Reproductive hormone levels and cognition in men: The European Male Ageing Study.

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ABSTRACT

Objective: It has been proposed that endogenous sex hormone levels may present a modifiable risk factor for cognitive decline. However, the evidence for effects of sex steroids on cognitive ageing is highly mixed. We therefore investigated associations between endogenous hormone levels, androgen receptor CAG repeat length, and cognitive decline in a large-scale longitudinal study of middle-aged and older men.

Methods: Men aged 40-79 years from the European Male Ageing Study (EMAS) underwent cognitive assessments and measurements of hormone levels at baseline and follow-up (mean = 4.4 years, SD ± 0.3 years). Hormone levels measured included total and calculated free testosterone and estradiol, dihydrotestosterone, luteinizing hormone, follicle-stimulating hormone, dehydroepiandrosterone sulphate and sex hormone-binding globulin. Cognitive function was assessed using the Rey-Osterrieth Complex Figure Copy and Recall, the Camden Topographical Recognition Memory and the Digit Symbol Substitution Test. Multivariate linear regressions were carried out to examine associations between baseline and change hormone levels, androgen receptor CAG repeat length, and cognitive decline.

Results: Statistical analyses included 1,827 and 1,423 participants for models investigating effects of hormone levels and CAG repeat length, respectively. In age-adjusted models, we found a significant association of higher baseline free testosterone (β=-0.001, p=0.005) and dihydrotestosterone levels (β=-0.065, p=0.003) with greater decline on Rey-Osterrieth Complex Figure Recall over time. However, these effects were no longer significant following adjustment for centre, health, and lifestyle factors. No relationships were observed between any other baseline and change hormone levels nor the androgen receptor CAG repeat length and cognitive decline.

Conclusions: In this large-scale prospective study there was no evidence for an association between endogenous sex hormone levels or CAG repeat length and cognitive ageing in men. These data suggest that sex steroid levels do not affect visuospatial function, visual memory, or processing speed in middle-aged and older men.

Keywords: Hormones

Cognition

Men

Aging

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1. Introduction

Cognitive decline is one of the most undesired aspects of growing old and is associated with high social and economic costs. In the absence of effective treatments there is growing interest in identifying modifiable risk factors and associated prevention strategies for cognitive dysfunction. Sex steroid levels, which undergo substantial changes across the lifespan, may present a potential target for such preventative approaches (e.g. Etgen et al., 2010). In men, ageing is accompanied by decreases in total testosterone (TT), free testosterone (FT), dihydrotestosterone (DHT), estradiol (E2), and dehydroepiandrosterone sulfate (DHEAS), whereas luteinizing hormone (LH), follicle-stimulating hormone (FSH), and sex hormone-binding globulin (SHBG) levels gradually increase over time (Hsu et al., 2015; Morley et al., 1997). Both androgen (Jänne et al., 1993; Simerly et al., 1990; Sarkey et al., 2008) and estrogen (González et al., 2007; Simerly et al., 1990; Cui, Shen, and Li, 2013; Saito & Cui, 2018) receptors are expressed throughout the brain, with a particular abundance in the hippocampus (Beyenburg et al., 2000; Höfer et al., 2013; Almey et al., 2015; Österlund & Hurd, 2001). Animal and cell culture studies suggest various neuroprotective effects of sex hormones, including neurogenesis, B-amyloid regulation, and protection against tau hyperphosphorylation, oxidative stress, and neuronal apoptosis (Pike et al., 2009; Hammond et al., 2001; Spence & Voskuhl, 2012; Norbury et al., 2003). Whilst these mechanisms remain to be elucidated in humans, it is physiologically plausible that endocrinological changes could affect cognitive ageing processes. However, evidence from observational studies for an association between sex steroid levels and cognitive decline is highly mixed.

Research to date has predominantly focused on the influence of testosterone levels, with some studies indicating a positive relationship between T and/or FT concentrations and global cognitive functioning (Yaffe et al., 2002; Hsu et al., 2015), memory (Moffat et al., 2002), and visuospatial cognition (Moffat et al., 2002). However, others have reported a negative (Martin et al., 2007), curvilinear (Barrett-Connor et al., 1999; Muller et al., 2005), or no relationship (Wolf et al., 2002; LeBlanc et al., 2010) between testosterone and cognition in men. Hormone replacement therapy studies investigating cognition in hypogonadal men have similarly reported contradictory findings (Borst et al., 2014; Cherrier et al., 2003; Cherrier et al., 2005; Huang et al., 2016; Lasaite et al., 2017;

Lu et al., 2006; Maki et al., 2007).

Fewer studies have been dedicated to the influence of estrogens, gonadotropins, and SHBG on cognitive ageing in men. Reports on the role of estrogen levels are inconsistent, with some suggesting a positive relationship with cognition (Hsu et al., 2015; Hogervorst et al., 2004) but others indicating a negative (Barrett-Connor et al., 1999; Fonda et al., 2005) or no association (Lessov-Schlaggar et al., 2005; Martin et al., 2007; Wolf & Kirschbaum, 2002). Similarly, higher SHBG or DHEAS levels have been linked to better cognitive function in some (LeBlanc et al., 2010; Hogervorst et al., 2010; Castanho et al., 2014), but not all studies (Martin et al., 2007; Lessov-Schlaggar et al., 2005). The potential role of gonadotropins remains underexplored, although preliminary studies have not observed any clear associations with cognition (Martin et al., 2007; Yeap et al., 2008).

Further complicating the relationship between sex steroids and cognition, genetic factors may affect hormone-related cognitive decline by influencing the number and activity of steroid receptors across different brain regions. For instance, long CAG repeat length in exon 1 of the androgen receptor (*AR*) gene is associated with reduced androgen sensitivity (Choong et al., 1996), and was related to greater decline of a range of cognitive tasks in 201 men in the Study of Osteoporotic Risk in Men (Yaffe et al., 2003). However, a study by Lehmann and colleagues (2003) suggested that men with short (≤20 repeats) rather than long CAG repeat lengths were more likely to have Alzheimer's disease (AD), whereas no association between CAG repeat length and AD was observed in a different cohort of older male and female adults (Ferrari et al., 2013). Recently, it has been proposed that CAG repeat length interacts with testosterone levels, such that a short repeat length exacerbates negative effects of persistent low FT levels on attention and processing speed (Tan et al., 2021).

Taken together, there is a need to clarify the potential contribution of different sex steroids to cognitive decline. In this large-scale longitudinal study we examine the relationship between the full hormonal profile and cognitive performance in ageing men. The multi-centre prospective European Male Ageing Study (EMAS) assessed physiological and cognitive ageing in a large cohort of middle-aged and older men (Lee et al., 2009). In *cross-sectional* analyses, there was a modest association between DHEAS levels and fluid cognition in the EMAS cohort (Lee et al., 2010). No further cross-

sectional correlations were found between hormone levels or CAG repeat length and cognitive performance. The aims of the present *prospective* study were (1) to explore associations between baseline reproductive hormone levels and change in different cognitive domains, (2) to assess the relationship between change in hormone levels and cognitive performance over time, and (3) to establish whether *AR* CAG repeat length affects the rate of cognitive decline in ageing men.

2. Material and methods

2.1 Participants

A detailed description of recruitment, response rates, and assessments in the EMAS cohort is provided elsewhere (Lee et al., 2009). Briefly, 3,369 men between the ages of 40 and 79 years were recruited from population registers by age-stratified random sampling. The eight participating centres were located in Leuven, Belgium; Manchester, UK; Florence, Italy; Malmö, Sweden; Santiago de Compostela, Spain; Tartu, Estonia; Łódź, Poland, and Szeged, Hungary. Participants were sent a postal questionnaire to gather information about demographic details, general health, and lifestyle factors, and were invited for further screening at a local clinic. Baseline assessments took place between 2003 and 2005, with follow-up measurements being conducted on average 4.4 (SD ± 0.3) years later. Written informed consent was given by all participants and ethical approval was obtained in accordance with local institutional requirements in each centre and the Declaration of Helsinki.

2.2 Questionnaire and anthropometric measurements

At the clinic, participants were asked to complete an interviewer-assisted questionnaire which included Beck's Depression Inventory-II (BDI-II; Beck et al., 1996) to assess depressive symptomatology as well as the Physical Activity Scale for the Elderly (PASE; Washburn et al., 1993). Physical abilities were measured using Reuben's Physical Performance Test (PPT; Reuben & Siu, 1990). Use of prescription and non-prescription medications was self-reported by the participants. Height and weight were measured with standard, calibrated instruments.

2.3 Hormone measurement and AR *CAG genotyping*

Morning phlebotomy was performed before 10.00 h to obtain a fasting blood sample at baseline. TT, total E2, and DHT were measured by gas chromatography-mass spectrometry (Labrie et al., 2006). Intra- and inter-assay coefficients of variations (CVs) were 2.9 and 3.4% for TT, 3.5 and 3.7% for E2, and 3.1 and 4.1% for DHT. The lower limit of quantification (LLOQ) was 0.17 nmol/L for TT, 7.34 pmol/L for E2, and 0.07 nmol/L for DHT (O'Connor et al., 2011). Measurement of LH, FSH, DHEAS, and SHBG levels was carried out using the Modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). The intra-and inter-assay CVs were 1.9 and 3.0% for LH, 1.8 and 5.3% for FSH, 1.1 and 4.1% for DHEAS, and 1.7 and 3.2% for SHBG. Detection limits were 0.10 U/L, 0.10 U/L, 0.003 μmol/L, and 0.35 nmol/L for LH, FSH, DHEAS, and SHBG, respectively (Eendebak et al., 2018; O'Connor et al., 2011; Wu et al., 2008). Calculated FT and free E2 levels were derived from TT and E2, SHBG, and albumin concentrations using Vermeulen's mass action equations and association constants (Huhtaniemi et al., 2009; Vermeulen et al., 1999). *AR* CAG repeat length PCR-based genotyping was performed in the laboratory of the Centre for Integrated Genomic Medical Research (CIGMR, University of Manchester) as described by Huhtaniemi and colleagues (2009). After completing PCR, samples were run on ABI PRISM 3100 Genetic Analyser and were genotyped using GeneScan analysis software (Applied Biosystems, Foster City, CA, USA). Allele frequencies were checked for consistency with HapMap data.

2.4 Cognitive assessment

Assessments of cognitive functioning were carried out during both baseline and follow-up visits. The EMAS cognitive test battery consisted of paper-and-pencil tasks which were selected with the aim of minimising cultural and linguistic differences between the centres. In order of administration, the tests used were: the Rey-Osterrieth Complex Figure (ROCF) test, the Camden Topographical Recognition Memory (CTRM) test, and the Digit Symbol Substitution Test (DSST). The ROCF Copy task is a measure of visuo-constructional ability (Rey, 1944) in which participants have to copy an abstract figure as accurately as possible within five minutes' time. Thirty minutes after completing the Copy task, participants were asked without prior warning to draw the figure from memory. This ROCF

Recall component assessed visual memory in addition to visuo-constructional functions. Scoring criteria for the ROCF Copy and Recall tasks were based on the original test procedure (Rey, 1944) with a maximum score of 36 for each task. The CTRM was designed to measure the recognition component of visual memory (Warrington, 1996). Participants were shown a sequence of 30 coloured photographs of urban scenes followed by a three-way forced recognition component. Each correct identification was awarded one point, with a maximum score of 30. Finally, the DSST was performed to assess processing speed and visual scanning (Uiterwijk, 2001). In this subtest of the Wechsler Adult Intelligence Scale (WAIS-III), participants were asked to substitute as many symbols for digits as possible within 60 seconds using a coding table. Higher scores reflect better performance on all four tasks.

2.5 Statistical analyses

Participants with missing cognitive or hormone data, pituitary, testicular or adrenal disease, or taking medications which can affect pituitary and testicular function (e.g. anti-androgens and anabolic steroids) were removed from the dataset. Paired *t*-tests and chi-squared (χ^2) tests were applied to examine differences in baseline characteristics of participants who completed the measurements at both time points and participants who were lost to follow-up. In addition, Kruskal-Wallis tests were used to assess whether changes in cognitive task performance differed across the eight research centres. Paired *t*-tests were conducted to evaluate change in cognitive scores between baseline and follow-up.

The core statistical analyses focused on associations of (1) baseline hormone levels and (2) normalized hormonal change ([follow-up – baseline] / baseline) with cognitive performance over time. As follow-up hormone levels were not available for E2 and DHT data, analyses of change in hormone levels were restricted to TT, FT, DHEAS, LH, FSH, and SHBG measures. Continuous change in cognitive function and hormone levels were calculated by subtracting baseline measurements from the follow-up values. Outcomes on the cognitive assessments were transformed to z-scores to facilitate comparison across task types. *AR* CAG repeat length was assessed as a continuous variable as well as in tertiles to account for potential non-linear effects.

Multivariate linear regressions were carried out for each hormone and *AR* CAG repeat length to examine their relationships with cognitive decline. The first set of models included hormone levels and age as the predictor variables, with change scores on all cognitive tasks as the outcome variables. Subsequently, an intermediate model was implemented with additional adjustments for the following potential confounding factors: education (years), body mass index (BMI; kg/m^2), BDI score, smoking status (current vs. non-smoker), alcohol consumption (<1 day/week vs. ≥1 day/week), psychotropic medication use (none vs. any), and centre. Finally, a fully-adjusted model was fitted including PASE score, PPT walking speed, and co-morbidities (0, 1, or 2 co-morbidities present at baseline) variables. The assessed co-morbidities were heart conditions, high blood pressure, stroke, diabetes, liver and kidney conditions, bronchitis, and cancer. As prior work has suggested that the optimal levels of sex steroids may vary by age (Martin et al., 2007), we also explored age-dependent associations in separate models with interaction terms of continuous hormone levels and age as a categorical variable (40-50 years, 50-60 years, 60-70 years, and 70+ years). Bonferroni corrections were carried out to adjust for multiple comparisons across the hormone profile, with the *p*-value threshold for significance being set at 0.0055. Main and interaction effects of CAG repeat length and androgenic hormone levels (TT, FT, DHT, and DHEAS) on cognitive decline were examined for all four cognitive tasks in multivariate linear regression models with a *p*-value threshold of 0.05. All results are expressed as unstandardized β-coefficients and 95% confidence intervals. Associations between hormone, *AR* CAG repeat length, and cognitive scores were also evaluated graphically in age-adjusted locally weighted scatterplot smoothing (LOWESS) plots. All analyses were completed using Stata software (Version 15.1).

3. Results

3.1 Study population

Of the 3,369 individuals taking part in the baseline assessments, 2,736 men (86.1% of survivors) returned for follow-up measurements. On average, participants who were lost to follow-up had lower cognitive scores, FT and DHEAS levels, and higher E2, LH, FSH, and SHBG levels at baseline than those who completed assessments at both time points (all p <0.05). Men with missing hormone data

(n=205), missing cognitive data (n=447), taking medications affecting pituitary or testicular function (n=206), or with pituitary, testicular, or adrenal disease (n=51) at baseline or follow-up were excluded from the analyses. Total samples of 1,827 and 1,423 participants were available for statistical analyses of sex steroids and *AR* CAG repeat lengths, respectively. Descriptive information of the study participants at baseline is displayed in Table 1. The mean age of the participants was 58.1 years, average BMI was 27.7 kg/m^2, and 38% of all participants had 1 or more co-morbidities at baseline. The mean (SD) androgen receptor CAG allele length was 22 (3) with a range of 7 to 39 repeats.

[Table 1 about here]

3.2 Change in cognitive performance

Mean changes in cognitive scores are shown in Table 2. Overall, participants' scores on the ROCF Recall and CTRM measures tended to increase over time, which is likely due to a practice effect on these assessments. In contrast, an average decline in scores was observed on the DSST outcome, which is less sensitive to practice effects. Linear regressions indicated that older age at baseline was associated with greater decline on the ROCF Copy (β=-0.006, p=0.009, ROCF Recall (β=-0.008, p<0.001), CTRM (β=-0.005, p = 0.015) and DSST (β=-0.010, p<0.001). Kruskal-Wallis tests revealed that decline on all cognitive tasks varied significantly by research centre (all p<0.001).

[Table 2 about here]

3.3 Baseline sex steroid levels and cognitive decline

The first set of analyses assessed the relationship between baseline hormone levels and cognitive changes over time. In age-adjusted multivariate linear regressions there was a significant negative association between FT, DHT, and change in ROCF Recall scores (Table 3). After correcting for potential confounders (age left education, BDI score, BMI, smoking, alcohol consumption, psychotropic drug use, and centre), there were no significant relationships between baseline hormone

levels and any of the cognitive tasks. Models including interaction terms between age group and hormone level indicated that there were no age-dependent differences in these associations, and LOWESS plots did not reveal any non-linear trends in the data (see Supplementary Material).

[Table 3 about here]

3.4 Change in hormone levels and cognition

Next, we tested whether change in hormone levels was associated with cognitive decline. Neither age-adjusted models nor models adjusting for multiple confounding variables showed any significant associations between change in hormone levels and cognition (Table 4). As with the baseline hormone analyses there was no evidence for age-dependent or non-linear associations with cognitive change (see Supplementary Material).

[Table 4 about here]

3.5 **AR** *CAG repeat length and cognitive decline*

Continuous *AR* CAG repeat length was not associated with decline on any of the cognitive tasks in age-, intermediate-, or fully-adjusted models. Similarly, when *AR* CAG repeat length was investigated in tertiles, no significant associations with cognitive change were found (see Table 5). Interaction effects of CAG repeat length and androgens TT, FT, DHT, and DHEAS on cognitive decline were not significant (Supplementary Material).

[Table 5 about here]

4. Discussion

4.1 Main findings

In this prospective cohort study of ageing European men, baseline and change in reproductive hormone levels did not predict cognitive decline over 4.4 years. In age-adjusted models, higher baseline FT and DHT levels were associated with greater decline on a task of visual memory. However, these effects were not maintained when adjusting for health, centre, and lifestyle factors. In

contrast with cross-sectional analyses of the EMAS cohort (Lee et al., 2010), no associations were observed between DHEAS levels and cognitive change. Finally, there was no evidence that *AR* CAG repeat length either directly affected cognitive functioning or modified relationships between hormone levels and cognition in men.

4.2 Endogenous hormone levels and cognitive decline

Evidence from longitudinal research to date on the links between variation in serum hormone levels and cognitive performance has been highly inconsistent. Whilst some observational studies reported associations between the androgens T (Hsu et al., 2015; Muller et al., 2005; Hogervorst et al., 2010), FT (LeBlanc et al., 2010; Yeap et al., 2008), or DHT (Hsu et al., 2015) and cognition in men, others were unable to replicate this finding (Kische et al., 2017) and/or instead noted significant relationships of estrogen (Geerlings et al., 2006) or SHBG (LeBlanc et al., 2010) and cognitive ageing. Possible explanations for the divergent findings across both cross-sectional and longitudinal studies include differences in population characteristics, sampling and analysis methodology, and baseline androgen levels. In addition, it can be hypothesized that effects of sex steroids may differ depending on the specific cognitive domain tested. Previous studies have frequently adopted global cognitive screening tools (e.g. Geerlings et al., 2006; Hsu et al., 2015; LeBlanc et al., 2010; Yeap et al., 2008) which could obscure more specific effects on cognitive function. The present findings suggest that androgen, estrogen, and gonadotropin hormone levels do not present a risk factor for declines in visuospatial function and processing speed in men. Further work may be needed to explore which, if any, domains are most affected by sex steroid levels.

4.3 Cognitive ageing and CAG repeat length

Replicating findings from the cross-sectional analyses of the EMAS cohort (Lee et al., 2010), we did not observe any effects of *AR* CAG repeat length on cognitive ageing in men. Although prior studies have found direct negative (Yaffe et al., 2003), positive (Lehmann et al., 2003), or no relationships (Ferrari et al., 2013) between CAG repeats and cognitive performance, interpretations have been limited by their cross-sectional nature. One previous study of young men demonstrated CAG repeat

length might modulate androgen-cognition associations rather than influencing cognitive decline directly. They reported that only men who presented with both low TT levels and long CAG repeat lengths showed better performance on a spatial navigation task (Nowak et al., 2014). In contrast, a study of older men reported a detrimental effect of low FT and short CAG repeat length on cognitive function (Tan et al., 2021). In the present work, however, interaction terms of CAG repeat length and androgen hormone levels were not significantly associated with cognitive decline. This outcome is perhaps not surprising, as previous research in the EMAS cohort indicated that weaker transcriptional activity of the *AR* due to longer CAG repeat length is mostly compensated for by higher TT levels (Huhtaniemi et al., 2009). The present study thus does not support an association between *AR* CAG repeat length and pre-specified domains of cognitive ageing in healthy men.

4.4 Strengths and limitations

A major strength of the EMAS study is the comprehensive assessment of hormone levels, which allowed us to explore the full endocrinological profile of the participants in relation to prospective changes in cognitive functioning. We were therefore able to assess the relationships between cognition an less-frequently studied hormones, including LH, FSH, and SHBG. In addition, gas chromatography-mass-spectrometry, which is considered to be the gold standard for steroid hormone measurements, was employed to determine TT, E2, and DHT levels. A further strength is the inclusion of participants across different age ranges. Although it has been suggested that hormone effects differ for younger and older adults, most studies to date have focused exclusively on adults >65 years (Hogervorst et al., 2010; Hsu et al., 2015; LeBlanc et al., 2010). Importantly, our findings did not demonstrate any differences across middle-aged and older participants.

We acknowledge several limitations to the present study. As FT concentrations were calculated using the Vermeulen method (Vermeulen et al., 1999) rather than measured directly, it is possible that our estimations of FT differ from the circulating steroid concentrations. However, previous studies have shown excellent correspondence between FT dialysis measurements and calculated FT (Fiers et al., 2018), and it is therefore likely that calculated values in the present sample accurately represent actual concentrations. In addition, it should be taken into account that serum sex

steroid levels can differ significantly from concentrations in the brain, limiting interpretations about the biological mechanisms underlying hormone levels and cognitive function. As studies comparing CSF sex steroid levels to serum levels suggest reasonable correlations between peripheral and central metabolomes (Nguyen et al., 2017), however, serum levels likely provide a suitable proxy measure. The cognitive tasks used were specifically selected to minimise potential cultural and linguistic differences between the participating centres. However, we cannot exclude the possibility that cognitive domains not measured here, such as verbal ability, would be differentially affected by sex steroids. A further limitation is that an average improvement rather than decline in absolute scores over time was evident for the ROCF and CTRM tasks. It is likely that: a) practice effects; and b) attrition of participants with low baseline cognitive scores, contributed to this change in performance. Whilst these factors were not unexpected and tend to be features of any cohort study, the apparent improvement in scores may have limited our ability to observe effects of reproductive hormone levels on cognition.

4.5 Conclusions

There was no evidence for associations between endogenous sex steroid levels and cognitive decline in the EMAS cohort. Furthermore, no relationships between the *AR* CAG repeat polymorphism and cognitive task performance were observed. These findings suggest that sex steroid levels do not affect visuospatial function, visual memory, or processing speed in middle-aged and older men.

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Table 1 Baseline characteristics of EMAS participants included in the analyses (N = 1,827)

	Mean (SD) or %
Age (years)	58.1 (10.5)
Age left education (years)	21.6 (7.2)
Beck Depression Inventory (BDI) score	6.3 (5.9)
Body Mass Index (kg/m^2)	27.7 (4.0)
Physical Performance Test (PPT) walking speed score	24.1 (2.4)
Physical Activity Scale for the Elderly (PASE) score	214.4 (88.1)
Current smoker (%)	19.3
Alcohol consumption (≥1 day/wk; %)	59.5
Any psychotropic drugs[a] (%)	4.9
Co-morbidities (%)[b]	
0 co-morbidities	61.5
1 co-morbidity	24.0
2 co-morbidities	14.5
Cognitive tasks (raw scores)	
ROCF Copy	34.1 (3.5)
ROCF Recall	18.1 (6.3)
CTRM	23.5 (4.4)
DSST	29.5 (8.2)
Hormone levels	
TT (nmol/l)	6.1 (1.5)
FT (pmol/l)	305.2 (86.4)
Total E2 (pmol/l)	74.2 (24.3)
Free E2 (pmol/l)	1.3 (0.4)
DHEAS (µmol/l)	4.9 (2.8)
DHT (nmol/l)	1.7 (1.1)
LH (IU/l)	5.8 (3.6)
FSH (IU/l)	7.8 (7.7)
SHBG (nmol/l)	41.8 (18.8)
AR CAG repeat length (n = 1,423)	22.1 (3.1)

[a] Anti-depressants, benzodiazepines, antipsychotics, and sedatives

[b]Co-morbidities considered were: heart conditions, high blood pressure, stroke, diabetes, liver and kidney conditions, bronchitis, and cancer.

Abbreviations: ROCF, Rey-Osterrieth Complex Figure; CTRM, Camden Topographical Recognition Memory; DSST, Digit Symbol Substitution Test; TT, total testosterone; FT, free testosterone; E2, estradiol; DHEAS, dehydroepiandrosterone sulphate; DHT, 5α-dihydrotestosterone; LH, luteinizing hormone; FSH, follicle stimulating hormone; SHBG, sex hormone-binding globulin.

Table 2 Mean changes in cognitive performance and sex steroid levels between baseline and follow-up (N = 1,827)

	Mean (SD) change	*t*-value	*p*-value
Cognitive scores			
ROCF Copy	0.0 (3.5)	0.21	0.834
ROCF Recall	0.6 (5.8)	479	<0.001
CTRM	0.2 (3.8)	2.13	<0.05
DSST	-0.9 (4.9)	7.74	<0.001
Hormone levels			
TT (nmol/l)	-0.4 (4.1)	4.15	<0.001
FT (pmol/l)	-14.9 (71.5)	8.88	<0.001
DHEAS (µmol/l)	-0.8 (1.4)	26.35	<0.001
LH (IU/l)	0.3 (2.4)	-4.75	<0.001
FSH (IU/l)	0.3 (2.9)	-4.45	<0.001
SHBG (nmol/l)	2.2 (10.8)	-8.86	<0.001

Cognitive scores represent z-scores of the follow-up – baseline score.

Abbreviations: ROCF, Rey-Osterrieth Complex Figure; CTRM, Camden Topographical Recognition Memory; DSST, Digit Symbol Substitution Test; TT, total testosterone; FT, free testosterone; DHEAS, dehydroepiandrosterone sulphate; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SHBG, sex hormone-binding globulin

Table 3 Baseline hormone levels and change in cognitive performance (n = 1,827)

	ROCF Copy	ROCF Recall	CTRM	DSST
Model 1[a]				
TT (nmol/l)	-0.003 (-0.010; 0.005)	-0.008 (-0.015; -0.000)	0.0035 (-0.004; 0.011)	0.005 (-0.003; 0.012)
FT (pmol/l)	-0.000 (-0.001; 0.000)	**-0.001 (-0.001; -0.000)***	0.000 (-0.000; 0.000)	0.000 (0.000; 0.001)
Total E2(pmol/l)	0.001 (-0.001; 0.003)	-0.001 (-0.003; 0.001)	-0.001 (-0.003; 0.001)	-0.001 (-0.003; 0.001)
Free E2(pmol/l)	0.059 (-0.050; 0.167)	-0.067 (-0.175; 0.041)	-0.071 (-0.180; 0.039)	-0.013 (-0.122; 0.096)
DHEAS (µmol/l)	0.003 (-0.016; 0.021)	-0.011 (-0.030; 0.008)	-0.020 (0.001; 0.039)	0.009 (-0.010; 0.028)
DHT (nmol/l)	-0.023 (-0.067; 0.021)	**-0.065 (-0.109; -0.022)***	0.052 (0.008; 0.097)	0.010 (-0.034; 0.054)
LH (IU/l)	0.005 (-0.009; 0.018)	0.004 (-0.010; 0.017)	-0.002 (-0.016; 0.011)	-0.014 (-0.028; -0.001)
FSH (IU/l)	0.000 (-0.006; 0.006)	0.003 (-0.003; 0.009)	-0.005 (-0.011; 0.001)	-0.002 (-0.008; 0.005)
SHBG (nmol/l)	0.001 (-0.002; 0.003)	-0.001 (-0.003; 0.002)	-0.000 (-0.003; 0.002)	-0.001 (-0.004; 0.002)
Model 2[b]				
TT (nmol/l)	-0.002 (-0.010; 0.006)	-0.008 (-0.016; -0.000)	0.003 (-0.005; 0.012)	-0.002 (-0.010; 0.006)
FT (pmol/l)	-0.000 (-0.001; 0.000)	-0.001 (-0.001; -0.000)	0.000 (-0.000; 0.001)	0.000 (-0.000; 0.001)
Total E2 (pmol/l)	0.001 (-0.001; 0.002)	-0.001 (-0.003; 0.001)	-0.001 (-0.003; 0.001)	-0.001 (-0.003; 0.001)
Free E2(pmol/l)	0.021 (-0.094; 0.136)	-0.957 (-0.169; 0.055)	-0.075 (-0.193; 0.042)	-0.060 (-0.174; 0.055)
DHEAS (µmol/l)	0.001 (-0.018; 0.021)	-0.012 (-0.031; 0.006)	0.014 (-0.005; 0.034)	0.014 (-0.005; 0.033)
DHT (nmol/l)	-0.029 (-0.073; 0.015)	-0.057 (-0.099; -0.014)	0.043 (-0.002; 0.088)	0.011 (-0.033; 0.055)
LH (IU/l)	0.004 (-0.010; 0.017)	-0.001 (-0.014; 0.012)	-0.003 (-0.016; 0.011)	-0.012 (-0.025; 0.002)
FSH (IU/l)	0.001 (-0.005; 0.008)	0.001 (-0.005; 0.007)	-0.005 (-0.011; 0.002)	-0.000 (-0.007; 0.006)
SHBG (nmol/l)	0.001 (-0.002; 0.004)	-0.000 (-0 003; 0.002)	-0.000 (-0.003; 0.003)	-0.002 (-0.005; 0.001)
Model 3[c]				
TT (nmol/l)	-0.000 (-0.009; 0.008)	-0.007 (-0.016; 0.001)	0.004 (-0.004; 0.013)	-0.007 (-0.009; 0.008)
FT (pmol/l)	-0.000 (-0.001; 0.000)	-0.001 (-0.001; 0.000)	0.000 (-0.000; 0.001)	0.000 (-0.000; 0.001)
Total E2 (pmol/l)	0.000 (-0.002; 0.002)	-0.001 (-0.003; 0.001)	-0.001 (-0.003; 0.001)	-0.001 (-0.003; 0.001)
Free E2(pmol/l)	-0.002 (-0.125; 0.121)	-0.042 (-0.161; 0.077)	-0.068 (-0.191; 0.056)	-0.059 (-0.180; 0.061)
DHEAS (µmol/l)	0.000 (-0.019; 0.020)	-0.010 (-0.030; 0.009)	0.014 (-0.006; 0.034)	0.015 (-0.005; 0.034)
DHT (nmol/l)	-0.032 (-0.078; 0.014)	-0.050 (-0.094; -0.005)	0.038 (-0.008; 0.085)	0.016 (-0.029; 0.061)
LH (IU/l)	0.003 (-0.011; 0.017)	-0.002 (-0.016; 0.011)	-0.001 (-0.015; 0.013)	-0.014 (-0.027; 0.000)
FSH (IU/l)	0.002 (-0.005; 0.008)	0.001 (-0.005; 0.007)	-0.005 (-0.012; 0.001)	-0.002 (-0.008; 0.005)
SHBG (nmol/l)	0.002 (-0.001; 0.005)	-0.001 (-0.003; 0.002)	0.000 (-0.003; 0.003)	-0.002 (-0.005; 0.001)

Data are beta coefficients (95% confidence interval); positive values indicate that higher baseline hormone values overall are associated with improvements (positive changes) in cognitive function; negative values indicate that higher baseline hormone values overall are associated with a worsening (negative changes) of cognitive function.

* p <0.0055

Abbreviations: ROCF, Rey-Osterrieth Complex Figure; CTRM, Camden Topographical Recognition Memory; DSST, Digit Symbol Substitution Test; TT, total testosterone; FT, free testosterone; E2, oestradiol; DHEAS, dehydroepiandrosterone sulphate; DHT, 5α-dihydrotestosterone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SHBG, sex hormone-binding globulin

[a] Adjusted for age

[b] Adjusted for age, age left education, BMI, BDI score, smoking (non- vs current), alcohol consumption (<1 vs ≥ 1 day/week), psychotropic drug use (none vs any), and centre

[c] Adjusted for age, age left education, BMI, BDI score, smoking (non- vs current), alcohol consumption (<1 vs ≥ 1 day/week), psychotropic drug use (none vs any), centre, PASE score, PPT walking speed, and co-morbidities (0, 1, or 2 co-morbidities)

Table 4 Change in hormone levels and cognitive performance (n = 1,827)

	ROCF Copy	ROCF Recall	CTRM	DSST
Model 1[a]				
TT (nmol/l)	-0.004 (-0.015; 0.007)	-0.002 (-0.013; 0.009)	0.003 (-0.009; 0.014)	0.014 (0.003; 0.025)
FT (pmol/l)	-0.000 (-0.001; 0.000)	-0.000 (-0.001; 0.001)	0.000 (-0.000; 0.001)	0.000 (-0.000; 0.001)
DHEAS (µmol/l)	-0.024 (-0.058; 0.010)	-0.002 (-0.036; 0.032)	-0.005 (-0.039; 0.030)	-0.005 (-0.039; 0.029)
LH (IU/l)	-0.012 (-0.032; 0.007)	0.006 (-0.014; 0.026)	-0.023 (-0.043; -0.003)	-0.007 (-0.027; 0.013)
FSH (IU/l)	-0.002 (-0.017; 0.014)	0.003 (-0.013; 0.018)	-0.007 (-0.022; 0.009)	0.003 (-0.013; 0.018)
SHBG (nmol/l)	-0.002 (-0.006; 0.002)	-0.001 (-0.005; 0.003)	0.001 (-0.003; 0.005)	0.004 (-0.001; 0.008)
Model 2[b]				
TT (nmol/l)	-0.002 (-0.013; 0.009)	-0.005 (-0.016; 0.006)	-0.002 (-0.014; 0.009)	0.011 (-0.001; 0.022)
FT (pmol/l)	0.000 (-0.001; 0.001)	0.000 (-0.001; 0.001)	0.000 (-0.001; 0.001)	0.000 (-0.000; 0.001)
DHEAS (µmol/l)	-0.019 (-0.053; 0.015)	0.002 (-0.031; 0.035)	0.005 (-0.030; 0.040)	-0.021 (-0.055; 0.013)
LH (IU/l)	-0.008 (-0.028; 0.011)	0.005 (-0.014; 0.024)	-0.021 (-0.041; -0.001)	-0.003 (-0.023; 0.016)
FSH (IU/l)	-0.002 (-0.017; 0.014)	-0.008 (-0.023; 0.008)	-0.004 (-0.020; 0.012)	0.006 (-0.010; 0.022)
SHBG (nmol/l)	-0.002 (-0.007; 0.002)	-0.003 (-0.008; 0.001)	-0.001 (-0.006; 0.003)	0.003 (-0.002; 0.007)
Model 3[c]				
TT (nmol/l)	-0.002 (-0.014; 0.009)	-0.006 (-0.018; 0.005)	-0.004 (-0.016; 0.008)	0.009 (-0.003; 0.020)
FT (pmol/l)	0.000 (-0.001; 0.001)	-0.000 (-0.001; 0.001)	-0.000 (-0.001; 0.001)	0.000 (-0.000; 0.001)
DHEAS (µmol/l)	-0.023 (-0.058; 0.013)	0.001 (-0.034; 0.035)	0.009 (-0.027; 0.045)	-0.020 (-0.054; 0.015)
LH (IU/l)	-0.007 (-0.028; 0.013)	0.004 (-0.016; 0.024)	-0.024 (-0.045; -0.003)	-0.006 (-0.027; 0.014)
FSH (IU/l)	-0.002 (-0.019; 0.014)	-0.008 (-0.024; 0.008)	-0.007 (-0.023; 0.010)	0.009 (-0.007; 0.025)
SHBG (nmol/l)	-0.003 (-0.007; 0.002)	-0.003 (-0.008; 0.001)	-0.001 (-0.006; 0.004)	0.002 (-0.002; 0.007)

Data are beta coefficients (95% confidence interval); positive values indicate that an increase in hormone values is associated with an improvement (positive change) in cognitive function; negative values indicate that a decrease in hormone values is associated with a worsening (negative change) in cognitive function.

Abbreviations: ROCF, Rey-Osterrieth Complex Figure; CTRM, Camden Topographical Recognition Memory; DSST, Digit Symbol Substitution Test; TT, total testosterone; FT, free testosterone; DHEAS, dehydroepiandrosterone sulphate; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SHBG, sex hormone-binding globulin

[a] Adjusted for age

[b] Adjusted for age, age left education, BMI, BDI score, smoking (non- vs current), alcohol consumption (<1 vs ≥ 1 day/week), psychotropic drug use (none vs any), and centre

[c] Adjusted for age, age left education, BMI, BDI score, smoking (non- vs current), alcohol consumption (<1 vs ≥ 1 day/week), psychotropic drug use (none vs any), centre, PASE score, PPT walking speed, and co-morbidities (0, 1, or 2 co-morbidities)

Table 5 Continuous and tertile CAG repeat length and change in cognitive performance (n = 1,423)

	ROCF Copy	ROCF Recall	CTRM	DSST
Model 1[a]				
CAG repeat length	-0.000 (-0.017; 0.017)	0.008 (-0.009; 0.024)	-0.006 (-0.023; 0.011)	-0.009 (-0.025; 0.007)
Tertile I	Reference	Reference	Reference	Reference
Tertile II	-0.084 (-0.206; 0.038)	-0.009 (-0.128; 0.110)	-0.031 (-0.152; 0.090)	0.003 (-0.113; 0.120)
Tertile III	0.044 (-0.093; 0.182)	0.038 (-0.097; 0.173)	-0.060 (-0.197; 0.077)	-0.086 (-0.217; 0.046)
Model 2[b]				
CAG repeat length	0.002 (-0.015; 0.019)	0.006 (-0.010; 0.022)	-0.004 (-0.021; 0.012)	-0.004 (-0.020; 0.012)
Tertile I	Reference	Reference	Reference	Reference
Tertile II	-0.083 (-0.201; 0.040)	0.014 (-0.102; 0.131)	-0.013 (-0.135; 0.110)	0.035 (-0.080; 0.149)
Tertile III	0.062 (-0.077; 0.201)	0.015 (-0.116; 0.147)	-0.055 (-0.194; 0.083)	-0.042 (-0.172; 0.088)
Model 3[c]				
CAG repeat length	0.003 (-0.014; 0.021)	0.008 (-0.009; 0.025)	-0.002 (-0.019; 0.016)	-0.004 (-0.021; 0.012)
Tertile I	Reference	Reference	Reference	Reference
Tertile II	-0.076 (-0.205; 0.053)	0.024 (-0.098; 0.147)	-0.009 (-0.136; 0.118)	0.023 (-0.095; 0.142)
Tertile III	0.064 (-0.081; 0.053)	0.032 (-0.105; 0.170)	-0.040 (-0.183; 0.104)	-0.054 (-0.187; 0.080)

Abbreviations: ROCF, Rey-Osterrieth Complex Figure; CTRM, Camden Topographical Recognition Memory; DSST, Digit Symbol Substitution Test

[a] Adjusted for age

[b] Adjusted for age, age left education, BDI score, BMI, smoking (non- vs current), alcohol consumption (<1 vs ≥ 1 day/week), psychotropic drug use (none vs any), and centre

[c] Adjusted for age, age left education, BDI score, BMI, smoking (non- vs current), alcohol consumption (<1 vs ≥ 1 day/week), psychotropic drug use (none vs any), centre, PASE score, PPT walking speed, and co-morbidities (0, 1, or 2 co-morbidities)