

## Hormone levels and cognitive function in postmenopausal midlife women

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### Abstract

Gonadal hormones may influence cognitive function. Postmenopausal midlife women in the population-based Melbourne Women's Midlife Health Project cohort were administered a comprehensive battery of neuropsychological tests on two occasions 2 years apart. Participants ( $n = 148$ , mean age 60 years) had undergone natural menopause and were not using hormone therapy. Estrone, total and free estradiol, and total and free testosterone levels were measured at time of the first testing. Principal-component analysis identified four cognitive factors. In multiple linear regression analyses, better semantic memory performance was associated with higher total ( $p = 0.02$ ) and free ( $p = 0.03$ ) estradiol levels and a lower ratio of testosterone to estradiol ( $p = 0.007$ ). There were trends for associations between better verbal episodic memory and lower total testosterone ( $p = 0.08$ ) and lower testosterone/estradiol ratio ( $p = 0.06$ ). Lower free testosterone levels were associated with greater 2-year improvement on verbal episodic memory ( $p = 0.04$ ); higher testosterone/estradiol predicted greater semantic memory improvement ( $p = 0.03$ ). In postmenopausal midlife women, endogenous estradiol and testosterone levels and the testosterone/estradiol ratio are associated with semantic memory and verbal episodic memory abilities.

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### 1. Introduction

The extent to which, and the circumstances under which, endogenous and exogenous ovarian steroids affect cognitive function after natural menopause remains controversial. Circulating levels of estradiol decline as women enter the

menopausal transition and drop to a low, steady level during the early postmenopause (Burger et al., 2007). In contrast, although testosterone declines during a woman's reproductive years, levels are generally unaffected by the natural menopausal transition (Burger et al., 2000; Davison et al., 2005). Memory complaints are highly prevalent during this midlife period (Woods et al., 2000). Some observational studies support a positive association between use of estrogen-containing hormone therapy and cognitive function in healthy women, but the results of randomized controlled trials have failed to show clear evidence of benefit (Hen-

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derson and Sherwin, 2007; Lethaby et al., 2008), and there is even some suggestion of mild detriment (Espeland et al., 2004; Maki et al., 2007). While this topic remains one of ongoing debate, the basic question of whether endogenous serum levels of these hormones influence cognitive performance remains uncertain.

In older postmenopausal women, relatively higher serum estradiol levels have been associated with better global cognitive function (Lebrun et al., 2005; Yaffe et al., 2000), episodic memory (Drake et al., 2000; Hogervorst et al., 2004; Wolf and Kirschbaum, 2002) and executive function (Wolf and Kirschbaum, 2002); however, findings are not consistent (Barrett-Connor, 1999; Carlson and Sherwin, 2000; Hogervorst et al., 2004). Relatively low estradiol levels in the late postmenopause have been linked to Alzheimer's disease risk in women (Hoskin et al., 2004; Manly et al., 2000), but again there are inconsistencies (Geerlings et al., 2003).

Few studies have investigated potential links between serum estrogens and cognitive function in recently postmenopausal women, even though the midlife period represents the time when women are most likely to use hormone therapy. Moreover, for this age group, studies have failed to find significant associations between cognitive function and total estradiol levels (Henderson et al., 2003; Herlitz et al., 2007; Luetters et al., 2007); associations with free estradiol are not reported. Most estradiol circulates tightly bound to sex hormone binding globulin (SHBG) or loosely bound to albumin. Free estradiol, which readily crosses the blood–brain barrier, is considered the biologically active form. Further, while the association between estrogen levels and cognitive function remains of strong interest, other gonadal hormones, such as testosterone may also be important (Drake et al., 2000; Hogervorst et al., 2010; Thilers et al., 2006; Wolf and Kirschbaum, 2002). Receptors for estrogen and androgen are found in distinct neuronal populations (Simerly et al., 1990), and laboratory studies indicate that both estrogens and androgens are neuroprotective and can influence brain function (Pike et al., 2009; Spencer et al., 2008). Moreover, estrogen and androgen receptors may interact directly, and expression of each may be influenced by estrogens and by androgens (Adesanya-Famuyiwa et al., 1999; Panet-Raymond et al., 2000; Sharma and Thakur, 2006).

Our study sought to determine whether endogenous levels of estrone, estradiol, or testosterone are associated with specific domains of cognitive function in postmenopausal midlife women. We were particularly interested in associations with verbal episodic memory, a cognitive domain where poor performance is especially linked to increased dementia risk (Chen et al., 2000; Small et al., 2000). Total and free estradiol and testosterone levels, the ratio of these two hormones, and estrone were tested for association with cognition and with cognitive change over a 2-year period.

## 2. Methods

### 2.1. Study population

Sampling procedures used to establish the Melbourne Women's Midlife Health Project cohort have been described (Dennerstein et al., 1993; Ryan et al., 2009). In brief, households were selected via random digital telephone dialing, and eligible women (Australian-born, aged 45–55 years and currently residing in Melbourne) provided written informed consent if they agreed to participate. The study protocol was approved by the University of Melbourne Human Research Ethics Committee, and procedures were followed in accordance with ethical standards of the National Health and Medical Research Council. Women who had menstruated in the three prior months, were not currently using oral contraceptives or hormone therapy, and had a uterus and at least one ovary were invited to participate in the longitudinal cohort. Compared with the rest of the survey population, the 438 women who agreed (response rate 56%) reported better self-rated health and were more likely to have a tertiary education, to exercise at least once a week, to have ever had a Papanicolaou smear, and to have undergone dilatation and curettage (Burger et al., 1995).

In Years 11 and 13, cohort members were administered a comprehensive neuropsychological battery to assess potential influences of reproductive status, hormone therapy, and other exposures on midlife cognitive skills. Although 317 women (85%) remained in the longitudinal cohort at the time of initial testing in Year 11 (i.e., baseline neuropsychological testing), present analyses were restricted to a subsample of 148 women. We excluded women if they did not participate in baseline cognitive testing ( $n = 60$ ), were not yet postmenopausal ( $n = 22$ ), reported surgical menopause ( $n = 40$ ), were current hormone therapy users ( $n = 33$ ), or were missing data for hormone levels ( $n = 7$ ) or other covariates ( $n = 7$ ).

### 2.2. Cognitive testing

The neuropsychological test battery was administered by two graduate psychologists. Tests included an adaption of the California Verbal Learning Test II (CVLT) (Delis et al., 2000) using semantically related words and a 10-item list learning task using unrelated words (Collie et al., 1999); the unrelated word list had been administered 3 years earlier as a solitary task (Henderson et al., 2003). Both tests assessed immediate (sum of three learning trials) and delayed recall. Immediate and delayed recognition trials (Faces I and II) from the Wechsler Memory Scale III were also administered (Wechsler, 1997). Other tasks were Letter-Number Sequencing and Block Design tests of the Wechsler Adult Intelligence Scale III (Wechsler, 1991), category fluency (animal naming) (Troyer, 2000), a 30-item version of the Boston Naming Test (Mack et al., 1992), the oral Symbol-Digit Modalities test (Smith, 1991), the Trail Making Test

Part B (Reitan, 1958), and Judgment of Line Orientation (Benton et al., 1978). Details regarding test administration and normative values in this population have been reported (Clark et al., 2004a, 2004b; Elkadi et al., 2006a, 2006b).

### 2.3. Hormone levels

Postmenopausal women provided fasting morning blood samples at the time of baseline neuropsychological testing. Blood samples collected in EDTA tubes were centrifuged and the serum stored immediately at  $-20^{\circ}\text{C}$ . Samples were transferred and stored for up to 1.5 years at  $-80^{\circ}\text{C}$ . Estrone, estradiol, testosterone, and SHBG were measured by validated immunoassays optimized for measurements in women.

#### 2.3.1. Estrone, total estradiol, and total testosterone

Serum was extracted with hexane-ethyl acetate (3 : 2) and extract purified by celite column partition chromatography before radioimmunoassay, using ethylene glycol as the stationary phase (Goebelsmann et al., 1974, 1979). Elution of estrone from the column was carried out with 15% ethyl acetate in isooctane. Assay sensitivity is 5 pg/mL; intra-assay coefficient of variation (CV) is 7.9% at 26 pg/mL; and interassay CV is 12% at 26 pg/mL. Estradiol was eluted with 40% ethyl acetate in isooctane. Assay sensitivity is 4 pg/mL; intra-assay CV is 8.9% at 14 pg/mL; and interassay CV is 14% at 14 pg/mL. Testosterone was eluted with 40% toluene in isooctane. Assay sensitivity is 1.5 ng/dL; the intra-assay CV is 7.0% at 14.3 ng/dL; and interassay CV is 10.4% at 6.1 ng/dL.

#### 2.3.2. SHBG

Solid-phase, two-site chemiluminescent immunoassay on the Immulite analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, CA) was used to measure SHBG. Sensitivity is 1 nmol/L; intra-assay and interassay CVs are 4.1% at 7.7 nmol/L and 5.8% at 13 nmol/L, respectively.

#### 2.3.3. Free estradiol and free testosterone

Free (nonprotein bound) estradiol and testosterone were calculated using a validated algorithm (Rinaldi et al., 2002) based on derived equations (Södergård et al., 1982; Vermeulen et al., 1999). The algorithm uses measured concentrations of total estradiol or testosterone and SHBG, an assumed average concentration of albumin (3.5 g/dL), and appropriate affinity constants of SHBG and albumin for estradiol or testosterone. The molar ratio of free testosterone to free estradiol was also calculated (free testosterone, nmol/L divided by free estradiol, nmol/L) ( $T/E_2$ ), which is almost identical to the molar ratio of total testosterone to total estradiol.

### 2.4. Other measures

Questionnaires administered at each follow-up interview gathered information on sociodemographic and lifestyle characteristics, aging-related problems and overall health. Age at natural menopause defined by 12 months of amenorrhea was calculated based on last reported menstruation,

in most instances validated by menstrual diaries (Taffe and Dennerstein, 2000). Body mass index ( $\text{kg}/\text{m}^2$ ) was calculated from measured height and weight. Information was recorded on self-reported education level, marital situation, smoking status, alcohol consumption over the last week and paid employment. Current level of physical activity was recorded as the frequency of engaging in exercise for fitness or recreational purposes (Netz et al., 2008). Women indicated whether they suffered from hot flushes or night sweats over the last 2 weeks. Chronic health conditions were identified based on physical measurements, self-report, and medication use. These included hypercholesterolemia, diabetes, hypertension, chronic asthma, heart disease, stomach or bowel ulcers, arthritis, rheumatism, cancer or migraine. A dichotomous variable was created to indicate whether a woman suffered from at least three of these health problems. Depressive symptoms were assessed using the 10-item Center for Epidemiology Studies Depression Scale (Radloff, 1977), and women scoring at least 10 from a maximum score of 30 were classified as having depressive symptoms (Andresen et al., 1994). Apolipoprotein E genotyping based on restriction enzyme isotyping was carried out from blood samples as previously described (Hixson and Vernier, 1990) at the University of Southern California (Los Angeles, CA), and participants carrying at least one copy of the  $\epsilon 4$  allele of the apolipoprotein E gene (*APOE*) were identified.

### 2.5. Statistical analysis

To reduce the number of cognitive outcomes examined and thus the risk of a Type 1 error, principal-component analysis was performed to identify groupings of correlated cognitive tests. The principal axis method was used to extract the components (eigenvalues  $> 1$ ), followed by a varimax rotation. In interpreting the rotated factor pattern, a test was said to load on a given component if the factor loading was .50 or greater for that component. An individual's factor-based score on each of the components so identified was calculated by converting the scores for each specific cognitive test to a normalized  $z$ -score using the group means and standard deviations, and then averaging individual  $z$ -scores within each component. As the Trail Making Test is a timed task, the scoring for this variable was reversed before normalization and averaging, so higher scores indicated better performance on all tests (recoded score = 241 minus original score, where 241 seconds = maximum time recorded + 1). The change in cognitive function over the 2-year period was calculated by subtracting each baseline test score from the corresponding score 2 years later.  $Z$ -scores were then derived for changes in individual test scores, and an average change score for each cognitive factor was calculated. Low change scores indicated a decline in cognitive function.

Initial analyses compared the characteristics of women at the time of baseline testing according to cognitive factor scores, using correlations and  $t$ -tests. Linear regression anal-

ysis was then used to model the association between individual hormone measures and cognitive function at baseline. The distribution of hormone levels could be approximately normalized using a log transformation. Analyses were adjusted *a priori* for factors previously reported to confound the association between hormone measures and cognition: age, education level, depressive symptoms and age at menopause. Analyses also considered the inclusion of additional covariates. For 108 women who underwent cognitive testing at both time points, similar adjusted analyses controlling also for baseline cognitive scores, examined the association between baseline hormone measures and the 2-year change in cognitive function. Two-way interaction terms were considered for hormone levels and age and education level. SAS version 9.1 (SAS Institute, Cary, NC) was used for statistical analyses with a two-tailed significance level of alpha less than 0.05.

### 3. Results

#### 3.1. Cross-sectional analyses

Characteristics of participants are summarized in Table 1, and hormone values are given in Table 2. Levels of estrone, total and free estradiol, and total and free testosterone were significantly, positively correlated (Table 3). The women had a mean age of 60 years; only six women were aged 65 or older. The average age at natural menopause was 53. Participants were in relatively good health. One third re-

Table 1  
Participant characteristics (n = 148)

Clinical or demographic feature <sup>a</sup>	Value	
	Mean (SD)	Range
Age, years	60 (2.4)	56–67
Age at menopause, years	53 (2.4)	47–62
Years since menopause	6.8 (2.3)	1.1–11.2
Body mass index, kg/m <sup>2</sup>	27 (5.3)	17–47
	Number	Percent
At least 12 y of education	54	37
Married or living with a partner	113	76
Current smoker	9	6
Weekly alcohol consumption		
None	26	18
1–7 drinks per week	80	54
8 or more drinks per week	42	28
Current paid employment		
Full time	75	51
Part-time/casual/occasional	54	37
Not working	19	13
Current physical activity		
Less than a few times each mo	21	14
1–3 times per week	59	40
At least 4 times each week	68	46
Hot flushes or night sweats, past 2 weeks	52	35
Past user of hormone therapy	52	35
Chronic health problems	24	16
Depressive symptoms	28	19
At least one copy of the <i>APOE</i> ε4 allele	49	34

<sup>a</sup> See text for details.

Table 2

Serum hormones values and sex hormone-binding globulin levels  
(n = 148)

Measure	Median	Interquartile range	Reference intervals
Sex hormone-binding globulin, nmol/L	42.7	31.8–57.0	17–95
Estrone, pg/mL	16.1	11.0–22.6	10–60
Total estradiol, pg/mL	13.4	10.7–16.4	<30
Free estradiol, pg/mL	0.33	0.26–0.45	0.06–0.75
Total testosterone, ng/dL	24.6	19.7–32.6	5–50
Free testosterone, pg/mL	5.2	3.8–6.3	0.6–6.7
Free testosterone/free estradiol, molar ratio	18.1	13.7–23.1	—

ported vasomotor symptoms, and the prevalence of depressive symptoms was low. Serum hormone levels indicated a postmenopausal population. Compared with women in the Year 11 cohort not included in this baseline analysis, participants were older ( $p = 0.02$ ), with lower levels of total estradiol ( $p < 0.001$ ) and free estradiol ( $p < 0.001$ ), and higher values for total ( $p = 0.05$ ) and free testosterone ( $p < 0.001$ ) and for T/E<sub>2</sub> ( $p < 0.001$ ). These observations reflect exclusion of pre- and peri-menopausal women. Included women performed better on the verbal episodic memory factor ( $p = 0.04$ ; see below) compared with excluded women but did not differ on other cognitive test results or general participant characteristics.

Principal-component analysis of neuropsychological test results resulted in a four-component solution, accounting for 63% of the variance. The identified cognitive factors were labeled as verbal episodic memory, visual episodic memory, semantic memory, and executive function–visuospatial skills (Table 4). Chronbach's alphas for component tests were 0.77 (factor 1), 0.73 (factor 2), 0.60 (factor 3), and 0.75 (factor 4). Factors were significantly correlated with one another ( $r$  values 0.27–0.34,  $p < 0.001$ ) with the exception of the correlation between visual memory and executive–visuospatial factors ( $r = 0.13$ ,  $p = 0.12$ ).

In linear regression models adjusted for age, age at menopause, education level, and depressive symptoms, a significant positive association was found between both total and free log estradiol levels and the semantic memory factor (Table 5). A highly significant negative association was also found between log T/E<sub>2</sub> and this factor, indicating that a higher level of free testosterone relative to free estradiol was associated with worse performance on semantic memory tasks. There were also trends towards higher values of log T/E<sub>2</sub> ( $p = 0.06$ ) and total testosterone ( $p = 0.08$ ) being associated with worse verbal episodic memory. No significant associations were found between any hormone value and either visual episodic memory or executive–visuospatial function. Controlling for covariates from Table 1 in these models did not modify the results. Removing mood from the regression models had essentially no effect on significant or near-significant associations reported in Table 5, and no new significant associations were revealed



Table 3

Pearson correlations and probability values among serum hormone values and sex hormone-binding globulin (n = 148)<sup>a</sup>

	Estrone	Total estradiol	Free estradiol	Total testosterone	Free testosterone	Free testosterone/free estradiol, molar ratio
Sex hormone-binding globulin	0.19	−0.27	−0.61	0.16	−0.36	0.37
	0.02	0.0009	<0.0001	0.06	<0.0001	<0.0001
Estrone		0.65	0.60	0.35	0.41	−0.23
		<0.0001	<0.0001	<0.0001	<0.0001	0.008
Total estradiol			0.92	0.39	0.49	−0.40
			<0.0001	<0.0001	<0.0001	<0.0001
Free estradiol				0.26	0.55	−0.46
				0.001	<0.0001	<0.0001
Total testosterone					0.86	0.69
					<0.0001	<0.0001
Free testosterone						0.48
						<0.0001

<sup>a</sup> Correlations are based on log values.

(data not shown). SHBG showed no independent association with any cognitive factor ( $p > 0.4$ ). Effects were not modified by age or education.

To assess further these identified associations, post-hoc analyses explored the relationship between hormone levels and the individual cognitive tests within these factors. With respect to the semantic memory factor, total and free estradiol (positively), as well as T/E<sub>2</sub> (negatively), were significantly associated with performance on the Boston Naming Test (Supplementary Table 1A). T/E<sub>2</sub> was also negatively associated with category fluency scores. The trends for associations between total testosterone levels and T/E<sub>2</sub> values with verbal episodic memory resulted from negative associations with CVLT immediate and delayed recall scores (Supplementary Table 1B).

### 3.2. Longitudinal analyses

Change in cognitive function was assessed after a 2-year follow-up period. Mean scores on all tests except the Boston Naming Test and Letter-Number Sequencing improved between the two test sessions, and the mean length of time to complete the Trail Making Test increased slightly. Both

higher total and free testosterone levels were associated with smaller mean improvement on the verbal episodic memory factor (Table 6), in particular the immediate and delayed recall tasks of the unrelated word list (Supplementary Table 2). There was also a significant positive association between T/E<sub>2</sub> and the change in semantic memory performance over time (Table 6); post hoc analysis showed only weak associations with the two neuropsychological tests comprising this factor (both  $p$  values = 0.16; data not shown). Removing mood from the regression models had essentially no effect on significant or near-significant associations in Table 6, and no new significant associations were revealed (data not shown). SHBG was not independently associated with change in cognitive factors ( $p > 0.1$ ). Effects were not modified by age or education.

## 4. Discussion

### 4.1. Relations between hormone measures and cognitive outcomes

In this well characterized, relatively young postmenopausal cohort, we examined associations between sex hor-

Table 4

Neuropsychological battery of tests, by cognitive factor (n = 148)

Cognitive factor	Test	Mean (SD)	Range
Verbal episodic memory	CVLT, immediate recall	26.8 (5.8)	13–39
	CVLT, delayed recall	9.0 (3.3)	0–16
	Unrelated word list, immediate recall	21.5 (3.0)	15–29
	Unrelated word list, delayed recall	5.8 (2.2)	0–10
Visual episodic memory	Faces, immediate recognition	35.1 (4.3)	25–44
	Faces, delayed recognition	36.6 (4.6)	20–46
Semantic memory	Boston naming test	27.4 (2.1)	21–30
	Category fluency (animal naming)	20.6 (5.5)	11–37
Executive function and visuospatial skills	Trail making test, part B	85.8 (30.1)	41–240
	Symbol-digit modalities test	50.5 (8.3)	29–70
	Letter-number sequencing	10.5 (2.4)	3–17
	Judgment of line orientation	53.8 (4.3)	40–60
	Block design	37.3 (11.0)	10–60

CVLT = California Verbal Learning Test, 2nd Ed.; SD = standard deviation.

Table 5

Linear regression analyses for the association between serum hormone values and cognitive factors at baseline (n = 148)<sup>a</sup>

Hormone measure	Verbal episodic memory			Visual episodic memory			Semantic memory			Executive function–visuospatial skills		
	$\beta$	SE	<i>p</i>	$\beta$	SE	<i>p</i>	$\beta$	SE	<i>p</i>	$\beta$	SE	<i>p</i>
Log estrone <sup>b</sup>	0.02	0.12	0.86	−0.12	0.14	0.41	0.08	0.12	0.52	−0.06	0.10	0.55
Log total estradiol	−0.003	0.19	0.99	−0.03	0.23	0.89	0.46	0.20	0.02*	0.19	0.16	0.26
Log free estradiol	0.01	0.15	0.94	0.01	0.19	0.98	0.36	0.16	0.03*	0.14	0.14	0.30
Log total testosterone	−0.25	0.14	0.08†	−0.25	0.17	0.15	−0.13	0.15	0.39	−0.06	0.13	0.63
Log free testosterone	−0.21	0.13	0.12	−0.19	0.16	0.24	−0.07	0.14	0.64	−0.04	0.12	0.73
Log (free testosterone/free estradiol)	−0.27	0.15	0.06†	−0.23	0.18	0.18	−0.41	0.15	0.007*	−0.18	0.13	0.17

SE = standard error.

<sup>a</sup> Adjusted for age, education, depressive symptoms and age at menopause.<sup>b</sup> 12 women were missing estrone measurements (n = 136).\* Probability  $p < 0.05$ ; †  $0.1 \leq p \leq 0.05$ .

mones—serum levels of estrone, total and free estradiol, and total and free testosterone, as well as T/E<sub>2</sub>—and four domains of cognitive function. In both cross-sectional and longitudinal analyses, principal findings concerned two of these domains: semantic memory and verbal episodic memory. There were no significant associations with hormone measures and visual episodic memory or executive–visuospatial factors. Estrone and SHBG were unrelated to cognitive measures. Associations with cognition were similar for free and total hormone levels, as these values were highly correlated.

Better semantic memory was associated with higher estradiol levels (total and free) and a lower ratio of testosterone to estradiol. There were also trends for higher total testosterone and higher T/E<sub>2</sub> to be associated with lower verbal episodic memory. Findings in 2-year longitudinal analyses showed both similarities and differences when compared with cross-sectional results. Higher total and free testosterone levels were associated with less improvement on verbal episodic memory, a finding that supports trends seen with baseline cognitive scores. There were no significant associations between estrogen levels and cognitive

change. Higher T/E<sub>2</sub> at baseline was associated with greater mean improvement in semantic memory over 2 years, which contrasts with cross-sectional findings of an inverse association between T/E<sub>2</sub> and semantic memory. This apparent discrepancy may be partially understood if relevant hormonal effects on brain function and thus cognitive performance are recognized as “activational” (and thus relatively short-lived) rather than “organizational” (Arnold, 2009). Hormone levels at one point may have limited predictive value for performance 2 years later, except to the extent that current levels correlate with future levels. Practice and ceiling effects may also have played roles. Mean scores on almost all tests improved over the 2 years, and practice effects made it more difficult to discern cognitive decline. Ceiling effects of one of the semantic memory tasks (Boston Naming Test) limited the ability of strong performers at baseline to improve scores 2 years later.

In prior analyses from the Melbourne Women’s Midlife Health Project cohort, we found no association between word-list learning for unrelated words and serum levels of total estradiol or the free estradiol index (Henderson et al., 2003). Two subsequent studies of midlife women (Herlitz

Table 6

Linear regression analyses for the association between serum hormone values and the 2 year change in cognitive function (n = 108)<sup>a</sup>

Hormone measure	Verbal episodic memory			Visual episodic memory			Semantic memory			Executive function–visuospatial skills		
	$\beta$	SE	<i>p</i>	$\beta$	SE	<i>p</i>	$\beta$	SE	<i>p</i>	$\beta$	SE	<i>p</i>
Log estrone <sup>b</sup>	−0.04	0.11	0.69	−0.01	0.13	0.92	0.00	0.13	0.99	0.03	0.08	0.68
Log total estradiol	−0.13	0.19	0.49	−0.23	0.22	0.31	−0.16	0.22	0.48	−0.09	0.14	0.53
Log free estradiol	−0.13	0.15	0.42	−0.18	0.18	0.33	−0.21	0.18	0.24	−0.14	0.11	0.21
Log total testosterone	−0.26	0.14	0.06†	−0.03	0.17	0.87	0.26	0.16	0.11	0.02	0.10	0.84
Log free testosterone	−0.27	0.13	0.04*	−0.05	0.16	0.76	0.11	0.15	0.46	−0.06	0.09	0.50
Log (free testosterone/free estradiol)	−0.20	0.14	0.16	0.10	0.17	0.55	0.36	0.16	0.03*	0.07	0.10	0.50

SE = standard error.

<sup>a</sup> Adjusted for age, education, depressive symptoms, age at menopause and baseline cognitive score; longitudinal analyses involved 40 fewer women, who did not participate in follow-up cognitive testing.<sup>b</sup> 10 women were missing estrone measurements (n = 98).\* Probability  $p < 0.05$ ; †  $0.1 \leq p \leq 0.05$ .

et al., 2007; Luetters et al., 2007) similarly failed to find significant associations between total estradiol and verbal episodic memory or verbal fluency. Our present findings involving free estradiol strengthen these earlier findings. None of these studies considered testosterone levels.

Other investigators have examined associations between estrogen levels and cognitive function in older postmenopausal women, with conflicting results (Barrett-Connor, 1999; Drake et al., 2000; Wolf and Kirschbaum, 2002). Likewise for testosterone, reports in older women, or in a mixed population of younger and older postmenopausal women, describe positive (Barrett-Connor, 1999; Carlson and Sherwin, 2000; Drake et al., 2000; Hogervorst et al., 2010; Wolf and Kirschbaum, 2002), negative (Hogervorst et al., 2004; Thilers et al., 2006) and null associations with cognitive function (Carlson and Sherwin, 2000; Drake et al., 2000; Hogervorst et al., 2004; Hogervorst et al., 2010). Variable results in part could be because levels of estradiol and testosterone were not considered together, because cognitive tasks differed, or because population characteristics differed.

As estradiol levels (Burger et al., 2007)—but not testosterone levels (Burger et al., 2000; Davison et al., 2005)—decline dramatically across the natural menopausal transition, the ratio of testosterone to estradiol will increase. In our study, high levels of testosterone relative to estradiol were negatively associated with both semantic memory and verbal episodic memory. Moreover, total and free testosterone predicted poorer verbal episodic memory performance over a 2-year period. Although there were no significant or near-significant associations between hormone values and visual episodic memory, total testosterone and T/E<sub>2</sub> showed similar effect sizes for both verbal and visual episodic memory (Table 5), suggesting that apparent deleterious cross-sectional associations between episodic memory and testosterone or T/E<sub>2</sub> are not necessarily modality specific.

Many prior studies infer hormone effects on a particular cognitive domain on the basis of a single cognitive test. We found significant associations for estradiol levels (positive) and T/E<sub>2</sub> values (negative) with semantic memory, as well as negative trends for total testosterone and T/E<sub>2</sub> with verbal episodic memory; post hoc analyses enabled us to examine which specific tests within these cognitive domains were primarily responsible for these associations.

Semantic memory refers to memory for generic, over-learned information, including memory for word names. For the semantic memory factor, higher total estradiol, higher free estradiol and lower T/E<sub>2</sub> were each significantly associated with Boston Naming Test scores (Supplementary Table 1A). Lower T/E<sub>2</sub> values were associated with category fluency. Trouble with word names is a common midlife complaint. Semantic memory involves widely distributed areas of cerebral cortex (Patterson et al., 2007). Estrogen receptor beta shows a wide distribution within Layer II–VI cortical neurons (González et al., 2007), pro-

viding a hypothetical link between menopausal estrogen loss and semantic memory symptoms. The association between estradiol and naming suggests the need to examine more closely the effects of menopause on this particular cognitive ability.

For the verbal episodic memory factor, negative associations for total testosterone and T/E<sub>2</sub> were identified for immediate and delayed recall tasks of the CVLT, whereas there were no associations for unrelated word list recall tasks (Supplementary Table 1B). Although performances on these memory tasks are correlated ( $r = 0.36$  and  $0.29$  for immediate and delayed recall tasks respectively,  $p$  values  $< 0.001$ ), psychomotor properties of the two tests differ. The CVLT word list is longer than the unrelated word list (16 vs. 10 words) and may, on this basis, provide a more precise estimate of episodic memory ability. Another key difference is that the CVLT is composed of groups of semantically related words. Recognizing the relation among CVLT words facilitates learning and recall, and implicates executive control processes in addition to memory skills. In fact, it is suggested that executive processing involving the prefrontal cortex is especially sensitive to estrogens (Joffe et al., 2006). However, we observed no significant associations between hormone measures and the executive–visuospatial factor in our cohort. It is interesting that negative associations between free and total testosterone and 2-year changes in verbal episodic memory were stronger for recall of unrelated words (Supplementary Table 2). The unrelated word-list learning task had been previously administered to cohort members as a solitary measure (Henderson et al., 2003), and speculatively, relevant memory processes may have been more closely linked to the stronger practice (learning) effect than to word list acquisition and recall within the short temporal window encapsulated within a test session.

More generally, post hoc findings emphasize the inherent limitation of generalizing associations based on an individual neuropsychological test—performance of which inevitably draws to some extent upon multiple attentional, perceptual, processing, and executive skills—to an entire domain of cognitive function.

#### 4.2. Limitations and strengths

The relatively modest sample size limits power to detect smaller associations between hormone measures and cognition, although sample sizes are larger than in many previous reports of postmenopausal women (Carlson and Sherwin, 2000; Drake et al., 2000; Hogervorst et al., 2004; Wolf and Kirschbaum, 2002). To reduce the likelihood of false-positive associations, primary analyses used principal-component analysis to reduce the number of cognitive end points. Some associations with hormone measures, however, could still have occurred by chance. It would be injudicious to generalize cognitive findings in healthy postmenopausal midlife women—where serum levels of estrone

and estradiol are relatively low—to clinical populations (for example, women with frank dementia) or to women with hormone levels above the range included in our sample (for example, women of reproductive age or postmenopausal women receiving exogenous estrogens or testosterone). Data concerning some covariates were self-reported and thus may be subject to recall bias and misclassification. Bias may have been introduced through the exclusion of women who did not complete the cognitive testing or who used hormone therapy. In longitudinal analyses, we examined cognitive change over a 2-year period, and for most tests the average score had improved. This practice effect made it more difficult to detect associations between hormone levels and age-related cognitive decline, particularly as women were relatively young (mean age 60 years) and healthy. Because we did not measure hormone levels at the time of the second testing, we were unable to examine associations between hormone changes and cognitive change. Finally, there may have been unrecognized confounding factors that were not considered in these analyses.

This study has a number of strengths. This is a population-based study of randomly recruited women, and analyses adjusted for a number of factors that could potentially confound the association between hormone levels and cognitive function. We have considered not only levels of estrone, total estradiol and total testosterone but also free estradiol and testosterone levels and the ratio between levels of free testosterone and free estradiol. Further, our broadly based neuropsychological battery permitted examination of a number of cognitive domains impacted by cognitive aging, some of which are suggested as sentinel markers of late-life dementia.

#### 4.3. Concluding perspectives

The relation among endogenous sex steroids, brain function and behavior is undoubtedly complex. Free steroid hormones rapidly cross the blood–brain barrier, and within the brain testosterone can be converted to estradiol by the enzyme aromatase (Boon et al., 2010). In addition, these hormones are neurosteroids (Mukai et al., 2006), subject to endogenous synthesis within the brain from cholesterol precursors. Serum levels may therefore reflect brain levels imprecisely.

Our results show that serum levels of estradiol or testosterone in isolation are less revealing than measures of both hormones. This finding is perhaps not surprising, given the potential for physiological interactions between estrogens and androgens on several levels (Adesanya-Famuyiwa et al., 1999; Boon et al., 2010; Panet-Raymond et al., 2000; Sharma and Thakur, 2006). The balance between estradiol and testosterone may be therefore important for some aspects of cognitive function in postmenopausal midlife women. The finding that the relation between gonadal hormones and cognition differs across cognitive domains indicates that a single global measure of cognition may thus

overlook important associations limited to a specific domain.

Results support the view that estradiol, within the range of endogenous levels examined in this study, is associated with better semantic memory—and perhaps naming ability more specifically—in postmenopausal midlife women.

Consistent with prior reports involving midlife and elderly women (Thilers et al., 2006) and surgically menopausal women (Möller et al., 2010), our results suggest also that relative androgen excess in midlife is negatively associated with performance on tasks of semantic and verbal episodic memory. Relatively high testosterone is linked to adverse cognitive outcomes in older women as well (Hogervorst et al., 2004). The association, while not necessarily causal, is of concern. Verbal episodic memory is sensitive to cognitive aging (Weintraub et al., 2009), and impaired performance in this domain is linked to development of Alzheimer's disease later in life (Chen et al., 2000; Small et al., 2000). Observed associations for endogenous testosterone levels do not necessarily predict effects of exogenous testosterone therapy administered at pharmacological doses, but findings suggest the need for further study when off-label testosterone therapy is considered for midlife women. Findings also suggest the need to consider the role of testosterone in designing and interpreting results of trials of estrogen therapy or estrogen-progestin therapy in midlife women.

More generally, our results highlight the importance of considering the relation between estradiol and testosterone rather than each hormone in isolation, of assessing specific cognitive skills rather than global cognition, and of using more than a single neuropsychological test to define performance within a cognitive domain. This approach may help resolve inconsistencies in the literature regarding associations between gonadal hormones, cognition, and cognitive aging after a woman's reproductive years.

#### Disclosure statement

JR, FZS, WJM, MSC, DK and VWH report no actual or potential conflicts of interest. LD has received lecture and consulting fees from, and has served on advisory boards for, Bayer Schering, Wyeth and Boehringer Ingelheim. CS has received honoraria from Pfizer and Lundbeck, and she may accrue revenue on a pending patent for a diagnostic assay for seizure prediction.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2010.07.014.

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Supplementary Table 1

Linear regression analyses for the association between select serum hormone values and specific cognitive tests (post hoc analyses,  $n = 148$ )<sup>a</sup>

Hormone measure				Boston naming test			Category fluency						
				$\beta$	SE	$p$	$\beta$			SE	$p$		
A. Semantic memory factor													
Log total estradiol				0.72	0.23	0.002*	0.19			0.25	0.44		
Log free estradiol				0.60	0.19	0.002*	0.11			0.21	0.60		
Log (free testosterone/free estradiol)				−0.44	0.18	0.02*	−0.39			0.19	0.05†		
Hormone measure		CVLT, immediate recall			CVLT, delayed recall			Unrelated word list, immediate recall			Unrelated word list, delayed recall		
		$\beta$	SE	$p$	$\beta$	SE	$p$	$\beta$	SE	$p$	$\beta$	SE	$p$
B. Verbal episodic memory factor													
Log total testosterone		−0.35	0.18	0.05†	−0.33	0.19	0.09†	−0.14	0.19	0.46	−0.17	0.19	0.36
Log (free testosterone/free estradiol)		−0.29	0.18	0.10†	−0.42	0.19	0.03*	−0.16	0.19	0.41	−0.13	0.19	0.50

CVLT = California Verbal Learning Test, 2nd Ed.; SE = standard error.

<sup>a</sup> Adjusted for age, education, depressive symptoms and age at menopause.\* Probability  $p < 0.05$ ; †  $0.1 \leq p \leq 0.05$ .

Supplementary Table 2

Linear regression analyses for the association between serum hormone values and the 2 year change in verbal episodic memory (post hoc analysis, n = 108)<sup>a</sup>

Hormone measure	CVLT, immediate recall			CVLT, delayed recall			unrelated word list, immediate recall			unrelated word list, delayed recall		
	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p
log total testosterone	−0.02	0.22	0.92	−0.03	0.21	0.88	−0.57	0.21	0.008*	−0.59	0.21	0.005*
log free testosterone	−0.09	0.21	0.69	−0.05	0.20	0.81	−0.50	0.20	0.01*	−0.60	0.19	0.003*

CVLT: California Verbal Learning Test, 2nd Ed.; SE = standard error.

<sup>a</sup> Adjusted for age, education, depressive symptoms, age at menopause and baseline cognitive score.\* Probability  $p < 0.05$ .