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Longitudinal Assessment of Serum Free Testosterone Concentration Predicts Memory Performance and Cognitive Status in Elderly Men

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Circulating testosterone (T) levels have behavioral and neurological effects in both human and nonhuman species. Both T concentrations and neuropsychological function decrease substantially with age in men. The purpose of this prospective, longitudinal study was to investigate the relationships between age-associated decreases in endogenous serum T and free T concentrations and declines in neuropsychological performance. Participants were volunteers from the Baltimore Longitudinal Study of Aging, aged 50–91 yr at baseline T assessment. Four hundred seven men were followed for an average of 10 yr, with assessments of multiple cognitive domains and contemporaneous determination of serum total T, SHBG, and a free T index (FTI). We administered neuropsychological tests of verbal and visual memory, mental status, visuomotor scanning and attention, verbal knowledge/language, visuospatial ability, and depressive symptomatology. Higher FTI was associated with better scores on visual and verbal memory, visuospatial functioning, and visuomotor scanning and a reduced rate of longitudinal decline in visual memory. Men classified as hypogonadal had significantly lower scores on measures of memory and visuospatial performance and a faster rate of decline in visual memory. No relations between total T or the FTI and measures of verbal knowledge, mental status, or depressive symptoms were observed. These results suggest a possible beneficial relationship between circulating free T concentrations and specific domains of cognitive performance in older men. (*J Clin Endocrinol Metab* 87: 5001–5007, 2002)

OLDER AGE IS associated with functional declines throughout the body, including some aspects of cognitive performance. Although only some elderly individuals develop dementia, declines in cognitive functioning impact daily living for many. However, there are individual differences in age-related cognitive changes, and the factors that contribute to this variability have not been well characterized. Recent evidence suggesting that age-related alterations in the endocrine environment (1) may modulate cognitive changes has generated considerable interest.

In women, there is evidence that postmenopausal estrogen replacement therapy (ERT) may exert beneficial effects on specific cognitive functions, most pronounced for verbal memory (2–6), and may reduce the incidence and delay the onset of Alzheimer's disease (7–9). In men, there is limited information on the effects of testosterone supplementation on cognition despite substantial declines in endogenous testosterone (T) with advancing age. Total T levels decline by as much as 50% from ages 30–80 yr (1), and as many as 68% of men over 70 yr can be classified as hypogonadal based on their calculated free T concentrations (10). These observations raise the question of whether the loss of androgens with age (the male andropause) may be associated with age-related declines in selected cognitive functions.

T manipulations both in the neonatal period and later in life have been shown unequivocally to exert behavioral effects in nonhuman species (11, 12). In humans, studies have focused on those abilities for which reliable sex differences have been reported, such as visuospatial ability (13). The organizational effects of T on visuo-spatial performance later in life have been suggested by enhanced performance in females exposed prenatally to excess androgens (14, 15) and reduced spatial performance in males with idiopathic hypogonadotropic hypogonadism (16). The majority of investigations examining the association between circulating T levels and cognition have been small studies in young adult men and women. The results of these studies have been somewhat mixed, with some studies reporting positive linear relationships (17, 18) and others reporting that moderate levels of T (an inverted U) may be associated with higher visuospatial cognitive performance (19–22).

Few studies have examined the association between T and cognition in older men. In one such study Barrett-Connor *et al.* (23) measured the association between baseline T and neuropsychological performance in 547 men between the ages of 59 and 89 yr. After controlling for a number of possible confounding variables, they found higher bioavailable T concentrations in men who scored better on a measure of long-term verbal memory. In three small, randomized, placebo-controlled clinical trials, T administration in older men

Abbreviations: BLSA, Baltimore Longitudinal Study of Aging; BMI, body mass index; BVRT, Benton Visual Retention Test; CVLT, California Verbal Learning Test; ERT, estrogen replacement therapy; FTI, free testosterone index; MMSE, Mini Mental State Exam; ROT, card rotations test; T, testosterone; TRAILSA, Trail Making Test Part A; TRAILSB, Trail Making Test Part B.

was reported to improve visuospatial performance (24, 25), verbal memory (25), and working memory (26).

In the present study we followed a sample of 407 men with age at baseline T assessment ranging from 50–90 yr for a mean duration of 9.7 yr. We collected multiple serum samples for determination of total T and SHBG and free T concentrations and obtained multiple cognitive measures across a variety of cognitive domains over the same time interval. We report here a prospective longitudinal study assessing the impact of long-term total and free T levels on neurocognitive function in this sample of community-dwelling men.

Subjects and Methods

Subjects

Subjects were male volunteers participating in the Baltimore Longitudinal Study of Aging (BLSA), a study performed by the NIA (27). Participants are generally healthy volunteers who return every 2 yr to the Gerontology Research Center of the NIA for comprehensive medical, physiological, and neuropsychological evaluations. Androgen data were available from a large pool of BLSA participants whose blood samples were assayed for androgen as part of a study of prostate health and disease. The present study restricted analyses to the subsample of men who were 50 yr or older and who had androgen measures that overlapped temporally with their cognitive assessments. There were 407 men who met these criteria. The mean age ± SD at baseline assessment was 64.07 ± 9.40 yr, and the duration of follow-up averaged 9.7 yr. Mean level of education was 16.75 ± 3.08 yr.

The focus of the present study was on the relationship between T and cognition in men without dementia. Therefore, subjects meeting National Institute of Neurological and Communicative Disorders and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable (n = 21) or possible (n = 12) Alzheimer's disease (28) at any visit were excluded from analyses. Additionally, 7 subjects with Parkinson's disease (with or without dementia), 4 with cerebrovascular disease (with or without dementia), 3 with other unspecified dementias, and 58 with cancer (non-skin) were excluded. This protocol was approved by the local institutional review board, and all subjects provided written informed consent to participate.

Procedure

Hormone determinations. Blood samples were collected prospectively at each visit starting in 1963 and stored at –70 C. As part of the BLSA prostate study, 3621 samples from the serum bank were analyzed to examine the longitudinal changes in serum T in 901 men (10). For each subject, samples selected for assay were those from the visits closest to 10, 15, and 20 yr before the most recent visit and then as many as were available within 10 yr from the most recent visit. Blood samples were drawn between 0600–0800 h after an overnight fast. All serum total T and SHBG measurements were performed at Covance Laboratories, Inc. (McLean, VA). The free T index (FTI) was calculated by dividing serum T by SHBG. The FTI, calculated from RIA of T and SHBG, has been shown to be well correlated with measures of free T by dialysis and bioavailable T by ammonium sulfate precipitation and is simpler to obtain (29). Both Vermeulen *et al.* (30) and Morley *et al.* (29) recently demonstrated that calculation of an index from T and SHBG, as determined by immunoassay, provides a rapid, simple, and reliable index of bioavailable T, comparable to measures of free T by dialysis or bioavailable T by ammonium sulfate precipitation. According to these researchers, this methodology is suitable for clinical determinations, except in pregnancy, where SHBG concentrations are very high.

Details of the hormonal assay have been published previously (10). T levels were determined in duplicate using ^{125}I double antibody RIA kits obtained from Diagnostic Systems Laboratories, Inc. (Webster, TX). Minimum detectable T levels averaged 0.42 nmol/liter, with intra- and interassay coefficients of variance, respectively, of 4.8% and 5.7% at concentrations of 7.74 and 7.29 nmol/liter, and 3.3% and 6.4% at concentrations of 44.7 and 42.9 nmol/liter. SHBG concentrations were measured using RIA kits purchased from Radim (Liege, Belgium), which employ ^{125}I-labeled SHBG and polyethylene glycol-complexed second antibody. The sensitivity of the SHBG assay was approximately 10 nmol/liter. The coefficient of variation at 5 nmol/liter was 22%, and that at 25 nmol/liter was 5%, with intra- and interassay coefficients of variance, respectively, of 3.4% and 10.8% at concentrations of 22 and 19 nmol/liter, and of 1.8% and 7.7% at concentrations of 117 and 118 nmol/liter.

Preliminary analysis of data from the samples stored between 1961 and 1995 revealed a significant increase in T level with length of storage independent of subject age. On investigation we were able to demonstrate that the increase was due to a date-related assay artifact. This increase in T with length of storage was linear, with a slow constant rate over time. A mixed effects model was used to adjust T for the date effect, with all values adjusted to 1995, the year when the samples were analyzed (10). Because this adjustment was linear and constant, it was unlikely to substantially alter the relative rank ordering of individuals within this sample.

Cognitive tests. The neuropsychological tests included in the present study were administered at the same BLSA visits as the blood sampling. The neuropsychological tests included: the Benton Visual Retention Test (BVRT) (31), a measure of short-term visual memory and visuoconstructional skills; the Mini Mental State Exam (MMSE) (32); semantic (FLUCAT) and phonemic (FLULET) word fluency (33); and the Trail Making Test Part A (TRAILSA) and Part B (TRAILSB) (34), measures of visuomotor scanning and attention. The California Verbal Learning Test (CVLT) (35) served as a measure of verbal learning and memory. Dependent measures used were the total number of words recalled over all five trials of list A (CVLT-A), free recall after a 20-min delay (CVLT-D), and recognition accuracy (CVLT-R). The card rotations test (ROT) (36) was administered as a visuospatial test of mental rotation of geometric shapes. Digit Span forward and backward (37) served as measures of attention/concentration. The Center for Epidemiologic Studies-Depression scale (38) was used as an index of symptoms of depression.

Cognitive tests in the BLSA are administered on a time- and age-based schedule, because the testing program has evolved over the course of the longitudinal study. Thus, not all subjects received all cognitive tests, and subjects may have had a variable number of observations for each test depending on the year and age at entry into the study. The BVRT was administered to participants every 6 yr from 1960 through 1991. Since 1991, subjects aged 50 yr and older received the BVRT every 2 yr, and since 1993, these subjects received the CVLT, Digit Span forward, and Digit Span backward every 2 yr. The MMSE, TRAILSA, TRAILSB, FLUCAT, and FLULET have been administered every 2 yr since August 1985 and March 1990, respectively, for subjects 70 yr and older and 60 yr and older. Final sample sizes are listed in the tables for each analysis.

Data analysis

To examine the effects of individual differences in T levels on cognitive function, baseline and mean T were investigated in relation to level of cognitive performance (cognitive status) and change in cognitive performance over time (change). For hormone measurements, baseline and within-individual mean over repeated evaluations (mean) were calculated for both T and FTI. For cognitive variables, cognitive status was measured by the within-individual mean performance across repeated test administrations, and where possible, cognitive change scores were calculated as annual rates of change from the within-individual slopes. The mean of cognitive measures was used to estimate cognitive status because this was thought to reflect the most reliable estimate of level of cognitive performance across the assessment interval. Annualized longitudinal rates of change were calculated for all subjects who had at least three observations over the T collection interval. Annual rates of change were calculated from the slope of test score *vs.* age at assessment. Longitudinal rates of change were available for the BVRT, MMSE, Center for Epidemiologic Studies-Depression scale, FLUCAT, FLULET, TRAILSA, and TRAILSB. For other cognitive tests, there were insufficient data for calculation of rates of change, and cognitive status alone was used as the outcome measure. There are reductions in the number of subjects in analyses involving rates of change due to our requirement for each subject to have at least three cognitive assessments for calculation of reliable annual rates of change.

The associations between T and FTI and neuropsychological out-

comes were examined using two different analytic approaches. In the first, multiple regressions were computed, treating all measures as continuous variables. The baseline (T and FTI) and mean (T and FTI) hormone concentrations were used to predict cognitive status (within-individual mean) and the rate of cognitive decline (annual rate of change). Age, years of education, smoking status (never, former, or current), body mass index (BMI), history of coronary heart disease (none, possible, and definite based on history and electrocardiogram), diabetes (normal *vs.* fasting blood sugar >125 mg/dl or being treated for diabetes mellitus), and alcohol consumption (<2 *vs.* >2 oz/d) were included as covariates in the regression models to control for medical and disease-related factors that could potentially influence hormonal values and/or cognitive test scores. In addition, for analyses involving rates of change, baseline cognitive values were entered as additional covariates to control for the fact that rates of change were negatively associated with baseline cognitive scores.

A second set of analyses investigated the effects of hypogonadism on neurocognitive performance. Men were classified as either hypogonadal or eugonadal according to criteria established previously (10). The definition of hypogonadism employed was statistical, as are all definitions currently employed clinically as well as for purposes of research. This is because the actual minimum levels of free or bioavailable T, below which physiological or functional deficits attributable to a lack of T action occur, have yet to be established for cognitive or indeed for any other function. Men were classified as hypogonadal if they had at least one FTI measurement that was below the 2.5th percentile for men aged 40 yr and younger. All other men were considered eugonadal. Because the men classified as hypogonadal (mean age, 68.9 ± 9.3 yr; n = 149) were significantly older at baseline than those classified as eugonadal (mean age, 60.7 ± 7.9 yr; n = 211), cognitive performance was standardized based on age-normed scores. All men were assigned z-scores that reflected how far their cognitive scores for each test deviated from their age decade-based norms. Similar approaches have been used in observational studies of ERT in postmenopausal women (5). These age-normed scores were then used for group comparisons. Using analysis of covariance to adjust for years of education, smoking status, BMI, coronary heart disease, diabetes, and alcohol consumption, hypogonadal and eugonadal men were compared on cognitive status. For comparisons involving rate of cognitive decline, raw scores rather than age-normed scores were used because age was not associated with rate of cognitive decline.

To reduce the undue influence of outliers, subjects with values greater than 3 SD from the mean on any outcome measure were excluded from analyses involving that variable.

Results

Associations between T and the FTI with cognition

Detailed cross-sectional and longitudinal analyses of age-related androgen declines have been reported previously for the full sample of 901 BLSA men (10). Consistent with these findings, mean T and FTI (±SD) in the present sample were 421.5 ± 100.7 and 5.4 ± 2.4) ng/dl, respectively, and the correlations with age were r = −0.30 ($P < 0.001$) for T and r = −0.54 ($P < 0.001$) for FTI. Table 1 presents the mean scores, mean annual rates of change, and the correlations of these measures with age for all cognitive variables assessed in this study.

Multiple regression analyses were performed with neuropsychological outcomes as dependent variables. Table 2 shows the standardized regression coefficients, adjusted for age, education, smoking status, BMI, coronary heart disease, diabetes, and alcohol consumption, for the effects of T and FTI levels (both baseline and mean) on cognitive status (mean) and annual rates of change (change). The results of the regression analyses indicated that T levels were largely

TABLE 1. Baseline performance, annual rates of change, and correlations with age for cognitive tests

Cognitive tests	Cognitive outcome measure (n)	Mean (SD) test score	Correlation with age
Memory			
BVRT ®	Baseline (320)	4.90 (2.80)	0.35[c]
	Change (113)	0.25 (0.52)	0.15
CVLT-A	Baseline (198)	47.57 (10.81)	−0.18[b]
CVLT-D	Baseline (198)	9.56 (3.45)	−0.17[b]
CVLT-R	Baseline (192)	92.28 (6.05)	−0.15[a]
Spatial ability			
ROT	Baseline (195)	80.03 (34.80)	−0.26[c]
Visuomotor scanning and attention			
TRAILSA ®	Baseline (257)	40.82 (15.07)	0.20[c]
	Change (86)	0.46 (3.66)	−0.12
TRAILSB ®	Baseline (255)	92.21 (36.80)	0.16[a]
	Change (86)	2.58 (7.27)	0.10
DIGFOR	Baseline (211)	8.34 (2.26)	−0.15[a]
DIGBAC	Baseline (211)	7.39 (2.36)	−0.13[a]
Verbal knowledge/language			
Vocabulary	Baseline (195)	37.1 (9.50)	−0.11
FLUCAT	Baseline (257)	15.16 (3.16)	−0.17[b]
	Change (97)	−0.41 (0.56)	−0.01
FLULET	Baseline (257)	15.30 (4.30)	−0.04
	Change (97)	−0.28 (0.67)	−0.03
Mental status			
MMSE	Baseline (255)	28.68 (1.24)	−0.20[c]
	Change (89)	−0.05 (0.42)	0.18
Depressive symptoms			
CES-D	Baseline (276)	5.18 (4.73)	0.01
	Change (102)	0.13 (1.33)	0.06

Data represent mean (SD). ® Reversed scoring scale, higher scores represent poorer performance; baseline, cognitive test score at baseline assessment; change, within-individual slopes calculated to assess the annualized rates of change.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

TABLE 2. Adjusted standardized regression coefficients for TT and the FTI with cognitive outcome as dependent measures

Cognitive test	Cognitive outcome measure (n)	T measure			
		TT		FTI	
		Baseline	Mean	Baseline	Mean
Memory					
BVRT ®	Status (317)	−0.014	0.077	−0.165[b]	−0.215[c]
	Change (111)	0.129	0.031	−0.279[b]	−0.308[b]
CVLT-A	Status (193)	−0.025	0.033	0.202[a]	0.274[c]
CVLT-D	Status (193)	0.024	0.050	0.127	0.178[a]
CVLT-R	Status (187)	−0.032	−0.004	0.137	0.179[a]
Spatial ability					
ROT	Status (190)	−0.087	0.029	0.174[a]	0.286[c]
Visuomotor scanning and attention					
TRAILSA ®	Status (254)	0.013	−0.035	−0.066	−0.145[a]
	Change (84)	−0.014	−0.033	−0.030	−0.087
TRAILSB ®	Status (252)	0.005	−0.058	−0.093	−0.175[b]
	Change (81)	0.177	0.130	0.148	0.088
DIGFOR	Status (206)	−0.077	−0.095	−0.095	−0.054
DIGBAC	Status (206)	−0.182[a]	−0.181[a]	−0.091	−0.108
Verbal knowledge/language					
Vocabulary	Status (190)	−0.137	−0.132	0.031	0.043
FLUCAT	Status (256)	−0.021	−0.022	0.010	0.042
	Change (97)	0.134	0.124	−0.093	−0.016
FLULET	Status (256)	−0.097	−0.090	−0.055	−0.026
	Change (97)	0.096	0.067	−0.118	−0.088
Mental status					
MMSE	Status (252)	0.003	0.026	0.026	0.104
	Change (87)	0.024	0.089	−0.187	−0.089
Depressive symptoms					
CES-D	Status (273)	0.003	0.038	−0.060	−0.044
	Change (100)	−0.114	−0.060	0.045	0.021

® Reversed scoring scale, higher scores represent poorer performance; Status, within-individual mean performance across repeated test administrations; Change, within-individual slopes calculated to assess the annualized rates of change.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

unrelated to cognitive performance. The only statistically significant results were negative associations between baseline T and mean T and the attention/concentration index (Digit Span backward).

In contrast, the FTI proved to be a robust predictor of neuropsychological outcomes in a number of cognitive domains, as shown in the last two columns of Table 2, which contain the multiply adjusted regression coefficients for the effects of the FTI (both baseline and mean) on all cognitive outcome variables. High levels of mean FTI were associated with more positive outcomes on measures of visual memory (BVRT); immediate, delayed, and recognition verbal memory (CVLTA, CVLTD, and CVLTR); visuomotor scanning (Trail A and Trails B); and visuospatial rotation (ROT). Similar results were obtained using baseline FTI, with high baseline FTI associated with more positive outcomes on measures of visual memory (BVRT), immediate verbal memory (CVLTA), and mental rotation (ROT). The FTI was not associated with performance on measures of verbal knowledge, general mental status, or depression. Analysis of those cognitive tests for which there were sufficient data to allow for longitudinal analysis of cognitive decline revealed that high levels of both baseline FTI and mean FTI were associated with a reduced rate of decline in visual memory (BVRT).

In addition, we calculated a rate of change in both T and FTI in subjects with at least three measures and used this variable to predict cognitive outcomes. This variable did not prove to be a significant predictor of either cognitive status or cognitive decline in our sample.

Effects of hypogonadism on neuropsychological outcomes

To assess the effects of hypogonadism on cognitive status and cognitive change, men classified as hypogonadal or eugonadal based on their FTI calculations were compared for cognitive status and cognitive decline using analysis of covariance (Table 3). Age-normed cognitive scores were used as dependent variables to control for the fact that men classified as hypogonadal were, on the average, 8 yr older at baseline.[1] Men classified as hypogonadal had lower cognitive status on measures of visual memory (BVRT), immediate verbal memory (CVLTA), delayed verbal memory (CVLTD), visuospatial rotation (ROT), and visuomotor scanning (TRAILS-A and TRAILS-B). Analysis of rate of cognitive decline indicated that hypogonadism was associated with increased cognitive decline on the measure of visual memory (BVRT). Measures of verbal knowledge, mental status, and depressive symptomatology were not significantly different between hypogonadal and eugonadal men.

[1] Analyses also were performed on the raw cognitive scores while incorporating age as a covariate in the model. This analysis yielded virtually identical results.

TABLE 3. Performance on cognitive measures as a function of gonadal status

Cognitive tests	Cognitive outcome (no. hypogonadal/ no. eugonadal)	Hypogonadal	Eugonadal
Memory			
BVRT ®	Status (134/188)	0.182 (1.05)	−0.134 (0.92)[c]
	Change (63/49)	0.377 (0.66)	0.130 (0.40)[b]
CVLT-A	Status (80/113)	−0.258 (1.09)	0.178 (0.89)[b]
CVLT-D	Status (80/113)	−0.181 (1.01)	0.113 (0.97)[a]
CVLT-R	Status (79/113)	−0.132 (0.96)	−0.082 (1.02)
Spatial ability			
ROT	Status (78/112)	−0.289 (0.88)	0.207 (1.03)[b]
Visuomotor scanning and attention			
TRAILSA ®	Status (111/143)	0.139 (1.05)	−0.124 (0.92)[a]
	Change (52/33)	0.403 (3.25)	0.547 (4.28)
TRAILSB ®	Status (111/143)	0.139 (1.11)	−0.119 (0.88)[b]
	Change (50/32)	1.77 (7.25)	3.21 (6.49)
DIGFOR	Status (87/119)	0.017 (1.03)	0.024 (0.96)
DIGBAC	Status (87/119)	0.120 (0.97)	−0.075 (1.01)
Verbal knowledge/language			
Vocabulary	Status (79/113)	0.086 (0.93)	−0.064 (1.04)
FLUCAT	Status (112/144)	−0.014 (0.95)	0.022 (0.97)
	Change (56/41)	−0.340 (0.58)	−0.500 (0.52)
FLULET	Status (112/144)	−0.020 (1.00)	0.002 (1.05)
	Change (56/41)	−0.234 (0.62)	−0.332 (0.74)
Mental status			
MMSE	Status (112/144)	−0.031 (0.95)	0.024 (1.05)
	Change (55/33)	−0.085 (0.73)	−0.118 (0.43)
Depressive symptoms			
CES-D	Status (115/163)	0.092 (0.99)	−0.045 (1.00)
	Change (54/46)	−0.099 (1.14)	0.159 (1.54)

Data represent mean (SD). ® Reversed scoring scale, higher scores represent poorer performance; Status, within-individual mean performance across repeated test administrations; Change, within-individual slopes calculated to assess the annualized rates of change.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

Discussion

The results of the present study demonstrate a substantial association between long-term endogenous serum FTI calculations and selected aspects of cognitive processing in nondemented older men, suggesting a protective effect on some aspects of cognition. Higher FTI values, albeit within the normal range, were associated with better performance on measures of visuospatial processing, visual memory, visuomotor scanning, and multiple measures of verbal memory. No substantial relationships were found between circulating T or FTI concentrations and measures of mental status, verbal knowledge, or depression. Moreover, when men were classified as either hypogonadal or eugonadal based on their FTI, hypogonadal men had lower cognitive status for measures of visual and verbal memory, visuomotor scanning, and visuospatial rotation.

In addition to substantial relationships between FTI and cognitive status, there was a significant association of FTI with rate of decline in visual memory. In this population, men with higher FTI concentrations exhibited reduced rates of decline on the BVRT. Mirroring this finding was the observation that men classified as hypogonadal showed a faster rate of cognitive decline on this test. This finding may be clinically significant, as accelerated rates of decline on the BVRT in BLSA participants predict a diagnosis of dementia and cognitive decline years later (39). Moreover, previous studies in the BLSA have shown that longitudinal decline on the BVRT is modulated by estrogen replacement status in women (4).

For virtually all tests for which we detected significant findings, the FTI proved to be a better predictor of performance than did total T concentrations. This finding provides indirect evidence that the associations observed are physiologically significant, as the FTI is a better predictor of true bioavailable T than is the total T measurement (30). Moreover, when multiple samples from each individual, rather than a single baseline hormone measurement, were used, the strengths of the associations were generally enhanced. We attribute the latter finding to the well known observation that multiple measurements of a variable increase the reliability of the measurement. In addition, whereas baseline T and FTI measures may be separated by a number of years from the cognitive assessments, the sequential T measures in our study bracketed in time the cognitive measures, ensuring that both cognitive and hormonal status variables were assessed contemporaneously.

Previous studies of endogenous T concentrations and cognition have yielded somewhat conflicting results, in part because these studies were performed in relatively small samples of college-age men and women who have not begun to show substantial age-related hormone depletion or cognitive decline (17–22). In the only other population-based study of the cognitive correlates of androgen loss in men, Barrett-Connor *et al.* (23) found that higher bioavailable T

was associated with better scores on measures of long-term memory. However, the latter study relied on measures of T at a single point in time to predict cognitive status years later, which may have reduced the likelihood of detecting associations between T status and some cognitive variables. The cognitive test battery employed in the present study contained only minimal overlap with the measures employed by Barrett-Connor *et al.*, making direct comparisons between the two studies difficult.

Numerous investigations support the biological plausibility of a protective effect of T on cognitive function. Studies in nonhuman species demonstrate that gonadal steroids affect the development and/or expression of sexual and maternal behavior, activity levels, aggression, juvenile play, motor activity, and learning and memory (11, 12, 40–42). Regions of the rat brain thought to subserve aspects of spatial learning, including the hippocampus, are targets of gonadal steroids (40, 43). In primates, androgens present in the prenatal or postnatal period affect learning abilities as well as maturation of the cortical regions subserving these abilities (44, 45). In adult animals, androgen receptors have been found in high concentration in hippocampal CA1 pyramidal cells (46). Androgen treatment may prevent *N*-methyl-D-aspartate excitotoxicity in hippocampal CA1 neurons (47) and may facilitate recovery after injury by promoting fiber outgrowth and sprouting in hippocampal neurons (48). Moreover, T administration increases nerve growth factor levels in the hippocampus, septum, and neocortex and induces an up-regulation of nerve growth factor receptors in the forebrain (49). Moreover, T is aromatized to estradiol within the brain, so that T also may exert its effects on the nervous system indirectly via estrogen receptor-mediated mechanisms. As circulating levels of estrogens are also reduced in older compared with younger men (23), the possibility that endogenous differences in estrogens contribute to our observed findings with respect to memory cannot be excluded. In contrast, age-associated changes in estradiol concentration cannot account for our findings for measures of spatial rotational ability, because increased estrogen is associated with reduced spatial ability in younger women (50) and has no apparent effect on spatial performance in older women (5).

Studies of postmenopausal women reveal protective effects of ERT on several cognitive domains (2–6), most notably verbal memory, and suggest that ERT may reduce the incidence and delay the onset of Alzheimer's disease (7–9). In the present study higher free T levels were associated with higher scores on numerous indexes of the CVLT, a measure of verbal learning and memory. Hypogonadism was also associated with poorer outcomes on this test. To our knowledge, there are no prospective studies investigating the possibility that, as with ERT, higher endogenous T or exogenous T supplementation may be associated with reduced incidence of Alzheimer's disease.

Although our data suggest a protective effect of T on neuropsychological performance in elderly men, our data cannot confirm a causal impact of T on neuropsychological outcome. It is possible, for example, that high-normal T concentrations in older men may be reflective of enhanced physical or mental health. Several features of our analyses mitigate against such an interpretation. Firstly, we controlled statistically for several health-related factors that may potentially affect T concentrations or cognitive performance, including smoking, alcohol use, BMI, heart disease and diabetes status, age, and education levels. Moreover, individuals with cancer (non-skin), dementia, or other neurological problems were excluded from the study. Most importantly, as noted above, the FTI showed much stronger associations with cognition than did total T, attesting to the relative specificity of the findings. The fact that the FTI, but not total T, showed significant relationships with cognitive performance and cognitive decline suggests that our results are not simply a result of the confounding influence of age, as both measures show a negative association with age. Nevertheless, because of the associational nature of our study, we cannot eliminate the possibility that low T/free T levels may serve as a marker for, rather than a causative factor in, age-related cognitive decline.

Currently, there is a discordance between the rapidly expanding number of studies of the possible neuroprotective effects of estrogens in postmenopausal women and the relative dearth of analogous research on the putative effects of T on brain function in older men. The results of the current study taken together with those of recent small scale testosterone intervention trials in elderly men suggest that the progressive physiological decline in T secretion in aging men contributes to selective losses in cognitive functions that may be reversed at least in part by T supplementation. Larger scale investigations are warranted to assess the safety of T administration, whether it can prevent or attenuate cognitive loss in healthy aging men and/or men with Alzheimer's disease, and whether neuroprotection is mediated primarily via androgenic or estrogenic mechanisms, or both. Such studies will also contribute to a fundamental understanding of the effects of sex steroid hormones on brain aging.

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