following 6-weeks of exogenous testosterone administration. On the other hand, a significant increase in scores on tests of verbal and in spatial memory failed to occur in men who had randomly received either a placebo or a low dose of testosterone which failed to significantly increase testosterone levels, or in men who randomly received a supraphysiological dose of testosterone which increased their testosterone levels into the supraphysiological range. This suggests that testosterone levels that fall into the middle of the average male range may be optimal for maximal performance on tasks of spatial abilities and, as a corollary, that testosterone levels above or below this mid-range may result in poorer performance on tests of spatial abilities. The fact that endogenous testosterone levels fell within the lower one-third of the normal male range in our sample of healthy older men may explain why we did not detect more significant hormone-cognition relationships. Moreover, 50% of the healthy elderly men in our sample (27/53) were hypogonadal, with morning levels of bioavailable testosterone below 3.8 nmol/l (Morales and Lunenfeld, 2002) which resulted in less variability in their testosterone levels thereby making it more difficult to find significant relationships between hormone levels and aspects of cognitive functioning.

Finally, a negative association between free testosterone levels and scores on a test of spatial visualization occurred in a study of 450 men aged 35–80 (Yonker et al., 2006). However, these participants were considerably younger (54 ± 13 years) than the men in the present study (69 ± 4 years) and the proportion of men who were found to be hypogonadal following hormonal assays was not reported. This is a potentially important omission since the variability in testosterone levels is reduced in hypogonadal men. Moreover, direct RIA methods for analyzing free testosterone levels, such as those used in their study, can be inaccurate (Rosner, 2001; Vermeulen et al., 1999).

The second hypothesis that total estradiol levels would be positively related to performance on tasks of verbal memory in our sample of healthy, older men was not supported; no significant associations between total estradiol levels and scores on tests of verbal memory were found. However, significant improvements in verbal memory scores following increases in circulating estradiol levels subsequent to exogenous testosterone treatment in older men have been reported in the literature, and may have occurred in those studies because some of administered testosterone was aromatized to estradiol (Cherrier et al., 2001, 2003, 2007). In contrast, when the association between endogenous levels of estradiol and performance on tests of verbal memory in older men was explored in correlational studies, no relationship between estradiol levels and verbal memory in men was evident (Carlson and Sherwin, 2000; Hogervorst et al., 2004; Kampen and Sherwin, 1996; Muller et al., 2005; Martin et al., 2007), consistent with the present findings. The absence of an estradiol–verbal memory association in men may be due to the fact that levels of endogenous estradiol in older men are very low. Indeed, in the present sample, mean estradiol levels of our healthy older men fell within the lower one-third of the normal male range, and suggests that significant relationships between endogenous estradiol levels and aspects of cognition are likely more difficult to detect in healthy older men whose endogenous estradiol levels remain relatively stable at low levels. Indeed, as men age and their endogenous sex steroid hormone levels decline, weaker hormone–cognition associations would be expected.

Importantly, the hospital laboratory that carried out our RIAs does not measure free or bioavailable estradiol levels. This raises the possibility that our failure to find a significant relationship between estradiol levels and verbal memory performance may have occurred because we were only able to measure total estradiol, as opposed to free or bioavailable estradiol. It is important to recall that the essential difference between total, free, and bioavailable estradiol is whether or not SHBG and plasma albumin values are taken into account in their derivation. The assay for total estradiol includes the amount of circulating estradiol that is bound and inactivated by SHBG, whereas free estradiol represents the amount of estradiol that is not bound to SHBG (and therefore free to exert biological activity) and bioavailable estradiol is the amount of estradiol not bound to either SHBG or to albumin in serum which is thought to represent the most meaningful estimate of estradiol in the tissues. This is even more important in older men since SHBG levels begin to increase gradually and continuously during young adulthood so that, by age 85 years, SHBG levels are nearly double the values seen in 25 year old men (Vermeulen et al., 1996). These known age-related increases in SHBG levels would compromise the validity of total estradiol values whereas measures of either free or bioavailable estradiol which correct for the higher SHBG levels in older men would be more accurate (Vermeulen, 1996). Indeed, it is likely that these steroid dynamics may underlie the conflicting findings between studies that investigated sex steroid hormones and cognition in men, and would be a particular problem with regard to the interpretation of studies that assayed only levels of total testosterone and estradiol. Although this would appear to strengthen the argument that measures of free or bioavailable sex steroid hormone levels would be more reliable in investigations of hormone–cognition relationships (Moffat et al., 2002; Thilers et al., 2006; Yaffe et al., 2002), it is counterintuitive that a recent cross-sectional study evaluating cognition in 198 older men with or without prostate cancer, found few differences in cognition when total or bioavailable testosterone levels were used (Alibhai et al., 2009). Once again, this may have occurred because associations between endogenous sex steroid hormones and cognition may be difficult to detect in older men, even when bioavailable fractions of sex hormones are measured, because of their very low circulating levels.

Finally, the prediction that a significant positive association would be evident between testosterone levels and working memory performance was partially supported by our findings of significant curvilinear relationships between working memory performance and both free and bioavailable testosterone levels. Few studies have investigated the relationship between testosterone and working memory/executive function in older men. In two large correlational studies, negative associations were found between testosterone and working memory/executive function such that both higher free and total testosterone levels were associated with poorer executive function performance in 1046 middle aged and elderly men aged 35–80 (Martin et al., 2007) and a negative association between total, but not bioavailable, testosterone levels and working memory function was found in a sample of 198 older men aged 50–87 (Alibhai et al., 2009). Conversely, in a placebo-controlled treatment study, increases in free testosterone levels following testosterone treatment resulted in improved working memory performance in 10 older men aged 61–75 (Janowsky et al., 2000). Similarly, Muller et al. (2005) found a significant positive linear association between total testosterone levels and executive function in a sample of 400 men aged 40–80 and both

higher total and bioavailable testosterone levels were associated with better performance on executive function in a subgroup of older men aged 71–80. Additionally, a trend for a quadratic relationship between bioavailable testosterone levels and executive function was found in their sample of middle aged and elderly men (Muller et al., 2005). Given the contradictory nature of extant research findings on testosterone and working memory/executive function, it is difficult to reconcile the current finding with the existing literature. Clearly, more research is needed to determine the effect of testosterone on frontal lobe-mediated cognitive functions in older men.

The major limitation of our study was its correlational design which does not allow cause-and-effect statements. On the other hand, it provides further information on the relationship between endogenous sex hormone levels and cognitive functions in healthy older men. Because our sample was predominantly Caucasian, it is not known whether these findings would generalize to other racial/ethnic segments of the population. Moreover, although none of the men in our study were apparently obese, body mass index (BMI) was not measured and could be a confounding variable since BMI is inversely correlated with both total and free testosterone levels in both obese and non-obese men (Svartberg et al., 2004; Vermeulen et al., 1996) and BMI is inversely associated with verbal memory performance in men (Cournot et al., 2006).

In summary, the major findings of this study were that significant quadratic associations were found between both free and bioavailable testosterone levels and working memory function in our sample of elderly men. However, no other relationships were evident between either estradiol or testosterone levels and any of the other cognitive functions evaluated. Interestingly, the results of our study are consistent with those of Alibhai et al. (2009) who concluded that sex steroid hormones did not generally appear to be significantly associated with cognitive function in their sample of older men, with the possible exception of working memory. However, because a considerable proportion of the healthy elderly men in our sample met criteria for hypogonadism, it is possible that their low hormone levels may have mitigated against finding significant linear relationships with their cognitive performance. Since 34–68% of older men in their 60s and 70s can be expected to be hypogonadal when free testosterone index criteria are used (Harman et al., 2001), their reduced variability in sex steroid hormone levels is an inherent challenge in the effort to evaluate their hormone–cognition relationships and probably explains some of the discrepancies in the literature. It would therefore be helpful if future studies report the proportion of men in their samples who meet standards for hypogonadism using a standardized set of criteria (e.g., ISSAM guidelines; see Morales and Lunenfeld, 2002; or Wang et al., 2008). In addition, large-scale longitudinal studies in which free and bioavailable measures of testosterone and estradiol are measured in narrower age ranges of healthy older men are needed to further evaluate the impact of declining gonadal hormone levels on cognitive aging in men.

Acknowledgments

This study was supported by an operating grant from the Canadian Institutes of Health Research (No. MOP-77773) awarded to Dr. Barbara B. Sherwin and by a Postgraduate Scholarship (PGS-A) awarded to Rose H. Matousek by

the Natural Sciences and Engineering Research Council (NSERC). We would also like to acknowledge and thank Professor Rhonda Amsel, McGill University, for statistical consultation, and the men who participated in this study.

References

- Alibhai SMH, Mahmoud S, Hussain F, Nagalie G, Tannock I, Tomlinson G, Fleshner N, Krahn M, Warde P, Klotz L, Breunis H, Leach M, Canning SD. Levels of sex hormones have limited effect on cognition in older men with or without prostate cancer. *Crit Rev Oncol Hematol*. 2009; 73:167–175. [PubMed: 19346137]
- Anderton BH. Ageing of the brain. *Mech Ageing Dev*. 2002; 123:811–817. [PubMed: 11869738]
- Beck, A., Steer, R. Patent No. San Antonio, TX: The Psychological Corporation; 1993.
- Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-Hikim I, Shen R, Storer TW. Testosterone dose–response relationships in healthy young men. *Am J Physiol Endocrinol Metab*. 2001; 281:E1172–E1181. [PubMed: 11701431]
- Braunstein, M. The testes. In: Greenspan, F., Forsham, P., editors. *Basic and Clinical Endocrinology*. Appleton-Century-Crofts; Connecticut: 1994.
- Bremner WJ, Vitiello MV, Prinz PN. Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. *J Clin Endocrinol Metab*. 1983; 56:1278–1281. [PubMed: 6841562]
- Carlson LE, Sherwin BB. Higher levels of plasma estradiol and testosterone in healthy elderly men compared with age-matched women may protect aspects of explicit memory. *Menopause*. 2000; 7:168–177. [PubMed: 10810962]
- Cherrier MM, Asthana S, Plymate S, Baker L, Matsumoto AM, Peskind E, Raskind MA, Brodtkin K, Bremner W, Petrova A, LaTendresse S, Craft S. Testosterone supplementation improves spatial and verbal memory in healthy older men. *Neurology*. 2001; 57:80–88. [PubMed: 11445632]
- Cherrier MM, Craft S, Matsumoto AH. Cognitive changes associated with supplementation of testosterone or dihydrotestosterone in mildly hypogonadal men: a preliminary report. *J Androl*. 2003; 24:568–576. [PubMed: 12826696]
- Cherrier MM, Matsumoto AM, Amory JK, Asthana S, Bremner W, Peskind ER, Raskind MA, Craft S. Testosterone improves spatial memory in men with Alzheimer disease and mild cognitive impairment. *Neurology*. 2005a; 64:2063–2068. [PubMed: 15985573]
- Cherrier MM, Matsumoto AM, Amory JK, Ahmed S, Bremner W, Peskind ER, Raskind MA, Johnson M, Craft S. The role of aromatization in testosterone supplementation—effects on cognition in older men. *Neurology*. 2005b; 64:290–296. [PubMed: 15668427]
- Cherrier MM, Matsumoto AM, Amory JK, Johnson M, Craft S, Peskind ER, Raskind MA. Characterization of verbal and spatial memory changes from moderate to supraphysiological increases in serum testosterone in healthy older men. *Psychoneuroendocrinology*. 2007; 32:72–79. [PubMed: 17145137]
- Christiansen K, Knussmann R. Sex hormones and cognitive functioning in men. *Neuropsychobiology*. 1987; 18:27–36. [PubMed: 3444523]
- Cournot M, Marquié JC, Ansiau D, Martinaud C, Fonds H, Ferrières J, Ruidavets JB. Relation between body mass index and cognitive function in healthy middle-aged men and women. *Neurology*. 2006; 67:1208–1214. [PubMed: 17030754]
- Ekstrom, R., French, J., Harman, H., Derman, D. Princeton Patent No. Educational Testing Service; 1976.
- Evans DA, Hebert LE, Beckett LA, Scherr PA, Albert MS, Chown MJ, Pilgrim DM, Taylor JO. Education and other measures of socioeconomic status and risk of incident Alzheimer disease in a defined population of older persons. *Arch Neurol*. 1997; 54:1399–1405. [PubMed: 9362989]
- Feldman HA, Longcope C, Derby CA, Johannes CB, Araujo AB, Coviello AD, Bremner WJ, McKinlay JB. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts Male Aging Study. *J Clin Endocrinol Metab*. 2002; 87:589–598. [PubMed: 11836290]

- Ferrini RL, Barrett-Connor E. Sex hormones and age: a cross-sectional study of testosterone and estradiol and their bioavailable fractions in community-dwelling men. *Am J Epidemiol.* 1998; 147:750–754. [PubMed: 9554416]
- Folstein M, Folstein S, McHugh P. Mini mental state: a practical method for grading the cognitive state of patients for the clinician. *J Psych Res.* 1975; 12:196–198.
- Fonda SJ, Bertrand R, O'Donnell A, Longcope C, McKinlay JB. Age, hormones, and cognitive functioning among middle-aged and elderly men: cross-sectional evidence from the Massachusetts male aging study. *J Gerontol A Biol Sci Med Sci.* 2005; 60:385–390. [PubMed: 15860479]
- Gordon HW, Lee PA. A relationship between gonadotropins and visuospatial function. *Neuropsychologia.* 1986; 24:563–576. [PubMed: 3095677]
- Gouchie C, Kimura D. The relationship between T levels and cognitive ability patterns. *Psychoneuroendocrinology.* 1991; 16:323–334. [PubMed: 1745699]
- Gray A, Feldman HA, McKinlay JB, Longcope C. Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts male aging study. *J Clin Endocrinol Metab.* 1991; 73:1016–1025. [PubMed: 1719016]
- Halari R, Hines M, Kumari V, Mehrotra R, Wheeler M, Ng G, Tonmoy S. Sex differences and individual differences in cognitive performance and their relationship to endogenous gonadal hormones and gonadotropins implications for schizophrenia. *Schizophr Bull.* 2005; 31:511.
- Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. *J Clin Endocrinol Metab.* 2001; 86:724–731. [PubMed: 11158037]
- Hogervorst E, De Jager C, Budge M, Smith AD. Serum levels of estradiol and testosterone and performance in different cognitive domains in healthy elderly men and women. *Psychoneuroendocrinology.* 2004; 29:405–421. [PubMed: 14644069]
- Hooven CK, Chabris CF, Ellison PT, Kosslyn SM. The relationship of male testosterone to components of mental rotation. *Neuropsychologia.* 2004; 42 (6):782–790. [PubMed: 15037056]
- Janowsky JS, Oviatt SK, Orwoll ES. Testosterone influences spatial cognition in older men. *Behav Neurosci.* 1994; 108:325–332. [PubMed: 8037876]
- Janowsky JS, Chavez B, Orwoll E. Sex steroids modify working memory. *J Cogn Neurosci.* 2000; 12:407–414. [PubMed: 10931767]
- Kampen DL, Sherwin BB. Estradiol is related to visual memory in healthy young men. *Behav Neurosci.* 1996; 110:613–617. [PubMed: 8889006]
- Kaufman JM, Vermeulen A. The decline of androgen levels in elderly men and its clinical and therapeutic implications. *Endocr Rev.* 2005; 26:833–876. [PubMed: 15901667]
- Manni A, Partridge W, Cefalu W, Nisula B, Bardin C, Santner S, Santen RJ. Bioavailability of albumin bound testosterone. *J Clin Endocrinol Metab.* 1985; 61:705–710. [PubMed: 4040924]
- Mann VA, Sasanuma S, Sakuma N, Masaki S. Sex differences in cognitive abilities: a cross-cultural perspective. *Neuropsychologia.* 1990; 28:1063–1077. [PubMed: 2267058]
- Martin D, Wittert G, Burns N, Haren M, Sugarman R. Testosterone and cognitive function in ageing men: data from the Florey Adelaide Male Ageing Study (FAMAS). *Maturitas.* 2007; 57:182–194. [PubMed: 17287097]
- McKeever WF, Deyo RA. Testosterone, dihydrotestosterone, and spatial task performances of males. *Bull Psychon Soc.* 1990; 28:305–308.
- Moffat SD, Hampson E. A curvilinear relationship between testosterone and spatial cognition in humans: possible influence of hand preference. *Psycho-neuroendocrinology.* 1996; 21:323–337.
- Moffat SD, Zonderman AB, Metter EJ, Blackman MR, Harman SM, Resnick SM. Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men. *J Clin Endocrinol Metab.* 2002; 87:5001–5007. [PubMed: 12414864]
- Morales A, Lunenfeld B. Investigation, treatment and monitoring of late-onset hypogonadism in males: official recommendations of the International Society for the Study of the Aging Male. *Aging Male.* 2002; 5:74–86. [PubMed: 12198738]
- Morley JE, Kaiser FE, Perry HM, Patrick P, Morley PMK, Stauber PM, Vellas B, Baumgartner RN, Garry PJ. Longitudinal changes in testosterone, luteinizing hormone, and follicle-stimulating hormone in healthy older men. *Metab Clin Exp.* 1997; 46:410–413. [PubMed: 9109845]

- Muller M, Aleman A, Grobbee DE, de Haan EHF, van der Schouw YT. Endogenous sex hormone levels and cognitive function in aging men—is there an optimal level? *Neurology*. 2005; 64:866–871. [PubMed: 15753424]
- O'Connor DB, Archer J, Hair WM, Wu FCW. Activational effects of testosterone on cognitive function in men. *Neuropsychologia*. 2001; 39:1385–1394. [PubMed: 11585606]
- Owen AM. The functional organization of working memory processes within human lateral frontal cortex: the contribution of functional neuroimaging. *Eur J Neurosci*. 1997; 9:1329–1339. [PubMed: 9240390]
- Pardridge WM. Transport of protein-bound hormones into tissues in vivo. *Endocr Rev*. 1981; 2:103–123. [PubMed: 7028469]
- Pardridge W, Mietus L. Transport of steroid hormones through the rat blood–brain barrier: primary role of albumin-bound hormone. *J Clin Invest*. 1979; 64:145–154. [PubMed: 447850]
- Perry P, Lund B, Arndt S, Holman T, Bever-Stille K, Paulsen J, Demers LM. Bioavailable testosterone as a correlate of cognition, psychological status, quality of life, and sexual function in aging males: implications for testosterone replacement therapy. *Ann Clin Psychiatry*. 2001; 13:75–80. [PubMed: 11534928]
- Piaget, J., Inhelder, B. *The Child's Conception of Space*. Routledge & Kegan Paul; London: 1956.
- Raz, N. Aging of the brain and its impact on cognitive performance: integration of structural and functional findings. In: Craik, FIM., Salthouse, TA., editors. *Handbook of Aging and Cognition II*. Erlbaum; Mahwah, NJ: 2000. p. 1-90.
- Raz N. Aging, sexual dimorphism, and hemispheric asymmetry of the cerebral cortex: replicability of regional differences in volume. *Neurobiol Aging*. 2004; 25:377–396. [PubMed: 15123343]
- Raz N, Gunning FM, Head D, Dupuis JH, McQuain J, Briggs SD, Loken WJ, Thornton AE, Acker JD. Selective aging of the human cerebral cortex observed in vivo: differential vulnerability of the prefrontal gray matter. *Cereb Cortex*. 1997; 7:268–282. [PubMed: 9143446]
- Rosner W. Errors in the measurement of plasma free testosterone. *J Clin Endocrinol Metab*. 1997; 82:2014–2015. [PubMed: 9177424]
- Rosner W. An extraordinarily inaccurate assay for free testosterone is still with us. *J Clin Endocrinol Metab*. 2001; 86:2903. [PubMed: 11397908]
- Salat DH, Kaye JA, Janowsky JS. Prefrontal gray and white matter volumes in healthy aging and Alzheimer Disease. *Arch Neur*. 1999; 56 (3):338–344.
- Senanarong V, Vannasaeng S, Pongvarin N, Ploybutr S, Udompuntharak S, Jamjumras P, Fairbanks L, Cummings JL. Endogenous estradiol in elderly individuals: cognitive and noncognitive associations. *Arch Neurol*. 2002; 59:385–389. [PubMed: 11890841]
- Shan ZY, Liu JZ, Sahgal V, Wang B, Yue GH. Selective atrophy of left hemisphere and frontal lobe of the brain in old men. *J Gerontol: Biol Sc*. 2005; 60A:165–174.
- Shepard RN, Metzler J. Mental rotations of three-dimensional objects. *Science*. 1971; 171:701–703. [PubMed: 5540314]
- Sherwin BB, Henry JF. Brain aging modulates the neuroprotective effects of estrogen on selective aspects of cognition in women: a critical review. *Front Neuroendocrinol*. 2008; 29:88–113. [PubMed: 17980408]
- Shute VJ, Pellegrino JW, Hubert L, Reynolds RW. The relationship between androgen levels and human spatial abilities. *Bull Psychon Soc*. 1983; 21:465–468.
- Sih R, Morley JE, Kaiser FE, Perry HM, Patrick P, Ross C. Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. *J Clin Endocrinol Metab*. 1997; 82:1661–1667. [PubMed: 9177359]
- Silverman D, Choi J, Phillips K. Testosterone levels and spatial ability in men. *Psychoneuroendocrinology*. 1999; 24:813–822. [PubMed: 10581652]
- Sodergard R, Backstrom T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 β to human plasma proteins at body temperature. *J Steroid Biochem Mol Biol*. 1982; 16:801–810.
- Svartberg J, von Mühlen D, Sundsfjord J, Jorde R. Waist circumference and testosterone levels in community dwelling men: the Tromsø Study. *Eur J Epidemiol*. 2004; 19:657–663. [PubMed: 15461197]

- Thilers PP, MacDonald SWS, Herlitz A. The association between endogenous free testosterone and cognitive performance: a population-based study in 35 to 90 year-old men and women. *Psychoneuroendocrinology*. 2006; 31:565–576. [PubMed: 16487665]
- Vandenberg SG, Kuse AR. Mental rotations, a group test of three-dimensional spatial visualization. *Percept Mot Skills*. 1978; 47:599–604. [PubMed: 724398]
- Van Strien JW, Weber RFA, Burdorf A, Bangma C. Higher free testosterone level is associated with faster visual processing and more flanker interference in older men. *Psychoneuroendocrinology*. 2009; 34:546–554. [PubMed: 19042092]
- Vermeulen A. Decreased androgen levels and obesity in men. *Ann Med*. 1996; 28:13–15. [PubMed: 8932500]
- Vermeulen A, Verdonck L, van der Straeten M, Orie N. Capacity of testosterone-binding globulin in human plasma and influence of specific binding of testosterone on its metabolic clearance rate. *J Clin Endocrinol Metab*. 1969; 29:1470–1480.
- Vermeulen A, Kaufman JM, Giagulli VA. Influence of some biological indexes on sex hormone-binding globulin and androgen levels in aging or obese males. *J Clin Endocrinol Metab*. 1996; 81:1821–1826. [PubMed: 8626841]
- Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab*. 1999; 84:3666–3672. [PubMed: 10523012]
- Vermeulen A, Kaufman JM, Goemaere S, Van Pottelberg I. Estradiol in elderly men. *Aging Male*. 2002; 5:98–102. [PubMed: 12198740]
- Wang C, Nieschlag E, Swerdloff R, Behre H, Hellstrom W, Gooren L, Kaufman J, Legros J, Lunenfeld B, Morales A. Investigation, treatment, and monitoring of late-onset hypogonadism in males: ISA, ISSAM, EAU, EAA, and ASA recommendations. *Eur Urol*. 2008; 55:121–130. [PubMed: 18762364]
- Wechsler, D. Patent No. New York: The Psychological Corporation; 1981.
- Wechsler, D. Patent No. San Antonio, TX: The Psychological Corporation; 1987.
- Wechsler, D. Patent No. San Antonio, TX: The Psychological Corporation; 1997.
- Wolf OT, Preut R, Hellhammer DH, Kudielka BM, Schurmeyer TH, Kirschbaum C. 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. *Biol Psychiatry*. 2000; 47:650–654. [PubMed: 10745058]
- Yaffe K, Lui LY, Zmuda J, Cauley J. Sex hormones and cognitive function in older men. *J Am Geriatr Soc*. 2002; 50:707–712. [PubMed: 11982672]
- Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, Leirer VO. Development and validation of a Geriatric Depression Screening Scale: a preliminary report. *J Psychiatr Res*. 1983; 17:37–49.
- Yonker JE, Eriksson E, Nilsson LG, Herlitz A. Negative association of testosterone on spatial visualization in 35 to 80 year old men. *Cortex*. 2006; 42:376–386. [PubMed: 16771043]
- Zmuda JM, Cauley JA, Kriska A, Glynn NW, Gutai JP, Kuller LH. Longitudinal relation between endogenous testosterone and cardiovascular disease risk factors in middle-aged men—a 13-year follow-up of former Multiple Risk Factor Intervention Trial participants. *Am J Epidemiol*. 1997; 146:609–617. [PubMed: 9345114]

Table 1

Means and standard deviations (SD) of demographic characteristics of the sample of healthy older men ($n=54$).

	Mean	SD	Range
Age (years)	68.55	4.39	61–77
Education (years)	15.15	3.34	9–22
Individual income (CDN dollars)	\$40, 141	\$3, 360	<\$15,000–>\$60,000
Household income (CDN dollars)	\$ 41,581	\$3, 390	<\$15,000–>\$60,000
MMSE	29.34	0.92	26–30
BDI	4.31	3.58	0–11
GDS	3.26	3.04	0–11
BAI	2.04	2.63	0–12

Note. MMSE=Mini Mental State Examination; BDI=Beck Depression Inventory; GDS=Geriatric Depression Scale; BAI=Beck Anxiety Inventory.

Table 2

Means and standard deviations (SD) of total T, free T, bioavailable T, total estradiol, albumin and SHBG levels of the entire sample of healthy older men ($n=54$) with normal ranges.

	Mean	SD	Normal range ^a
Total T (nmol/l)	9.74	2.31	9.0–25.0
Free T (pmol/l)	190.49	82.59	182–670
Bioavailable T (nmol/l)	3.97	1.69	3.7–13.8
Total E ₂ (pmol/l)	78.11	34.87	<191
Albumin (g/l)	44.85	2.13	37–48
SHBG (nmol/l)	41.42	19.00	12–52

Note. T= Testosterone; Bio= Bioavailable; E₂ =Estradiol; SHBG=Sex-Hormone Binding-Globulin.

^aFor men aged >60 years.

Table 3

Means and standard deviations (SD) for the psychological and cognitive test measures of the entire sample of healthy older men ($n=54$).

	Means	SD
Beck Depression Inventory ^a	4.31	3.58
Geriatric Depression Scale ^a	3.26	3.04
Mental Rotation Total Correct	3.42	2.37
Mental Rotation Time (s) ^a	380.33	92.39
Paper Folding Total Correct	3.69	1.81
Water Level Total Correct	3.47	1.92
Block Design Scaled Score	12.19	2.24
Digit Symbol Scaled Score	10.98	2.09
Letter–Number Sequencing Scaled Score	12.49	1.97
Logical Memory Immediate %	44.44	15.57
Logical Memory Delayed %	39.30	18.02
Verbal-Paired Associates Immediate Recall Trial 1	5.96	2.67
Verbal-Paired Associates Immediate Recall Trial 2	8.38	2.90
Verbal-Paired Associates Immediate Recall Trial 3	10.11	2.79
Verbal-Paired Associates Delayed Recall	8.84	3.26
Verbal-Paired Associates Total Recall Score	33.29	10.29

^aLower scores indicate better performance.

Table 4

Hierarchical regression analysis results for free testosterone and working memory.

	<i>R</i>	<i>R</i> ²	Adjusted <i>R</i> ²	Sum of squares	<i>df</i>	Mean square	<i>F</i>	<i>F</i> change	Sign. <i>F</i> change
Regression ^a	.198	.039	.001	2.071	2	1.036	1.037	1.037	.362
Residual				50.929	51	.999			
Regression ^b	.248	.061	.005	3.255	3	1.085	1.090	1.189	.281
Residual				49.745	50	.995			
Regression ^c	.427	.183	.116	9.683	4	2.421	2.738*	7.272	.001
Residual				43.317	49	.884			

Dependent Variables: Spatial composite score.

* *p*<0.05.

^a Predictors: SHBG, Education, Age.

^b Predictors: SHBG, Education, Age, Free Testosterone.

^c Predictors: SHBG, Education, Age, Free Testosterone, Free Testosterone (Quadratic Term).