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BRIEF REPORT

Interaction between Testosterone and Apolipoprotein E ϵ4 Status on Cognition in Healthy Older Men

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Context: Reduced testosterone levels have been implicated as a potential causative factor in cognitive decline with older age. Men who possess the apolipoprotein E (APOE) ϵ4 allele have an increased risk of developing Alzheimer's disease; however, no studies have examined whether the influence of testosterone on cognition in healthy older men may be modulated by this genetic predisposition.

Objective: The objective of the study was to investigate the association between serum testosterone concentrations and cognitive performance in healthy older men, taking into account APOE ϵ4 status.

Design: This was a cross-sectional study conducted from 2003 to 2004.

Setting: The study population consisted of community-dwelling males residing in Perth, Western Australia.

Participants: Healthy men over 55 yr, free of cognitive impairment and dementia (n = 45), were included in the study.

Main Outcome Measures: Participants had fasting early morning blood samples for testosterone and SHBG and were assessed for mood as well as indices of general cognition, verbal and visual memory, executive functioning, working memory, and attention.

Results: There was a significant interaction between calculated free testosterone (FT) and APOE ϵ4 on general cognition ($P = 0.01$) and executive functioning, working memory, and attention ($P < 0.01$). Higher levels of FT were associated with better general cognition in non-ϵ4 carriers ($P = 0.01$). By contrast, in ϵ4 carriers higher FT levels were associated with lower scores on tests of executive functioning, working memory, and attention ($P = 0.02$). In men at increased risk for Alzheimer's disease, higher testosterone levels were not associated with better cognitive function.

Conclusions: Cross-sectional and prospective studies of testosterone and cognition in older men should take into account APOE ϵ4 status. **(*J Clin Endocrinol Metab* 91: 1168–1172, 2006)**

LOWER TESTOSTERONE concentrations have been associated with lower scores on tests of cognitive ability (1, 2) and the subsequent development of Alzheimer's disease (AD) (3). Testosterone deficiency modulates neuronal processes capable of increasing the risk for AD (4), and beneficial effects of testosterone supplementation have been reported on specific parameters of cognition in older men (5, 6). The apolipoprotein E (APOE) ϵ4 allele is thought to be the major genetic risk factor for AD (7) and may independently modulate normal age-related cognitive change (8). An association among low testosterone, APOE ϵ4, and AD has been reported (9), but it is not known whether testosterone and APOE ϵ4 interact to modulate cognition in men before the development of AD or whether differential effects of testosterone on cognition exist that are influenced by the absence or presence of ϵ4. We investigated the association among testosterone concentrations, genetic risk for AD, and cognitive abilities in healthy older men.

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Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E; CAMCOG-R, Cambridge Cognitive Assessment-revised; D-KEFS, Delis and Kaplan Executive Function System; EFWMA, executive functioning, working memory, and attention; FSIQ, full-scale intelligence quotient; FT, free testosterone; MANCOVA, multivariate analysis of covariance; VR, visual reproduction; WMS-III, Wechsler Memory Scale-Third Edition.

Patients and Methods

Patients

Our institutional ethics committee approved this study and informed consent was obtained from participants. Community-dwelling men over 55 yr of age with no history of cognitive impairment, dementia, or other major medical illness were recruited. Exclusion criteria were hormone-suppressive therapy, testosterone therapy, medications known to affect bone metabolism within the past 3 months, significant prostate disease, abnormal liver function, hypothyroidism, major sleep disturbance, consumption of alcohol greater than 21 U/wk, psychiatric disorders, stroke,

head injuries, scores 80 or less on the Cambridge Cognitive Assessment-revised (CAMCOG-R) (10) (identifying frank cognitive deficits), and 1.5 SD or greater below the mean for age on measures of verbal learning and memory (identifying mild cognitive impairment). After these exclusions there were 45 healthy men studied.

Procedures

Information concerning: current medical problems, medications, lifestyle factors, and years of formal education (cumulative years of primary, secondary, and tertiary education) were obtained, and blood pressure and body mass index (kilograms per square meter) was measured. Blood samples were collected between 0830 and 1030 h after overnight fasting. A standard breakfast (energy value 938 kJ) was provided before cognitive testing of constructs cited as relevant in the AD literature (11) and parameters influenced by testosterone concentrations (1, 2, 5, 6).

Biochemical assessments. Total testosterone concentrations were determined by coated tube RIA (Coat-A-Count, Diagnostic Products Corp.-BioMediq, Doncaster, Australia) with coefficient of variation being 8.9% at 2.5 nmol/liter and 8.5% at 14 nmol/liter. SHBG concentrations were determined via chemiluminescent immunoassay on an Immulite 2000 analyzer (Diagnostic Products Corp.-BioMediq) with coefficient of variation of 6.8% at 5.1 nmol/liter and 9.3% at 82.5 nmol/liter. Free testosterone (FT), specifically the fraction not bound to either SHBG or albumin, was calculated using a previously validated method (12).

APOE genotyping. Peripheral blood leukocyte DNA was extracted and APOE genotyping was performed as previously described (13).

Mood assessments. Depressive symptomatology was assessed via the Geriatric Depression Scale (14). Current anxiety levels were assessed via the State Anxiety Scale from the Spielberger State Trait Anxiety Inventory (15).

Neuropsychological tests

The National Adult Reading Test (16) was administered to derive an estimate of full-scale intelligence quotient (FSIQ) to control for differences in reading and estimated intellectual ability. The following tests were administered to obtain the listed cognitive constructs:

1. General cognition. CAMCOG-R (10) total score was analyzed to obtain an index of performance on orientation, memory, language, praxis, attention, tactile perception, calculation, abstract thinking, and visual perception tasks.

2. Immediate memory. Immediate recall for verbal information on Wechsler Memory Scale-Third Edition (WMS-III) (17) Wordlists and visual information on the visual reproduction (VR) subtests was analyzed.

3. Delayed memory. Comprised delayed recall, recognition, and percentage retention of verbal and visual information over a 25-min delay period (via the WMS-III Wordlists and VR subtests respectively). Scores on VR copy condition were used to control for differences in visuospatial ability between participants and as a statistical covariate in the visual memory data analyses.

4. Executive functioning, working memory, and attention (EFWMA). The Delis and Kaplan Executive Function System (D-KEFS) Color-Word Interference Test (18), color-naming, word-reading, inhibition, and inhibition-switching components were administered in series to obtain measures of executive functioning. Completion times for inhibition and inhibition switching were analyzed. To assess attention and working memory, total correct responses on the WMS-III letter number sequencing (17) were analyzed.

5. Verbal fluency. Total correct responses on the D-KEFS verbal fluency test (18) (letter fluency, category fluency, and category switching conditions) and the percentage switching accuracy and errors made across all three conditions were analyzed.

A priori composites were calculated for each construct by converting raw scores for each participant on each test to age-adjusted scaled scores according to established norms (17, 18) and averaging the scaled scores. Composites reflecting broader constructs are more appropriate measures of cognitive ability and exhibit higher reliability than the individual components represented by single neuropsychological tests (19). These constructs were used as dependent variables in hierarchical linear regression analyses to examine the FT by APOE ϵ4 status interaction on cognition.

Statistical analyses

Data were analyzed using the Statistical Package for Social Sciences (version 11.5; SPSS Inc., Chicago, IL). Cohort characteristics were explored via one-way ANOVA for continuous data and using the Pearson method for 2×2 contingency tables (χ^2) for categorical data. Homogeneity of variance and normality of data distributions were evaluated for FT and neuropsychological data in the groups [ϵ4 carriers (ϵ4+) *vs.* noncarriers (ϵ4−)]. Analysis of covariance/multivariate analysis of covariance (MANCOVA) was conducted to compare the effect of ϵ4 status (absence *vs.* presence) on FT, measures of mood, and cognitive performance, separately. Where appropriate, age and/or VR total copy score served as statistical covariates. The interaction between FT and ϵ4 status on composites of cognitive functioning was further examined via hierarchical linear regression analyses. Main effects involving FT concentrations were subsequently explored via Pearson's correlation coefficient (r).

Results

Characteristics of the cohort stratified by ϵ4 status are reported in Table 1. The ϵ4+ group was significantly older than the ϵ4− group [$F_{(1, 43)} = 5.75$, $P = 0.02$]. The groups were similar for years of education, estimated FSIQ, body mass index, blood pressure, cigarette and alcohol use, current medications, and medical history. SHBG was higher in the ϵ4+, compared with ϵ4− group (44.4 ± 16.4 *vs.* 34.5 ± 14.0 nmol/liter, $P = 0.04$); however, FT was similar in both groups (0.27 ± 0.06 *vs.* 0.26 ± 0.07 nmol/liter, respectively).

Evaluation of group differences

Analysis of covariance was performed using FT as the dependent variable and ϵ4 status as the independent variable, with age as a statistical covariate. Age significantly influenced FT [$F_{(1, 42)} = 8.79$, $P = 0.01$]. After adjusting for age, there was no significant effect of ϵ4 status on FT [$F_{(1, 42)} = 2.95$, $P = 0.09$]. Unadjusted group means and SDs for ϵ4+ and ϵ4− groups for FT are presented in Table 1.

Mood

MANCOVA was performed to examine group differences on anxious and depressed mood. After adjusting for age, there was no significant effect of ϵ4 status on depressed and anxious mood ($F_{(2, 41)} = 1.18$, $P = 0.32$). The statistical covariate age did not influence mood ($F_{(2, 41)} = 0.08$, $P = 0.92$).

Cognitive performance

Separate MANCOVAs were performed using the individual measures of cognitive functioning as dependent variables, with age and VR total copy score as statistical covariates where appropriate. The ϵ4+ and ϵ4− groups performed similarly on general cognition (CAMCOG-R total score), visual memory (WMS-III VR immediate and delayed recall, percentage retention, and recognition indices), EFWMA (WMS-III letter number sequencing total recall and D-KEFS inhibition and inhibition-switching completion times), and verbal fluency (D-KEFS verbal fluency test indices).

TABLE 1. Characteristics of healthy older men stratified by APOE ϵ4 status

Variable	ϵ4+ (n = 16)	ϵ4− (n = 29)	*P* value
Age (yr)[a]	74.31 ± 5.82	69.52 ± 6.71	0.02
Education (yr)[a]	13.31 ± 4.11	11.41 ± 3.27	0.10
NART estimated premorbid FSIQ[a]	118.19 ± 6.66	120.62 ± 18.50	0.62
Body mass index (kg/m^2)[a]	26.79 ± 2.94	28.85 ± 3.68	0.06
Medical history (%)			
Diabetes	12.50	13.79	0.90
Cardiovascular risk factors[b]	25.00	24.14	0.95
Hypertension	43.75	27.59	0.27
Current medication (%)			
Antihypertensives	18.75	17.24	0.90
Anticoagulants	25.00	17.24	0.53
Nonsteroidal antiinflammatories	43.75	34.48	0.54
Sedatives/hypnotics	6.25	13.79	0.27
Lifestyle factors			
Alcohol use (standard drinks/day)[a]	1.22 ± 1.21	1.63 ± 1.11	0.26
Cigarette smoking (%)			0.97
Current	6.25	6.90	
Never	62.50	58.62	
Ever	31.25	34.48	
Blood pressure (mm Hg)[a]			
Systolic	141.08 ± 25.69	145.71 ± 20.57	0.51
Diastolic	79.73 ± 11.78	82.14 ± 8.93	0.44
Biochemical assessments			
Calculated free testosterone (nmol/liter)[a, c]	0.27 ± 0.06	0.26 ± 0.07	0.09

NART, National Adult Reading Test.
[a] Mean ± SD.
[b] Individuals with a history of heart disease, hypercholesterolemia, and statin use.
[c] Age-adjusted ANCOVA.

Significant group differences emerged on verbal memory performance. MANCOVA was performed using raw scores on the WMS-III Wordlists immediate and delayed recall, percentage retention, and recognition indices as dependent variables. Age significantly influenced these dependent variables [$F_{(4, 39)} = 3.42$, $P = 0.02$]. After adjusting for age, the overall MANCOVA was significant with respect to the influence of ϵ4 status [$F_{(4, 39)} = 3.22$, $P = 0.02$] on these four measures of verbal memory. Because the overall MANCOVA was significant, subsequent analysis of each individual dependent variable showed significant differences in scores between ϵ4+ and ϵ4− groups, on delayed recall [$F_{(1, 42)} = 9.47$, $P < 0.01$] and percentage retention [$F_{(1, 42)} = 8.31$, $P < 0.01$] indices of the WMS-III Wordlists.

The interaction between ϵ4 and FT on cognitive performance in the cohort was explored via hierarchical linear regression analyses, using FT, ϵ4 status, and ϵ4 × FT as predictors of the following: 1) CAMCOG-R total score; 2) immediate memory; 3) delayed memory; 4) EFWMA; and 5) verbal fluency constructs. Hierarchical linear regression models are presented in Table 2. In line with the previous MANCOVA analyses, ϵ4 status was significantly related to delayed memory performance. The ϵ4 × FT interaction was significantly related to performance on the CAMCOG-R and EFWMA. These ϵ4 × FT interactions were subsequently explored within ϵ4+ and ϵ4− groups by calculating separately the correlation coefficient (*r*).

The linear relationship between CAMCOG-R scores and FT within the ϵ4− and ϵ4+ groups is summarized in Fig. 1, A and B, respectively. CAMCOG-R scores positively correlated with FT ($r = 0.46$, n = 29, $P = 0.01$) in the ϵ4− group. There was significant negative correlation between FT concentrations and EFWMA in the ϵ4+ group ($r = -0.58$, n = 16, $P = 0.02$), as shown in Fig. 1, C and D.

Discussion

We systematically investigated the interaction between ϵ4 status and testosterone concentrations on cognition in apparently healthy older men. No difference in FT between the groups was noted in this cohort. Consistent with previous findings (20), we observed that APOE ϵ4 carriers had lower

TABLE 2. Hierarchical linear regression models for composites of cognitive constructs

	Unstandardized β	SE	t	*P* value
General cognition				
ϵ4 status	−0.69	1.60	−0.43	0.67
FT	16.42	11.45	1.44	0.16
ϵ4xFT	−65.16	23.96	−2.72	0.01
Immediate memory				
ϵ4 status	−1.19	0.75	−1.58	0.12
FT	3.76	5.40	0.70	0.49
ϵ4xFT	−14.39	12.06	−1.19	0.24
Delayed memory				
ϵ4 status	−1.54	0.65	−2.38	0.02
FT	4.76	4.64	1.03	0.31
ϵ4xFT	−8.88	10.46	−0.85	0.40
EFWMA				
ϵ4 status	−0.55	0.72	−0.76	0.45
FT	−3.04	5.16	−0.59	0.56
ϵ4xFT	−32.57	10.58	−3.08	<0.01
Verbal fluency				
ϵ4 status	−0.56	0.54	−1.03	0.31
FT	0.66	3.87	0.17	0.86
ϵ4xFT	−11.49	8.62	−1.33	0.19

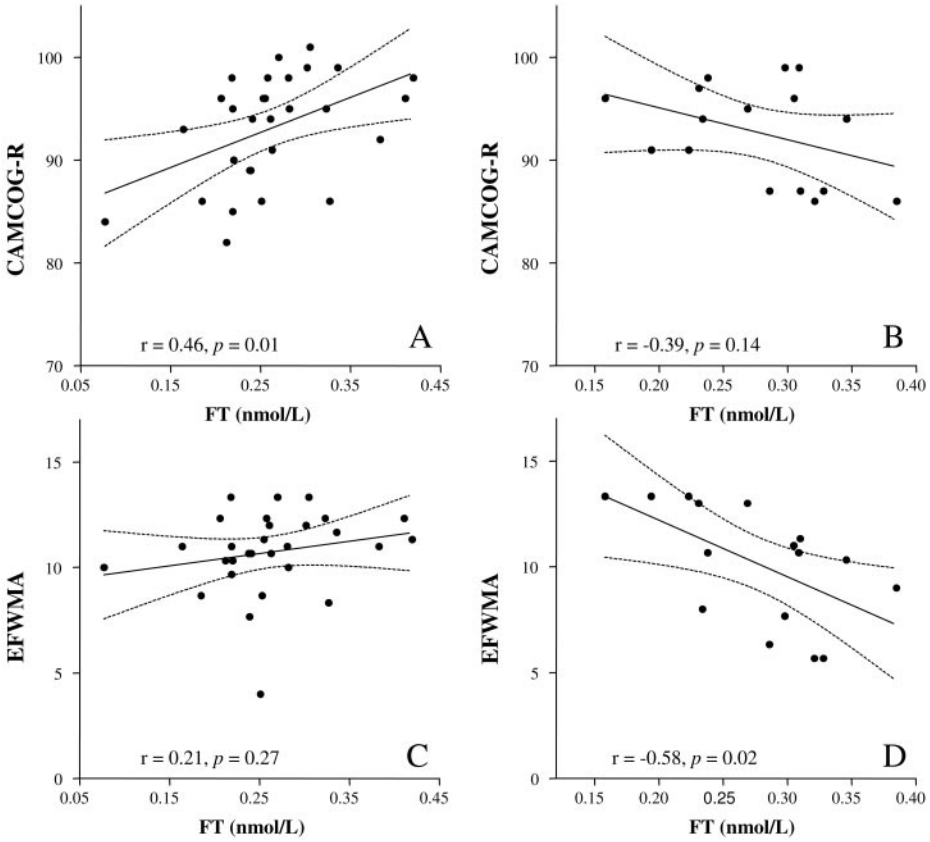

FIG. 1. Scatterplots of calculated FT *vs.* composites of cognitive constructs. Regression line and 95% confidence intervals as well as Pearson correlation coefficient (*r*) are shown. A, FT *vs.* CAMCOG-R scores for the ϵ4− group. B, FT *vs.* CAMCOG-R scores for the ϵ4+ group. C, FT *vs.* the EFWMA composite for the ϵ4− group. D, FT *vs.* the EFWMA composite for the ϵ4+ group.

scores on delayed recall for verbal information compared with non-ϵ4 carriers.

The novel finding is the interaction between FT and ϵ4 status on general cognition and EFWMA. In cognitively healthy older men without the ϵ4 allele, FT levels were positively correlated with general cognitive ability (*i.e.* CAMCOG-R scores). By contrast, in men who possess the ϵ4 allele, FT levels correlated negatively with EFWMA. These statistically significant observations are of potential clinical relevance as testosterone supplementation has been postulated to have beneficial effects on cognition (5, 6); however, this was not addressed here. Because higher testosterone concentrations were associated with lower performance on EFWMA in ϵ4 carriers, testosterone supplementation may not be beneficial in this group (who are at higher relative risk for AD). It may be necessary to stratify men by ϵ4 status when examining the relationship between testosterone and cognition or when designing interventional trials of testosterone replacement for the prevention of cognitive decline in older men.

Particular strengths of this study were the careful case selection, allowance for potential confounders including mood, detailed neuropsychological assessments, and the examination of a cohort free of cognitive impairment and dementia. Additionally, composite measures of cognitive performance were determined *a priori* and used to increase the validity of our analysis. Limitations of this study are the relatively small sample size, the cross-sectional nature, and the need to adjust for age differences between the groups. A larger study with follow-up to provide a longitudinal or prospective component would be highly desirable to extend our present findings.

Briefly, we observed an interaction between testosterone and APOE ϵ4 on cognition in healthy older men. In men not possessing an ϵ4 allele, higher testosterone concentrations were associated with better general cognition. However, higher testosterone concentrations in men who possessed an ϵ4 allele had lower performance on executive functioning, working memory, and attention tasks. APOE ϵ4 status should be taken into account when investigating the relationship between androgens and cognition in older men.

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References

1. **Moffat SD, Zonderman AB, Metter EJ, Blackman MR, Harman SM, Resnick SM** 2002 Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men. J Clin Endocrinol Metab 87:5001–5007
2. **Yaffe K, Lui LY, Zmuda J, Cauley J** 2002 Sex hormones and cognitive function in older men. J Am Geriatr Soc 50:707–712
3. **Moffat SD, Zonderman AB, Metter EJ, Kawas C, Blackman MR, Harman SM, Resnick SM** 2004 Free testosterone and risk for Alzheimer disease in older men. Neurology 62:188–193
4. **Gouras GK, Xu H, Gross RS, Greenfield JP, Hai B, Wang R, Greengard P** 2000 Testosterone reduces neuronal secretion of Alzheimer's β-amyloid peptides. Proc Natl Acad Sci USA 97:1202–1205
5. **Cherrier MM, Asthana S, Plymate S, Baker L, Matsumoto AM, Peskind E, Raskind MA, Brodkin K, Bremner W, Petrova A, LaTendresse A, Craft S** 2001 Testosterone supplementation improves spatial and verbal memory in healthy older men. Neurology 57:80–88
6. **Kenny AM, Bellantonio S, Gruman CA, Acosta RD, Prestwood KM** 2002 Effects of transdermal testosterone in cognitive function and health perception in older men with low bioavailable testosterone levels. J Gerontol 57A:M321–M325
7. **Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD** 1993 Apolipoprotein E: high-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer's disease. Proc Natl Acad Sci USA 90:1977–1981
8. **Deary IJ, Whiteman MC, Pattie A, Starr JM, Hayward C, Wright AF, Carothers A, Whalley LJ** 2002 Cognitive change and the APOE ϵ4 allele. Nature 418:932
9. **Hogervorst E, Lehmann DJ, Warden DR, McBroom J, Smith AD** 2002 Apolipoprotein E ϵ4 and testosterone interact in the risk of Alzheimer's disease in men. Int J Geriatr Psychiatry 17:938–940
10. **Roth M, Tym E, Mountjoy CQ, Huppert FA, Hendrie H, Verma S, Goddard R** 1986 CAMDEX: a standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. Br J Psychiatry 149:698–709
11. **Locascio JJ, Growdon JH, Corkin S** 1995 Cognitive test performance in detecting, staging, and tracking Alzheimer's disease. Arch Neurol 52:1087–1099
12. **Vermeulen A, Verdonck L, Kaufman JM** 1999 A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84:3666–3672
13. **Burkhardt MS, Foster JK, Laws SM, Baker LD, Craft S, Gandy SE, Stuckey BGA, Clarnette R, Nolan D, Hewson-Bower B, Martins RN** 2004 Oestrogen replacement therapy may improve memory functioning in the absence of APOE ϵ4. J Alzheimers Dis 6:221–228
14. **Brink TL, Yesavage JA** 1982 Somatoform disorders: differentiation of conversion, hypochondriacal, psychophysiologic, and related disorders. Postgrad Med 72:189–194, 196, 198
15. **Spielberger CD, Gorsuch RL, Lushene R, Vagg PR, Jacobs GA** 1970 Spielberger State Trait Anxiety Inventory. Menlo Park, CA: Mind Garden Inc.
16. **Nelson HE** 1982 National Adult Reading Test (NART). Windsor, Berkshire, UK: The NFER-Nelson Publishing Co.
17. **Wechsler D** 1997 Wechsler Memory Scale-III. San Antonio TX: The Psychological Corp.
18. **Delis DC, Kaplan E** 2001 Delis-Kaplan Executive Function System (D-KEFS). San Antonio TX: The Psychological Corp.
19. **Ossenkopp KP, Mazmanian DS** 1985 The measurement and integration of behavioural variables: aggregation and complexity as important issues. Neurobehav Toxicol Teratol 7:95–100
20. **Bondi MW, Salmon DP, Monsch AU, Galasko D, Butters N, Klauber MR, Thal LJ, Saitoh T** 1995 Episodic memory changes are associated with the APOE-ϵ4 allele in nondemented older adults. Neurology 45:2203–2206