



Published in final edited form as:

Clin Endocrinol (Oxf). 2010 March ; 72(3): 393–403. doi:10.1111/j.1365-2265.2009.03692.x.

Association between sex steroids and cognition in elderly men

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SUMMARY

Objective—Examine the association of cognitive function with sex steroid and sex hormone binding globulin (SHBG) levels among elderly men.

Design—Prospective cohort study, The Osteoporotic Fractures in Men Study (MrOS), consisting of 5995 US community dwelling men 65 years or older.

Patients—1602 men chosen randomly from MrOS cohort for sex steroid level measurements by Mass Spectrometry (MS) at baseline. 2623 MrOS participants with sex steroids measured using RIA were also examined.

Measurements—Baseline and follow-up (4.5 years later) performance on two cognitive tests: Trails B (executive function and motor speed) and 3MS (global cognitive function). Baseline total testosterone and estradiol were measured by MS. Free testosterone (free-T) and free estradiol (free-E) were calculated. SHBG was measured by radioimmunoassay. Data were analyzed using linear regression.

Results—Baseline free-T and free-E levels were not associated with cognitive performance or change in cognition, following adjustment for age, education, race, health status and alcohol use. Baseline SHBG levels were inversely associated with follow-up trails B ($p=0.03$) and 3MS performance ($p=0.02$). Higher SHBG was associated with an increased risk of cognitive decline. Total sex steroid levels were not associated with cognitive performance.

Conclusions—Despite large numbers of participants and rigorous sex steroid measurements, we did not observe an association between cognition and either testosterone or estradiol levels. We conclude that endogenous sex steroids in the normal range are not related to executive function or global cognitive function in elderly men. High SHBG deserves further examination as a risk factor for cognitive decline.

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Competing interests/financial disclosure:

ESL: Nothing to disclose, PYW: Nothing to disclose, JSJ: Nothing to disclose, MBN: Nothing to disclose, HAF: Nothing to disclose, KY: Served on the Women's Health Initiative OSMB, LMM: Nothing to disclose, JAL: Nothing to disclose, MLS: Nothing to disclose, ESO: Nothing to disclose

Keywords

Cognition; Testosterone; Estradiol; SHBG; Aging

INTRODUCTION

In men, aging is accompanied by decreases in free testosterone (free-T) levels, with free-T falling by as much as 40% from age 40 to 70 years of age.¹⁻⁴ Aging is also accompanied by declines in memory^{5, 6} and deterioration in psychomotor speed and cognitive flexibility.⁷ With evidence that free-T may have maintenance and neuroprotective actions in brain regions critical for these cognitive domains,⁸⁻¹¹ the decrease in testosterone production in later decades of life may be an important contributor to cognitive aging.

Studies examining the relationship between sex steroids and cognition in older men have reported conflicting findings. In some, higher free (non protein bound) or bioavailable (loosely protein bound) T levels were associated with better cognitive performance;¹²⁻¹⁴ whereas, in others either no association¹⁵⁻²¹ or a negative association between free-T and cognitive performance has been reported.¹⁴ Total testosterone has been less closely associated with cognitive performance.^{12, 13, 20} One study noted that higher total and bioavailable estradiol levels were associated with worse cognitive performance on a measure of global cognition (MMSE); while on a measure of executive function (Trails B) there was a U shaped relationship.²⁰ However, other studies have not corroborated these findings.^{12, 14, 15, 18}

The disparate results may be due to differences in study populations and methodologies. Sex steroid's effects on cognition may be moderated by age-related vulnerability of the brain to cognitive decline, e.g. testosterone levels may be associated with cognition in older but not younger men.¹² Second, there may be critical testosterone levels below or above which cognition is impaired so that only men with very low or very high T levels might experience cognitive impairment.¹³ Finally, the timing of sex steroid measurements in relationship to cognitive testing may be important. Studies with sex hormone levels drawn years prior to cognitive testing^{15, 20} may fail to find an association because testosterone level at the time of cognitive testing is critical.

Unraveling the relationship between sex steroids and cognitive function in men as they age is an important public health issue. The Institute of Medicine issued a report in 2003 highlighting the need for more information about the association between sex steroids and cognitive aging.²² The present study examines data on sex steroids and cognitive performance in a large group of men aged 65 or older in the The Osteoporotic Fractures in Men (MrOS) study. The objectives of our study were to 1) determine the relation between cognitive function, measured specifically by the modified mini-mental status (3MS) and Trails B tests, and testosterone, estradiol and sex hormone binding globulin (SHBG) at a single point in time, 2) to determine if any single baseline measure of the sex steroid or SHBG was associated with future cognitive performance or cognitive decline during follow-up, and 3) to examine whether there was any evidence that a specific level (or threshold) of the sex steroid or SHBG was associated with cognition scores.

STUDY DESIGN AND METHODS

Participants

MrOS is a multi-center prospective study of risk factors for and consequences of vertebral and non-vertebral fractures in older men. The design of MrOS has been described in detail

elsewhere.^{23, 24} Briefly, the MrOS cohort consists of 5995 community dwelling men aged 65 and older. Eligibility criteria were: (1) age \geq 65 years, (2) ability to walk without the assistance of another, and (3) absence of bilateral hip replacements. Approximately 1000 participants were recruited at each of six academic medical centers: Birmingham, AL, Minneapolis, MN, Palo Alto, CA, Pittsburgh, PA, Portland, OR, and San Diego, CA. Recruitment efforts included community mailings and a variety of community outreach and educational activities.²⁴ The Institutional Review Board at each center approved the study protocol, and written informed consent was obtained from all participants.

Participants completed two study visits, which averaged 4.5 years apart. At baseline, participants completed questionnaires regarding medical history, highest level of schooling completed, mental functioning (included mood-oriented items) and physical health (Medical Outcomes Study 12-Item Short Form [SF-12]), and lifestyle characteristics and had fasting morning serum collected.²³ Participants were asked about prescription drugs they took daily or almost daily over the past month including benzodiazepines, narcotics, corticosteroids, selective serotonin reuptake inhibitors, trazodone, or tricyclics. Participants completed cognitive tests at the baseline and follow-up visit.

In this analysis we excluded participants taking exogenous androgens or antiandrogens, as our goal was to examine the association between endogenous sex steroid levels and cognition. Only men with complete data for specified sex hormone levels and cognitive test results were eligible (Fig.).

Cognitive measures

To assess cognitive function, participants completed Part B of the Trail Making Test (Trails B)²⁵ and the Modified Mental State Examination (3MS).²⁶ Trails B is a test of executive function and motor speed.^{27, 28} Executive function includes those brain processes that guide action in accordance with internally generated ideas (i.e. planning and strategizing). The participant sequentially connects a series of dots shifting between numbers and letters (e.g. 1 to A to 2 to B...). Time to complete the test is measured in seconds and faster times indicate better performance. If the subject did not complete the trail within 5 minutes or made 5 mistakes, the test was stopped and a score of 300 seconds was recorded.

3MS is a screening measure for dementia that includes questions in a variety of cognitive domains. It includes the Mini Mental Status Exam (MMSE).²⁹ The maximum score for 3MS is 100 and higher scores indicate better performance.

Sex steroid assays

Primary sex steroid cohort (Mass Spectrometry cohort)—Baseline fasting morning blood was collected for all participants. Serum was prepared immediately after phlebotomy and stored at -70°C . A random sample of 1602 men was chosen to have sex steroids measured by Gas Chromatographic Mass Spectrometry (GC/MS). Total serum testosterone and estradiol were measured using a combined GC negative ionization tandem mass spectrometry (GC/NCI/MS/MS) and liquid chromatographic electrospray tandem mass spectrometry (LC/ESI/MS/MS) bioanalytical method (Taylor Technology; Princeton, NJ, USA). A $1/(\text{concentration})^2$ weighted least squares regression procedure was used to fit a linear function to the calibration data. The lower limit of detection for estradiol is 0.625 pg/mL (2.29 pmol/l), and for testosterone is 25.0 pg/mL (0.09 nmol/l). Duplicate aliquots from each participant's serum were assayed, and the two results were averaged. The intra-assay coefficient of variation (CV) for testosterone was 2.5%, interassay CV 6.0%; the estradiol intra-assay CV was 6.4%, interassay CV 10.1%. Serum SHBG concentrations were measured using an Immulite Analyzer with chemiluminescent substrate (Diagnostic

Products Corp.; Los Angeles, CA, USA). The standard curve ranged from 0.2 to 180 nm/l. The SHBG intra-assay CV was 4.4%, interassay CV 6.0%. Albumin values for the free hormone calculations were obtained from baseline serum using routine colorimetric methods (interassay CV 2.0%). Calculation of the free fractions of testosterone and estradiol use the method described by Södergard et al.³⁰

Secondary sex steroid cohort (RIA cohort)—To be able to compare our results to previous studies which used radioimmunoassay (RIA) to measure sex steroids, we also evaluated the association between sex steroids and cognition in 2623 participants who were selected using a stratified sampling design to have sex steroids measured by RIA. Strata were clinic site (each of six sites), race (white or nonwhite), and availability of a complete set of skeletal imaging procedures (to be used in future analyses of the effects of sex steroids on skeletal change).⁴ Sex hormones were measured using solid-phase ¹²⁵I RIA (Testosterone; Diagnostic Products Corp., Los Angeles, CA) or ultra sensitive RIA (Estradiol; Diagnostic Systems Laboratory Inc., Webster, TX).⁴ 1139 participants had sex steroid levels measured by both RIA and GC/MS. Testosterone levels by RIA and GC/MS were very highly correlated ($r=0.92$). Estradiol levels were modestly correlated ($r=0.52$).

Statistical analysis

The analysis quantified the relationship between sex steroid levels and cognitive performance as measured by Trails B and 3MS. We hypothesized that free serum sex steroids would be the most accurate reflection of brain levels as unbound hormones can cross the blood brain barrier.³¹ We first excluded men who were taking androgens or antiandrogens at baseline ($n=93$), with missing free-T and/or free-E values ($n=124$), or with missing values for cognitive testing at baseline ($n=34$ for Trails B and $n=1$ for 3MS). Pearson correlation coefficients were calculated between sex steroids and cognitive performance. We then applied non-parametric generalized additive models (GAMs) to sex steroid variables and cognition scores (adjusted for age and study site) in order to identify and characterize the appropriate functional form (i.e. linear vs. nonlinear). We generated plots showing the measured data, the fitted model and the trend. These plots illustrated how well the data fit the model and also allowed the visual assessment of any non-linear trends. The GAM model did not suggest any statistically significant nonlinear relationship; therefore we explored the association between sex steroids and cognition further with linear regression. Before applying linear regression models, residuals were examined for non normality visually using quantile-quantile (Q-Q) and histogram plots. Generalized Linear Models were constructed by using sex steroids in quartiles and adjusted only for age. Age-adjusted least-squares means and 95% CIs were calculated.

The dependent variables were baseline, follow-up and change in cognitive test score and the independent variables were the continuous values of free-T, free-E, and SHBG. We also examined the association using quartiles of sex steroids as the independent variable. Because the results were similar, we present only the significance values for the analyses using the continuous sex steroid values. We repeated the above analyses using total sex steroid levels as independent variables.

We next examined whether free-T, free-E, or SHBG were risk factors for cognitive decline. Because the definition of cognitive decline by 3MS is not established, we defined decline on the 3MS as either a 5 or more³⁴ or 10 or more³² decrease in score. For Trails B, decline was considered to be the worst decile of change between visits.³³ For the MrOS cohort, this translated into a greater than 49 second increase in Trails B performance. Men who were considered to have cognitive decline at baseline (Trails B performance >250 seconds or 3MS score <80) were excluded from these analyses. We used log binomial regression to

estimate regression parameters, and used a robust variance estimation procedure to get their standard errors and perform significance testing. The independent variable, continuous sex hormones, were fitted to estimate the adjusted risk ratios (expressed per 1 standard deviation as the unit change in sex steroid or SHBG) and 95% confidence intervals of significant cognitive decline. We repeated the log-binomial regression models using quartile of sex steroid or SHBG. Because the results were similar, we present the data using the continuous sex steroid values.

We considered all the demographic (age, clinic site, race, BMI, highest level of education obtained), lifestyle (alcohol use, smoking), physical and mental health (SF-12), physical activity (Physical Activity Scale for the Elderly [PASE]), self-reported healthcare provider diagnosed medical conditions (Parkinson's disease, stroke, non-skin cancer), and medication (benzodiazepines, narcotics, corticosteroids, selective serotonin reuptake inhibitors, trazodone, or tricyclics) variables known to influence sex steroid measures and cognition test scores as potential confounding factors. During descriptive analyses, we determined which confounding factors varied according to both sex steroid level and cognitive score. These identified confounders were added into the model individually in the multivariable analysis and compared with the crude model that contained only baseline sex steroid quartiles. We took 10% change in the measure of association to identify confounding variables. For SHBG analyses, we also covaried by free-T and free-E to evaluate the independent effect of SHBG.

Finally, to enable comparisons of results to previous studies using RIA, we evaluated the association between cognitive performance and free sex steroid levels as measured by RIA. Because MrOS participants whose serum was used for sex steroid measurement by RIA were selected using a stratified sampling design, we included the strata [clinic site (each of six sites), race (white or nonwhite), and availability of a complete set of skeletal imaging procedures (to be used in future analyses of the effects of sex steroids on skeletal change)] as covariates in the analyses. To determine the robustness of our findings we performed sensitivity analyses. We compared those with the lowest free-T levels (lowest 2.5%) to those with higher free-T levels to determine if the lowest T levels impart a specific increased risk. Lastly, we examined the association between sex steroids and SHBG in the oldest men (those >74 years of age). SAS v9.1 software (Statistical Analysis System, Cary, NC) was used for all analyses.

RESULTS

Study population

Sex steroid data by MS were available on 1602 participants. After exclusions based on medical conditions or medication use, missing values for free-T or free-E, or missing values for cognitive assessments (Fig.), 1364 and 1397 participants were included in the baseline cross-sectional Trails B and 3MS analyses, respectively. Deaths (N=140), lack of participation in the follow-up clinic visit (N=202), or missing Trails B score at the follow-up visit (N=42) resulted in further sample size reductions, so that 980 and 1022 participants were included in the longitudinal Trails B and 3MS analyses. Men who completed cognitive testing at both visits had significantly higher baseline scores than those who did not ($p < 0.0001$). In addition, men who completed cognitive testing at both visits had slightly higher free-T at baseline than men who did not [$p = 0.01$; 0.28 ng/dL (N= 980) vs. 0.26 ng/dL (N = 384)]. The study participants were aged 73.6 years on average and generally well-educated, with approximately 52% having received college or higher degrees.

Men in the higher free-T quartiles were younger, had lower BMI's, were more educated, and had higher self-rated health and physical summary scores (all p values < 0.001). Race was not

evenly distributed among free-T quartiles ($p=0.04$; Table 1); however, the differences were not systematic. Participants in the higher free-E quartiles tended to be younger ($p=0.08$), heavier ($p<0.001$) and less likely to have smoked ($p<0.02$) or to consume alcohol ($p<0.04$; data available from corresponding author upon request). Men in the highest quartile of SBHG were younger ($p<0.0001$), weighed less ($p<0.0001$), and were less likely to smoke ($p<0.03$; data available from corresponding author upon request).

Cognitive performance and sex steroid characteristics of participants

Baseline performance on Trails B and 3MS was related to age (all p values <0.0001); older men had worse cognitive performance. Participants did not show a decline in Trails B performance between visits (4.5 years apart); in fact there was a slight, nonsignificant improvement in performance, which is presumably a practice effect. (Table 2) 3MS performance declined between visits (Table 2). 57 (4.2%) participants achieved the maximum possible score (at floor) for Trails B. 59 (4.2%) obtained the maximum score (at ceiling) for 3MS. Participants at floor or ceiling were included in the analyses. Pearson correlations between serum levels of sex steroids and SHBG (free-T and SHBG: $r = 0.16$; free-E and SHBG: $r = -0.14$) and between free-T and free-E ($r = 0.53$) were modest.

Association between baseline sex steroids and Trails B

There was no association between baseline free-T level and Trails B performance at either the baseline or follow-up visits in the age or multiple-variable adjusted models (Table 3A). Baseline free-T level was also not associated with change in Trails B performance between visits. Total-T showed no significant associations with Trails B performance at baseline, at follow-up, or with change in performance from baseline to follow up (all p values ≥ 0.63 ; data available from corresponding author upon request).

Baseline free-E levels (Table 3A) and total-E levels (all p values ≥ 0.10 ; data available from corresponding author upon request) were not associated with Trails B performance at either visit or with change in performance.

Relationship between baseline sex steroids and 3MS

Baseline free-T levels were not associated with 3MS performance at baseline or follow-up or with change in 3MS performance (Table 3B). Baseline total-T levels were also not associated with 3MS performance (all p values ≥ 0.29 ; data available from corresponding author upon request). Neither baseline free-E (Table 3B) nor total-E levels (all p values ≥ 0.10 ; data available from corresponding author upon request) were associated with either 3MS performance or change in 3MS performance.

Association between SHBG and cognitive performance

In the multiple-variable adjusted model, increasing baseline SHBG levels were associated with worsening performance on Trails B and 3MS at follow-up ($p=0.03$ and $p=0.02$, respectively; Table 4A and 4B). When the association was corrected for free-T and free-E, it became stronger ($p=0.006$ and $p=0.01$; Table 4A and 4B). Trails B and 3MS performance worsened as SHBG levels increased. There was no association between baseline SHBG level and baseline cognitive performance or change in cognitive performance in the multiple-variable adjusted model.

However, when free-T and free-E were added to the model, higher SHBG was associated with worse baseline Trails B performance ($p=0.02$; Table 4A) and more decline on 3MS performance between visits ($p=0.01$; Table 4B). The results are similar when further adjusted for insulin levels.

Effects of sex steroids and SHBG on cognitive decline

We examined whether free sex steroid and SHBG levels were risk factors for cognitive decline (Table 5), defined as a 49 second or more increase in Trails B performance³³ or either a 5 or greater³⁴ or 10 or greater point decline on 3MS.³² Free-T and free-E levels were not associated with cognitive decline as measured by Trails B or 3MS. However, every SD increase in SHBG was associated with a 1.18-fold higher risk of cognitive decline on Trails B (95% CI 1.01, 1.37) and a 1.07 (for 5 point decline) to 1.25-fold (for 10 point decline) higher risk of cognitive decline on 3MS (95% CI 0.97, 1.19 for 5 point decline and 95% CI 1.06, 1.49 for 10 point decline) after multiple-variable adjustment. The risks increased slightly after further adjustment for free-T and free-E [RR 1.23 (95% CI 1.07, 1.41) for Trails B; RR 1.13 (95% CI 1.02, 1.26) for 3MS by 5 point decline; and RR 1.32 (95% CI 1.11, 1.58) for 3MS by 10 point decline]. This translated into those in the highest SHBG quartile having a 1.8 fold increased risk of cognitive decline on Trails B (RR 1.81; 95% CI 1.05–3.14) and a 2-fold increased risk of a 10 point decline on 3MS (RR 2.02, 95% CI 1.0–4.07) compared to those in the lowest quartile. Adjustment for insulin levels did not alter the results.

Sensitivity Analyses: RIA cohort

We also examined the association between cognitive performance and sex steroids as measured by RIA. As in the GC/MS cohort, there was no association between free-T and free-E and cognitive performance. However, the association between Trails B performance and SHBG was stronger in the larger RIA cohort. We found that higher SHBG was associated with worse performance on Trails B at both the baseline and follow-up visit ($p=0.002$ and $p<0.001$, respectively) and that those with higher SHBG had more decline on Trails B between visits ($p=0.01$). There was a borderline ($p=0.05$) association between SHBG and 3MS at baseline in the RIA cohort.

Men with lowest T levels

To examine whether there was a threshold effect, we divided men into hypogonadal [free T in lowest 2.5% (mean free-T=0.1 ng/dl (0.0 nmol/L); N=430) and eugonadal (mean free-T=0.3 ng/dl (0.1 nmol/L); N=1321)] categories. Hypogonadal and eugonadal men had similar baseline and follow-up Trails B and 3MS performance and comparable change in cognitive performance over time.

Additional sensitivity analyses

We examined the relationship between free sex steroids and cognitive performance in the oldest group of participants (age ≥ 75 years; N=518). The findings in the oldest men were similar to the results in men overall. When only men who had cognitive testing performed at both visits (longitudinal cohort) were analyzed, the results were essentially unchanged. Adding physical activity to the models did not change the results.

DISCUSSION

We found no evidence of a relationship between testosterone or estradiol and cognition as measured by Trails B or 3MS. Higher baseline SHBG was associated with worse follow-up cognitive performance and more cognitive decline.

Despite cellular and animal data suggesting sex steroids act in brain regions important for cognition,^{8–11, 35–38} clinical studies are conflicting and thus inconclusive. Of seven^{12–15, 17, 20, 21} previous studies, only the Baltimore Longitudinal Study of Aging (BLSA) found an association of free-T with Trails B.¹³ In Rancho Bernardo, a U-shaped relationship was reported between total estradiol and Trails B;²⁰ however, other studies did not confirm

this.^{12, 14, 15, 17} In both BLSA and Rancho Bernardo, sex steroids were measured using RIA, which is inaccurate at low values.^{39, 40}

One study found that higher bioavailable T levels were less likely to be associated with low MMSE scores¹² but others have not.^{13, 15, 17, 18, 20, 21} Although in Rancho Bernardo higher estradiol was associated with worse MMSE performance,²⁰ other studies have not confirmed this.^{12, 14, 15, 17, 18} However, mean MMSE scores were close to the upper limit (27–29 out of 30), which could have precluded finding a significant association (ceiling effect). Only 4% of our participants were at ceiling; their mean score was 94 (maximum 100). 3MS significantly declined over time, suggesting adequate sensitivity to detect cognitive changes. Therefore, our null findings are unlikely from ceiling effects.

We did not find worse cognition in hypogonadal men. This contrasts with BLSA where men with the lowest 2.5% bioavailable-T, as measured by RIA, had lower Trails B scores.¹³ BLSA participants were approximately 10 years younger than MrOS participants. We were unable to evaluate whether each participant had their own critical level below which cognition is affected.

We examined the association of sex steroid levels with cognition in the oldest men, i.e. those >74 years of age, because sex steroids may affect aging brains differently. Although one study found a positive linear association between testosterone and Trails B in the oldest group,¹² we did not find different effects in the oldest group compared to the entire cohort.

Previous studies have found no association between total or free testosterone and Trails B or MMSE performance assessed 1–8 years later.^{13, 15, 20} Estradiol was associated with prospective MMSE and Trails B performance in Rancho Bernardo.²⁰ In our study, free-T and free-E levels did not predict cognition 4.5 years later or cognitive performance change over that time period. In BLSA, higher testosterone was associated with poorer visual memory but not executive function decline over 10 years.¹³ Together our data suggest that currently circulating free-T and free-E levels do not influence future cognition or change in cognition; however, we did not measure testosterone levels at follow-up and only MMSE, and not Trails B, performance declined significantly during the study.

Previous data on the association between SHBG and cognition are scant.^{14, 15, 17} Higher SHBG levels were recently shown to be associated with increased dementia risk.⁴¹ SHBG likely has effects beyond its carrier protein role by amplifying sex steroid sufficiency or deficiency⁴² or having independent effects.⁴³ SHBG has been shown to stimulate a membrane-associated receptor in the brain⁴³ and could thereby directly influence intracellular signaling. Such signaling may require SHBG-sex steroid interactions^{42, 44} or use a megalin-mediated endocytic pathway involving unbound SHBG.^{42, 45} However, SHBG could be a marker for other factors affecting cognition. Lower insulin, lower IGF-1, and aging increase SHBG, while obesity, frailty, and nutritional status decrease SHBG.^{46, 47} In our study, we examined physical health, self-rated health, BMI, age, and insulin levels as confounders and did not find they altered the association. A possible association between SHBG and cognitive function is intriguing and should be explored in future studies.

To our knowledge, this is one of the largest studies to address associations between cognition and sex steroids in elderly men and the first to use GC/MS to measure sex steroids. GC/MS is reasonably accurate at the low concentrations seen in elderly men.^{39, 40} Cognitive testing was carefully performed and potentially important confounding variables, including age, education, medications, medical history, mental functioning, and physical health, were evaluated. Many men were over age 80 years, a segment of the population that is increasing steadily but has not been well studied.

There are several limitations. The primary outcome of MrOS was fractures, not cognition. For Trails B, we did not utilize a control for speed (Trails A). We could not have distinguished effects on executive function from speed. Because there was no decline on Trails B, detecting associations between cognitive change and sex steroids was difficult. 3MS was not normally distributed and 3MS change was narrowly distributed. Sex steroids could be related to cognitive domains not tested in this study (visuo-spatial function or memory).^{48–51} For longitudinal analyses, we excluded men without follow-up cognitive measures. Participants who were not studied may have had less cognitive decline, biasing our results to the null. We did not measure inflammatory markers, which can influence cognition. Free sex steroid levels were calculated and not directly measured;³⁰ however, calculation using mass action equations is comparable to direct measurement by equilibrium dialysis.^{52, 53} Finally, we did not measure hormone level changes.

In conclusion, testosterone and estradiol were not related to cognitive performance in a large cohort of elderly men. The large sample size and accurate sex steroid levels make it unlikely we missed important associations between native, endogenous sex steroid levels and cognitive performance on Trails B or MMSE in elderly men. High SHBG deserves further examination as a risk factor for cognitive decline.

Acknowledgments

The Osteoporotic Fractures in Men (MrOS) Study is supported by National Institutes of Health funding. The following institutes provide support: the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Institute on Aging (NIA), the National Center for Research Resources (NCRR), and NIH Roadmap for Medical Research under the following grant numbers: U01 AR45580, U01 AR45614, U01 AR45632, U01 AR45647, U01 AR45654, U01 AR45583, U01 AG18197, U01-AG027810, and UL1 RR024140.

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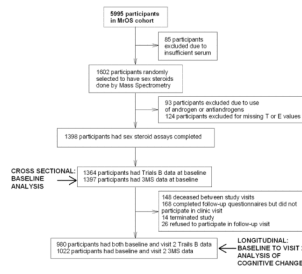


Figure 1. Design for the MrOS sex steroids and cognitive performance study

Table 1

Baseline Characteristics by Free Testosterone* Quartile

Baseline Characteristics	Quartiles of Free T (range in nmol/l)				P value [†]
	Q1 (lowest) N = 371 (0.00 – 0.22)	Q2 N = 296 (0.23 – 0.26)	Q3 N = 382 (0.27 – 0.32)	Q4 (highest) N = 315 (0.33 – 1.36)	
Age group [Number (% [§])]					0.0002
65 – 69 yrs	87(23.5)	85(28.7)	124(32.5)	119(37.8)	
70 – 74 yrs	100(27.0)	87(29.4)	101(26.4)	89(28.3)	
75 – 79 yrs	99(26.7)	85(28.7)	86(22.5)	69(21.9)	
BMI [Mean (SD)]	28.8 (4.2)	27.6 (3.7)	27.0 (3.2)	26.2 (3.2)	<0.0001
Caucasian race [Number (% [§])]	346 (93.3)	263 (88.9)	352 (92.2)	281 (89.2)	0.12
College graduate [Mean (SD)]	167 (45.0)	145 (49.0)	212 (55.5)	187 (59.4)	0.0007
Self-rated health [Number (% [§])]					< 0.0001
Excellent	108 (29.1)	111 (37.5)	117 (30.6)	131 (41.6)	
Fair/Poor/Very poor	75 (20.2)	39 (13.2)	69 (18.1)	26 (8.3)	
Physical Activity Scale for the Elderly (PASE) [Mean (SD)]	138.7 (67.4)	145.6 (70.0)	151.3 (72.8)	152.3 (64.9)	0.03
Physical Summary Scale [Mean (SD)]	46.6 (11.4)	49.6 (10.0)	48.2 (10.5)	51.1 (8.7)	<0.0001
Mental Summary Scale [Mean (SD)]	55.5 (7.3)	55.5 (6.9)	55.8 (6.4)	56.1 (6.1)	0.78
Ever smoked [Number (% [§])]	241 (65.0)	198 (66.9)	235 (61.5)	184 (58.4)	0.13
Alcohol consumption [Number (% [§])]					0.29
None	137 (36.9)	98 (33.2)	116 (30.4)	92 (29.2)	
≥14 drinks/week	48 (12.9)	34 (11.5)	45 (11.8)	45 (14.3)	
Free T nmol/l [Mean (SD)]	0.17 (0.05)	0.25 (0.01)	0.29 (0.02)	0.40 (0.10)	<0.0001
Free E pmol/l [Mean (SD)]	1.7 (0.6)	1.9 (0.5)	2.0 (0.6)	2.4 (0.9)	<0.0001
Total T nmol/l [Mean (SD)]	8.6 (3.0)	12.3 (2.5)	15.2 (2.8)	21.1 (5.6)	<0.0001
Total E pmol/l [Mean (SD)]	68.2 (26.4)	78.2 (22.1)	84.4 (25.1)	104.2 (37.6)	<0.0001
SHBG nM [Mean (SD)]	45.2 (20.4)	46.0 (18.7)	50.7 (17.7)	56.9 (19.6)	<0.0001

* Baseline characteristics by Free Estradiol and SHBG quartiles are available from corresponding author upon request

⁷ P values for categorical variables were compared using chi-square tests for categorical variables; p values for continuous variables with normal distributions were calculated using one-way ANOVA; p values for variables with skewed distributions were calculated using **Kruskal Wallis** tests.

⁸ Refers to percentage of men in each quartile with that characteristic

Table 2

Cognitive performance at baseline and follow-up in men who had testing performed at both visits

	Baseline	Follow-up	p value
Trails B mean completion time(sec) (SD)	124.59 (51.91)	121.96 (56.60)	0.09
3MS score, mean (SD)	94.05 (5.23)	92.30 (7.17)	0.0001

Table 3

Table 3A. Association Between Sex Steroids and Trails B performance

	Quartile 1 (lowest) Baseline Free Testosterone (0.00 – 0.22 nMol/l) N = 371*	Quartile 2 Baseline Free Testosterone (0.23 – 0.26 nMol/l) N = 296*	Quartile 3 Baseline Free Testosterone (0.27 – 0.32 nMol/l) N = 382*	Quartile 4 (highest) Baseline Free Testosterone (0.33 – 1.36 nMol/l) N = 315*	Age adjusted p value [†]	Multiple-variable adjusted p value ^{‡§}
Baseline Trails B mean completion time (sec), age adjusted (95% CI)	153.2 (147.1, 159.4)	146.2 (139.1, 153.3)	147.7 (141.4, 153.9)	140.1 (133.1, 147.1)	0.11	0.44
Follow-up Trails B mean completion time (sec), age adjusted (95% CI)	143.8 (135.4, 152.3)	142.2 (133.8, 150.7)	139.5 (130.4, 148.5)	138.0 (129.0, 147.0)	0.22	0.48
Change in mean Trails B completion time, baselineto follow up (sec), age adjusted (95% CI)	2.3 (–5.3, 10.0)	2.8 (–4.8, 10.3)	2.9 (–5.2, 11.0)	3.2 (–4.8, 11.3)	0.59	0.52
	Quartile 1 (lowest) Baseline Free Estradiol	Quartile 2 Baseline Free Estradiol	Quartile 3 Baseline Free Estradiol	Quartile 4 (highest) Baseline Free Estradiol	Age adjusted p value [†]	Multiple-variable adjusted p value ^{‡§}
	(0.1 – 1.5 pMol/l) N = 342*	(1.5 – 1.9 pMol/l) N = 334*	(1.9 – 2.3 pMol/l) N = 346*	(2.3 – 9.6 pMol/l) N = 342*		
Baseline Trails B mean completion time (sec), age adjusted (95% CI)	142.6 (136.1, 149.2)	151.8 (145.2, 158.4)	147.0 (140.4, 153.5)	149.3 (142.6, 156.0)	0.40	0.46
Follow-up Trails B mean completion time (sec), age adjusted (95% CI)	136.0 (127.4, 144.6)	145.7 (136.8, 154.5)	138.4 (129.9, 147.0)	145.8 (137.1, 154.5)	0.72	0.69
Change in mean Trails B completion time, baselineto follow up (sec), age adjusted (95% CI)	6.8 (–1.0, 14.6)	0.3 (–7.7, 8.2)	2.2 (–5.4, 9.9)	1.4 (–6.5, 9.2)	0.27	0.26

Table 3B. Association Between Sex Steroids and 3MS performance

	Quartile 1 (lowest) Baseline Free Testosterone (0.00 – 0.21 nMol/l) N = 322*	Quartile 2 Baseline Free Testosterone (0.22 – 0.26 nMol/l) N = 365*	Quartile 3 Baseline Free Testosterone (0.27 – 0.31 nMol/l) N = 338*	Quartile 4 (highest) Baseline Free Testosterone (0.32 – 1.36 nMol/l) N = 372*	Age adjusted p value [†]	Multiple-variable adjusted p value ^{‡§}
Baseline 3MS score, age adjusted mean (95% CI)	91.6 (90.8, 92.3)	92.2 (91.5, 92.9)	92.5 (91.8, 93.3)	92.3 (91.6, 93.0)	0.77	0.33
Follow-up 3MS score, age adjusted mean (95% CI)	89.2 (88.2, 90.2)	89.6 (88.6, 90.5)	90.1 (89.1, 91.1)	89.7 (88.7, 90.7)	0.68	0.85
Change in 3MS score, baseline to follow up, adjusted mean (95% CI)	-3.3 (-4.1, -2.6)	-3.1 (-3.9, -2.4)	-2.9 (-3.7, -2.1)	-3.0 (-3.8, -2.2)	0.69	0.50
	Quartile 1 (lowest) Baseline Free Estradiol (0.1–1.5 pMol/l) N = 350*	Quartile 2 Baseline Free Estradiol (1.5–1.9 pMol/l) N = 342*	Quartile 3 Baseline Free Estradiol (1.9–2.3 pMol/l) N = 351*	Quartile 4 (highest) Baseline Free Estradiol (2.3 – 9.6 pMol/l) N = 354*	Age adjusted p value [†]	Multiple-variable adjusted p value ^{‡§}
Baseline 3MS score, age adjusted mean (95% CI)	92.6 (91.9, 93.3)	91.9 (91.2, 92.6)	92.0 (91.3, 92.7)	91.9 (91.2, 92.6)	0.16	0.20
Follow-up 3MS score, age adjusted mean (95% CI)	90.0 (89.0, 91.0)	89.3 (88.3, 90.3)	89.5 (88.5, 90.4)	89.6 (88.6, 90.6)	0.83	0.75
Change in 3MS score, baseline to follow up, adjusted mean (95% CI)	-3.2 (-3.9, -2.4)	-2.8 (-3.5, -2.0)	-3.1 (-3.8, -2.3)	-3.5 (-4.3, -2.7)	0.31	0.32

Note: Lower Trails B score indicates better performance. Higher 3MS score indicates better performance.

* Refers to N for visit 1. Number decreases for follow-up visit. For Trails B: free-T quartiles at follow-up: 248, 274, 229, and 247; free E: 248, 248, 251, 251. For 3MS: free-T quartiles at follow-up: 256, 277, 237, and 252; free-E: 252, 260, 256, 254.

[†] P value calculated based on continuous sex steroid measures entered as independent variables in linear regression models.

[§] Multiple-variable adjusted model adjusted for age group, education level (complete high school or less vs. complete college or more), race, self-rated health and alcohol consumption

Table 4

Table 4A. Association Between SHBG and Trails B Performance

	Quartile 1 (lowest) SHBG (12.2–35.7 nM) N = 343*	Quartile 2 SHBG (35.8–46.6 nM) N = 339*	Quartile 3 SHBG (46.7–59.2 nM) N = 342*	Quartile 4 (highest) SHBG (59.3–179.0 nM) N = 340*	Age adjusted p value [†]	Multiple-variable adjusted p value ^{‡§}	Adjusted for free-T and free-E ^{†§}
Baseline Trails B mean completion time (sec), age adjusted [†] (95% CI)	147.2 (140.3, 154.2)	142.4 (135.7, 149.2)	150.6 (144.0, 157.2)	149.3 (143.0, 155.6)	0.19	0.10	0.02
Follow-up Trails B mean completion time (sec), age adjusted [†] (95% CI)	138.0 (128.9, 147.0)	133.8 (124.8, 142.8)	142.1 (133.5, 150.8)	146.8 (138.6, 155.1)	0.05	0.03	0.006
Change in mean Trails B completion time, baseline to follow up (sec), age adjusted [†] (95% CI)	-2.5 (-10.6, 5.6)	1.2 (-6.9, 9.4)	3.4 (-4.4, 11.1)	6.4 (-1.0, 13.9)	0.14	0.25	0.27

Table 4B. Association Between SHBG and 3MS Performance

	Quartile 1 (lowest) SHBG (12.2–35.7 nM) N = 350*	Quartile 2 SHBG (35.8–46.5 nM) N = 350*	Quartile 3 SHBG (46.7–59.1 nM) N = 347*	Quartile 4 (highest) SHBG (59.2–179.0 nM) N = 350*	Age adjusted p value [†]	Multiple-variable adjusted p value ^{‡§}	Adjusted for free-T and free-E ^{†§}
Baseline 3MS score, age adjusted mean [‡] (95% CI)	92.5 (91.8, 93.3)	91.8 (91.1, 92.6)	92.1 (91.4, 92.8)	92.1 (91.4, 92.8)	0.54	0.56	0.50
Follow-up 3MS score, age adjusted mean [‡] (95% CI)	90.4 (89.3, 91.4)	89.9 (88.8, 90.9)	89.8 (88.8, 90.8)	88.8 (87.9, 89.8)	0.02	0.02	0.01
Change in 3MS score, baseline to follow up, adjusted mean [‡] (95% CI)	-2.8 (-3.6, -2.0)	-2.8 (-3.6, -2.0)	-2.8 (-3.5, -2.0)	-3.7 (-4.5, -3.0)	0.06	0.07	0.01

Note: Lower Trails B score indicates better performance. Lower 3MS score indicates worse performance.

* Refers to N for visit 1. Number decreases for follow-up visit. For SHBG and Trails B: 248, 251, 250, 249. For SHBG and 3MS: 257, 253, 256, 256.

[†]P value calculated based on continuous sex steroid measures entered as independent variables in linear regression models.

[§]Multiple-variable adjusted model adjusted for age group, education level (complete high school or less vs. complete college or more), race, self-rated health and alcohol consumption

[‡]Multiple-variable adjusted means (95% CI) are as follows: Baseline Trails B (sec) Q1 162.4 (151.9, 172.8), Q2 159.9 (149.2, 170.7), Q3 166.8 (156.4, 177.3), Q4 166.4 (156.3, 176.6); Follow-up Trials B (sec) Q1 152.8 (139.7, 165.9), Q2 151.5 (137.9, 165.1), Q3 158.2 (145.0, 171.3), Q4 163.1 (150.0, 176.1); Change in mean Trails B completion time Q1 0.3 (-12.2, 12.7), Q2 3.9 (-9.0, 16.8), Q3 5.3 (-7.1, 17.7), Q4 8.2 (-4.2, 20.6); Baseline 3MS score Q1 89.7 (88.5, 90.8), Q2 88.7 (87.5, 89.8), Q3 89.1 (88.0, 90.3), Q4 89.1 (88.0, 90.3); Follow-up 3MS score Q1 86.6 (85.0, 88.1), Q2 85.7 (84.1, 87.3), Q3 85.8 (84.3, 87.4), Q4 85.0 (83.5, 86.5); Change in mean 3MS score Q1 -4.1 (-5.3, -2.8), Q2 -4.1 (-5.4, -2.8), Q3 -4.0 (-5.3, -2.8), Q4 -4.9 (-6.2, -3.7).

Table 5

Risk of cognitive decline according to sex hormone or SHBG quartile

RR (95% CI)	Baseline Sex Steroid or SHBG	
	Free Testosterone	Free Estradiol SHBG
Relative Risk of clinically important decline (>49 seconds) in Trails B per SD change in sex steroid or SHBG		
Age-adjusted	0.89 (0.74, 1.07)	0.97 (0.82, 1.16)
Multiple-variable adjusted*	0.87 (0.73, 1.05)	0.95 (0.80, 1.14)
Multiple-variable and free T, free E adjusted	N/A	N/A
		1.23 (1.07, 1.41) [†]
Relative Risk of clinically important decline in 3MS (-5 as the cut point) per SD change in sex steroid or SHBG		
Age-adjusted	0.94 (0.83, 1.07)	1.06 (0.97, 1.17)
Multiple-variable adjusted*	0.94 (0.82, 1.07)	1.07 (0.97, 1.19)
Multiple-variable and free T, free E adjusted	N/A	N/A
		1.13 (1.02, 1.26) [†]
Relative Risk of clinically important decline in 3MS (-10 as the cut point) per SD change in sex steroid or SHBG		
Age-adjusted	1.06 (0.87, 1.28)	1.14 (0.99, 1.31)
Multiple-variable adjusted*	1.07 (0.87, 1.31)	1.15 (0.99, 1.35)
Multiple-variable and free T, free E adjusted	N/A	N/A
		1.32 (1.11, 1.58) [†]

Footnote for Table 5:

Note: For Trails B analyses, the size of the analytic sample is 944, after 36 men with baseline completion time ≥ 251 seconds were excluded. The size of the 3MS analytic sample is 1001, after 21 men with baseline 3MS score < 80 were excluded.

* Multiple-variable RR adjusted for age groups, race, education level (complete high school or less vs. complete college or more), self-reported health and alcohol consumption

[†] These RR per SD change in SHBG translate into the following relative risks when highest SHBG quartile is compared to lowest SHBG quartile: 1.81 (95% CI 1.05, 3.14) fold increased risk of clinically important decline on Trails B; 1.39 (95% CI 0.99, 1.96) fold increased risk of 5 point decline 3MS; 2.02 (95% CI 1.00, 4.07) fold increased risk of 10 point decline on 3MS.