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CLINICAL STUDY

Endogenous hormones, androgen receptor CAG repeat length and fluid cognition in middle-aged and older men: results from the European Male Ageing Study

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Abstract

Objective: Data remain divergent regarding the activational effects of endogenous hormones on adult cognitive function. We examined the association between cognition, hormones and androgen receptor (*AR*) CAG repeat length in a large cohort of men.

Design: Community-based, cross-sectional study of 3369 men aged 40–79 years.

Methods: Cognition tests were the Rey-Osterrieth Complex Figure, Camden Topographical Recognition Memory and Digit-Symbol Substitution. A fluid cognition (FC) *z*-score was computed from the individual tests. Testosterone, oestradiol (OE_2) and 5α-dihydrotestosterone were measured by gas chromatography–mass spectrometry; DHEAS, LH, FSH and sex hormone-binding globulin (SHBG) by electrochemiluminescence. Free testosterone and OE_2 were calculated from total hormone, SHBG and albumin. CAG repeat lengths were assayed by PCR genotyping.

Results: Total testosterone and free testosterone were associated with higher FC *z*-scores, LH and FSH with lower FC *z*-scores in age-adjusted linear regressions. After adjusting for health, lifestyle and centre, a modest association was only observed between DHEAS and a lower FC *z*-score ($\beta = -0.011$, $P = 0.02$), although this was driven by subjects with DHEAS levels > 10 μmol/l. Locally weighted plots revealed no threshold effects between hormones and FC. There was no association between CAG repeat length and FC *z*-score after adjustment for age and centre ($\beta = -0.007$, $P = 0.06$), nor any interaction effect between CAG repeat length and hormones.

Conclusion: Our results suggest that endogenous hormones are not associated with a vision-based measure of FC among healthy, community-dwelling men. Further studies are warranted to determine whether 'high' DHEAS levels are associated with poorer performance on a broader range of neuropsychological tests.

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Introduction

Although ageing is associated with functional declines in cognitive performance, these changes vary greatly between individuals, with only a proportion ultimately developing dementia (1). Nonetheless, declining cognitive performance severely abrogates quality of life and increases disability and dependency in an increasing

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number of elderly people (2, 3). An improved understanding of the factors which impact on age-related declines in cognitive performance may elucidate the underlying aetiologies and help develop strategies to maintain function and quality of life in the elderly.

In men, ageing is associated with changes in the levels of a number of hormones. Androgens, such as testosterone and DHEAS, decline progressively with age in healthy men from the 4th decade (30–39 years) onwards, while LH, FSH and sex hormone-binding globulin (SHBG) increase (4–7). Although testosterone and other neuroactive steroid hormones have been shown to play important organisational roles in the early development of cognitive and behavioural sexual dimorphisms in animal models (8), it remains unclear whether age-related changes in these hormone levels modulate cognitive functioning in normally ageing men (9).

Activational effects of endogenous hormones on cognition in adult men reported in the literature are inconsistent (9). For example, Martin and co-workers found that in men aged between 35 and 80 years, higher levels of both total testosterone and free testosterone were associated with poorer verbal memory and executive function, and faster processing speed (10). Thilers and co-workers described a positive relationship between free testosterone and both visuo-spatial and episodic memory performance in men, with a greater positive influence with increasing age (11). In contrast, data from the Massachusetts Male Ageing Study (MMAS) found no association between various hormones, including testosterone, oestrone, DHEAS and LH, and cognition when confounding factors were considered (12).

The decline in cognitive ability through middle to old age is evidently a complex multifactorial process that is influenced by educational, occupational, lifestyle and genetic factors (13, 14). If endogenous androgens impact on cognitive function, it is possible that genetic variations such as the androgen receptor (*AR*) gene CAG repeat polymorphism may directly or indirectly modulate these relationships. ARs are widely distributed throughout the brain, including learning and memory critical regions such as the hippocampus (15) and cerebral cortex (16). Longer CAG repeat length in exon 1 of the X-linked *AR* gene has been associated with decreased androgen sensitivity, (17) and epidemiological and clinical studies have suggested that variability within the normal range of CAG repeat length modulates androgen action (18). A recent study by Yaffe *et al*. showed that longer CAG repeat lengths were associated with lower cognitive functioning and poorer performance on a single dementia screening measure in older men (19). However, because of the feedback regulation within the endocrine axis which automatically compensates for any deficits in hormone action by adjusting the level of secreted hormones, it is important to examine the relationships of endogenous androgen levels and the *AR* CAG repeat polymorphism simultaneously to better characterise their influence on cognitive function in men. We used baseline data from the European Male Ageing Study (EMAS), a multicentre study of ageing among community-dwelling middle-aged and older men, to investigate the relationships between hormones and *AR* CAG repeat length with cognitive function.

Methods

Subjects and study design

Men aged 40–79 years were recruited from population registers for participation in EMAS in eight European centres: Florence (Italy); Leuven (Belgium); Lodz (Poland); Malmö (Sweden); Manchester (UK); Santiago de Compostela (Spain); Szeged (Hungary); and Tartu (Estonia). EMAS is a prospective, non-interventional cohort study of male ageing. There are two phases; a cross-sectional survey was undertaken between 2003 and 2005 with a follow-up investigation planned for completion in 2009. For the baseline survey, stratified random sampling was used with the aim of recruiting equal numbers of men into each of four age bands (40–49, 50–59, 60–69 and 70–79 years). The overall response rate for participation in the baseline phase was 41%. Further details of the sampling and recruitment procedures have been described previously (20). Ethical approval for the study was obtained in accordance with local institutional requirements in each centre, with written informed consent obtained from all the participants.

Assessments

Subjects were invited by letter to attend a screening visit at a local clinic. Included in the letter was a short questionnaire that the subjects were asked to complete and that gathered information on sociodemographic, general health, medical conditions, medications, smoking and alcohol consumption. Co-morbid conditions included self-reported heart condition, high blood pressure, diabetes, cancer, stroke, prostate disease, liver disease, kidney disease, thyroid disorders, bronchitis, asthma and epilepsy. The details of questionnaire standardisation and validation have been described previously (20).

Men who agreed to participate subsequently attended a hospital research clinic to complete an interviewer-assisted questionnaire which included the Beck Depression Inventory-II (BDI) to measure depressive symptomology (21). Height and weight were measured using standard procedures, and body mass index (BMI) defined as weight (kg) divided by the square of height (m) was measured. Medication use was corroborated by examination of prescriptions and drugs brought to the clinic for that purpose.

Blood sampling, hormone assays and AR *CAG repeat genotyping*

Morning phlebotomy was performed before 1000 h to obtain a fasting blood sample. Measurement of total testosterone, total oestradiol (OE_2) and 5α-dihydrotestosterone (DHT) was carried out by gas chromatography–mass spectrometry (GC–MS) (22). LH, FSH, DHEAS and SHBG levels were measured using the Modular E170 platform electrochemiluminescence immunoassays (Roche Diagnostics) as described previously (23). Free testosterone and OE_2 levels were derived from total hormone, SHBG and serum albumin concentrations (24). Further details of hormone assays and coefficients of variation have been reported previously (22, 23).

Genotyping of the *AR* CAG repeat was performed in the laboratory of the Centre for Integrated Genomic Medical Research (CIGMR, The University of Manchester) using the PCR and fluorescently-labelled primers as described previously (25). Post PCR, samples were run on an ABI Prism 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, USA) and were genotyped using GeneScan analysis software (Applied Biosystems), and allele frequencies were checked for consistency with HapMap data.

Cognitive function assessment

The cognitive domains assessed in this study were spatial awareness, memory and processing speed, i.e. domains traditionally attributed to 'fluid cognition' (FC) (26). The EMAS battery of cognitive tests was specifically selected on the basis that they could be standardised across different centres and applied to individual subjects independent of culture and language. They were (in the order they were administered) the Rey-Osterrieth Complex Figure (ROCF) test, the Camden Topographical Recognition Memory (CTRM) test and the Digit-Symbol Substitution (DSST) test. Higher scores for each test reflect better cognitive performance.

Copying and delayed reproduction of the ROCF were used as a measure of visual perceptual abilities, memory and executive function (27). The copying score measures overall visuo-constructional ability, while the delayed recall is more indicative of visual memory. The ROCF scoring criteria used here was based upon Osterreith's original test procedure (27), which defined 18 units of the drawing, assigning point values of 0–2 to each unit depending upon the degree to which the units are correctly drawn and placed. Each element of the ROCF test has a score range of 0–36. Subjects were requested to complete the copy and the 30-min delayed recall elements, but they were not pre-informed that there would be a memory component to the test. The CTRM test was developed in order to measure the recognition component of visual memory retrieval, tapping into the cortical component of visual memory (28). The CTRM involves the presentation of 30 colour photographs of urban scenes, each for 3 seconds, followed by a three-way forced recognition component. The CTRM has a score range of 0–30. The DSST test is a subtest adopted from the Wechsler Adult Intelligence Scales and provides a reliable measure of cognitive processing speed, visual scanning and memory (29). The test sheet consists of nine symbols corresponding with nine digits followed by 13 rows of symbols with blank spaces below them. Subjects were asked to make as many correct digit-for-symbol substitutions as possible within a 1-min period.

The raw cognitive scores were converted to standardised scores (*z*-scores) using the mean and S.D. from the baseline measures for all the subjects. The *z*-scores were then averaged to derive a single measure considered to mainly, though not uniquely, reflect FC. This approach has been used in several previous studies characterising cognitive function (30–32).

Analysis

Statistical analyses were performed using Intercooled Stata version 9.2 (StataCorp, College Station, TX, USA). Subjects with either missing cognitive or hormone data, prevalent pituitary or testicular disease or those using medications which could directly impact upon pituitary/testicular function (23) were excluded from this analysis. Cognitive outcomes, hormone levels, age, age at which education was left, the BDI score and BMI were treated as continuous variables. *AR* CAG repeat lengths were examined as continuous variables or classified into quintiles.

Linear regression was used to determine the association of hormones and SHBG levels (independent variables) with the FC *z*-score (dependent variable) after adjusting for age. Adjustments were subsequently made for education, depression, BMI, smoking, alcohol consumption, psychotropic drug use and centre. Regression analyses of the association between CAG repeat lengths and FC *z*-score were adjusted for age and centre. Effect modification by CAG repeat length was also assessed by inclusion of interaction terms between the androgenic hormones (total testosterone, free testosterone, DHT or DHEAS) and CAG repeat length in the regression models. Results are expressed as unstandardised β-coefficients (β) and 95% confidence intervals.

The association of hormone and SHBG levels, and *AR* CAG repeat length with the FC *z*-score was also evaluated graphically (while simultaneously adjusting for age) using the locally weighted scatterplot smoothing (LOWESS) technique (33). This approach, where linear regression is applied repeatedly to sequential small sections of the covariate–outcome relationship, is primarily exploratory. By reducing the influence of outliers, this technique provides a smooth fit to the data so that overall relationships and any thresholds or

inflection points can be more readily identified. Importantly, it makes no *a priori* assumptions as to the best model to describe the covariate–outcome relationship across the entire data range.

Results

Cohort characteristics

A total of 153 subjects with incomplete cognitive and/or hormone data, and 143 subjects with known pituitary or testicular diseases, or using medications which could affect pituitary/testicular functions or sex steroid clearance (23) were excluded from this analysis. The baseline characteristics of the remaining 3073 men are shown in Table 1. The number of subjects in each age decade was $n=759$ (40–49 years), $n=839$ (50–59 years), $n=774$ (60–69 years and $n=701$ (70–79 years). Among those reporting one or more morbidities ($n=1501$), the four most common conditions were hypertension (57%), heart condition (32%), prostate disease (20%) and diabetes (15%).

The mean hormone levels, *AR* CAG repeat length and cognitive test scores are shown in Table 2. Total testosterone ($r=-0.03$, $P=0.08$), free testosterone ($r=-0.37$, $P<0.001$) and DHEAS ($r=-0.52$, $P<0.001$) showed negative, and LH ($r=0.30$, $P<0.001$), FSH ($r=0.31$, $P<0.001$) and SHBG ($r=0.36$, $P<0.001$) showed positive cross-sectional relationships with age (23), with no significant association between CAG repeat length and age (25). Total OE_2 ($r=0.12$, $P<0.001$) and DHT ($r=0.06$, $P<0.01$) were positively associated with age. As reported previously (25), CAG repeat length was positively associated with total and free levels of testosterone and OE_2, and it was inversely associated with LH (all $P<0.05$, data not shown).

Table 1 Baseline characteristics of European Male Ageing Study (EMAS) cohort ($n=3073$).

	Mean (S.D.) **or %**
Age at interview (years)	59.5 (10.9)
Age left education (years)	20.9 (7.6)
Beck Depression Inventory (BDI)	6.8 (6.4)
Body mass index (kg/m^2)	27.7 (4.1)
BDI-II depression category (score range)	
Minimal depression (0–13)	87.1%
Mild–borderline (14–19)	8.6%
Moderate–extreme (20–56)	4.3%
Current smoker	21.1%
Alcohol consumption $\geq$1 day/week	57.1%
One or more morbidities	49.6%
Any psychotropic drugs[a]	7.1%

[a]Includes anti-depressants, anti-psychotics and sedatives.

Table 2 Baseline hormone, *AR* CAG repeat length and cognitive scores.

	Mean (S.D.)
Hormones	
Testosterone (nmol/l)	16.5 (5.9)
Oestradiol (pmol/l)	73.9 (25.0)
5α-dihydrotestosterone (nmol/l)	1.3 (0.6)
DHEAS (μmol/l)	4.6 (2.8)
LH (IU/l)	6.2 (4.3)
FSH (IU/l)	8.5 (8.8)
Sex hormone-binding globulin (nmol/l)	42.5 (19.4)
AR CAG repeat length	22.9 (3.2)
Cognitive test scores	
ROCF copy	33.4 (4.4)
ROCF recall	17.1 (6.6)
Camden topographical memory	22.8 (4.7)
Digit-symbol substitution	27.8 (8.8)

AR, androgen receptor; ROCF, Rey-Osterrieth Complex Figure.

Cognitive tests

As reported previously, there were significant between-centre differences in cognitive test scores with centres in northern and western Europe (Leuven, Manchester, Malmö) scoring highest for all the tests, while centres in Southern Europe scored lower (34). All four scores were inversely associated with higher age (34). The distribution of the FC *z*-score is shown in Fig. 1 using a smoothed histogram (Epanechnikov kernel density plot) (35), and it achieved a near normal distribution in our study population. The internal homogeneity of the FC *z*-score was assessed by Cronbach's coefficient α. The overall α was 0.74 indicating a good degree of intercorrelation among the four items.

Hormones and cognition

Results of the multiple linear regressions of the FC *z*-score versus hormones (including SHBG) are shown in Table 3. Models are presented adjusting for age only or age, education, depression, BMI, smoking, alcohol consumption, morbidities, psychotropic drug use and centre. In all cases, age was independently associated with the FC *z*-score in all linear models shown in Table 3 ($P<0.001$, data not shown). In age-adjusted models, higher levels of total testosterone ($\beta=0.006$, $P=0.004$) and free testosterone ($\beta=0.007$, $P<0.001$) were significantly associated with a higher FC *z*-score, while higher levels of LH ($\beta=-0.008$, $P=0.01$) and FSH ($\beta=-0.003$, $P=0.04$) were associated with a lower FC *z*-score. However, after adjustment for age, centre and the other confounders, an independent and negative association was only observed between DHEAS and the FC *z*-score ($\beta=-0.011$, $P=0.014$). There were no independent associations between any of the other hormones or SHBG and the FC *z*-score (all $P>0.05$) in the fully adjusted regression models. When the multivariable-adjusted regression analyses were restricted to older subjects ($\geq$60 years), with the FC *z*-score as the

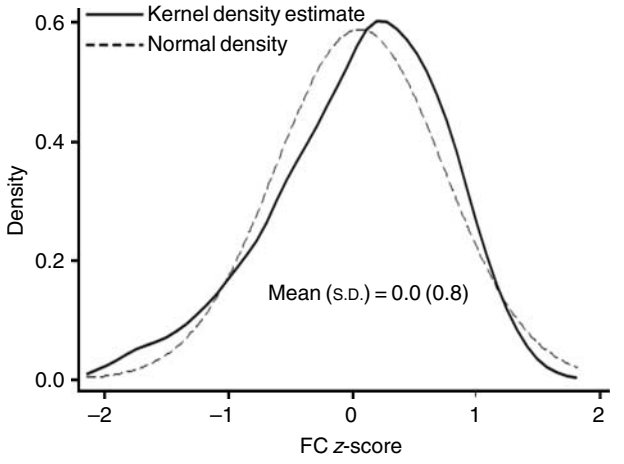

Figure 1 Distribution of fluid cognition (FC) *z*-score. Curves represent smoothed histograms (Epanechnikov kernel density plot (35)); density refers to the area under the curve (equal to 1).

outcome, broadly the same patterns of association were observed as in Table 3 (all $P>0.05$, data not shown), with the lack of an independent association between DHEAS and the FC *z*-score most likely due to reduced statistical power. We also performed additional exploratory analyses to examine whether hormone–cognition associations differed among men with a potentially affected/morbid hypothalamic–pituitary–testicular axis, i.e. obesity (BMI ≥ 30 kg/m^2) and/or self-reported diabetes ($n=852$). No significant associations between any of the hormones and the FC *z*-score were observed in this subgroup of men in the fully adjusted linear regression models (all $P>0.05$, data not shown).

Figure 2 shows the results from the age-adjusted LOWESS analyses. In addition to there being no distinct associations between hormone levels (including SHBG) and the FC *z*-score, the LOWESS plots did not reveal any obvious threshold level effects. Although no inflection was apparent in the association between total testosterone and the FC *z*-score, when we considered 'hypogonadal' levels of testosterone, i.e. total testo-sterone <10.5 nmol/l = 'hypogonadal' ($n=419$) versus ≥ 10.5 nmol/l = 'eugonadal' ($n=2654$; reference group) and repeated the multivariable linear regression using total testosterone as an independent variable dichotomised around this level, we again found no significant association with the FC *z*-score ($\beta=0.053$, $P=0.11$).

To explore further the relationship between DHEAS and cognitive functions, we repeated the multivariable-adjusted linear regression using the raw scores from each of the four individual cognitive tests as dependent variables (see Table 4). Increasing DHEAS levels were independently associated with the CTRM ($\beta=-0.089$, $P=0.005$) and DSST scores ($\beta=-0.136$, $P=0.013$). When DHEAS was treated as a quintiled categorical variable in the same regression models (Table 4), significant associations were observed between the subjects in the 4th ($\beta=-0.595$, $P=0.03$) and 5th ($\beta=-0.841$, $P=0.002$) quintiles of DHEAS for the CTRM score, and the 5th quintile only ($\beta=-1.226$, $P=0.01$) for the DSST score (with the 1st quintile as the reference group).

AR *CAG repeat length and cognition*

The results from the regression models exploring the association between *AR* CAG repeat length and the FC *z*-score are summarised in Table 5. An increasing CAG repeat length was not independently associated with the FC *z*-score in both unadjusted ($\beta=-0.009$, $P=0.06$) and age- and centre-adjusted ($\beta=-0.007$, $P=0.06$) linear regressions. Potential effect modification of the CAG repeat length – FC *z*-score association by age was explored by including an interaction term (CAG repeat length × age decade) in the linear regressions (all $P_{\text{interaction}}>0.1$, data not shown). In addition, when we restricted our unadjusted regression analysis to older

Table 3 Association between fluid cognition (FC) *z*-score (dependent variable) and serum hormone levels: multiple regression analyses.

	Fluid cognition *z*-score	
Hormone	Age[a]	Full model[b]
β-coefficient (95% CI)		
Total testosterone (nmol/l)	0.006 (0.002, 0.010)†	−0.003 (−0.007, 0.001)
Free testosterone (10 pmol/l)	0.007 (0.004, 0.010)‡	−0.002 (−0.005, 0.000)
Total OE_2 (pmol/l)	0.001 (−0.001, 0.002)	0.000 (−0.001, 0.001)
Free OE_2 (pmol/l)	0.050 (−0.003, 0.104)	−0.004 (−0.056, 0.047)
DHT (nmol/l)	0.000 (−0.039, 0.038)	−0.034 (−0.073, 0.005)
DHEAS (μmol/l)	−0.009 (−0.019, 0.001)	−0.011 (−0.020, −0.002)*
LH (IU/l)	−0.008 (−0.013, −0.002)†	−0.002 (−0.008, 0.003)
FSH (IU/l)	−0.003 (−0.006, −0.001)*	−0.001 (−0.004, 0.002)
SHBG (nmol/l)	−0.001 (−0.002, 0.001)	−0.001 (−0.002, 0.001)

OE_2, oestradiol; DHT, 5α-dihydrotestosterone; SHBG, sex hormone-binding globulin. *$P<0.05$, $^{\dagger}P<0.01$, $^{\ddagger}P<0.001$.
[a]Adjusted for age only.
[b]Adjusted for age, age at which full-time education was left, BDI score, BMI, smoking (non- versus current), alcohol consumption (<1 vs ≥1 day/week), morbidities (none versus any), psychotropic drug use (none versus any) and centre.

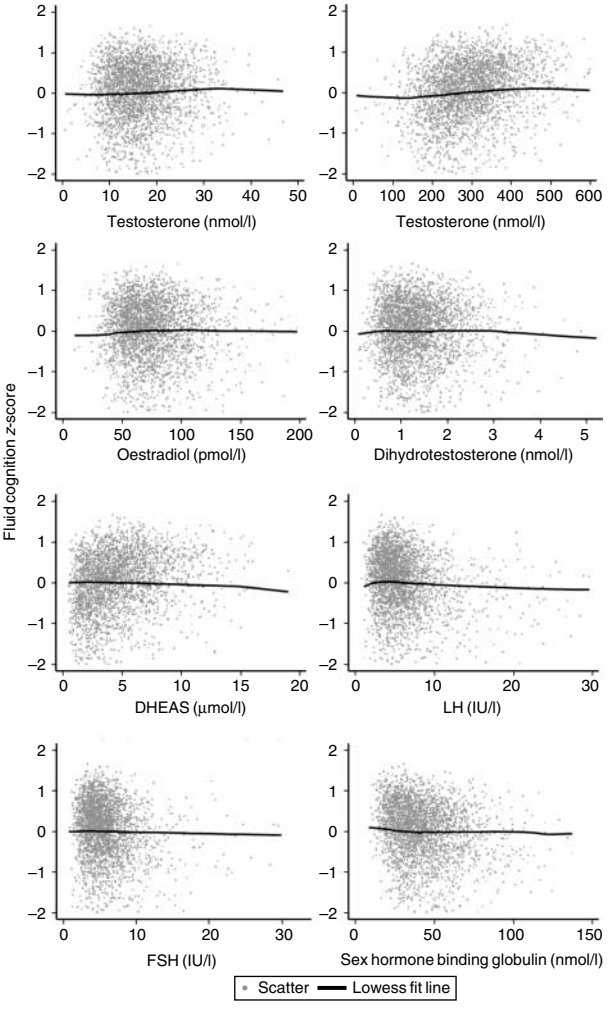

Figure 2 Relationship between sex hormone levels and FC *z*-score: LOWESS plots adjusted for age.

men ($\geq$60 years; $n=1475$), we found no evidence of a significant association between CAG repeat length and the FC *z*-score ($\beta=-0.011$, $P=0.12$). The LOWESS plot (see Fig. 3) supported the linear regression models in terms of there being no obvious association between the FC *z*-score and CAG repeat length, and it provided no evidence of any obvious threshold effect.

Modulation of hormone–cognition association by AR *CAG repeat length*

To explore any potential effect modification of hormone – FC *z*-score relationships by CAG repeat length, linear regressions were performed (adjusted for age and centre) including an interaction term between CAG repeat length and androgenic hormone (total testosterone, free testosterone, DHT or DHEAS). In all cases, no significant CAG repeat length by hormone interaction effects were observed (all $P>0.1$, data not shown).

Discussion

Main findings

In this population-based study of middle-aged and older European men, we investigated whether endogenous hormone levels and *AR* CAG repeat length were associated with cognitive functions that are known to decline with increasing age. Overall, our data provided no consistent evidence of either linear or threshold relationships between hormone levels and cognitive performance. Lower levels of total testosterone and free testosterone, and higher levels of LH and FSH were significantly associated with poorer FC *z*-scores, after adjustment for age. However, following adjustment for other potential confounders, the only statistically significant association was between higher levels of DHEAS and a lower FC *z*-score. Longer *AR* CAG repeat lengths were not independently associated with a lower FC *z*-score, and this polymorphism did not have a modulating effect on the relationship between androgenic hormones and cognition.

Previous studies of androgens and cognitive function

Previous observational studies have demonstrated positive relationships between androgens and measures of spatial visualisation, memory and attention (11, 36), while others have shown negative associations (10, 37). However, numerous studies have failed to find any relationship between androgens, OE_2 or gonadotrophins and tests of visuo-spatial ability, working memory and processing speed among men (12, 38–41). Some data exist from interventional trials supporting a positive effect of testosterone supplementation on spatial and verbal memory among both healthy, older men (42) and men with Alzheimer's or mild cognitive impairment (43). However, other interventional studies suggest that short-term, significant changes in sex hormone status do not modify normal day-to-day cognitive function in men (44). Several factors might explain these discrepant findings. Many studies have been limited by small sample sizes, failure to adjust for multiple confounders, utilisation of diverse cognitive tests (different domains may be differentially associated to hormones) and use of immunoassays to measure hormones (questionable precision and lack of standardisation) (9, 45).

Previous observational studies have tended to suggest that cognitive performance is not associated with DHEAS levels (12, 46, 47), while interventional studies of DHEA replacement in patients with adrenal deficiency have not shown any benefits on cognitive function (48, 49). Although we observed an inverse relationship between higher DHEAS levels and our composite measure of cognitive function, the magnitude of the linear association was small ($\sim$0.01 S.D. decrease in the FC *z*-score per 1 μmol/l increase in DHEAS). A recent

Table 4 Association between cognitive test scores and serum DHEAS levels: multiple regression analyses.

	ROCF copy	ROCF recall	CTRM	DSST
β-coefficient[a] (95% CI)				
DHEAS (μmol/l)	−0.061 (−0.122, 0.000)	0.023 (−0.069, 0.114)	−0.089 (−0.151, −0.027)†	−0.136 (−0.239, −0.033)†
DHEAS quintiles (μmol/l)				
I (0.5–2.2)	Reference	Reference	Reference	Reference
II (2.3–3.4)	−0.095 (−0.558, 0.368)	−0.315 (−1.009, 0.378)	−0.369 (−0.838, 0.101)	−0.637 (−1.413, 0.139)
III (3.5–4.8)	−0.227 (−0.708, 0.255)	0.012 (−0.710, 0.733)	−0.429 (−0.917, 0.060)	−0.207 (−1.015, 0.600)
IV (4.9–6.7)	−0.092 (−0.605, 0.422)	−0.176 (−0.946, 0.594)	−0.595 (−1.116, −0.074)*	−0.635 (−1.497, 0.226)
V (6.8–24.6)	−0.475 (−1.007, 0.058)	−0.028 (−0.826, 0.770)	−0.841 (−1.382, −0.301)†	−1.226 (−2.119, −0.332)*

*$P<0.05$, †$P<0.01$.
[a]Adjusted for age, age at which full-time education was left, BDI score, BMI, smoking (non- versus current), alcohol consumption (<1 vs ≥1 day/week), morbidities (none versus any), psychotropic drug use (none versus any) and centre.

longitudinal analysis of a Taiwanese cohort provided evidence that the relationship between DHEAS and a composite measure of cognitive function may have an inverted U-shape (50). Goldman & Glei reported an increase in cognitive impairment both at low (<33 μg/dl or <0.9 μmol/l) and at high levels of DHEAS (>218 μg/dl or >5.9 μmol/l) (50). Our data suggest that the overall negative associations seen between DHEAS and the ROCF copy, CTRM and DSST scores in age-adjusted linear regressions were primarily driven by men with DHEAS levels ≥6.8 μmol/l (250 μg/dl) (see 'quintile' regression results; Table 4). When we repeated the multivariable regressions with DHEAS as a continuous variable and excluded the subjects with DHEAS levels above 6.8 μmol/l, the associations with the ROCF copy, CTRM and DSST scores were attenuated and were no longer significant (all $P>0.05$, data not shown). Restricting the exclusion criteria to those subjects with DHEAS levels above 10 μmol/l (370 μg/dl), or equivalent to the 95th percentile for our data, gave broadly the same results (all $P>0.3$, data not shown). The fact that we did not find a positive association between DHEAS and cognitive performance may in part be due to the higher levels of DHEAS in our sample of men (10–90th percentiles = 1.6–8.3 μmol/l) compared with Goldman's (10–90th percentiles = 0.9–4.9 μmol/l) (50). DHEAS levels have been shown to decrease progressively with age in healthy men from the 4th decade onwards (4, 5), reflecting the higher levels found in our cohort (40–79 years) as compared with Goldman's sample (≥54 years). Indeed, if we are observing only the upper part of an inverted U-shaped relationship across the DHEAS range with cognition, this may explain the lack of any positive relationships in our cohort. However, Goldman & Glei used different cognitive tests compared with ours and also utilised a composite count of cognitive impairment (50) (derived from the number of cognitive tasks completed incorrectly), making direct comparisons with our composite cognitive score problematic. A caveat is that we did not obtain independent measures of either stress or glucocorticoid levels in this study. This may be important in interpreting the relationship between DHEAS and cognition as cognitive decline, and frailty in elderly adults is reported to be significantly related to the DHEAS-to-cortisol ratio but not to DHEAS alone (51).

The continuing difficulties in determining the relationships between endogenous hormones and cognitive function in adults have resulted in a number of investigators exploring whether non-linear or quadratic models better describe these relationships (52–55). However, our LOWESS analyses, which were not pre-specified in terms of the function to fit the model to the data, did not contradict our use of single linear functions to best describe the relationships between hormone levels and cognitive performance. A number of

Table 5 Influence of androgen receptor (*AR*) CAG repeat length on fluid cognition (FC) *z*-score.

	Fluid cognition *z*-score	
	Unadjusted	Full model[a]
β-coefficient (95% CI)		
Number of CAG repeats (7–40)	−0.009 (−0.018, 0.001)	−0.007 (−0.014, 0.000)
Quintiles (CAG repeat range)		
I (7–20)	Reference	Reference
II (21–22)	−0.049 (−0.135, 0.037)	−0.035 (−0.103, 0.033)
III (23–24)	−0.071 (−0.165, 0.022)	0.015 (−0.088, 0.059)
IV (25–25)	−0.002 (−0.115, 0.110)	0.001 (−0.088, 0.090)
V (26–40)	−0.073 (−0.168, 0.022)	−0.077 (−0.152, −0.002)

[a]Adjusted for age and centre.

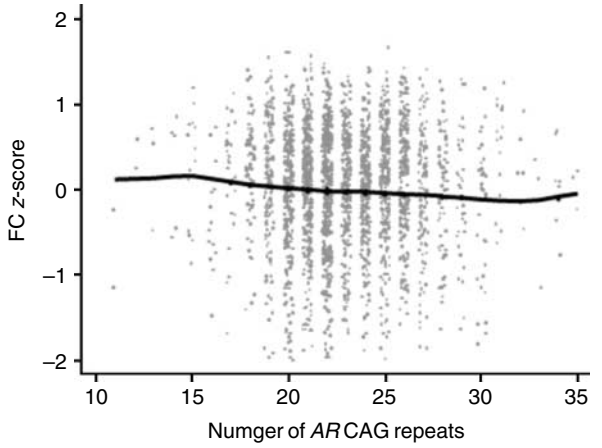

Figure 3 CAG repeat length and fluid cognition *z*-score: LOWESS plot adjusted for age.

studies have also suggested that significant associations between sex hormones and cognitive function may be limited to older men (50, 51, 53), although we found no evidence to support this in our data.

Previous studies of AR *CAG repeat length and cognitive function*

We are aware of only one previous community-based study that investigated the association between the *AR* CAG repeat polymorphism and cognitive function in men. Yaffe and co-workers showed that a longer CAG repeat length in exon 1 of the *AR* gene was associated with poorer performance on the Mini-Mental Status Examination, Trails B test and DSST among 301 older (mean age 73 years) men (19). In contrast, we did not find an independent association between CAG repeat length and a lower FC *z*-score either in our entire sample (see Table 5) or when the analysis was restricted to older men ($\geq$60 years) as in Yaffe's study. To compare more directly with Yaffe's data, we also explored the relationship between CAG repeat length and the DSST score in unadjusted regression models. In common with the FC *z*-score, we found no significant association between the DSST and CAG repeat length either in the entire sample ($\beta=-0.054$, $P=0.3$) or in men aged $\geq$60 years ($\beta=-0.023$, $P=0.8$). We have previously shown that weaker transcriptional activity of the *AR* with longer CAG repeat lengths appears to be totally or nearly totally compensated for by higher testosterone levels (25), and it is perhaps unsurprising that we failed to observe any independent association between CAG repeat length and cognition given the lack of any significant associations between either testosterone or OE_2 and the FC *z*-score. Nonetheless, further genetic association studies are needed to verify the relationship (or lack thereof) between this polymorphism and specific domains of cognitive function.

To our knowledge, there have been no previous studies exploring the influence of the *AR* CAG repeat length as a potential effect modifier of the association between hormones and cognition. In relation to human behaviour, data from the MMAS showed that depression was significantly and inversely associated with total testosterone in men with shorter (8–20), but not with moderate and longer (21–40), CAG repeats (56). Although we found that the length of the *AR* CAG repeat was not an effect modifier of the association between the hormones and the FC *z*-score, we cannot exclude the possibility that different relationships may exist with other cognitive domains.

Strengths and limitations

The main strengths of our study are that it is based on a large sample of community-dwelling men, and used uniform methods to assess cognitive function, hormone levels and putative confounders. In addition, it employed a state-of-the-art GC–MS methodology for measuring serum testosterone, OE_2 and DHT levels. Practical limitations inherent to the EMAS study have been described previously (20), although a number of specific factors need to be considered here. The overall response rate for participation in the study was 41%. Those who participated may have differed with respect to levels of cognitive function and also sex hormone status than those who did not participate, and some caution, therefore, is needed in interpreting these data. The main findings were based on internal association among responders which reduces the risk that selection factors had any important effect on these results. Our analysis was restricted to age-sensitive, vision-based tests of cognitive function. This was primarily to reduce language and cultural effects inherent in many text-based tests but was also due to the practical constraints of a large observational study that was not a neuropsychology-based project but a multidimensional exploration of ageing in males in Europe. The fact that we did not utilise the more conventional hormonally sensitive tests of cognitive function (e.g. spatial ability and verbal fluency) (9) may explain the absence of independent associations with sex hormones. In addition, although we derived a composite cognitive score that we consider mainly taps into 'fluid intelligence', crystallised intelligence is also likely to impact upon performance in the four cognitive tests used here. The cross-sectional data cannot offer any explanation for the observed associations with the possibility that some of our findings may be due to unmeasured factors and/or residual confounding. Ultimately, causality can only be supported with intervention trials. Finally, our results were obtained from a male, predominantly Caucasian European population and should be extrapolated beyond this setting with care.

Conclusion

Our results suggest that endogenous hormone levels are not markedly associated with performance on a composite measure of FC in a sample of healthy, community-dwelling middle-aged and older European men. DHEAS levels were inversely associated with FC, although this relationship was weak and appeared to be particularly influenced by the subjects with the highest levels of this adrenal androgen. The clinical significance of this finding is currently unclear. The CAG repeat polymorphism of the *AR* gene was not associated with cognitive performance in our sample, nor did it not modulate any observed effects of androgen levels on cognition. Future studies are warranted to ascertain whether high levels of DHEAS have detrimental effects on neuropsychological performance on a more extensive battery of cognitive tests, or if they are associated with adverse longitudinal changes in specific domains of cognitive performance during normal ageing.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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