

Gonadotropins and Cognition in Older Women

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Abstract. Recent research studies associate elevated gonadotropin levels with dementia. Specifically, an age associated increase in levels of luteinizing hormone has been linked to an increased risk of Alzheimer's disease. The objective of this study was to investigate the association between gonadotropin levels and cognition in older, healthy postmenopausal women. Cognitive functioning was compared with plasma levels of estradiol, luteinizing hormone, follicle stimulating hormone, A β 40 and APOE genetic status in 649 community-dwelling, non-demented older women residing in Western Australia. High endogenous luteinizing hormone levels were associated with a lower cognitive score, especially in older women and in those women that were depressed. Unexpectedly, disproportionately well preserved cognitive functioning was found for the oldest women who had high endogenous levels of follicle stimulating hormone. The findings indicate that gonadotropins can impact upon cognitive functioning in older postmenopausal women, and that luteinizing hormone and follicle stimulating hormone may exert contrasting effects. Taken together, the findings have important implications for the development of possible preventive strategies for dementia.

Keywords: Amyloid- β 40, APOE, Cambridge Cognitive Examination, estradiol, follicle stimulating hormone, gonadotropin releasing hormone, luteinizing hormone

INTRODUCTION

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Alzheimer's disease (AD) is a neurodegenerative disorder characterised by amyloid- β (A β) deposition in and around cerebral neurons, neuronal death and atrophy of the brain causing loss of cognitive functions

including memory. Age [30] and genetic variations, specifically possession of the apolipoprotein $\epsilon 4$ allele (APOE $\epsilon 4$, reviewed in [37]), are significant risk factors for AD in the majority of the population. Early reports have shown that the incidence of AD is higher in women than in men [16], notwithstanding the fact that life expectancy is longer, on average, in women. Furthermore, it has been shown that two pathological hallmarks of AD, neurofibrillary tangles and amyloid senile plaques, are more substantial in women with AD (particularly APOE $\epsilon 4+$ women) compared to men with the disease [11]. The higher incidence of AD in women may possibly be due to a rapid decline in the steroid hormone estrogen after menopause [7,12, 33,46].

An age associated increase in levels of gonadotropins has additionally been linked to an increased risk of developing AD. More specifically, recent reports have shown that, compared to age-matched control subjects, plasma luteinizing hormone (LH) levels are elevated in AD subjects [3,21]. Elevated levels of gonadotropins have also been found in individuals with Down's syndrome, where dementia and Alzheimer-like symptoms usually develop with advancing age [4]. Further, there is an increase in LH immunoreactivity compared with age-matched controls in the pyramidal neurons of the AD brain – a cell type that is reliably affected in AD [5]. Coupled with our recent finding that LH modulates amyloid- β protein precursor (A β PP) processing and A β generation *in vitro* [3, 4], and that gonadotropin releasing hormone (GnRH) agonists decrease A β levels in mouse brain [35], these findings suggest the menopause/andropause-related increases in gonadotropins are a physiologically relevant signal that potentially modulates neurodegeneration in the aging brain.

It should be noted that the regulation of the pituitary gonadotropins follicle stimulating hormone (FSH) and LH is under the control of complex feedback loops within the hypothalamic-pituitary-ovary axis that, in turn, regulate estrogen levels. In pre-menopausal women, circulating levels of steroid hormones regulate LH/FSH secretion via hypothalamic feedback. After menopause, a reduction in circulating levels of estrogen induces a loss of negative feedback, resulting in increased gonadotropin levels [17]. The sensitivity to negative feedback of the hypothalamic-pituitary axis is also reduced in postmenopausal women, possibly due to an aging hypothalamus [26].

Although, as previously noted, low levels of estrogen and high levels of gonadotropins have been asso-

ciated with cognitive deficits in dementia cases, it still remains to be determined if the changes in one or a combination of these hormones can influence cognitive functioning for older, healthy postmenopausal women whose cognitive status lies within the normal range. The main objective of this study was, therefore, to ascertain whether estrogen, FSH and LH levels were associated with lowered cognitive functioning in older women. Given established associations with AD [2, 45,24,25] and complaints of impaired memory capacity [14], possible associations between APOE $\epsilon 4$ status, plasma A β concentration and cognitive functioning were also investigated.

MATERIALS AND METHODS

Subjects

The study received approval from the University of Western Australia's ethics committee. All participants in the study were capable of providing informed consent. The study was conducted in accordance with the guidelines in the Declaration of Helsinki. Related to the availability of an existing cohort, all women recruited for this study were healthy, non-demented and over the age of 70.

Subjects were recruited between May and October 2002 from participants available for testing from a population-based study conducted at Sir Charles Gardner Hospital (Perth, Western Australia) investigating calcium intervention and bone fractures (CAIFOS). In order to eliminate significant major confounds from this investigation, participants were excluded from this study if a) they has been previously receiving bone active agents, including calcium supplements, or b) they were receiving hormone replacement therapy. The Cambridge Cognitive Examination (CAMCOG) [34] – a very widely used, objective measure of cognitive status – was used to evaluate cognitive capacity in this study. Potential participants were excluded from the study if they scored less than 80 on the CAMCOG (suggestive of impaired cognitive functioning), or if they manifested significant illness which made it unlikely that they would be able to complete the longitudinal components of the study. Notwithstanding these exclusion criteria, a random selection of suitably healthy, non-demented women over the age of 70 on the electoral roll in Western Australia received a letter inviting them to join the original CAIFOS study. (Over 98% of women in this age range are listed on the electoral

roll.) 18% of these women responded positively. Of the total of 1460 women recruited to CAIFOS, 649 individuals were randomly chosen to participate in the current cognitive study.

Informed consent was obtained for the current study at the time of a hospital visit for the CAIFOS study. Subjects were later contacted by telephone, and a time arranged for them to attend the hospital for cognitive testing. To maximise participant numbers, home visits were organised for participants who were unable to attend at the hospital. The majority of interviews were conducted at the School of Medicine and Pharmacology, Sir Charles Gairdner Hospital, Perth, Australia.

Data collection

Cognitive performance was measured using the Cambridge Cognitive Examination (CAMCOG) component of the Cambridge Mental Disorder of the Elderly Examination (CAMDEX [29]), administered by a trained assessor (first author). CAMCOG scores can range from a minimum of 0 to a maximum of 107 points, with the lowest end of the continuum representative of the highest degree of cognitive compromise (typical clinic cut-off scores are applied in a range between 80–85).

Demographic variables

Age, years since menopause, parity, BMI [mass/height (kg/m^2)] and education were determined via a questionnaire administered at baseline. Years since menopause were calculated for each patient using reported age at last menstrual period. Age at hysterectomy and/or ovariectomy (if conducted) or the onset of hot flushes (if observed) was also recorded. Height and weight were measured at baseline with patients in light clothing and without shoes. Education (measured as the age when each woman left school) was coded as low (≤ 15 years of age), medium (16–18 years) and high (≥ 19 years). At the commencement of the study, each subject was asked to record any adverse events resulting in the attendance of a health professional in a diary (with assistance, as appropriate). The diaries were collected at four-month intervals, and all ongoing adverse events were coded using the International Classification of Primary Care (ICPC2 Plus©) system (a database of disease coding) and CAPS© (a database of pharmaceuticals, developed and supplied by the Family Medicine Research Unit, Department of General Practice, University of Sydney). Adverse events were

grouped according to the 17 categories identified by the ICPC2 Plus system [6]. A blood sample (18mls) was taken during the cognitive assessment visit. This was used to measure plasma estradiol (E2; the major circulating form of estrogen), FSH and LH, APOE $\epsilon 4$ status and A $\beta 40$, which (together with CAMCOG score) were the variables of primary interest in the current investigation.

Laboratory procedures

The plasma from the blood samples collected in tubes containing EDTA was separated by centrifugation at $130 \times g$ for 15 minutes, aliquoted, stored at -20°C and batched for analysis. With respect to E2, inter-assay CV was 6.6% at a mean of 101 pmol/L ($n = 17$) with an analytical sensitivity of 5 pmol/L. (This degree of sensitivity is necessary when assaying E2 in older postmenopausal women, in which the concentration of E2 is likely to be low.) FSH and LH levels were measured by radioimmunoassays at the Department of Clinical Biochemistry, PathCentre, Perth, Western Australia. Plasma A β concentrations were measured using the Enzyme-Linked Immunosorbent Assay (ELISA), as described previously [27,28]. Apolipoprotein E genotyping was performed by Polymerase Chain Reaction (PCR) amplification using the protocol detailed by Hixson and Vernier [20], with oligonucleotide primers described by Wenham and colleagues [44]. It was possible to ascertain APOE status for 618 of 649 participants (95.2%; not obtained for 31 subjects), of which 138 were APOE $\epsilon 4+$ (APOE $\epsilon 4$ group; 22.3%) and 480 were APOE $\epsilon 4-$ (non-APOE $\epsilon 4$ group; 77.7%).

Statistical analysis

Data were analysed using SPSS (Version 11). In addition to descriptive statistics, Pearson's tests were used to assess associations between variables. Independent sample *t* tests were used to compare group means. It was established statistically that CAMCOG score was not influenced by whether women were taking calcium as part of the CAIFOS study, or by whether women had received hysterectomy or ovariectomy. Multiple linear regression analyses were next undertaken to assess the relationship between E2, FSH, LH, APOE status, A $\beta 40$ (predictor variables) and cognitive performance (CAMCOG score; outcome variable). Age and education were used as statistical covariates, given the established relationship between these variables and age-related cognitive functioning. A diagnosis of

Table 1

Descriptive statistics of the participant sample. CAMCOG (Cambridge Cognitive Examination), E2 (estradiol), FSH (follicle stimulating hormone), LH (luteinizing hormone), A β 40 (amyloid- β 40), BMI (Body Mass Index), Parity (number of children)

Variable	n	Min.	Max.	Mean	SD
CAMCOG	649	67	106	95.4	5.3
E2 (pmol/l) ²	645	5	143	28.7	16.1
FSH (IU/l)	611	1.4	172.0	55.1	24.0
LH (IU/l)	615	0.6	146.4	39.6	21.9
Age (years)	649	75	87	80.0	2.6
years since menopause	614	13	48	26.6	6.4
A β 40 (pg/ml)	603	0.3	246.1	81.4	42.8
BMI	619	16.3	48.5	26.9	4.3
Parity	648	0	9	3.0	1.7

¹ $P > 0.05$ for all comparisons of means.

² t test performed using logarithm transformed dependent variable.

depression was also considered a relevant variable in this study, given the potential influence of depressive symptomatology on age-related cognitive status. All tests were conducted and validated by experienced biostatisticians (KJ and SD).

RESULTS

Sample characteristics

Descriptive statistics are listed in Table 1. The prevalence of statin use was 28.8%, hypertension 52.1%, cerebrovascular event 6.6%, diabetes 8.5% and depression 10.3%. It was noted that E2, FSH and LH were poorly correlated: $r = -0.12$ for E2 and FSH, $r = 0.09$ for E2 and LH and $r = 0.13$ for FSH and LH.

CAMCOG scores

Among the continuous variables of interest (E2, FSH, LH, APOE status, A β 40, age, education and CAMCOG score), there were significant positive correlations between CAMCOG score and FSH (Pearson's $r = 0.08$, $p < 0.05$) and education ($r = 0.18$, $p < 0.001$), and significant negative correlations between CAMCOG score and LH ($r = -0.15$, $p < 0.001$), E2 ($r = -0.103$, $p < 0.01$) and age ($r = -0.21$, $p < 0.001$). There were no significant associations between APOE ϵ 4 status, plasma A β 40 concentration and CAMCOG score.

In order to examine the factors underlying the observed correlations in more detail, multiple linear regression was undertaken. The results of these analyses are presented in Table 2. Among the variables of interest, FSH ($p < 0.001$) and LH ($p < 0.01$) were

identified as statistically significant factors associated with CAMCOG performance, as were the covariates age ($p < 0.05$) and education ($p < 0.001$). With respect to the predictor variables for which significant main effects were identified, three additional interaction terms were statistically significant: i) age and FSH ($p < 0.005$), ii) age and LH ($p < 0.005$) and iii) LH and depression ($p < 0.01$).

Predicted CAMCOG values were calculated using the relevant equation for the CAMCOG regression model that is shown in Table 2. Education was the only main effect not to be included in an interaction term. Relative to individuals with low education, CAMCOG scores were approximately 1.7 and 3.9 points higher for those with medium and high levels of education, respectively ($P < 0.001$), after adjustment for the significant effects of FSH, LH, age and depression.

As noted earlier, analysis of Pearson's correlations (including partial correlations) indicated that there was a positive relationship between FSH and CAMCOG, and a negative relationship between LH and CAMCOG. However, both relationships showed significant interactions such that they became more pronounced with increasing age. In addition, the effect of LH on CAMCOG performance became more pronounced in the presence of depression.

To appreciate the interaction of FSH and age on CAMCOG scores in this sample, consider the following: non-depressed women aged 84 with low education, with an average level of LH (50th percentile) and with low (10th percentile), average (50th percentile) or high (90th percentile) levels of FSH manifested CAMCOG scores, on average, of 92.0, 94.1 and 96.5, respectively (compared with scores of 96.1, 96.5 and 96.9, respectively, for similar women aged 77).

Table 2

Results of multiple linear regression analysis showing statistically significant factors (alpha = 0.05) impacting on CAMCOG (Cambridge Cognitive Examination) scores and relevant Pearson's correlation¹. FSH (follicle stimulating hormone), LH (luteinizing hormone)

Variables	Coefficient	Standard Error	Pearson's R	P
Constant	132.824	17.193	–	<0.001
FSH	–0.680	0.248	0.083	0.006
LH	0.772	0.257	–0.154	0.003
Age	–0.482	0.215	–0.205	0.025
Education medium ²	1.669	0.445	0.178	<0.001
high ²	3.947	0.608	–	<0.001
FSH*age	0.009	0.003	–	0.004
LH*age	–0.010	0.003	–	0.002
LH*depression	–0.071	0.026	–	0.007

¹R² = 0.181, adjusted R² = 0.169, F_{9,596} = 14.649, P < 0.001.

²relative to low education.

The interaction of LH and age on CAMCOG is demonstrated when considering the following: non-depressed women with low education and with an average level of FSH (50th percentile), aged 84 years (90th percentile) and with low (10th percentile), average (50th percentile) and high (90th percentile) LH values manifested average CAMCOG scores of 95.2, 94.1 and 91.8, respectively (compared with scores of 96.4, 96.5 and 96.6, respectively, for similar women aged 77). With respect to the interaction between LH and depression, consider that depressed women (with the same demographic characteristics) and aged 84 years manifested average CAMCOG scores of 95.6, 93.4 and 88.6, respectively (as compared to those of women aged 77, with scores of 96.9, 95.8 and 93.4, respectively).

DISCUSSION

In the current study, cognitive performance using the CAMCOG component of CAMDEX was assessed in a group of 649 healthy, non-demented older women, ranging in age from 75 to 87 years. None of the cognitive scores obtained in this study breached the typical clinically employed CAMCOG cut-off scores; that is, individuals in this sample were not manifesting dementia (by definition, with respect to the inclusion criteria for the study).

Multiple linear regression analysis was used to examine the association between CAMCOG performance and several key variables (APOE status, plasma levels of estradiol, LH, FSH and A β 40, age, education and depression rating). The findings of this study indicate that FSH and LH may significantly modulate cognitive functioning in postmenopausal women, in combination with the factors of age and depression. These results are

of potential clinical relevance. No significant associations were observed between APOE ϵ 4 status, plasma A β 40 concentration and cognitive functioning.

The findings of this study indicated that, in this cohort, level of education was positively associated with cognitive status, with age manifesting a negative association. These results concur with many studies that show that cognitive status positively correlates with years of education over the lifetime, whereas increasing age is associated with deleterious changes in several elements of cognitive functioning [41].

The hormones of the hypothalamic-pituitary-gonadal axis and certain steroid hormones have previously been linked to the incidence of AD. More specifically, low levels of estradiol and high levels of the gonadotropins LH and FSH have previously been associated with an increased risk of developing dementia in postmenopausal women [3,21,45]. In the current study, the levels of LH and FSH were significant associated with CAMCOG scores, but in opposite directions. More specifically, higher levels of LH were associated with lower levels of CAMCOG performance in these women. Furthermore, this association was modulated by age, such that the impact of LH in lowering CAMCOG performance was more exaggerated in older women. These results would appear to concur with other studies showing elevated levels of LH in AD patients [3,21]. Moreover, LH levels have been reported to be elevated in the cytoplasm of pyramidal neurons in AD brains compared to age-matched controls [5]. LH preferentially influences the metabolism of A β PP processing towards the amyloidogenic pathway, whereby A β is generated from A β PP via a proteolytic series of events catalyzed by β - and γ -secretase [4]. Taken together with the findings of previous studies [9,10], our results suggest that high levels of plasma LH may be

a significant factor mediating cognitive status in older postmenopausal women.

The findings of the current study indicate that the negative association that was observed between LH and CAMCOG performance is modulated by depression, such that the impact of LH in lowering CAMCOG performance was more exaggerated in women with a history of depression. Depressive symptoms have been associated with cognitive impairment in the elderly [31], and are commonly found in patients with AD [13]. Furthermore, patients with AD and at least one APOE ϵ 4 allele have been reported to have a threefold increase in signs of depression and psychosis [32]. Considering issues related to mood and affect more generally, Uday et al. have recently shown a relationship between GnRH and fluoxetine effects on anxiety-like behaviour, which may be relevant in the context of the present findings regarding depression and LH levels [39].

In contrast to an increase in LH levels, high plasma levels of FSH were associated with improved CAMCOG scores in the women assessed in this study. This association was also modulated by age, such that the impact of FSH-associated improvement in CAMCOG was greater in older women. These results do not concur with earlier findings obtained in studies of AD patients which indicated that high levels of FSH confer an increased risk for developing dementia [3,21]. However, these previous studies refer to patients in whom a diagnosis of AD has already been made, and offer relatively small sample sizes (ranging from 69 to 284). Conversely, some other studies in non-AD patients have shown improvements in cognitive functioning with higher FSH levels [18,19]. Taken together with our current findings, these latter studies indicate that previous findings in the field may need to be revised: FSH may in fact be neuroprotective rather than exerting deleterious effects on cognitive functioning – at least in postmenopausal women who are not clinically demented.

The link between activin and FSH is perhaps relevant to consider with respect to the novelty of these findings, since activins have the ability to stimulate FSH production [40]. It was recently found that activin A levels continually rise with age, with augmented levels in the last decades of life [1]. However, the role of activin and FSH in cognition and memory is largely an unexplored area. It is possible that postmenopausal rises in FSH and activin levels are inter-related [42]. Interestingly, activin has also been shown to affect neuronal viability, independent of FSH. *In vitro* studies in cultured neurons have revealed that activin A increases survival

rate, and it may protect against neurotoxic damage [22]. Furthermore, the release of activin A has been shown to be a functionally significant component of the neuronal response to brain injury [23]. The question therefore appears to remain open as to whether the neurocognitive correlates of FSH observed in the current study are independently mediated via FSH, or whether they are linked to activin-related mechanisms.

In our group of postmenopausal women, multiple regression analysis indicated that endogenous estradiol level was not a statistically significant predictor of CAMCOG score. The relationship between estrogen and age-related cognitive capacity represents a controversial area of research. Previously, no association between estradiol levels and cognition was found prior to and following the administration of Premarin (a conjugated estrogen homologue) for one year to 120 postmenopausal women diagnosed with AD [38]. However, in recent studies the use of exogenous estrogen homologues has been associated with cognitive impairment in some postmenopausal women [15,36]. Our own findings are intriguing, because the primary analysis indicated that estradiol was significantly negatively correlated with CAMCOG performance (Pearson's correlation coefficient = -0.103 , $p = 0.007$). However, estradiol failed to reach statistical significance when entered into the multiple regression model (specifically, $p = 0.070$; i.e. estradiol was the strongest predictor of CAMCOG scores after age, education, LH and FSH). Given the direction of the observed correlation between estradiol and CAMCOG, these findings would appear to indicate that, if anything, higher endogenous estradiol levels are more likely to have an adverse effect on cognitive status in older postmenopausal women. It is perhaps worthy of note in this context that the postmenopausal levels of estradiol observed in the current study (<130 pg/ml), although variable, were – collectively – significantly lower than previously reported pre-menopausal levels (200–400 pg/ml [8]). Furthermore, given differences in concentration and/or biochemical properties of endogenous versus exogenous estrogen, it is questionable whether the current findings can be generalized to estrogen administration, especially where exogenous agents are homologues derived from non-human species [38].

In conclusion, this study has shown that plasma levels of endogenous gonadotropins (LH and FSH) are significantly associated with cognitive capacity in older non-demented postmenopausal women, but estradiol and A β 40 levels are not. The correlations observed between LH, FSH and CAMCOG score were modulated

by age, and the relationship between LH and CAMCOG was further modulated by depression. Although high levels of LH have previously been associated with AD in men and women, this study is the first to report that elevated levels of endogenous LH are associated with decreased cognitive functioning in non-demented, older postmenopausal women. Furthermore, this is the first report of a positive association between endogenous levels of FSH and cognitive functioning. These findings are supportive of other reports indicating that the physiological significance of gonadotropins transcends their reproductive function, and that plasma gonadotropins are important biological markers of age-related cognitive status [43,47]. Taken together with previous findings in the literature, the results obtained in this study suggest that specific gonadotropin-related agents are involved in regulating cognitive functioning in older women and, potentially, in influencing the risk of dementia. In future work, the analysis of GnRH (responsible for modulating the release of FSH and LH from the anterior pituitary) would help to inform the significance of the present findings.

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