Cognition, mood, and physiological concentrations of sex hormones in the early and late postmenopause

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Variations in the hormonal milieu after menopause may influence neural processes concerned with cognition, cognitive aging, and mood, but findings are inconsistent. In particular, cognitive effects of estradiol may vary with time since menopause, but this prediction has not been assessed directly using serum hormone concentrations. We studied 643 healthy postmenopausal women not using hormone therapy who were recruited into early (<6 y after menopause) and late (10+ y after menopause) groups. Women were administered a comprehensive neuropsychological battery and assessed with the Center for Epidemiologic Studies Depression Scale. They provided serum for free estradiol, estrone, progesterone, free testosterone, and sex hormone binding globulin measurements. Cognitive outcomes were standardized composite measures of verbal episodic memory, executive functions, and global cognition. Covariate-adjusted linear regression analyses were conducted for each hormone separately and after adjustment for other hormone levels. Endogenous sex steroid levels were unassociated with cognitive composites, but sex hormone binding globulin was positively associated with verbal memory. Results for early and late groups did not differ significantly, although progesterone concentrations were significantly positively associated with verbal memory and global cognition in early group women. Hormone concentrations were not significantly related to mood. Results fail to support the hypothesis that temporal proximity to menopause modifies the relation between endogenous serum levels of estradiol and verbal memory, executive functions, or global cognition. Physiological variations in endogenous postmenopausal levels of sex steroid hormones are not substantially related to these aspects of cognition or mood; positive associations for progesterone and sex hormone binding globulin merit additional study.

Nuclear and nonnuclear receptors for estrogens, androgens, and progesterone are widely distributed in the brain and expressed within discrete neural populations (1–5), and sex steroids may influence brain function through other mechanisms. Variations in the hormonal milieu after menopause have the potential to affect cognitive function and mood as well as physiological processes linked to cognitive aging and late-life disorders, such as Alzheimer's disease (6, 7). A number of studies have examined associations between serum concentrations of sex steroids and cognition after menopause. Most have focused on 17β-estradiol (E2) (8), which is produced cyclically by the ovaries in women of reproductive age. After menopause, the depletion of ovarian follicles leads to permanent reductions in circulating levels of E2 as well as estrone (E1) and progesterone (P4). Findings related to cognition are inconsistent because of, in part, limited measures of cognitive abilities, measurement of only a single sex steroid, and restricted age range. Age is potentially quite important, because some hormonal effects on cognitive outcomes are proposed to vary by age or timing in relation to the final menstrual period (9–12). The timing, or critical window, hypothesis is best developed with regard to exogenous E2; no research has examined this hypothesis in relation to endogenous hormone concentrations.

The ongoing Early versus Late Intervention Trial with Estradiol (ELITE) targets two groups of women: women in early postmenopause and women in late postmenopause (*Protocol Plan*). The randomized interventions are oral E2 or placebo, and the goal is to assess whether time since menopause (as represented by postmenopause group) modifies the effect of E2 therapy on specified health outcomes, including cognitive change (ELITE-Cog). This large trial provides the additional opportunity to test in a well-characterized cohort of postmenopausal women the hypothesis that the relation of endogenous E2 levels to cognitive skills differs by temporal proximity to menopause and assess other hormone associations with cognition and mood.

In younger women, verbal episodic memory is reported to be sensitive to estrogen effects (13, 14), and verbal memory impairment is potentially important, given its association with Alzheimer's disease risk (15). Episodic memory depends on integrity of the hippocampus and adjacent medial temporal lobe structures (16), and studies in rodents document robust effects of E2 on hippocampal synaptic plasticity, hippocampal long-term potentiation, and hippocampal-mediated cognitive behaviors (17, 18).

Here, we report—for early and late postmenopausal women not using hormone therapy—prerandomization associations between verbal memory and other cognitive measures and serum concentrations of E2, E1, P4, and testosterone (T) as well as sex hormone binding globulin (SHBG). At the same time, we also

Significance

Hormone variations after menopause may influence brain processes concerned with cognition and mood. Effects may differ for exposures near menopause compared with much later. We addressed this prediction using serum concentrations of endogenous estradiol, estrone, progesterone, and testosterone in 643 healthy women not using hormone therapy (early group, <6 y after menopause; late group, 10+ y). In combined analyses, hormone levels were unrelated to verbal memory, executive functions, global cognition, or mood. For serum estradiol (our primary focus), the relation did not differ between postmenopause groups. In early group women, progesterone levels were associated with better memory and global cognition; this finding merits additional study. Results help clarify cognitive effects of physiological concentrations of sex steroids after menopause.

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examined the relation between serum hormone concentrations and mood or possible depression. Our primary hypothesis was that the association of E2 levels with verbal episodic memory would differ between postmenopause strata, with higher concentrations associated with better memory performance in the early group but not the late group.

Results

ELITE participants ranged in age from 41 to 84 y; 1 of 643 participants did not have cognitive testing at baseline but was included in analyses of mood and possible depression. Other women underwent cognitive assessment and contributed serum for sex hormone and SHBG analyses (Table 1). Two-thirds of

Table 1. Characteristics of ELITE-Cog participants at baseline

Characteristic	Early postmenopause (n = 271)	Late postmenopause (n = 372)	P
Mean age, y*	55.4 (4.1)	65.4 (6.0)	<0.001
Race or ethnicity			
White, non-Hispanic	174 (64.2%)	266 (71.5%)	
Black	24 (8.9%)	35 (9.4%)	
Hispanic	41 (15.1%)	49 (13.2%)	
Asian	32 (11.8%)	21 (5.7%)	
Other	0	1 (0.3%)	
Education			0.05
Less than high school	1 (0.4%)	2 (0.5%)	
High school or some college	75 (27.7%)	137 (36.8%)	
College graduate	195 (72.0%)	233 (62.6%)	
Age at menopause, y (269, 344)	51.9 (3.7)	47.9 (5.8)	<0.001
Years since menopause, median, IQR (269, 344)	3.5 (1.9, 5.0)	14.3 (11.5, 18.7)	<0.001
Type of menopause			
Natural	262 (96.7%)	312 (83.9%)	<0.001
Surgical	9 (3.3%)	60 (16.1%)	
Hot flashes, any within previous month (243, 324)	173 (71.2%)	157 (48.5%)	<0.001
Mean number per day for women with any hot flashes			
Mild	1.1 (1.7)	1.0 (1.4)	0.33
Moderate	1.1 (2.0)	1.2 (2.0)	0.83
Severe	0.62 (2.4)	0.55 (1.6)	0.74
Past use of hormone therapy, yes	138 (50.9%)	321 (86.3%)	<0.001
Current lipid-lowering medication, yes	40 (14.8%)	88 (23.7%)	0.005
Current use of antihypertensives, yes	50 (18.5%)	107 (28.8%)	0.003
Smoking history			0.19
Current	11 (4.1%)	11 (3.0%)	
Former	89 (32.8%)	147 (39.5%)	
Never	171 (63.1%)	214 (57.5%)	
Moderate or vigorous physical activity, hours during past week†	7.0 (8.4)	5.5 (6.2)	0.01
Alcohol consumption, weekly estimate			0.62
None	135 (49.8%)	194 (52.2%)	
>0–1 unit (15 g)/d	105 (38.8%)	127 (34.1%)	
>1–2 units/d	23 (8.5%)	39 (10.5%)	
>2 units/d	8 (3.0%)	12 (3.2%)	
Body mass index, kg/m^2	27.2 (5.4)	27.4 (5.4)	0.65
Systolic blood pressure, mmHg	117 (13)	119 (12)	0.06
Diastolic blood pressure, mmHg	76 (7)	74 (7)	0.001
Hemoglobin A1c, % (269, 369)	5.5 (0.5)	5.7 (0.4)	0.002
Intelligence quotient estimate‡	108 (12)	108 (11)	0.97
Mood (CES-D), median, IQR	6 (2, 12)	5 (2, 11)	0.074
Possible depression§	52 (19.2%)	61 (16.4%)	0.36
ApoE ε4 carrier status¶ (257, 354)	74 (28.8%)	111 (31.4%)	0.50
Serum concentrations, median, IQR			
Total E2, pg/mL	8.0 (5.8, 11.4)	7.7 (5.8, 10.5)	0.17
Free E2, pg/mL (268, 370)	0.20 (0.14, 0.31)	0.18 (0.13, 0.26)	0.04
E1, pg/mL (269, 372)	29.1 (22.7, 38.1)	27.7 (20.2, 37.1)	0.08
P4, ng/mL (271, 370)	0.2 (<0.2, 0.3)	0.2 (<0.2, 0.3)	0.12
Total T, ng/dL (269, 372)	20.5 (15.5, 27.9)	21.6 (14.1, 30.6)	0.44
Free T, pg/dL (268, 370)	3.8 (2.9, 5.5)	3.9 (2.8, 5.7)	0.86
SHBG, nmol/L (268, 370)	46.1 (32.5, 61.1)	51.1 (37.7, 66.9)	0.01

Mean (SD) or number (%) except where indicated. When data were missing, numbers of participants in each postmenopause group contributing data are given in parentheses. ApoE, Apolipoprotein E; CES-D, Center for Epidemiological Studies Depression scale; IQR, interquartile range.

*Ranges were 42–72 y in the early group and 41–84 y in the late group.

†Hours of moderate or vigorous physical activity (three or more standard metabolic equivalents) during the past 7 d.

‡Estimated from Wechsler Test of Adult Reading scores.

§Possible depression is suggested by CES-D scores of at least 16.

¶At least one ε4 allele.

participants were college graduates, and about one-third were members of a racial or ethnic minority. Women in the late postmenopause group were, on average, 10 y older and somewhat less well-educated than women in the early group. They were also less likely to have recorded hot flashes within the preceding 1 mo and more likely to have undergone surgical menopause and used hormone therapy.

Sex steroid levels were positively correlated (Table S1). SHBG concentrations increased slightly with age ($r = 0.14$) and were inversely related to body mass index ($r = -0.46$). In the late group, the median serum concentration of SHBG was higher, and the free E2 (but not total E2) concentration was lower. Concentrations of E1, total E2, P4, and T (free and total) were similar in both groups. Because of very high correlations between free and total E2 and between free and total T, subsequent analyses considered only free, unbound levels of these hormones, presumed to represent the biologically active fractions.

Women in the two postmenopause strata did not differ in estimated intelligence or mood (Table 1). Most neuropsychological tests showed expected age effects, with early postmenopausal women performing better than women in late postmenopause (Table S2). For cognitive composites, these differences represented standardized differences of 0.25 for verbal episodic memory, 0.46 for executive functions, and 0.23 for global cognitive function.

Estrogen Levels and Cognition. In both postmenopause groups, there was not a significant relation between serum concentrations of free E2 and verbal episodic memory or between free E2 and executive functions or global cognition (Table 2). Findings for E1 were similar. Adjusting for levels of P4, free T, and SHBG did not alter these results. As previously reported (19), free E2 was significantly positively associated with semantic memory (naming scores) among women in the early postmenopause group, with differences between the first and fourth quartiles of free E2 corresponding to about 0.4 SDs in naming performance (*SI Materials and Methods, Other Analyses* and Table S3).

Other Cognitive Outcomes. Concentrations of P4 and free T were not significantly associated with cognitive composite scores (Table 2). For P4, there was a trend for a difference between postmenopause groups, and in the early group, the P4 level was significantly positively associated with both verbal memory and global cognition. In addition, the concentration of SHBG was significantly positively associated with verbal memory performance, with similar effects in both postmenopause groups (Table 2). Time since menopause adjusted for age was unrelated to cognitive function (Table 3). Free T has been negatively linked to verbal fluency (20); we were unable to confirm this association (*SI Materials and Methods, Other Analyses*).

Mood and Possible Depression. For both early and late postmenopause groups, mood and possible depression were generally unrelated to hormone measures (Table 4). However, effects of free E2 on mood differed significantly between the two postmenopause groups, and the association between higher E2 concentrations and better mood in the early group showed a trend to significance.

Sensitivity Analyses. Excluding 28 women who reported premature or late menopause did not alter the significance of findings reported in Tables 2, 3, and 4 except in three instances, where previously nonsignificant trends ($P < 0.1$) were significant at $P < 0.05$. Interaction P values were significant for P4 associations with verbal episodic memory and global cognition (Table S4). Years since menopause adjusted for age were negatively related to executive functions ($\beta = -0.03$, SE = 0.01, $P = 0.03$, interaction $P = 0.71$).

Discussion

Principal Findings. As inferred from our results on semantic memory, variations in serum concentrations of free E2 within the low but normal physiological range characteristic of postmenopause may affect cognition. However, among 643 healthy community-dwelling postmenopausal women not using hormone therapy, we failed to confirm our primary hypothesis that the relation between free E2 and verbal episodic memory is modified by postmenopause group. Consistent with this finding, years since menopause adjusted for age were unrelated to verbal memory. We also failed to confirm significant relations between serum concentrations of free E2, E1, P4 or free T and cognitive outcomes in the combined group of early and late postmenopausal women. SHBG was an exception, and SHBG levels were positively associated with verbal memory. Sex steroid and

Table 2. Associations between serum hormone concentrations and cognitive composite scores

	Verbal episodic memory				Executive functions				Global cognition			
Hormone measure	β	SE	*P*	Interaction *P*	β	SE	*P*	Interaction *P*	β	SE	*P*	Interaction *P*
Free E2	−0.06	0.09	0.52	0.34	−0.02	0.08	0.84	0.41	0.03	0.10	0.80	0.80
E1	0.02	0.12	0.86	0.46	−0.01	0.11	0.95	0.60	0.01	0.15	0.95	0.97
P4	0.08	0.09	0.42	0.06*	−0.10	0.10	0.30	0.21	0.20	0.12	0.11	0.07*
Early group	0.31	0.15	0.03†‡						0.49	0.19	0.01†‡	
Late group	−0.09	0.14	0.48						−0.01	0.16	0.96	
Free T	−0.11	0.10	0.28	0.39	−0.03	0.09	0.75	0.99	0.02	0.12	0.88	0.57
SHBG	0.27	0.12	0.02†§	0.42	0.04	0.11	0.74	0.13	0.16	0.14	0.25	0.88

Values for steroid hormone and SHBG concentrations were log-transformed before analyses. Sample sizes for hormone measures ranged from 627 to 637. All analyses were adjusted for age. Other adjustments were as follows: free E2 (race/ethnicity, antihypertensive use, smoking history, systolic blood pressure, and hemoglobin A1c level); E1 (race/ethnicity, education, statin use, smoking history, physical activity, alcohol use, systolic blood pressure, hemoglobin A1c level, mood, and apolipoprotein E genotype); P4 (smoking history, systolic blood pressure, and apolipoprotein E genotype); free T (race/ethnicity, antihypertensive use, education, alcohol use, systolic blood pressure, hemoglobin A1c level, mood, and apolipoprotein E genotype); and SHBG (race/ethnicity, education, and systolic blood pressure). Additional adjustment for the presence of other hormone concentrations (except E2 when E1 was the predictor variable and E1 when E2 was the predictor variable) had no substantial effect.

*0.05 ≤ interaction $P < 0.1$ (interaction $P < 0.05$ in analyses that excluded women with premature or late menopause; in the text).

†Probability $P < 0.05$.

‡Among women in the early postmenopause group, the mean standardized difference between the fourth (highest) and first (lowest) quartiles of P4 for the verbal memory composite score was 0.30 ($P = 0.08$); for global cognition, the mean standardized difference was 0.41 ($P = 0.01$).

§Among women in both postmenopause groups combined, the mean standardized difference between the fourth and first quartiles of SHBG for verbal memory was 0.24 ($P = 0.03$).

Table 3. Years since menopause adjusted for age in relation to cognitive composite scores

Cognitive composite	β	SE	*P*	Interaction *P*
Verbal episodic memory	−0.01	0.01	0.24	0.50
Executive functions	−0.02	0.01	0.08*	0.79
Global cognition	−0.01	0.01	0.42	0.85

Analyses of 612 women were adjusted for age, education, and mood.
*$P = 0.03$ in analyses that excluded women with premature or late menopause (in the text).

SHBG concentrations were not significantly related to mood or possible depression.

Cognitive outcomes. Associations between serum estrogen levels (either E1 or E2 measured as total E2, bioavailable E2, free E2, or the free E2 index) and cognition have been considered in several relatively large studies ($n > 100$) of postmenopausal women (8), where cognitive end points have sometimes been limited. Among these studies, most focus on predominately younger postmenopausal women or predominately older postmenopausal women. None have distinguished between younger and older groups when both were included and thus, do not address the question of whether cognitive effects might differ based on age or time from menopause. None of these larger studies of E1 or E2 have examined both P4 and T, and when effects of one hormone are analyzed, potential confounding effects of other hormones are rarely considered.

Although beneficial (19, 21, 22) and deleterious (23, 24) associations are described for E1 or E2 and specific cognitive measures, findings in most human studies (19, 21–31) are largely consistent with our null results for an association between endogenous estrogens and cognitive composite scores. These generally nonsignificant findings in younger and older postmenopausal women contrast with results of studies in rodents and nonhuman primates. Here, E2 affects cognitive performance, synaptic plasticity, and neurophysiological properties in a manner that can vary with age or the interval between ovariectomy and hormone replacement (10, 32–35). Effects are tissue-specific and may reflect, in part, the increased degradation of estrogen receptor-α that occurs with aging or after ovariectomy (36, 37). Even in the animal literature, however, estrogen effects on cognition depend on the experimental paradigm. E2 may modulate different neural systems differently (38), and cognitive enhancement can be obscured by environmental factors that modify hormonal effects in naturalistic settings (39, 40).

The relation between endogenous P4 and cognition has been examined infrequently after menopause, and to our knowledge, it has only been examined in older postmenopausal women. In the Leisure World cohort, the serum P4 concentration was negatively associated with performance on a clock drawing task (25); in a smaller study, serum P4 was unrelated to several cognitive end points (41). The interaction between postmenopause groups showed a trend to significant difference; we found that serum P4 was positively related to verbal memory and global cognition in postmenopausal women closer to menopause.

Findings from human research on serum T and cognition are variable. T levels (measured as total, bioavailable, or free T) have been linked to better global cognition (Mini Mental State examination) in older women (26) and poorer verbal fluency in premenopausal and postmenopausal women (20). The absence of other cognitive associations is also reported (22). We identified no associations between free T and composite cognitive outcomes.

SHBG is a carrier protein that binds E2 and T. In addition to its transport function in the blood, SHBG may play a role in hormone uptake into neurons (42). Some studies in postmenopausal women report no relation to cognitive abilities (19, 25). However, SHBG levels are linked to increased risk of Alzheimer's disease in men and women (43) and cognitive decline in older men (44). We found that serum concentrations of SHBG were associated with better verbal memory, and adjustments for sex steroid levels did not alter this relation.

Mood and Possible Depression. Lower serum levels of E1 and E2 (29) and higher levels of free T (45) have been associated with depressive symptoms in older postmenopausal women. Other investigators, however, identified no relation between levels of estrogen (46–49) or T (46, 48) and mood or depression. We found that the association between E2 and mood differed between postmenopause groups, but the trend for better mood in the early group was not significant. Our results for mood or possible depression, thus, support the absence of an important relation with levels of these endogenous hormones. Reasons for discrepant findings are unclear, although for midlife women, fluctuations in estrogen levels over time may be more closely

Table 4. Associations between hormone measures and mood or possible depression

	Mood (CES-D score)				Possible depression (CES-D ≥16)			
Hormone measure	β	SE	*P*	Interaction *P*	Odds ratio	95% CI	*P*	Interaction *P*
Free E2	−0.07	0.10	0.48	0.046*	1.12	0.80–1.57	0.52	0.17
Early group	−0.23	0.13	0.07					
Late group	0.20	0.15	0.19					
E1	0.07	0.14	0.63	0.22	1.44	0.88–2.35	0.15	0.42
P4	0.10	0.11	0.38	0.60	1.23	0.84–1.82	0.29	0.45
Free T	0.05	0.12	0.69	0.79	1.04	0.68–1.59	0.86	0.62
SHBG	0.05	0.14	0.73	0.052†	1.18	0.73–1.91	0.50	0.52
Early group	0.27	0.19	0.17					
Late group	−0.17	0.19	0.38					

Values for steroid hormone and SHBG concentrations were log-transformed before analyses. Mood was analyzed after square root transformation of CES-D scores. Sample sizes for hormone measures ranged from 633 to 641. All analyses were adjusted for age. Other adjustments were as follows: free E2 (race/ethnicity and smoking history); E1 (no other adjustments); P4 (smoking history); SHBG (race/ethnicity, antihypertensive use, smoking history, and alcohol use); and free T (race/ethnicity, smoking history, alcohol use, and apolipoprotein E genotype). Additional adjustment for the presence of other hormone measures (except E2 when E1 was the predictor variable and E1 when E2 was the predictor variable) had no substantial effect. CES-D, Center for Epidemiologic Studies Depression scale; CI, confidence interval.
*$P < 0.05$.
†$0.05 \leq$ interaction $P < 0.1$.

linked to depressed mood and depression than absolute levels per se (47, 48).

We also identified no relation between serum concentrations of P4 or SHBG and mood or possible depression. In one study, SHBG levels were not associated with mood (46), but neither SHBG nor P4 has been well-studied in this regard.

Conclusions

For postmenopausal women close to or remote from the time of menopause, our results fail to support the view that endogenous estrogen exposures as measured by serum concentrations are directly related to verbal episodic memory. Findings were similar for executive functions and global cognition, suggesting the absence of a critical window, during which time serum levels of E2 (or E1) are substantially related to these areas of cognitive function within the range of physiological levels that characterize postmenopause. In the early postmenopause group, we were able to replicate the reported association in midlife women between levels of free E2 and naming performance (19), but smaller effects, if present for other domains, might not have been detected.

In the early postmenopause group, P4 concentrations were significantly associated with both better verbal memory and global cognition, although early and late groups showed only a trend to difference in primary analyses. In rodents, P4 is neuroprotective and can enhance cognition, but P4 can also antagonize neural effects of E2 (50–52). These cognitive findings in the early group merit replication and additional study. Other suggestive, but unpredicted results pertain to SHBG in both younger and older postmenopausal women. The positive association with verbal memory, which implies that SHBG may influence brain processes involved in learning and memory, requires confirmation.

There are limitations to these findings. Because sex steroid hormones (53, 54) and possibly SHBG (55, 56) can be synthesized within the CNS, blood levels may imprecisely reflect concentrations within the brain. Environmental and lifestyle factors that promote cognitive skills could otherwise obscure cognitive effects mediated by E2 (39, 40). Our results do not apply to men, women of reproductive age, or women with dementia, because these groups are not represented in the ELITE cohort. Cognitive and mood associations for free E2, E1, and P4 pertain to the physiological range of endogenous exposures normally encountered by postmenopausal women and not higher exposures resulting from hormone therapy or higher physiological levels in normally cycling women. Cross-sectional hormone associations may better reflect short-term activational effects on cognition mediated by changes in electrical properties and synaptic plasticity than possible long-term processes mediated by organizational changes within the brain.

Our study of healthy postmenopausal women has important strengths. Foremost was the ability to examine within a large, well-characterized voluntary cohort whether time from menopause modified associations between concentrations of endogenous free E2 and verbal episodic memory, executive functions, or global cognition. We found that it did not. Results from the ongoing ELITE clinical trial should clarify whether cognitive effects of exogenous E2 exposures are modified by time from menopause. Our measure of global cognition was derived from a comprehensive neuropsychological battery rather than a short screening instrument. Use of verbal memory and executive function composites rather than single psychometric test scores enhances the validity of these measures. Multiple hormones were analyzed for each participant. Because we were able to examine other sex steroids in relation to cognition and mood, we had the opportunity to explore whether the apparent effect of a particular hormone differed when other hormone measures were considered in the same analysis. Here, findings suggest that relations of sex hormones and SHBG to cognitive function and mood were independent of influences of other levels.

Materials and Methods

A detailed description of the methods is available in *SI Materials and Methods*.

Design Overview and Participants. ELITE was designed as a randomized, double-blind trial of oral E2 or matched placebo (*Protocol Plan*); 643 women were recruited into early and late postmenopause groups. Women in the early group were within 6 y of their final menstrual period (natural menopause) or bilateral oophorectomy (surgical menopause). Women in the late postmenopause group were at least 10 y beyond their final menstrual period or bilateral oophorectomy.

Cognitive Assessment. Cognitive skills were assessed at baseline with a comprehensive neuropsychological battery that emphasized standardized tests sensitive to age-associated change in middle-aged and older adults (57, 58) (Table S2). To guard against the type I error in modified intention-to-treat analyses, end points in the ELITE-Cog trial focus on cognitive composites for verbal episodic memory, executive functions, and overall cognitive performance (global cognition), which were calculated as the average of component standardized scores weighted by the inverse intertest correlation matrix (58).

Mood and Possible Depression. Mood was assessed with the 20-item Center for Epidemiological Studies depression scale analyzed continuously and dichotomously using a standard cut point (59) to indicate possible depression.

Sex Steroid and SHBG Concentrations. E2, E1, T, P4, and SHBG levels were measured in serum as described (*SI Materials and Methods*).

Statistical Analyses. The associations between individual serum hormone levels and cognitive measures and mood—and the relation between years since menopause and cognitive function—were modeled separately using multivariable linear regression analysis. The relation between hormone levels and possible depression was similarly examined by logistic regression. Associations between individual hormones or SHBG and cognitive end points were modeled first without and then adjusting for other hormone measures. Our primary hypothesis was based on differences in association for free E2 and verbal episodic memory, where we predicted an interaction based on postmenopause group for free E2. Results for all hormones are shown separately within each postmenopause stratum if postmenopause group modified the relation significantly ($P < 0.05$) or showed a trend toward significant modification ($0.05 \leq P < 0.1$). Results are otherwise given for early and late groups combined.

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