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HORMONAL AND NON-HORMONAL FACTORS ASSOCIATED WITH COGNITION IN POST-MENOPAUSAL WOMEN

By

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ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia world-wide accounting for more than two thirds of all dementia cases. AD is characterised by the presence of extracellular amyloid plaques, neurofibrillary tangles and congophillic amyloid angiopathy in the brain tissue of affected individuals. Of these neuropathological features the extracellular amyloid plaques are the most characteristic containing a peptide termed amyloid- beta (Aβ); the major protein component of these structures. In addition a number of genetic risk factors for AD have been identified. Of these the ε4 allele of the apolipoprotein E (*APOE*) gene found on chromosome 19 is considered to be the main genetic risk factor attributing to about 40-60% of all AD cases in most populations. Although there is strong evidence that genetic risk factors play an important role in AD they do not actually trigger the disease process.

Deficits in memory and learning are the most common clinical signs of AD in the initial stages of the disease. Neuropsychological tests such as the CAMCOG and California Verbal Learning Test (CVLT) are important diagnostic tools used for the assessment of cognition. The CAMCOG is an accurate and efficient measure of global cognitive ability, while the CVLT is more specific to areas of cognition influenced in the early stages of the disease such as verbal memory.

Substantial evidence indicates that changes in sex hormones following menopause in women are important factors in AD. Specifically, the reduced levels of oestrogen in post-menopausal women have been linked to cognitive decline and an increased risk of dementia. In addition the elevated level of the gonadotropins, a characteristic of the post-menopausal period, have been implicated with the disease. Numerous non-hormonal factors such as age and education may also be associated with the development and progression of cognitive decline.

This study endeavored to investigate the hormonal and non-hormonal factors associated with memory and cognitive decline in postmenopausal women between 75 and 87 years of age. The cross sectional findings demonstrate for the first time that increased plasma gonadotropin levels are associated with altered cognition, as found in a large sample cohort of approximately 600 elderly women. In particular, individuals with elevated LH were associated with a decline in CAMCOG score in non-*APOE* ε4 subjects. Conversely plasma FSH concentration was linked with increased CAMCOG score independent of *APOE* genotype. Age and levels of Aβ40 were related to a decline in memory function, whereas education had a positive effect on cognition. Longitudinal findings on the CAMCOG cohort indicated that the baseline result was the main factor associated with 12 month score with 57 % of individuals showing an improvement over the one year interval between assessments. The cross sectional results of the CVLT cohort of approximately 400 post-menopausal women show that age, education and levels of plasma Aβ40 were again main factors in determining score on specific parameters of the CVLT confirming the findings from the CAMCOG cohort. Longitudinal reports also indicate that baseline score was the only significant main factor on CVLT results after 12 months. A relationship between oestrogen and cognition was not evident in both the CAMCOG and CVLT cohorts. The results of this study may assist in a greater understanding of the hormonal, biochemical and environmental factors in cognitive decline, which may prove invaluable in facilitating earlier diagnosis, treatment and prevention of AD.

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ABBREVIATIONS

Aβ	Amyloid beta peptide
AD	Alzheimer's disease
APOE	Apolipoprotein E (gene)
ApoE	Apolipoprotein E (protein)
APP	Amyloid precursor protein
BMI	Body Mass Index
BSA	Bovine serum albumin
CAA	Congophillic amyloid angiopathy
CAIFOS	Calcium Intake Fracture Outcome Study
CAMCOG	Cambridge Cognitive Examination
CAMDEX	Cambridge Examination for Mental Disorders of the Elderly
^{o}C	Degrees Celsius
CDR	Clinical Dementia Rating
CSF	Cerebrospinal fluid
CT	Computed tomography
CVLT	California Verbal Learning Test
CVS	Cardio-vascular event
DDW	Double de-ionised water
df	Degrees of freedom
DMSO	Dimethyl sulphoxide
DNA	Deoyxribose Nucleic Acid
E2	Endogenous oestrogen
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
EOAD	Early-onset Alzheimer's disease
FAD	Familial Alzheimer's disease

FSH	Follicle stimulating hormone
GDS	Global Deterioration Scale
hCG	Human chorionic gonadotropin
HRP	Horse radish peroxide
HRT	Hormone replacement therapy
HPO	Hypothalamic-pituitary-ovarian axis
IQR	Inter-quartile range
LDL	Low density lipoprotein
LH	Luteinising Hormone
LOAD	Late-onset Alzheimer's disease
LRP	Low density lipoprotein receptor related protein
MCI	Mild cognitive impairment
MLR	Multiple linear regression
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NFT	Neurofibrillary tangles
NGF	Nerve growth factor
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with 0.05% Tween 20
PCOS	Polycystic ovary syndrome
PCR	Polymerase chain reaction
PCV	Packed cell volume
PET	Positron emission tomography
PS1	Presenilin 1
PS2	Presenilin 2
RIA	Radioimmunoassay

RPM	Revolutions per minute
RT	Room temperature
SCN	Suprachiasmatic nucleus
SD	Standard deviation
TBS	Tris buffered saline
TBST	Tris buffered saline with 0.05% Tween 20
TMB	Tetramethyl-benzidine
Tween 20	Polyoxyethylene (20) sorbitan monolaurate

DECLARATION

This is to certify that

(i) The thesis comprises only my original work.

(ii) Due acknowledgements are made in the text to all other materials used.

(iii) The thesis has not previously been accepted for any other degree in this or another institution.

(iv) The thesis has been substantially accomplished during enrolment in the degree.

(v) The thesis is less than 50,000 words in length, exclusive of tables, illustrative matter and appendices.

Mark Anthony Rodrigues

(B.Sc.)

Chapter 1 INTRODUCTION

Alzheimer's disease was first recognized in 1906 by Alois Alzheimer, a German physician. He identified an 'unusual disease of the cerebral cortex' which affected a woman in her fifties, causing memory loss, disorientation, hallucinations and ultimately her death at age 55. Examination of her brain revealed neuropathological lesions with characteristic senile plaques and neurofibrillary tangles. Since this initial description, Alzheimer's disease has been acknowledged as a progressive loss of cognitive and intellectual function leading to dementia with characteristic amyloid neuropathology in the brains of affected individuals (Gilman, 1997).

It is estimated that more than 22 million individuals world-wide will have Alzheimer's disease by 2025 and on average 10 % of people over 65 are afflicted with this disorder. These statistics rise to over 50% by the age of 85 years. Currently in Australia, an estimated 160,000 people are diagnosed with some form of dementia with the figure predicted to rise to 500,000 by the year 2041 (Jorm, 2001). Of these about two thirds suffer from AD (Boss, 2000). Dementia is the third leading cause of disease for older women behind ischemic heart disease and stroke (Mathers et al., 2000).

1.1 Pathological Characteristics of AD

1.1.1 Macroscopic characteristics

The major macroscopic changes that occur in the AD brain include widening of the sulci and narrowing of the gyri in the frontotemporal areas as well as enlargement of the ventricles (Refer to Fig 1.1; Brun, 1983). CT scans of the very early stages of AD indicate atrophy of the medial temporal lobe including the hippocampus and the amygdala. It has been reported that brain atrophy progresses from the temporal to the frontal lobe and then to generalised cerebral cortices (Maruyama et al., 1995).

Ventricular size progressively decreases as AD develops (Brun, 1983). Recently, the measure of the medial temporal lobe has been utilised as an aid to diagnosis or for monitoring disease progression (Denihan et al., 2000). Studies have found that the average rate of atrophy of the medial temporal lobe was 15.1 % per year in histopathologically confirmed AD patients as compared to 1.5% in healthy aged controls (Jobst et al., 1994).

1.1.2 Microscopic Pathology; neurofibrillary tangles, senile plaques and cerebral amyloid angiopathy

Alzheimer's disease neuropathology is primarily characterised by the presence of extracellular and intracellular amyloid deposits termed neuritic or senile plaques (NP) (Masters et al., 1995; Iwamoto et al., 1996) and neurofibrillary tangles (Gilman, 1997) which are found in selected areas of the brain mainly associated with memory and learning. Neuro-degeneration in AD can be attributed to a pathological state independent of aging (Maccioni et al., 2001; Johnson et al., 2000). However others suggest that AD is a relatively early but inevitable consequence of normal aging (Anderton, 2002). While this issue remains to be resolved it is interesting to note that although AD is age-related there are a considerable number of elderly people who escape the disease.

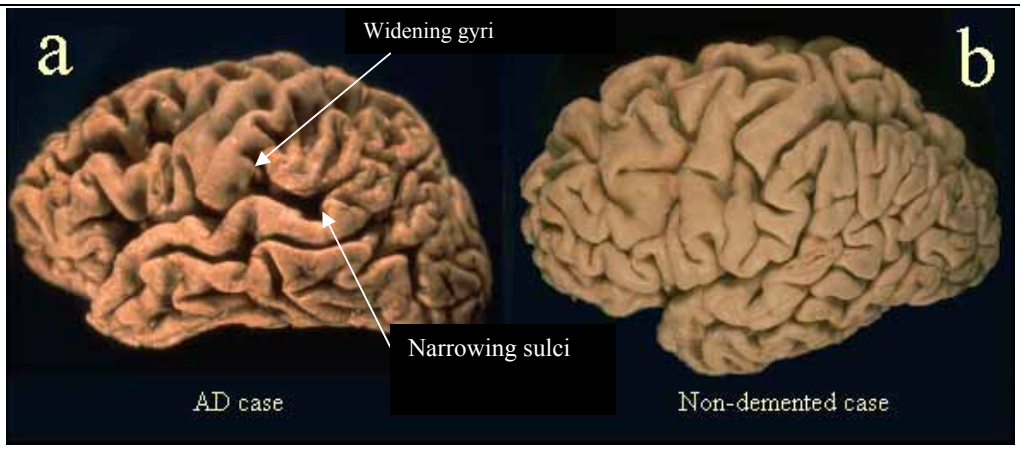

Fig 1.1: Macroscopic characteristics of AD (a) versus non-demented brain (b)

Comparison of the AD brain to normal brain. AD brain has characteristic widening of the gyri and narrowing of the sulci.

The presence of intraneuronal cytoskeletal neurofibrillary tangles (NFT) results from the aggregation of abnormally phosphorylated tau protein (Tapiola et al., 1997; Refer to Fig 2). In healthy neurons, microtubules are parallel structures that are involved in cell motility, transport, shape and mitosis and tau protein supports these normally parallel structures in the polymerisation process (Avila et al., 2004; Schraen-Maschke et al., 2004; Goedert, 1993). Normally the equilibrium between phosphorylation and de-phosphorylation of tau modulates the cytoskeleton influencing the morphology of the axon (Maccioni et al., 2001). The hyper-phosphorylation of tau protein, characteristic of AD pathology, reduces the binding of tau to microtubules and hence creates the characteristic filamentus structures known as paired helical filaments (PHF; Goedert, 1993). The de-polymerisation of microtubules leads to the degeneration of dendrites, axons and cell cytoskeleton. PHF's are the basic constituents of NFT (Gilman, 1997) as are neuropil threads found in the neuropil of the grey matter as well as peripheral organs (Avila et al., 2004). A key characteristic of AD is the specific pattern of NFT related lesions in the brain (Braak et al., 1997). This pattern of lesions tends to be symmetrical

in both hemispheres and is divided into six stages. These are the transentorhinal stages I and II, the limbic stages III and IV and the neocortical stages V and VI (Braak et al., 1997). The first two stages in the transentorhinal region is not considered as being associated with dementia as it is seen in normal aging whereas stages 3 to 6 are associated with increasing levels of cognitive decline (Anderton, 2002; Braak et al., 1997).

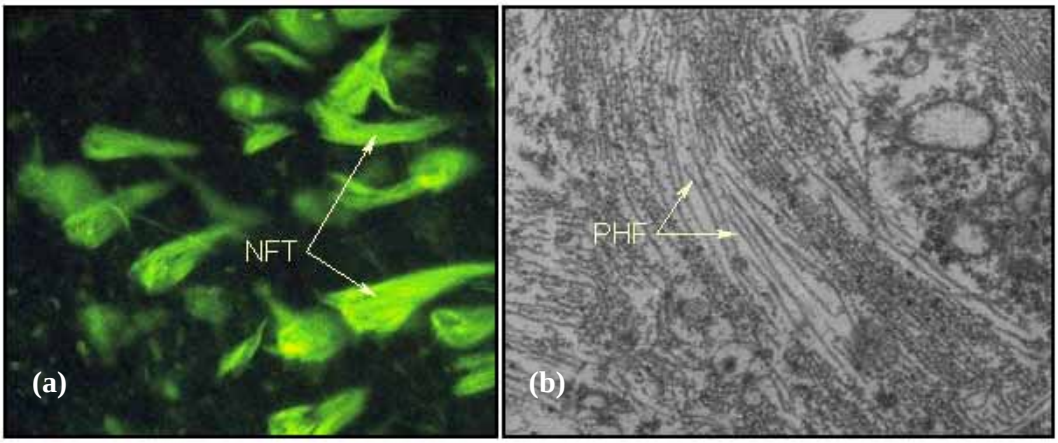

Fig 1.2: Neurofibrillary tangles (NFT) and Paired Helical Filaments (PHF)

Pathological hallmarks of AD include neurofibrillary tangles (a) described as filamentous structures arranged in bundles within affected neurons. The tangles are composed of paired helical filaments (b) that consist of tau.

Senile plaques in the brain are formed by the deposition of fibrils of extracellular aggregates of 42/43 amino acid peptide termed amyloid-beta (Aβ). Amyloid plaques consist of a central core of radiating amyloid fibrils surrounded by dystrophic neurites and activated glial cells (Brun, 1983). These plaques are classified either as diffuse (amorphous deposition of Aβ) or compact or neuritic (thought to be the primary cause of cerebral damage; Yamaguchi et al., 1990). The Aβ peptide is a fragment derived from the proteolytic processing of its parent molecule, amyloid precursor protein (APP).

Under silver stained light microscopy neurotic plaques present as a central core of Aβ surrounded by swollen abnormal neurites (Mathis et al., 2004; Anderton, 2002; Refer to Fig 1.3). Upon staining with congo red, the characteristic birefringence can be seen under polarised light (displaying the antiparallel beta-sheet conformation) and using transmission electron microscopy reveals the characteristic fibrillar nature of the plaque cores (Morris, 1997) which reflect the distinguishing pattern of Aβ aggregation. Neuritic plaques are surrounded by dystropic neurites, astrocytes and microglia and thought to develop from diffuse plaques (Delaere et al., 1993). Small numbers of neuritic plaques are present in the normal aged brain but in AD their numbers are greatly increased. (Mathis et al., 2004).

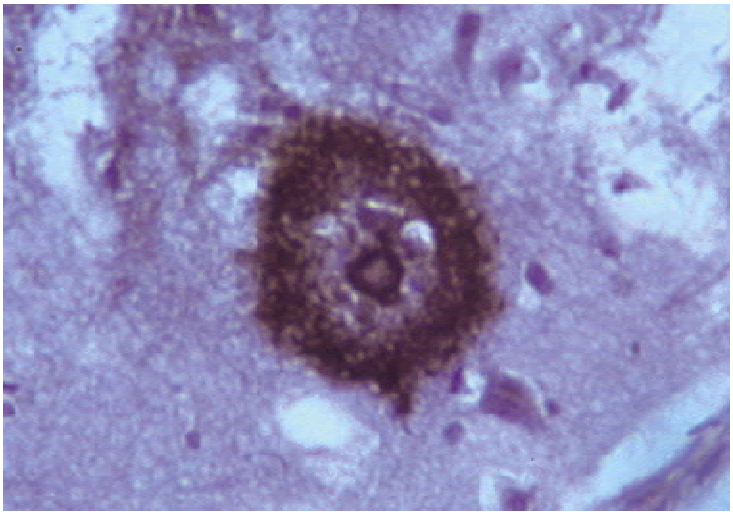

Fig 1.3: Neuritic plaque

Section of cerebral cortex displaying neuritic plaques with the characteristic dense core of amyloid.

The third neuropathological hallmark of AD is Congophillic Amyloid Angiopathy (CAA) which is the accumulation of Aβ peptide in the walls of capillaries and arteries within the brain and the leptomeningeal arteries (Weller et al., 2000; Refer to figure 1.4). The pattern of deposition of Aβ in senile plaques and in CAA suggests that the

peptide accumulates in pericapillary and periarterial interstitial fluid drainage pathways (Weller et al., 2000). CAA can increase the frequency of intracerebral hemorrhage due to the weakening of the vessel wall (Aβ replaces smooth muscle tissue) and hinders the elasticity of the arteries playing a significant role in the clinical pathology of AD (Weller et al., 2000). The effect of CAA is particularly pronounced in patients with Icelandic hemorrhage and Dutch hemorrhage, both of which have mutations within the Aβ region resulting in senile cerebral hemorrhage (Revesz et al., 2002, 2003; Frangione et al., 2001).

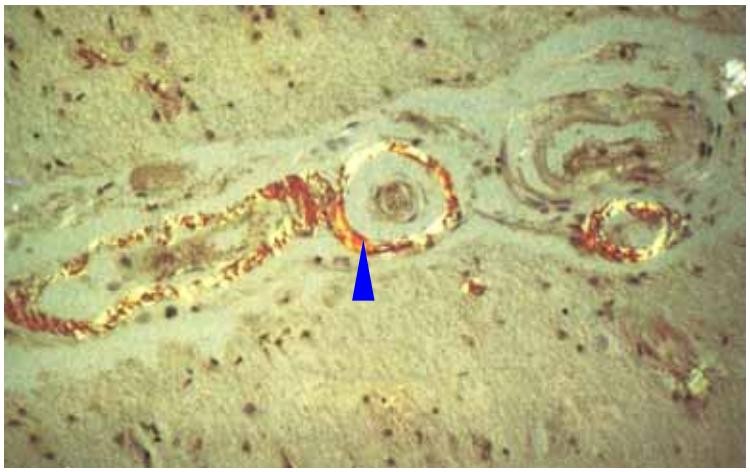

Figure 1.4: Congophillic amyloid angiopathy

Cross-section of the cerebral arteries of the brain displaying the characteristic staining of the arterial walls with congo red, representative of amyloid deposition (blue arrow).

1.2 Clinical Characteristics and Diagnosis of AD

The neuropathological changes in the AD brain are reflected by deterioration in clinical functions (Pietrini et al., 2000). The earliest clinical change is subtle decline in memory functions where consciousness is unaffected. (Braak et al., 1997). Typical memory problems include difficulty in learning and recalling new information (Small et al., 1997). Skills such as dealing with personal finances, performing household tasks or orientation depreciate. Personality begins to change as well as mental reasoning and

language skills beginning with anomia (inability to remember the names of things, people, or places) and progressing to fluent aphasia (an impairment of language, affecting the production or comprehension of speech; Bullock et al., 2003). Executive functioning also degenerates affecting judgment, insight and planning skills (Duke et al., 2000). The patient is usually unaware that their cognitive abilities are compromised (Bullock et al., 2003).

Neuropsychiatric symptoms may begin with apathy (Landes et al., 2001) and progress to hallucinations or delusions. With progression of AD, depression is a major factor (Peskind et al., 2003) as is agitation and frustration. Motor skills are relatively intact until the later stages of the disease where there is progressive gait disturbances and limited body movement and the body starts to suffer from hypokinetic, hypertonic syndrome (rigidity of muscle tone; Miyoshi et al., 2004). Patients typically die of bronchitis or pneumonia (Kalia, 2003). The speed and severity of the disease varies among AD patients with individuals usually surviving between 7 to 10 years following initial diagnosis (Crawford and Goate, 1992).

Definite antemortem diagnosis still requires further research and development however a clinical diagnosis based on exclusion criteria is reasonably accurate when undertaken by geriatricians with access to appropriate diagnostic facilities (McKhann et al., 1984). These tests involve the history of the patient including assessment of daily activities, changes in cognitive functioning and neurological assessments. Neurological examinations can indicate certain changes characteristic of AD or dementia and may display as changed gait, muscle tone abnormalities or existence of tremor (Miyoshi et al., 2004). Neuropsychological tests are an efficient and popular method for assessing cognitive function. A mental status examination such as the ' Mini- Mental State Examination' is of particular importance in the diagnosis of dementia where learning, recall, language, visuospatial, calculating and abstraction skills are assessed (Lorentz et

al., 2002). Laboratory tests can indicate the current metabolic status that may contribute to cognitive decline (Growdon, 1999). Neuro-imaging techniques such as magnetic resonance imaging (MRI ; Avila et al., 2004) or computed tomography (CT; Lee et al., 2003) can exclude other forms of dementia or identify treatable causes of dementia (tumors, ischemic disease). These techniques may be useful in differentiating between areas of the brain that are normal and areas that have atrophied. Hippocampal atrophy can be used to aid diagnosis of AD (Decarli et al., 1990). The diagnosis of AD falls into 3 groups; probable, possible or definite as defined by the National Institute of Neurological and Communitive Disorders and Stroke (NINCDS) (McKhann et al., 1984). Generally the first two groups can be determined using antemortem diagnostic techniques but the third 'definite' group can only be determined by examining the brain during autopsy for characteristic macroscopic and microscopic pathology of AD.The current criterion for the pathological diagnosis of AD post-mortem requires the presence of NFT and neuritic plaques in levels higher than age matched normal controls (McKhann et al., 1984).

1.3 Factors Implicated in the Pathogenesis of AD

1.3.1 Beta Amyloid (Aβ) protein

Aβ deposition is purported to play an essential role in the pathogenesis of AD (Masters et al., 1995; Sandbrink et al., 1997) and is a proteolytic fragment of an integral membrane glycoprotein known as Amyloid Precursor Protein (APP; Hartmann et al., 1996). The two major forms of Aβ are Aβ– 40 (terminates at Val40 of the Aβ sequence) and the longer and more amyloidogenic form Aβ- 42 (terminates at Ala 42 of the Aβ sequence; Iwatsubo et al., 1994) and are responsible for the formation of plaques. Immunochemical studies suggest Aβ42 precedes Aβ40 in plaque deposition (Iwatsubo et al., 1995). Approximately ninety percent of Aβ in the brain is Aβ40, with the

remaining 10 % Aβ 42 (Chapman et al., 2001). Aβ is constitutively secreted by neuronal cells and is found in the cerebrospinal fluid (CSF) as well as in the blood where it can accumulate in cerebral capillaries, arterioles and venules (Hampel et al., 2004; Maccioni et al., 2001). Increased Aβ levels is more strongly correlated with AD than amyloid plaque load (Fonte et al., 2002) and in the frontal cortex this increase occurred without neurofibrillar pathology (Naslund et al., 2000) indicating that increased Aβ levels precedes NFT formation. While the brain contributes to circulating Aβ in the blood other peripheral sources such as platelets are equally important (Borroni et al., 2002).

The 'amyloid cascade hypothesis' described by Hardy., et al (1992) states that aggregated forms of Aβ in the brain parenchyma trigger a cascade of pathological events that lead to AD pathology, neuronal loss and the associated cognitive decline. There is overwhelming genetic evidence in support of increased Aβ production being causative in familial AD (FAD; Revesz et al., 2003; Rocchi et al., 2003; Kowalska et al., 2004). Mutations in the genes that code for APP as well as PS1 and PS2 result in elevated Aβ levels in the brain of affected subjects. Currently less than 5% of all AD cases are associated with these mutations but for sporadic cases of AD that represent the vast majority of sufferers, other factors are involved. In addition, it could be argued that Aβ may not be the only factor in the pathogenesis of AD because cognitively normal subjects have also exhibited Aβ plaques in their brains. (Maccioni et al., 2001). On the other hand the latter finding does not necessarily negate the significance of the amyloid cascade hypothesis as it has not been ruled out that these apparent control subjects may actually have progressed to AD/dementia had they lived longer.

1.3.2 Amyloid Precursor Protein (APP)

APP is the precursor to Aβ, the main component of senile plaques. APP is expressed primarily in neurons but is also found in many different tissues in healthy individuals (Turner et al., 2003). The function of APP is still unclear but there is evidence to indicate that it is an important part of an intricate protein signaling network (Nishimoto et al., 1993) and associated in the regulation of non-neuronal cell mitosis (Popp et al., 1996). It may also play a role in the normal maintenance, growth, morphology and functional plasticity of nerve cells as well as in learning and memory (Bayer et al., 2001). In neurons it is mainly localized in synapses enabling neuronal communication (Chapman et al., 2001). The non-neuronal isoform of APP which contains the Kunitz protease inhibitor domain (KPI) is involved in cellular adhesion and inhibition of Serine proteases (Festoff et al., 2001).

The full length APP is a glycoprotein comprising a 770 amino acid polypeptide chain, characterized by a large ectodomain, a transmembrane domain and a small endodomain (Sisodia and Price, 1995). The gene coding for APP lies on chromosome 21 and undergoes proteolysis, by a set of proteases namely, α, β and γ secretase, through two mutually exclusive pathways, (Selkoe, 2001). The non-amyloidogenic pathway involves the cleavage of Aβ by α- secretase, between residues 16 and 17 of the Aβ sequence thus precluding Aβ formation. Instead, this pathway results in the liberation of a secreted form of APP (sAPP) and a membrane bound C-terminal fragment of APP which contain residues 18-42 of the Aβ sequence (Sisodia & Price, 1992). Cleavage of APP by α-secretase is regulated by protein kinase C or other factors such as hormones (Buxbaum et al., 1993; Bayer et al., 2001). The amyloidogenic pathway involves β secretase which cleaves APP at the N- terminus of the Aβ domain to release a truncated APPs product and amyloidogenic C-terminal fragments. Subsequent cleavage by γ-secretase releases

Aβ40 or variant Aβ42 (Naslund.et al., 2000). Recently, two β- secretase have been cloned and identified (as (BACE) 1 and 2 for β site APP cleaving enzyme) and belong to the aspartyl- protease family (Vassar, 2001). Over-expression of BACE was found to correlate with increased cleavage of APP and hence may be an important factor in AD pathogenesis. The gamma site-processing enzyme has not yet been identified but over-whelming evidence suggests that presenilins, together with other interacting proteins (APH-1, nicastrin and PEN-2) facilitate γ-secretase cleavage of APP (Xia, 2003).

Pathologic mutations in APP results in the higher production rates or self- aggregation of Aβ producing the greater amyloid plaque load seen in FAD pathology (Marechal et al., 2003). The majority of autosomal dominant mutations in APP are present within or around the β and γ -secretase cleavage sites (Okochi & Takeda, 2004). For example, two point mutations at amino acids 670 and 671, also known as the 'Swedish' mutation, results in a five to eight fold increase in the production of Aβ40 and Aβ42 (Johnston et al., 1994). The 'London' mutation (Val-lle) and 'Indiana' mutation (Val- Phe) at amino acid 717 increases the production of Aβ42 only (Chapman et al., 2001). Pathologically, individuals possessing mutations in APP exhibit neuro-pathological features identical to that seen with sporadic cases of AD, though FAD is usually associated with an earlier age of onset (Marechal et al., 2003).

1.3.3 Presenilin 1 / Presenilin 2

Mutations in the presenilins (PS) are associated with the autosomal dominant form of FAD (Checler, 1999; Czech et al., 2000). Two presenilin genes have been identified; presenilin 1 (PS1) located on chromosome 14 and presenilin 2 (PS2) found on the long arm of chromosome 1. PS1 is thought to participate in APP processing and reports suggest that PS1 mutants have increased Aβ production and higher ratio of Aβ42: Aβ40

(Martins et al., 1995, Xia et al., 1997). PS1 may have a role in protein and membrane trafficking and in signal transduction during development, apoptosis and possibly cellular calcium ion homeostasis (Marjaux et al., 2004). The majority of mutations that have been discovered in the PS1 gene are missense in nature and completely penetrant. The I4S5 and Glu318GLY mutations are exceptions (Rossor et al., 1996; Hutton and Hardy, 1997; Helisalmi et al., 2000).

PS2 which is identified on chromosome 1q42.1, has about a 60 % homology with PS1 and has slightly differing pattern of transcription, mostly found in cardiac and skeletal muscle as well as the pancreas (Marjaux et al., 2004). In the brain, PS2 is expressed less homogeneously than PS1. Different missense mutations in PS2 have been found including the 'Volga German ancestry' (Asn141Ile; Levy-Lahad et al., 1995) and the Italian pedigree (Met239Val; Rogaev et al., 1995) and are rarely found in the general population. Age of onset of the disease in PS2 cases range from 40 to 85 years (Marjaux et al., 2004).

1.3.4 Apolipoprotein E (APOE) as a risk factor for AD

1.3.4.1 APOE and its isoforms

APOE allele polymorphism is the most prevalent risk factor in late onset AD (Smith, 2002; Farrer et al., 1997), with the *APOE* ε4 allele associated with both familial and sporadic cases of AD (Rocchi et al., 2003). The risk of AD is increased and the age of onset reduced in individuals who are homozygous for the *APOE* ε4 allele (Corder et al., 1993; Frisoni et al., 1995; Poirier et al., 1993).

The mature form of ApoE protein is a single glycosylated 37-kDa polypeptide of 299 amino acids (Poirier et al., 2000). The gene coding for this protein is located on chromosome 19 (Shih et al., 2000). Three common allelic variations of the human *APOE* gene were found by iso-electric focusing namely; ε2, ε3 and ε4 (Zannis et al.,

1982). The ε2 isoform differs from ε3 by an Arg to Cys change at residue 158. The ε4 isoform has a single change at residue 112 where Cys is substituted for Arg. Six possible APOE genotypes exist and the allele frequency in different populations are variable with some Asian populations exhibiting an ε4 frequency as low as 8%, whereas some African and Australian aboriginal populations exhibiting frequencies as high as 40% (Smith, 2002).

1.3.4.2 Distribution and function of ApoE in the CNS

ApoE plays an essential role in the transport of cholesterol and phospholipids through its influence on plasma lipoproteins and is an important factor in lipid homeostasis especially in regulating the levels of low-density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol (Davignon et al., 1999). ApoE is synthesized by the liver and brain (Mahley, 1988), skin, macrophages and steriodogenic organs (Norum et al., 1983). This protein has a unique role among the apolipoproteins as it coordinates the movement of cholesterol in nervous tissue for the maintenance of myelin and neuronal membranes in the peripheral nervous system (Mahley, 1988).

The natural variation found in *APOE* conveys an effect on longevity, with the ε4 allele associated with increased AD pathology and cardiovascular disease (Poirier, 1995). The *APOE* ε2 allele is associated with reduced risk of AD and cardiovascular disease, and it has been suggested that it exerts a protective effect against the delirious effects of dementia. This protection, however, may vary among different populations (Martins et al., 1995). *APOE* genotype not only influences incidence and age of onset but also has a critical effect on AD pathology and progression (Smith, 2000). Studies suggest that ε4 carriers have an increased Aβ burden within the brain with some cases showing an increase in neurofibrillary tangles (Ohm et al., 1999).

1.3.4.3 APOE and its role in AD

Several mechanisms of ApoE action have been proposed that may account for its association with AD. Firstly, ApoE modulates the metabolism of Aβ which influences clearance and aggregation rates (Bales et al, 2002). In animal models, ApoE has been correlated with brain cholesterol which itself is correlated to Aβ accumulation (Refolo et al., 2000). A study by Naslund et al (1995) indicated that ApoE co-localised with Aβ in senile plaques within brain tissue from AD cases. Aβ polymerisation and aggregation has been shown to be enhanced by ApoE (Sanan et al., 1994). Conflicting results from various studies exist on the influence of the ε4 isoform on cellular uptake of Aβ with some studies suggesting this isoform is correlated with increased Aβ uptake, whereas others have shown there is less Aβ cellular uptake compared to other isoforms (Yang et al., 1999; Cole et al., 1999). ApoE may play an important isoform specific role in the deposition of dense core plaques, as shown in mouse models by its action on both aggregation and clearance of Aβ (Smith, 2002).

There is emerging evidence that ApoE may also influence neurite extension, tau phosphorylation and neuronal survival (Mahley, 1996). *In vitro* studies show that ε3 promotes neurite extension whereas ε4 is neutral or inhibitory to primary neurons and neuronal cell lines (Mahley & Huang, 1999). ApoE may have an isoform specific effect on the binding to tau (Fleming et al., 1996) or on endocytic trafficking which may account for endocytic abnormalities that precede Aβ deposition in AD brain (Cataldo et al., 2000). Increased levels of ApoE may have a detrimental influence on the neuronal cell system promoting cytotoxicity or neurite degeneration (Hashimoto et al., 2000). This however changes with native ApoE where *APOE* ε2 can have the most protective effect from cytotoxicity and ε4 the least protective (Mahley & Huang, 1999). There is evidence indicating that aging APOE ε4 transgenic mice have greater memory deficits

(Montine et al., 1999). APOE may have allele specific effects on microglial activation in AD brains (Egensperger et al., 1998) or an inhibitory effect on activation of cultured glial cells by Aβ peptides (Hu et al., 1998).

1.4 Other genetic and non-genetic risk factors and protective factors

Several risk factors for AD have been identified which include family history, age, APOE genotype and Down's syndrome. AD is an age-associated disease with several studies reporting that prevalence increases with older age (Ott et al., 1995; Yoshitake et al., 1995). There is some evidence that head injuries increase the risk of AD (Mortimer et al., 1991), exposure to solvents (Kukull et al., 1995), diabetes (Yoshitake et al., 1995), family history of Parkinson disease (van Duijn et al., 1991) and depression (Jorm et al., 1991; Devanand et al., 1996). Females also have a higher risk for developing the disease and this risk is markedly increased following menopause, indicating that sex hormones may play an important role in AD. There has been considerable research on the sex hormone oestrogen with conflicting results, while to date, limited attention has been given to the gonadotropins. The following section will address the role of oestrogen and the gondotropins in cognition and the pathogenesis of AD.

1.5 The Influence of Oestrogen on Alzheimer's Disease

1.5.1 Oestrogen and menopause

Oestrogen is a steroid sex hormone that controls female reproductive processes and is responsible for the feminine secondary sexual characteristics (Birkhauser et al., 2000). Three oestrogens occur naturally in humans, oestrone, oestradiol and oestriol with 17 β oestradiol the major secretory product and most potent steroid biologically (Morley et al., 2000). The oestrogens are produced by the theca interna and granulosa cells of the ovary, the corpus luteum and the placenta and their rate of production is controlled by the gonadotropins, LH and FSH via the hypothalamic-pituitary-ovarian axis (HPO)

(Morley et al,. 2000). Most oestrogen is produced by the ovary while small amounts also originate from conversion of androgens to oestrogen in peripheral adipose tissue (Simpson, 2003).

Menopause marks the permanent cessation of menses in women due to a diminished number of ovarian follicles and failure of follicular maturation (Gosden et al., 1985). The average age of onset of menopause is approximately 51 years of age (Birkhauser et al., 2000; Sherwin, 2003). Menopause is accompanied by a variety of physiological and psychological symptoms including hot flushes, mood changes, depression, insomnia, apprehension and memory deficits (Genazzani et al., 1997; Halbreich, 1997). Although menopause results in the marked reduction in gonadal oestrogen production, low levels of oestrogen are still produced by the aromatization process in adipose tissue with levels relatively unaffected by age and time (Cauley et al., 1989). However, this low level of oestrogen production by non-gonadal tissue may be insufficient to prevent the cognitive decline that is noticeably increased in some women following menopause (Costa et al., 1997; Paganini-Hill & Henderson, 1994). Furthermore, this group of women are at a higher risk of developing AD (Sherwin et al., 2002; 2003).

1.5.2 Oestrogen: Mechanisms of action

Oestrogen receptors have been identified in various tissues of the brain including the hypothalamus, pituitary gland, hippocampus, cerebral cortex, midbrain and brain stem. (Pfaff, 1983). There are two forms of intracellular oestrogen receptors; ER – α and ER – β (reviewed by McEwen & Alves, 1999). Distribution analysis of ER- α within the brain shows that the pituitary, hypothalamus, amygdala and hypothalamic preoptic area have high levels and the hippocampus, midbrain and cerebral cortex have lower levels. A number of isoforms of ER- β are differentially expressed in the brain and other tissues (McEwen & Alves, 1999) including the hypothalamus and midbrain (Shughrue

et al., 2000; Kuiper et al., 1997). Oestrogen receptors are found in the cell nuclei of target cells (Kuiper et al., 1997) and in extranuclear sites such as axon terminals, dendritic spines and glial processes (Milner et al., 1999) suggesting that brain cells have mechanisms that allow for rapid synaptic oestrogen synthesis. Studies have shown that oestrogen regulates synaptic connectivity in the hypothalamus and hippocampus *in vivo (*Carrer et al., 1982) involving the interactions of GABAergic and brain-derived neurotropic factor (Murphy et al., 1998).

Hormonal steroidal action has been classified as 'genomic' in nature (gradual in onset and prolonged action) or 'non-genomic' (rapid onset and short duration; McEwen et al., 1978). Direct genomic mechanism processes involves the nuclear form of oestrogen receptors (ER –α or ER –β; Umayahara, 1994; Paech et al., 1997). Indirect genomic processes involve the activation of ER linked to secondary messenger systems such as cAMP/ protein kinase. Oestrogen is thought to play a role in neuroprotection and works in concert with other neuroprotective agents such as neurotropins. Oestrogen at high concentrations is also known to act at the non-genomic level where it exhibits anti-oxidant activity (Stein & Hoffman, 2003).

1.5.3 Neuroprotective effects of oestrogen

Numerous studies have investigated the influence of varying concentrations of 17 α and 17 β oestradiol on neurons (Sawada & Shimohama, 2003; Segal & Murphy, 2001; Behl, 2002; Carrer, 2003). Oestrogen plays a pivotal role in the maintenance and function of the neuronal circuits of the brain and a multitude of studies have focussed on this significant aspect of oestrogen action (reviewed by Sherwin, 2003). Oestrogen has been shown to maintain important regions of the brain such as the hippocampus and basal forebrain as well as the dopaminergic, serotonergic and noradrenergic systems (McEwen, 2001) and provide resistance to neuronal damage by maintaining synaptic

connections. Several authors (Chung et al., 1988; 1993; Lustig 1994; Keefe et al., 1994) have reported that oestrogen can induce extension of neuronal processes and increase formation of synapses between nerve cells. Oestrogen may also influence neurotransmitters like acetylcholine as found in the basal forebrain region and supply information to the hippocampus and cortex (Chung et al., 1988). This may be achieved by oestrogen's influence on nerve growth factor (NGF) in the basal forebrain an area responsible for learning and memory (Toran - Allerand et al., 1992). McEwen and Alves (1999) found that oestrogen blocks the effects of neurotoxic and excitotoxic agents that may be associated with AD. Furthermore, oestrogen administration to male rats with cerebral artery occlusion by Silastic implants reduced the resultant lesion size (Hawk et al., 1998). Oestrogen has been found to increase cerebral blood flow (Belfort et al., 1995; Ohkura et al., 1995) and may influence use of glucose in the brain (Bishop & Simpkins, 1995; Namba & Sokoloff, 1984). Overall, there is substantial evidence implicating the role of oestrogen as a neuroprotective agent in the brain.

1.5.4 Neurotrophic effects of oestrogens

Neurotropic factors increase the growth and survival of neurons (Lee & McEwen, 2001). Oestrogens have a similar action to neurotropins, its receptors co-localise with neurotrophic receptors in the cerebral cortex, hypothalamus, hippocampus and sensory ganglia which help to explain the reciprocal regulation between oestrogen and neurotropin action at gene transcription level. Oestrogen seems to promote dendritic spine formation (Gibbs, 1998; Singh & Schweitzer, 1995) and neurite growth and reverse de-afferented neurons in the brain of mouse models (Gould et al., 1990).

Steroid	Influence	Brain Area Affected	Reference
17 β- estradiol	Increases IGF	Hypothalamus	Garcia-Segura et al.,1996
17 β- estradiol	Neurite growth	Hypothalamus	Toran – Allerand et al., 1992
17 β- estradiol	Neurite growth	Hypothalamus	Lorenzo et al., 1992
17 β- estradiol	Increase survival rate of neurites	Hypothalamus	Ferreira et al., 1991
17 β- estradiol	Neurite outgrowth	Neocortical cells	Diaz et al., 1992
17 β- estradiol	Neuronal differentiation	Cortical neurons	Zhang et al., 2000
17α and β-estradiol	Increase Bcl-x	Dorsal root ganglia	Patrone et al., 2000
17 β- estradiol	Increase BDNF	Hippocampus	Dittrich et al., 1999

Table 1.1: Oestrogen and its neurotrophic effects.

1.5.5 Oestrogen and Alzheimer's disease

Evidence in the literature suggests that lowered oestrogen levels in post-menopausal women is associated with cognitive decline contributing to dementia of which nearly two thirds is accounted for by AD (Boss, 2000). There is evidence that the risk for AD is reduced in post-menopausal women utilising oestrogen replacement treatments (Tang et al., 1996; Kawas et al., 1997; Paganini-Hill and Henderson, 1994). Hormone replacement therapy (HRT) use in 8877 women was investigated and it was reported that oestrogen users had one third less risk of developing AD than those who never used oestrogen supplements. This risk decreased with increasing dose of HRT and longer duration of use (Paganini- Hill and Henderson, 1994). The risk was also lowered with factors that increase endogenous oestrogen such as increased weight and younger age at menarche. A study by Tang et al., (1996) investigated the rate of AD in a cohort of 1282 non-demented elderly individuals. Oestrogen users had a half the risk of developing AD over non-users with only 167 patients developing dementia after follow-up. Interestingly an *APOE* dependent effect of oestrogen replacement therapy on memory and learning was found in healthy post-menopausal women (Burkhardt et al., 2004). Specifically it was found that in subjects with the *APOE* ε4 allele, HRT use had no effect on cognition. On the other hand, subjects who were non-*APOE* ε4 and on HRT significantly improved their cognitive ability.

The therapeutic effect of oestrogen on cognition has been observed in post-menopausal women suffering from AD (Asthana et al., 1999; Honjo et al., 1989; Ohkura et al., 1995). Honjo et al., (1989) examined the influence of oral HRT on symptoms of dementia from seven AD individuals over a 6 week treatment period with conjugated oestrogens. Six patients displayed significant improvement during cognitive testing with no improvement in the control cohort. Mortel et al., (1995) reported that oestrogen treatment was associated with reduced risk of dementia in a group of 306 post-

menopausal women. This contrasts with reports from recent randomised control studies. In the Alzheimer's disease Co-operative Study (Mulnard et al., 2000) forty-two AD patients received oestrogen therapy for one year where an initial beneficial effect of the sex hormone was seen but no long term effect was observed. Another study by Henderson et al., (2000) looked at HRT treatment on forty-two mild to moderate AD patients and controls over 4 to 16 weeks of treatment. No treatment effect was discovered. Considering the significance of the larger sample studies, HRT use may have little association with cognitive ability in AD patients. Furthermore, a recent large double blind placebo control study has shown an increased frequency of dementia in the oestrogen treatment group (Rapp et al., 2003; Shumaker et al, 2003). However these studies are not conclusive as they have significant limitations including co-morbidities in cohort subjects, length and type of treatments, age of commencement and dosage levels.

Limited data exists on the association between endogenous levels of oestrogen on memory and cognitive decline. Higher levels of peripheral oestrogen have been related to reduced risk of cognitive decline in post-menopausal women (Yaffe et al., 2000). A study on the effects of endogenous levels of oestrogen on specific aspects of memory has shown that natural levels of the sex hormone specifically influence verbal memory and frontal lobe functionality (Wolf & Kirschbaum, 2002). Lower scores on the MMSE were reported on individuals with lower endogenous oestrogen levels. Collectively, the published literature indicates that endogenous oestrogen plays a role in cognition although further evidence is required to ascertain the effect of oestrogen independent of HRT.

1.5.6 Aβ and Oestrogen

Oestrogen may have a role in reducing the levels of Aβ, a key molecule in the pathogenesis of AD (for recent review see Selkoe, 2001). The effect of oestrogen on Aβ was first recognised in non-neuronal (Chang et al., 1997; Gandy & Petanceska, 2001) and neuronal cell cultures (Xu et al., 1998) as well as animal models showing increased Aβ deposition in ovariectomized female animals (Petanceska et al., 2000). *In-vitro* studies using 17 β- oestradiol have shown to up-regulate the levels of soluble APP (Jaffe et al., 1994; Vincent et al., 2000) therefore alleviating the toxicity of Aβ to neuronal cells. Studies on cultured human cortical cells found that oestrogen treatment enhanced Aβ uptake and clearance (Li et al., 2000). Furthermore, Zheng et al., (2002) showed that *in vivo* oestrogen depletion could lead to accumulation of Aβ in the CNS in mouse models. This situation was reversed through oestrogen administration. Collectively these studies indicate that oestrogen may reduce Aβ associated toxicity by decreasing its levels or enhancing its clearance.

1.6 Gonadotropins and AD

1.6.1 The gonadotropins; luteinising and follicle simulating hormones

Reproductive function in both males and females is regulated by two hormones contained in the hypothalamic-pituitary-ovarian (HPO) axis; follicle stimulating (FSH) and luteinising hormones (LH) released from the anterior pituitary gland in response to the release of gonadotropin releasing hormone (GnRH) from the hypothalamus (Lathe, 2001). FSH is a glycoprotein comprising two subunits, alpha and beta. LH is also a glycoprotein and has an identical alpha subunit to FSH, however its β subunit is unique (Burns & Matzuk, 2002). FSH facilitates the development of the ovaries and stimulates the production of oestradiol, initiates development of ovarian organs, controls sexual maturation and is required for normal sexual performance (Burns & Matzuk, 2002).

FSH is regulated by hypothalamic feedback with circulating steroid hormones. Prior to ovulation high oestrogen levels promote FSH release through a positive feedback relationship. After ovulation, FSH levels are reduced via a negative feedback mechanism that is triggered by increased oestrodiol and progesterone concentrations. In addition, levels of FSH can be reduced via a hormone secreted by the ovaries termed inhibin (Burns & Matzuk, 2002). Secretion is pulsatile with pronounced cyclic fluctuations at particular times of the menstrual cycle. After menopause basal levels of FSH gradually rise.

LH increases during the ovulatory phase of the menstrual cycle indicating imminent ovulation. This gonadotropin promotes production of oestrogen and progesterone by the corpus luteum. LH like FSH is regulated by positive and negative feedback relationships with oestrogen and progesterone and secretion is pulsatile in nature. Basal levels of LH increase gradually after age 35 and continue to rise for several years after menopause (Burns & Matzuk, 2002).

1.6.2 LH/FSH and AD

Elevated levels of the gonadotropins were first reported by Bowen et al., (2000) in male subjects with dementia in long term care facilities. A subsequent larger study confirmed these findings in AD patients (Short et al., 2001). The latter authors stated that the low-density lipoprotein receptor related protein (LRP) is upregulated by LH and FSH, which is known to be associated with plaque formation in AD. LRP may have the capacity to influence both the production and the clearance of Aß by influencing APP processing (Kounnas et al., 1995; Ulery et al., 2000). Since LRP is known to be up-regulated by increased LH and FSH in mouse testicular granulosa cells (Foster et al., 1993) the latter authors postulated that the risk of AD due to high LH levels is mediated via its action on LRP (Short et al., 2001).In addition anti-gonadotropin agents such as leuprolide acetate

were found to reduce Aβ 40/42 in the brains of mice models (Bowen et al., 2004).

Oestrogen levels fall in post-menopausal women resulting in increased levels of the gonadotropins and may explain their increased frequency of dementia and AD. Replacement of circulating levels of oestrogen via HRT may reduce levels of LH and FSH and thus protect against AD. However given the failure of the recent trials with oestrogen replacement therapy (Rapp et al., 2003; Shumaker et al., 2003) the clinical significance of increased gonadotropin levels in the pathogenesis of AD warrants further investigation and may enable the development of important therapeutic approaches to treating AD.

1.7 Mild Cognitive Impairment as a Predictor of AD

Increasing attention has been placed on age related memory decline where a need exists for identifying cases of cognitive impairment that present without the signs of clinical diagnosed dementia. Traditionally, cognitive impairment without dementia has been considered to be a normal outcome of aging (Petersen et al., 2001). Terms such as 'benign senescent forgetfulness' (Kral, 1962) or 'age associated memory impairment' (Crook et al., 1992) were used to describe the memory decline common in elderly individuals but with symptoms that fall within considered limits of normal aging (Burns & Zaudig, 2002). Recent studies have suggested that individuals with demonstrated but subtle memory deficits have been shown to have a higher risk of future neurodegenerative disease (Petersen et al., 2001; Burns & Zaudig, 2002). The transitional state between normal aging and dementia has broadly been defined as mild cognitive impairment (MCI) with patients having a memory complaint and objective memory impairment relative to their age and education (Petersen et al., 2001). Emerging evidence suggest that individuals displaying signs of mild cognitive impairment (MCI) go on to develop dementia in the future (Blanchet et al., 2002) and

progress to probable AD at a considerably accelerated rate. Therefore this transitional period has been recognized as a suitable intervention time for treatment and therapy techniques for dementia patients.

MCI has been defined as a slight impairment in cognitive function (typically memory), with otherwise normal performance (Petersen et al., 2001). MCI patients have a condition that is different from normal aging. The criteria for definitive AD have been well established utilising neuro-pathological techniques post-mortem. Criteria also exist for probable AD with the relationship between clinical and pathological results leading to a high percentage rate of correct diagnosis. However, no international diagnostic criteria has been established for MCI (Lautenschlager et al 2001). Other forms of MCI have been recognized but problems with memory deficits such as those that lead to AD have been termed amnestic MCI (Burns & Zaudig, 2002). These patients suffer from subjective memory complaints with objective memory impairment, compared to age and education matched controls, but who perform satisfactorily in tasks of daily living and do reasonable well in general cognitive scales. Studies have found that compared with healthy aged matched controls who typically convert at 1 to 2% per year, affected individuals (MCI) will progress to AD at a rate of 10 to 15% per year (Burns & Zaudig, 2002). Other studies have suggested rates as high as 25% (Bozoki et al., 2001). Not all MCI individuals develop AD, with some progressing to another form of dementia or others with no significant deterioration in memory even after several years (Petersen et al., 2001).

Currently two main rating scales are in use to differentiate between normal aging individuals and patients with signs of dementia and AD. The Clinical Dementia Rating (CDR) is a scale with a range of measures from normal (CDR 0) through to severe dementia (CDR 3) with MCI equivalent to CDR 0.5. Another scale is the Global Deterioration Scale (GDS) which has several stages of measure from GDS 1 (normal)

to GDS 7 (severe dementia) with MCI ranging at GDS 2 or 3 (Petersen et al., 2001). Memory deficit in MCI is determined by scores greater than 1.5 standard deviations below that of age appropriate norms on measures of episodic memory but other measures of MCI may be harder to define in a clinical setting (Morris et al., 2001). Memory complaints are also a significant factor in MCI (Burns & Zaudig, 2002). The temporal factor surrounding the slow development of MCI hinders its identification especially when symptoms echo that of very mild dementia. One of the main problems in the diagnosis of AD, mild cognitive impairment and other deficits in memory lies in the long prodromal period in the development of the disease. The current criteria used for diagnosis of MCI can quite easily cause misclassification due to this long prodromal period and therefore more sensitive techniques may be needed to predict future manifestation of MCI or other forms of dementia. Retrospective studies of individuals with the symptoms of AD have reported slight impairments in episodic memory up to 20 years before diagnosis (Elias et al., 2000). These results suggest improvements in diagnostic procedure will give a much earlier indication of deficits in memory. Extended observational periods due to the slow rate of decline may assist in diagnosis, and are essential for early detection.

The retrieval and encoding of memory especially episodic memory may be an important factor in the diagnosis of MCI. Episodic memory is defined as the recollection of past events and problems in retrieval and encoding of episodic memory are early symptoms of cognitive decline (Collie et al., 2000). A study by Wang et al., (2001) involving MCI patients and controls looked at episodic memory and the ability to encode and retrieve information. They found a significant decline in orientation, praxis and language after using the CAMCOG measure in MCI patients when compared to controls. An episodic memory measure involving pictures and words found that MCI patients were more vulnerable to impairment in encoding this form of memory. The first stages of AD are

neuro-pathologically recognised in the entorhinal cortex (layer II) which is responsible for neuronal impulses to the hippocampus and is the neural base of encoding of episodic memory (Wang et al., 2001). Significant loss and atrophy of the entorhinal cortex is a feature in MCI as well as AD patients (Kordower et al., 2001). Taken together, these results suggest that the MCI condition is different from the clinical and pathological symptoms of normal aging in the brain and may be a transition phase to AD.

Neuro-imaging techniques can be used to differentiate between the many forms of dementias and can also be a tool to differentiate change and to substantiate the diagnosis of cognitive decline based on neuro-cognitive assessment. Structural imaging techniques such as CT (computed tomography) or MRI (magnetic resonance imaging) can indicate areas of atrophy in the brain especially in the hippocampus or entorhinal cortex (Lee et al., 2003). Studies have shown atrophy of the hippocampus can predict the rate of conversion from MCI to AD (Jack et al., 1995).

Functional neuro-imaging techniques such as PET (positron emission tomography) or MRS (magnetic resonance spectroscopy) scans can assist in identifying early metabolic deficits in the temporoparietal region especially in familial cases of AD (DeSanti et al., 2001; Petersen et al., 1999). A metabolic reduction in the cingulate cortex was also found in patients with a genetic risk of AD (Minoshima et al., 1997). Very few studies have concentrated on the pathological characteristics of MCI but most suggest that patients with clinically diagnosed MCI display NFT in the hippocampus and entorhinal cortex (reviewed by Lopez & De Kosky, 2003). Subjects with no detectable cognitive decline have also been shown to possess AD pathology (Price et al., 2001). This evidence supports the idea that individuals with MCI will probably have some aspects of AD neuropathology with very early cases of the disease exhibiting no clinically defined features (Burns & Zaudig, 2002).

Other research has concentrated on biomarkers that may assist in the prediction of AD especially in MCI patients. Classic markers such as the *APOE* ε4 allele have been used in many studies, with most showing an association with impaired memory (Petersen et al., 1999). Some studies have looked at APP abnormalities in MCI patients and have found alterations in platelet APP as an event in early AD pathology and suggest that measuring APP may be useful for predicting pre-clinical AD in MCI patients (Padovani et al., 2002). Brain oxidative stress was investigated in MCI patients and was found to be increased in MCI individuals when compared to controls (Pratico et al., 2002). Isoprostane was used as a marker for *in-vivo* lipid peroxidation that gave an indication of oxidative damage in the brain. This was found to be highly associated with other biomarkers of AD and suggests that measuring isoprostane levels may in combination with other diagnostic techniques be a useful tool to predict AD (Pratico et al., 2002).

1.8 Neuro-Cognitive Assessment

1.8.1 CAMDEX and CAMCOG

The Cambridge Cognitive Examination (CAMCOG), the cognitive section of the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX), is a neuropsychological test for the assessment of cognitive deficits in older people (Roth et al., 1986). It was designed to assess the array of cognitive functions required for diagnosis of dementia, differentiate between differing dementias and allow for comparison with other neuropsychological tests. The examination assesses specific cognitive functions including memory (remote, recent, semantic episodic), language, executive functioning, perception and attention (Roth et al., 1986). The CAMCOG examination also incorporates all items of the Mini-Mental State Examination (MMSE) as well as the Abbreviated Mental Test (Hodkinson, 1972) and scores derived from CAMCOG provide subscale measures for the different cognitive functions assessed. As

with most neuropsychological assessments, test scores are influenced by education, age, sex and socio-demographic factors (Roth et al., 1986).

Huppert et al., (1995) state that CAMCOG scores are effective in differentiating between demented and non-demented individuals. Compared to the MMSE, the CAMCOG suffers from reduced ceiling and floor effects (i.e. when individuals score higher or lower than the boundaries of the test), and socio-demographic variables can exert a significant influence on test scores (Huppert et al., 1995).

Some studies suggest that the CAMCOG examination lacks precision and is incapable of identifying the early signs of dementia. Nielsen et al., (1999) reported that CAMCOG was sufficiently adept to identify cognitive changes in early dementia but was not able to identify individuals who would later develop AD or dementia. However other reports have found that CAMCOG can be considered a moderately efficient measure to differentiate dementia patients from normal individuals (Lozano-Gallego et al., 1999; Lindeboom, 1993).

1.8.2 California Verbal Learning Test

The California Verbal Learning Test (CVLT) is a popular research and clinical instrument that measures key constructs in cognitive psychology such as repetition learning, serial position effects, semantic clustering, intrusions and proactive interference (Delis et al., 1997). It provides a learning curve, defines learning strategies and gives an indication of short and long term retention of verbal memory (Delis et al., 1997). It consists of five readings of a 16 word shopping list. Subjects are required to recall words after brief and extended time intervals. A distraction list of alternate words is also given. The test concludes with a recognition trial.

CVLT test scores are differentially influenced by many degenerative diseases of the brain. AD patients exhibited an 80% loss of word recall after delay (Delis et al., 1992). Perseveration rates were higher and learning rates were significantly lower in AD patients (Massman et al., 1996) and discriminability between early AD and depression was also noted on CVLT scores (Delis et al., 1992). A decline in verbal memory measured by the CVLT was noted by Lange et al., (2002) in non-demented older adults and mild AD patients, effectively predicting conversion to AD 1 to 2 years before the onset of clinical dementia characteristics. List learning and recall was compromised in AD patients in a study using the CVLT (Fox et al., 1998). A study to measure episodic memory changes in *APO*E ε4 individuals found that the CVLT was sensitive at picking up the subtle changes that occur prior to development of AD. Non-demented individuals were divided into *APOE* ε4 positive and negative groups with positive groups performing significantly poorer, on scores of nine CVLT parameters (Bondi et al., 1995). Libon et al., (1998) found that higher scores on the CVLT discriminability index were associated with increased size of the hippocampus and parahippocampal gyrus allowing for discrimination between AD and other neuro-degenerative disorders such as ischemic vascular dementia.

1.9 Aims of the Project and potential significance

The main purpose of this study is to investigate the influence of endogenous sex hormone levels on cognition in elderly post-menopausal women, not on hormone replacement therapy, longitudinally over a 12 month period. The major genetic and biological markers of AD will also be incorporated to evaluate their contribution to the effects of the sex hormones on cognitive decline.

This thesis aims to address the following hypotheses:

1) *"Higher levels of endogenous oestrogen in post-menopausal women are associated with better scores on two standard measures of cognition while considering the influence of specific biochemical factors"*

2) "Higher levels of the gonadotropins in post-menopausal women are associated with reduced scores on two standard measures of cognition while considering the influence of specific biochemical factors"

To achieve these goals the project has been divided into the following specific objectives:

1) Determine if cognitive performance in the CAMCOG and CVLT measures varies with endogenous plasma oestrogen levels.

2) Explore the relationships, if any, between plasma Aβ concentrations and *APOE* genotype with endogenous plasma oestrogen and cognitive measures.

3) Determine whether plasma concentrations of luteinizing and follicle stimulating hormones have an influence on cognitive performance measures.

4) Determine if variables such as age, education, body mass index, parity, family and educational history and statin (HMG-CoA reductase inhibitor) use are associated with cognition and memory.

5) Examine the change in cognitive performance over a 12-month period considering endogenous oestrogen levels and other biochemical factors.

Examining the role of sex hormones on cognition in post-menopausal women will provide the basis for developing effective diagnostic and therapeutic approaches for AD. There is much debate on the influence oestrogen has on cognition and its interaction with *APOE* genotype. The role of oestrogen in cognitive decline and AD is still poorly understood although recent clinical trials with HRT suggest that its therapeutic potential is of little benefit and in fact increases the risk of dementia in some subjects. Unfortunately these HRT studies are fraught with many confounding factors including co-morbidities in study subjects, duration of treatments, age of commencement and nature of hormone preparations. The current study benefits from evaluation of endogenous plasma oestrogen levels in a large cohort of post-menopausal women and thus has the potential for more clearly enhancing our current understanding of the role of this hormone in cognition. Furthermore this study will provide new knowledge on the role of the gonadotropins in cognition. To date current knowledge on these hormones are limited to a handful of recent publications which are based on small sample sizes that make the clinical significance of these finding questionable. The current study with its relatively large sample size therefore has the potential for clearly determining whether the gonadotropins and/or oestrogen play a role in cognition.

Chapter 2 METHODS AND MATERIALS

2.1 Study Design

This study assessed cognitive function and biological variables in post-menopausal, non-demented women who were currently not using any form of oestrogen-replacement therapy. Biological variables measured included 1) plasma oestradiol levels; defined as E2 in the results chapter and oestrogen throughout the thesis 2) *APOE* genotype 3) Plasma luteinising hormone 4) Plasma follicle stimulating hormone 5) Plasma beta-amyloid levels (Aβ40). Demographic information was ascertained during baseline cognitive assessment, or from existing databases kindly provided by the 'Calcium Intake Fracture Outcome Study' (CAIFOS), School of Medicine and Pharmacology, UWA, Sir Charles Gardner Hospital. Information included age, occupation, educational level, medical history, family history and drug intake. Baseline cognitive assessment involved the use of either the CAMCOG section of the CAMDEX or the CVLT (California Verbal Learning Test) with a sample of blood taken for biochemical and genetic analysis. A follow-up assessment of cognitive function was undertaken one year later again using CAMCOG /CVLT measure and acquisition of a second blood sample.

2.2 Ethics Approval

This study was carried out in accordance with procedures of the University of Western Australia human research ethics committee, and written consent was received from all subjects before participation in the study.

2.3 Patient Recruitment

Potential participants were identified through an existing 'Calcium and Bone Density' database (School of Medicine and Pharmacology) and sent a consent form outlining the

study objectives, expectations, confidentiality and ethics details. The form invited subjects to be included in the present investigation while also fulfilling requirements for the Calcium and Bone Density study (CAIFOS). Consenting individuals were later contacted by telephone and a time arranged for a visit to the hospital.

Approximately one year later, subjects who completed the baseline cognitive test were invited to take part in a second assessment coinciding with their annual visit for the 'Calcium and Bone Density' study. To maximise participant numbers, home visits were organised for participants who were unable to come to the hospital or could not honour the allocated interview times.

The majority of interviews were administrated at the School of Medicine and Pharmacology, Sir Charles Gairdner Hospital, Perth, Australia. On the allocated day for subject interviews, arriving participants were provided with identification stickers. A suitable private room was arranged for conducting cognitive testing and for the removal of blood samples. They were informed of study objectives and explained of confidentiality aspects with the assurance that all data obtained was treated as 'highly confidential' by the researcher. Some interviews were arranged to take place at the subject's home. Consent forms and other pertinent information were sent out to all participants.

2.4 Study Sample and Sub-Samples

The study sample consisted of 1065 mainly Caucasian post-menopausal women at baseline testing between the ages of 75 and 87 years of age. Of these, the vast majority (98%) were Caucasian. All participants were derived from a larger sample of volunteers currently participating in a longitudinal study to investigate the influence of calcium supplements on bone density in post-menopausal elderly females. Participants in this study were recruited with the kind assistance of A/Professor Richard Prince, from Sir

Charles Gairdner Hospital Unit (School of Medicine and Pharmacology) University of Western Australia (UWA), Western Australia.

The main cohort was separated into different sub-samples depending on the type of cognitive assessment given. The CAMDEX cohort included at baseline 651 healthy, non-demented post-menopausal women who were administered the CAMCOG assessment. Baseline testing took place between May and November 2001. A repeat visit approximately 12 months later was arranged with 455 participants returning for their follow-up CAMCOG assessment, and collection of a second blood sample.

The CVLT cohort included at baseline 413 healthy, non-demented, post-menopausal women who were tested using the CVLT measure. Baseline testing took place between November 2001 and April 2002. Approximately one year later the same subjects (n=234) were tested again with the CVLT measure.

2.5 Existing Participant Information

Volunteer information such as medical history, performance on the Mini-Mental State Examination (MMSE; Folstein, & McHugh, 1975) prior to baseline testing, occupation details, years since menopause, Body Mass Index and details on statin (HMG-CoA reductase inhibitor) use was made available from the existing database.

2.6 Selection Criteria

Participants were selected for the study if they met the following criteria: 1) obtained higher scores than 24 on the MMSE, 2) not undergoing any form of oestrogen replacement therapy.

2.7 Data Collection

2.7.1 Clinical assessment

The cognitive assessment of participants was taken in the form of an interview at baseline utilising either the CAMCOG section of the CAMDEX (Roth et al., 1986) for the CAMCOG cohort or the California Verbal Learning Test (CVLT) for the CVLT cohort. CAMDEX is a diagnostic assessment that provides a way to identify dementia, and to differentiate it from other common confounding disorders and the normal processes of ageing. The CAMCOG section incorporates tests of memory, language, praxis, calculation, abstract thinking, visual perception, attention and orientation. Demographic information as well as details such as medical history and drug intake was also recorded. Scores range from 0 to 106 with a score of 80 and above considered non-demented. The CAMCOG also incorporates the MMSE as part of its evaluation.

The CVLT cohort underwent assessment using the CVLT, a comprehensive neuropsychological test that thoroughly evaluated learning and memory of verbal material. Participants were read out a series of 16 items (list A) (4 examples in 4 unrelated semantic categories) in the guise of a 'shopping list' following standard CVLT protocol and asked to remember and recall as many items as they could after presentation. This was performed over 5 learning trials. The participant was then asked to remember and recall a second list of 16 words (List B; an interference list) and subsequently try to recall the items from List A (short delay recall). They then were asked to recall List A items by semantic category (short delay cued recall) after which a 20 minute distraction period took place. During this period the MMSE was administrated and the blood sample taken. After the 20 minute interval, the participants were asked to again recall List A (Long delay recall) and then by semantic category

(Long delay cued recall). Finally, the participants were asked to identify List A items from a longer series of words read out (recognition).

2.7.2 Blood sample collection

Blood was collected using standard venipuncture techniques into two EDTA 9 ml tubes (Interpath Services, West Heidelberg, Vic, Australia) pre-treated with prostaglandin E (PGE) solution (40ul of PGE1 stock (500 ug/ml) and 50ml of 100% ethanol). Tubes were spun at 800 RPM in an Eppendorf 5810R centrifuge for 10 minutes with no brakes. The top fraction (plasma) was transferred to fresh containers and spun at 1500 RPM for 15 minutes (full brakes), for platelet separation. Packed cell volume (PCV) was set aside for later processing. Plasma was then stored in 5ml tubes or 1.5ml eppendorf at -20C. Platelet pellet was re-suspended in TBS and washed for 5 minutes at 1500RPM and then stored in TBS at -20C. For leucocyte separation PCV (approximately 4mls per tube) was mixed with 3ml of 0.9% saline and 2 ml of dextran solution (1 litre equals 50g dextran, 7g NaCl, 0.02 % w/v Sodium azide) and left to separate for approximately 30 minutes. The top faction was then removed and spun at 1000 RPM for 5 minutes. Supernatant was discarded and the pellet washed a further two times in 0.9% saline solution. The resulting leucocyte pellet was resuspended in foetal calf serum (CSL Pty LTD, Parkerville, Vic, Australia) including 10 % v/v Dimethyl sulphoxide (DMSO) and stored at -80 C until required for DNA extraction or subsequent further analysis.

2.8 Indirect Enzyme Linked Immunosorbent Assay [ELISA] For Aβ Determination

2.8.1.1 Preparation of solutions/buffers

- PBS powder was dissolved to 1 L volume in DDW to yield a 10x stock solution. A working solution of 1 litre was prepared by mixing 100 mL of stock PBS

solution with DDW to a final volume of 1 L, after the pH was adjusted to 7.4 using 1 M NaOH or 1M HCl.

- ELISA washing buffer [Phosphate buffered saline with Tween-20 (PBST) was prepared by addition of 0.05% Tween-20 to a working solution of PBS and ensuring the final pH remained at 7.4. This solution was stored in a refrigerator at 4°C.

- The ELISA coating buffer was prepared by the addition of 10ml of 50mM carbonate/bicarbonate buffer and 25 µl primary antibodies (6E10) for each ELISA plate. The 50mM carbonate/bicarbonate buffer consisted of 0.26g of Na_2CO_3, 0.21g of $NaHCO_3$, with addition of DDW make up to the total volume of 50ml and pH adjusted to 9.6 using HCL.

- The ELISA blocking buffer was made up by dissolving 0.25g of bovine serum albumin (BSA: Sigma Chemical Co., USA) into 25ml of PBST for each ELISA plate.

- To prepare ELISA standards, the 1mg/ml $A\beta_{40}$ were diluted into PBST to make Aβ40 concentrations ranging from 15.625 pg/ml to 1ug/ml.

- The ELISA detection antibody solution was prepared by the addition of 0.125g BSA, 12.5ml of PBST and 12.5µl of secondary antibodies (R208) for each plate.

- The Neutravidin HRP solution was prepared by the addition of 1.25µl of nHRP and 12.5ml of PBST.

2.8.1.2 ELISA protocol for Aβ determination

Firstly, 100µl of ELISA coating buffer (containing the primary antibodies) was added onto every well of ELISA plate and the plates were incubated overnight at 4°C. Secondly, the primary antibody coated plates were washed with PBST three times in plate washer (Bio-Rad Microtech Platewasher) to remove the unbound antibodies. The plate was blocked by the addition of 200µl of blocking buffer onto each well and incubated at RT for one hour. After blocking, the plates were washed with the same protocol as above in a plate washer and then 100µl of samples or standards were loaded onto each well. The plates were then incubated at RT for two hours and kept at 4°C overnight. On the third day, the unbound peptides were removed by washing three times in the plate washer. After washing, 100µl of detection antibody solution was loaded onto each well and the plates were incubated at RT for one and a half hours. Another washing was performed to remove the unbound antibodies. Finally, 100µl neutravidin HRP solution was added and incubated for one hour at RT. Following the final wash, 100µl TMB/KGP (microwell peroxidase substrate system: purchased from Kirkegaard & Perry Laboratories, Inc. Maryland, USA) was added onto each well and incubated for about ten to fifteen minutes during which the ELISA plates were protected from the light. The reaction was stopped by the addition of 100µl of H_3PO_4 to each well and the colour change quantified at λ450 nm using a Bio-Rad Model 3550 Microplate reader.

2.9 Oestradiol Determination

Oestradiol levels were kindly performed by Dr John Beilby (The Western Australian Centre for Pathology and Research, Nedlands, Western Australia) using samples taken at the School of Medicine and Pharmacology, UWA where each subject had a blood sample collected in the morning after an overnight fast. Oestradiol was measured by (Radioimmunoassay) RIA (Orion Diagnostica, Espoo, Finland) with an analytical

sensitivity of 5 pmol/L. The inter-assay CV was 6.6% at a mean of 101 pmol/L (n=17) and 7.2% at a mean of 48 pmol/L (n=14). The intra-assay CV was 5.1% at a mean of 103 pmol/L (n=10) and 7.5% at a mean of 49 pmol/L (n=10).

2.10 LH/FSH Determination

Luteinising hormone and follicle stimulating hormone levels were again kindly determined by Dr John Beilby (The Western Australian Centre for Pathology and Research, Nedlands Western Australia) using an enzyme linked immunosorbent assay (ELISA). The microtiter plate is pre-coated with a monoclonal antibody specific for LH/FSH. Standards or samples are then added to the plate wells and LH/FSH if present, will bind to the antibody pre-coated on the wells. In order to quantitate the amount of LH/FSH present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for the hormone was added to each well to "sandwich" the LH/FSH immobilized on the plate. The plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain LH/FSH and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm.

2.11 Polymerase Chain Reaction

Apolipoprotein genotyping was performed by Polymerase Chain Reaction (PCR) amplification over 37 cycles on an Eppendorf Master-cycler Gradient using protocols described by Hixson & Vernier (1990). The oligonucleotide primers used are described by Wenham et al (1991). Four hours of digestion of the amplified product at 37.8°C was

then performed using 5.O U of the restriction enzyme Hhal (Fisher Biotech, Perth, W.A). The digested product was mixed with Ficoll loading buffer (1.5 g Ficoll, 0.02 Bromophenol Blue, 0.02g Xylene Cyanol, 10 ms 1x TE buffer- 10 mM Tris, 1mM EDTA). Products were shown by electrophoresence in 8% non-denaturing polyacrylamide gel in 1% TBE at 110V for one hour and stained with ethidium bromide for two to five minutes followed by de-staining with DDH20. Gels were visualised under ultra-violet light using a UV transilluminator to show patterns representing the characteristic DNA fragments of each of the different alleles.

2.12 Statistical Methods

The resulting data was analysed using the statistical software 'SPSS V11.0 for windows. Dependent variables investigated included CAMCOG score and 3 parameters of the CVLT (*SUM1to5* score, *DISCRIM* score, *FORGET* score). Independent variables include oestrogen levels, luteinising and follicle stimulating hormone levels, age, post-menopause years, Aβ40 levels, BMI, parity, statin use, depression, hypertension, cardio-vascular event, and diabetes. Descriptive statistics were performed for both CAMCOG and CVLT subsets at baseline and 12 month time-points. Univariate analysis was performed on the dependent variable (CAMCOG/CVLT measure) and each of the independent variables. Pearson's correlation coefficient and the age-adjusted regression were used to investigate the linear relationship. Histograms and scatterplots were utilisied to display the relevant data. Multiple Linear Regression was subsequently performed to investigate the relationship between the several independent variables and the dependent variable. Three modelling techniques were utilised; *backward elimination*, *stepwise forward* and *full model*. *Backward elimination* places all considered variables into the model. When a variable is above significance level (> 0.05) it is dropped and modelling continues until only significant variables are present. The *stepwise forward* model places the most significant variable in first and then the

second most significant variable, etc continuously reviewing the model until only significant variables are assembled. *The full model* is when all variables are placed into the model at once. The overall fit of the model was then examined by utilising the random scatter of residuals to give an indication of the presence of outliers. A scatterplot of the standardised residuals verus the standardised predicted values as well as histograms and boxplots were produced. Finally a plot of the regression of the residuals gave an indication of the normality of the dependent variable.

Table 2.1: Suppliers of Equipment and Reagents.

ITEM	SUPPLIER
Aβ 1-40 peptide	Keck Foundation Biotechnology Resource Laboratory, Yale University, CT, USA
Biotinylated monoclonal antibodies (R 208)	Kindly donated by Dr Pankaj Metha (Division of Immunology, Departmentt of Neurobiology, Institute for basic research in developmental disabilities, Staten Island, NY, USA.)
Mouse monoclonal antibody, 6E10	Signet Laboratories 180 Rustcraft Rd, Denham, MA, USA.
• Ethylenediaminetetra – acetic acid (EDTA) • Ethanol • NaOH • Methanol • Polyoxethylene (20) sorbitan monolaurate (Tween 20)	BDH Chemicals Australia Pty Ltd. Vic, Australia.
NaCl	Ajax Chemical , Auburn, NSW, Australia
• Aprotinin • Dimethyl sulphoxide (DMSO) • bovine serum albumin (BSA) • Anti-mouse/Horseradish peroxidase conjugate (HRP)	Sigma Chemical Company, St Louis, MO, USA.
Phosphate buffered Saline (PBS)	Fisher Biotec, Perth. Australia.
Biological Safety Cabinet, Class II	Email Westinghouse Pty. Ltd., NSW, Australia
TMB/KGP (microwell peroxidase substrate system)	Kirkegaard & Perry Laboratories, Inc. Maryland, USA
Bio-Rad Microtech Platewasher	Bio-Rad, Irvine, CA, USA.

Electronic balance ER 180A	A&D Company Ltd., Tokyo, Japan
Electronic balance Sartorius portable PT 1200	Sartorius AG, Göttingen, Germany
Foetal calf serum	CSL Biosciences, VIC, Australia
Millipore Milli-Q Plus DDW system	Millipore Corp., MA, USA
Rabbit anti-goat immunoglobulins (biotinylated)	DAKO Corp., CA, USA
Ultracentrifuge Beckman L8-70M and SW41 rotor	Beckman Coulter Inc., CA, USA
Vortex mixer	Thermolyne Corp., IO, USA
Centrifuge (bench top) “Super Minor”	MSE, England
Vacutainer tubes 9ml (EDTA)	Interpath Services, West Heidelberg Vic, Australia.

Chapter 3 RESULTS

3.1 CAMCOG Baseline

3.1.1 General Observations

After descriptive, univariate and multiple linear regression analysis, no significant association was found between oestrogen and the main dependent variable CAMCOG for both *APOE* ε4 and non-*APOE* ε4 subjects at baseline. Interestingly, high LH plasma levels were associated with lower scores of cognition with increasing age in non-*APOE* ε4 individuals. Conversely, higher levels of plasma FSH were associated with better scores on the CAMCOG again related to the age of the participant but independent of ε4 allele possession. This effect of FSH on cognition was more pronounced in statin users. A positive relationship was found between education and cognition in all subjects of this group. An age related decline in cognition in all baseline tested subjects was also evident. Levels of Aβ40 in plasma, parity and depression were found to have negative correlations with cognition.

3.1.2 Factors associated with cognition as measured by CAMCOG

The baseline CAMCOG cohort was comprised of 651 post-menopausal women aged from 75 to 87 years of age. Cognition was measured using the CAMCOG assessment, designed to test various components of memory and learning. The total score of 106 was calculated from the cumulative tally of all questions and was used as the sole dependent variable in this cohort. A total of 14 independent variables were considered for analysis and are described below.

Of the 651 female participants, one individual (2.2%) had an extremely low CAMCOG score of 38, indicative of dementia and, therefore, was excluded from the data analysis. A further case was excluded due to an outlying Aβ40 value of 894.0. A case summary for each of these two excluded cases is presented in Table 3.1.1.

3.1.3 Descriptive Statistics for Baseline CAMCOG cohort

The data were stratified by *APOE* ε4 status and the results reported accordingly. *APOE* ε4 status was known for 618 of the 649 (95.2%) eligible participants (Figure 3.3.1). Of these, 138 (22.3%) were classified *APOE* ε4 and 480 (77.7%) were non-*APOE* ε4.

3.1.4 Dependent Variable (Y), CAMCOG

The distribution of the CAMCOG values for non-*APOE* ε4 and *APOE* ε4 individuals are displayed in Figures 3.1.2 and 3.1.3 respectively. Descriptive statistics for CAMCOG by *APOE* ε4 status are presented in Table 3.1.2, as is for individuals whose *APOE* ε4 status was unknown. The difference between the mean CAMCOG score for non-*APOE* ε4 and *APOE* ε4 individuals was not significantly different (t_{616}=0.524; *P*=0.600).

3.1.5 Independent Variables

Descriptive statistics for the independent variables (X_i) with a continuous data format (age, menopause, oestrogen (E2), Luteinising Hormone (LH), Follicle Stimulating Hormone (FSH), amyloid-beta 1-40 (Aβ40), Body mass index (BMI), age and last year of formal education (education) are presented in Table 3.1.3, and the distribution of their values are displayed in the histograms in Figure 3.1.4. For each independent variable in Table 3.1.3, the mean values for non-*APOE* ε4 and *APOE* ε4 groups were compared; none were shown to be significantly different (*P*>0.05).

Case ID	CAMCOG	*APOEε4*	Age (years)	E2 (pmol/l)	LH (IU/l)	FSH (IU/l)	Meno-pause[a]	Aβ40 (pg/mol)	Statin	Education[b]	Parity	BMI
362	**91**	**3/3**	78	16	**59.1**	**59.6**	26	894.0	**Yes**	13	3	36.6
471	**38**	**3/3**	7	7	**39.9**	**75.0**	31	62.9	**No**	23	4	22.2

[a] years since menopause

[b] age at last year of formal education

E2 = oestrogen

Table 3.1.1: Summary of excluded cases

Case ID 362 was identified with an extremely high Aβ40 level and Case ID 471 included an extremely low CAMCOG score and hence both were deemed outliers.

Variable	n	Min.	Max.	Mean	SD	Median	IQR
Non-*APOE* ε4	480	67	106	95.4	5.4	96.0	6.0
***APOE* ε4**	138	75	105	95.2	5.4	96.0	7.0
Not genotyped	31	83	106	96.0	4.4	96.0	4.0
Total	649	67	106	95.4	5.3	96.0	6.0

SD = standard deviation

IQR = inter-quartile range

Table 3.1.2: Descriptive statistics of CAMCOG for *APOE* ε4, non-*APOE* ε4 and non-genotyped individuals

Minimum, maximum, mean, standard deviation, median and inter-quartile range for *APOE* ε4, non -*APOE* ε4, and non-genotyped subjects.

***APOE* Genotype in the CAMCOG Cohort** (N=618)

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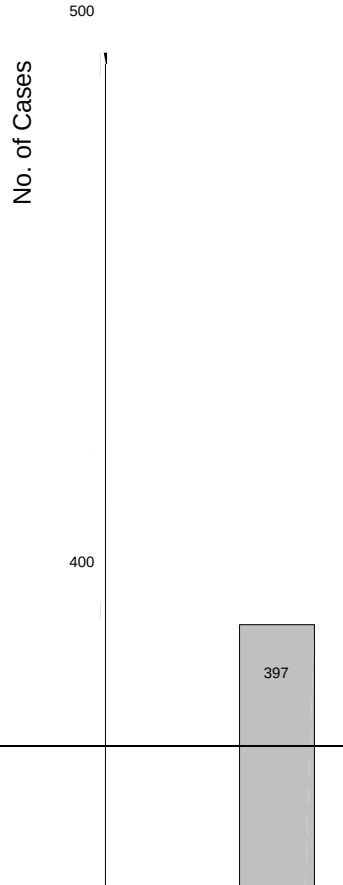


Figure 3.1.1: B

A bar chart repre type group.

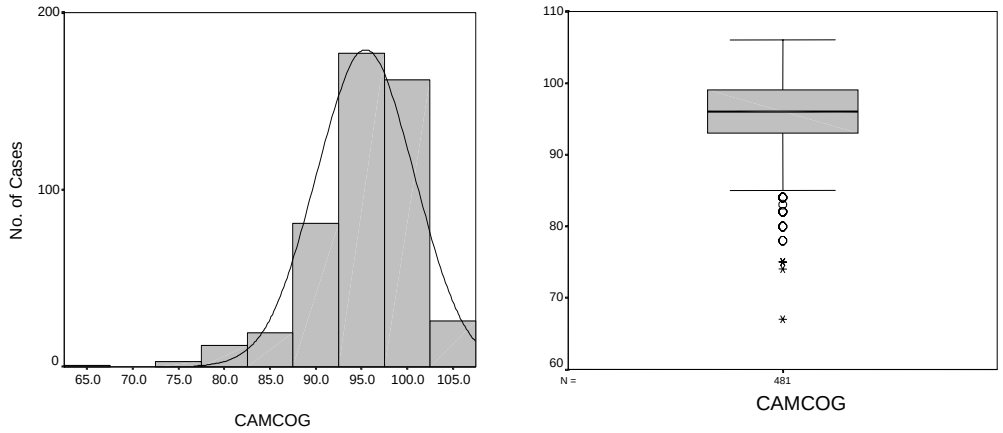


Figure 3.1.2: Histogram and boxplot of CAMCOG for non-*APOE* ε4 individuals

CAMCOG scores for 480 non-*APOE* ε4 subjects showing a normal distribution.

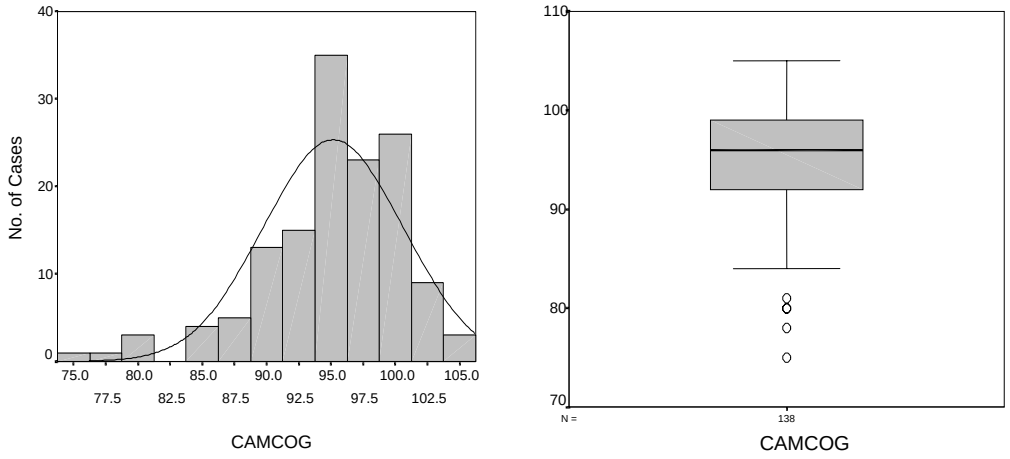


Figure 3.1.3: Histogram and boxplot of CAMCOG for *APOE* ε4 individuals

CAMCOG scores for 138 *APOE* ε4 individuals showing a normal distribution amongst cases.

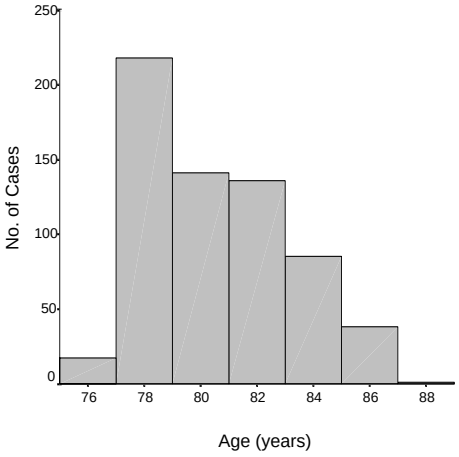
Variable		n	Min.	Max.	Mean	SD	Median	IQR
Age (yrs)	Non-*APOE* ε4	480	76	87	80.1	2.6	80.0	4.0
	APOE ε4	138	75	86	79.8	2.6	79.0	4.0
	total	618	75	87	80.0	2.6	80.0	4.0
Menopause (yrs)	Non-*APOE* ε4	459	13	48	26.8	6.5	26.0	7.0
	APOE ε4	132	16	45	26.1	5.8	25.0	7.8
	total	591	13	48	26.7	6.3	26.0	8.0
E2 (pmol/l)	Non-*APOE* ε4	476	5	143	28.9	15.8	26.0	17.5
	APOE ε4	138	5	132	27.4	16.6	23.0	16.0
	total	614	5	143	28.6	16.0	25.0	17.0
LH (IU/l)	Non-*APOE* ε4	457	0.6	146.4	39.0	21.7	33.3	23.1
	APOE ε4	132	7.2	125.6	42.5	22.5	38.7	27.6
	total	589	0.6	146.4	39.8	21.9	33.9	24.2
FSH (IU/l)	Non-*APOE* ε4	454	1.4	172.0	54.3	23.4	53.5	29.4
	APOE ε4	131	14.7	142.1	56.8	26.4	53.1	42.5
	total	585	1.4	172.0	54.9	24.1	53.4	32.5
Aβ40 (pg/mol)	Non-*APOE* ε4	446	0.3	240.1	82.4	42.7	79.0	60.4
	APOE ε4	131	3.8	230.6	76.4	38.6	72.0	48.9
	total	577	0.3	240.1	81.0	41.9	76.9	58.1
BMI	Non-*APOE* ε4	463	16.3	41.5	26.9	4.3	26.5	5.6
	APOE ε4	133	17.9	37.1	27.0	3.9	27.0	5.2
	total	596	16.3	41.5	26.9	4.2	26.6	5.5
Age at last year of formal education	Non-*APOE* ε4	476	9	74	17.9	9.4	15.0	3.0
(yrs)	APOE ε4	137	13	65	17.1	7.4	15.0	3.0
	total	613	9	74	17.7	9.0	15.0	3.0

Table 3.1.3: Descriptive statistics for independent variables

Minimum, maximum, mean, standard deviation (SD), median, inter-quartile range (IQR) for each continuous independent variable stratified by *APOE* ε4 possession.

Independent variables among both non-*APOE* ε4 and *APOE* ε4 individuals

a)



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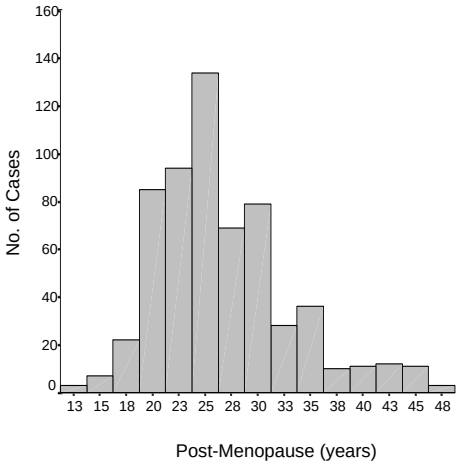
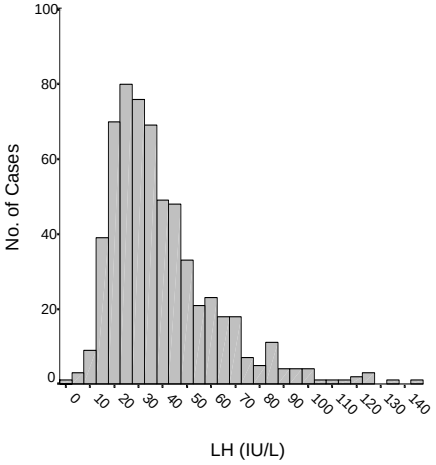


c)



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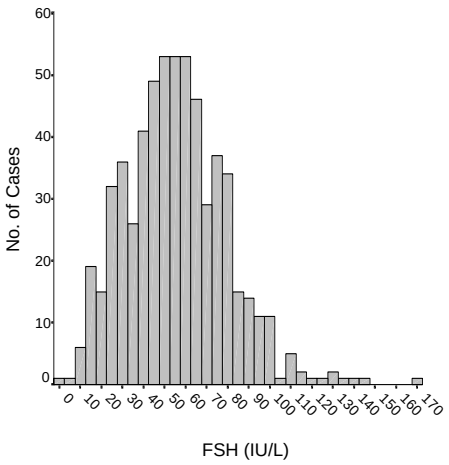


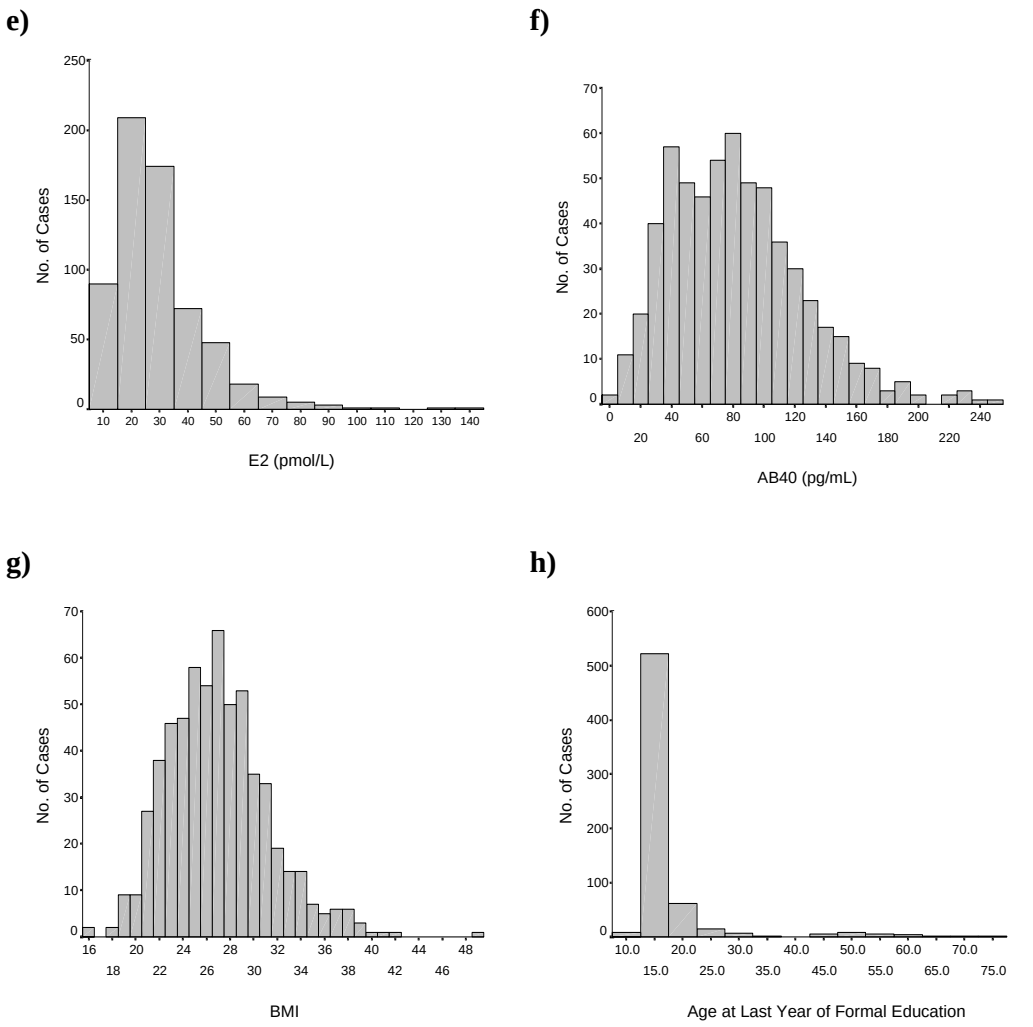


Figure 3.1.4: Histograms of independent variables among both non-*APOE* ε4 and *APOE* ε4 individuals

The distribution of variables age (a), post –menopause years (b), LH (c) and FSH (d) in both *APOE* ε4 and non-*APOE* ε4 individuals. All variables were deemed normal. The distribution of variables E2 (e), Aβ40 (f), BMI (g) and Age of last year of formal education (h) in both non-*APOE* ε4 and *APOE* ε4 individuals. The histogram depicting 'Age of Last Year of Formal Education' was deemed non-normal, and was converted into a categorical variable. E2, Aβ40, and BMI were classified as normal.

The variable, *Age at last year of formal education*, was extremely non-normal (Figure 3.1.4 h). As a consequence, it was recoded into a categorical variable, one in which was thought to best reflect the educational status of women in this age group. The new variable, *education*, has three categories: ≤15, 16-18 and 19 years of age, and will be termed here as low, medium and high, respectively. It was the intention that 1) the latter category represents women who pursued education beyond that of high school and includes those who recommenced education as mature age students and, 2) the base category represent women who undertook the minimalist of education required at the time. Accordingly, of the 476 non-*APOE* ε4 individuals, 53.2% were classified as having a low, 31.9% a medium and 14.9% a high level of education. Among *APOE* ε4 individuals, 78 (56.9%), 43 (31.4%) and 16 (11.7%) were classified as having low, medium and high levels of education, respectively. No association between *APOE* ε4 status and education was evident (χ^2 =1.077, 2df; *P*=0.584).

Parity is an ordinal variable and its distribution among non-*APOE* ε4 and *APOE* ε4 individuals (n=648) is shown in Figure 3.1.5. Parity ranged from 0 to 9 among the non-*APOE* ε4 individuals (n=479) and the mean and median values were 3.0 (SD 1.7) and 3.0 (IQR 2.0), respectively. Likewise, among the *APOE* ε4 individuals (n=138), parity ranged from 0 to 9 and the mean and median values were 3.0 (SD 1.7) and 3.0 (IQR 2.0). Parity was recoded into a categorical variable with four levels, *parity4*: 0-1, 2-3 and 4-5 and 6+. The distribution of non-*APOE* ε4 (n=479) and *APOE* ε4 individuals (n=138) by *parity 4* is shown in Table 3.1 4.

The prevalence of statin users, depression, hypertension, CVS event and diabetes were also examined in this investigation of CAMCOG among non-*APOE* ε4 individuals (Table 3.1.5). Out of 187 statin users, 28.5% were non-ε4 subjects with 29.7% possessing the ε4 allele. A total of 67 subjects were classed as depressed (after family

doctor consultation). Depression was shown to be significantly more prevalent in *APOE* ε4 than non-*APOE* ε4 individuals, 16.7% versus 8.8% (P=0.008). A total of 338 individuals from 649 subjects, had hypertension. Percentage-wise, 6.6% had a past cardio-vascular event. Diabetes (type 2) consisted of 8.5% of the total CAMCOG cohort.

Firstly, factors associated with CAMCOG were examined among non-*APOE* ε4 individuals using univariate analysis, followed by multiple linear regression. Thereafter, *APOE* ε4 individuals were separately but similarly investigated.

Parity among both non-*APOE* ε4 and *APOE* ε4 individuals

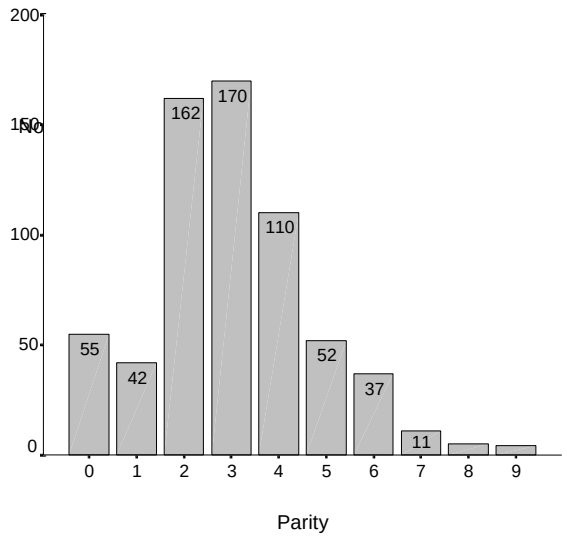


Figure 3.1.5 Bar chart illustrating parity among both non-*APOE* ε4 and *APOE* ε4 individuals

The rates of parity for the CAMCOG cohort (n=617). Of the 617 subjects, 170 had 3 offspring.

PARITY	0-1 n (%)	2-3 n (%)	4-5 n (%)	6+ n (%)	total n
Non-*APOE* ε4	76 (15.9)	230 (48.0)	131 (27.3)	42 (8.8)	479
APOE ε4	16 (11.6)	83 (60.1)	26 (18.8)	13 (9.4)	138

[a] χ^2=7.517, 3df; *P*=0.057

Table 3.1.4: Association between parity and *APOE* ε4 genotype[a]

48% of non-*APOE* ε4 subjects have 2-3 offspring. For *APOE* ε4 individuals 60.1% had 2-3 offspring, as opposed to only 9.4% having more than 6 children born.

Variable	Non-*APOE* ε4 n (%)	*APOE* ε4 n (%)	Total n (%)	χ^2 (df)	*P*-value
Statin	130 (27)	32 (23.1)	162 (28.8)	0.071	0.789
Depression	42 (8.8)	23 (16.7)	65 (10.3)	7.138	**0.008**
Hypertension	236 (49.1)	67 (48.5)	303 (52.1)	0.530	0.467
CVS event	28 (5.8)	9 (6.5)	37 (6.6)	2.226	0.136
Diabetes	44 (9.2)	7 (5)	51 (8.5)	1.579	0.209
n	**480**	**138**	**618**	**-**	**-**

3.1.5.1.1 Shading denotes significance ($P<0.05$)

Table 3.1.5: Prevalence of statin use, depression, hypertension, CVS and diabetes among non-*APOE* ε4 and *APOE* ε4 individuals

Prevalence of depression was found to be significantly different between non-*APOE* ε4 and *APOE* ε4 participants. All other factors were not significantly different between groups.

3.1.6 Non-APOE ε4 Individuals

3.1.6.1 Univariate Analysis

The relationship between the dependent variable, CAMCOG, and each of the independent variables was examined using univariate statistical methods for non-*APOE* ε4 subjects. Pearson's correlation coefficient (*r*) and the age-adjusted *r* were used to assess the linear relationship between each continuous independent variable and CAMCOG and were examined in Table 3.1.6. Age, post menopause years, LH, FSH, and oestrogen levels were found to be significantly related to cognition as measured by CAMCOG. These relationships are summarised by scatter plots as shown in figure 3.1.6.

The univariate relationship between CAMCOG and each of the categorical independent variables was also examined and the results are presented in Table 3.1.7. Parity, statin use, depression, hypertension, CVS event and diabetes were not significantly correlated to CAMCOG score. The variable *APOE* ε2 was added, and no significant relationship was found with CAMCOG. Significant mean differences in CAMCOG scores were found between education groups ($P<0.001$).

Variable	n	*r*	*P*-value	*Age-adjusted r*	*P*-value
Age (years)	480	-0.234	**<0.001**		
Post-menopause(years)	459	-0.088	**0.060**	0.016	0.740
LH (IU/l)	457	-0.157	**0.001**	-0.144	0.002
FSH (IU/l)	454	0.062	**0.184**	0.089	0.059
E2 (pmol/l)	476	-0.100	**0.029**	-0.103	0.025
Aβ40 (pgm/mol)	446	0.052	0.275	0.062	0.193
BMI	463	0.005	0.909	-0.006	0.905

r = regression coefficient

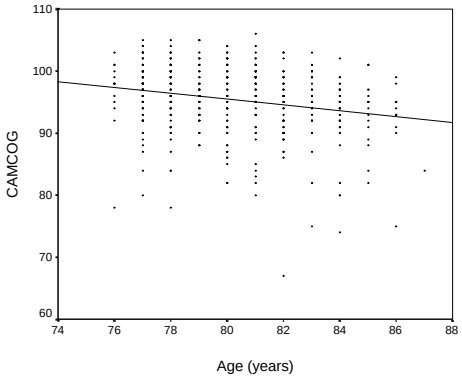
Shading denotes significance ($P<0.05$)

Table 3.1.6: Linear relationship between CAMCOG and continuous independent variables for non-*APOE* ε4 individuals

Age, LH, and E2 were found to have a significant linear relationship with CAMCOG score. All significant variables were found to have a negative linear relationship with the dependent variable.

Independent variables and CAMCOG scores for non-*APOE* ε4 individuals

a)



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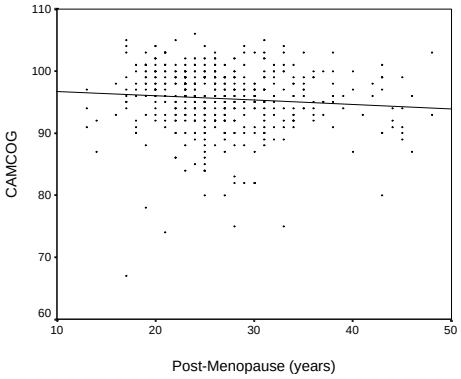

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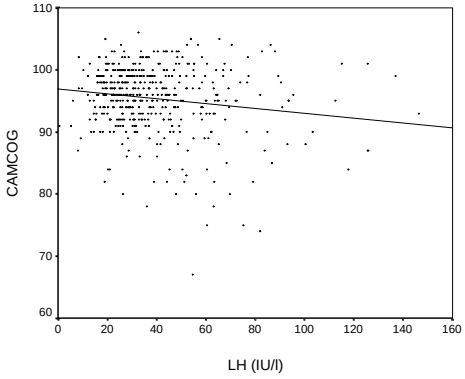

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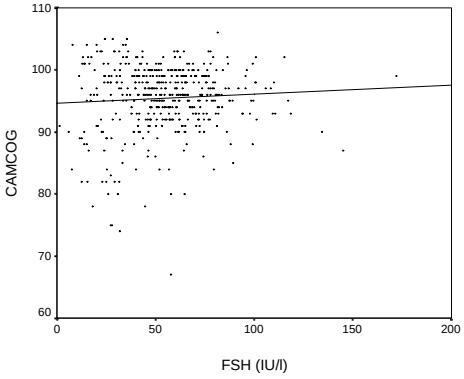


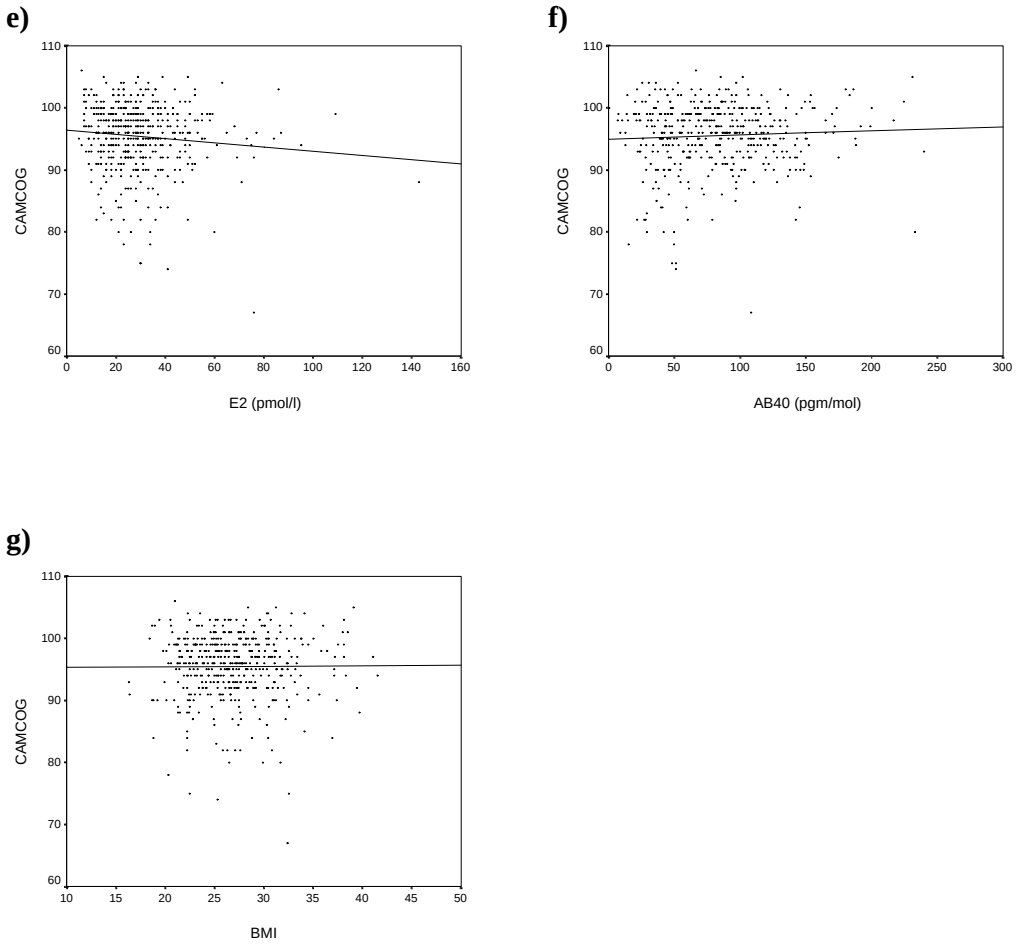


Figure 3.1.6: Scatter plots of independent variables and CAMCOG scores for non-*APOE* ε4 individuals

Scatterplots of age (a) showing a negative correlation with CAMCOG; post-menopause years (b), LH showing a negative correlation with CAMCOG (c), FSH (d), E2 showing a negative correlation with CAMCOG (e), Aβ40 (f) and BMI (g) against CAMCOG score.

Variable	Levels	n	Mean (SD)	Test Statistic	df	*P*-value
***APOE* ε2**	**no**	397	95.4 (5.5)	t=-1.005	139.053	0.317
	yes	83	95.9 (4.9)			
Education:	**low**	253	94.3 (5.7)	F=18.285	2,473	**<0.001**
	medium	152	96.2 (4.5)			
	high	71	98.2 (4.2)			
Parity:	**0-1**	76	96.1 (5.4)	F=0.787	3,475	0.502
	2-3	230	95.4 (5.4)			
	4-5	131	95.0 (5.5)			
	6+	42	95.6 (4.3)			
Statin	**no**	343	95.4 (5.3)	t=0.001	478	0.999
	yes	137	95.4 (5.6)			
Depression	**no**	438	95.5 (5.2)	t=0.581	45.86	0.564
	yes	42	94.9 (6.7)			
Hypertension	**no**	236	95.6 (5.6)	t=0.661	478	0.509
	yes	244	95.3 (5.1)			
CVS event	**no**	452	95.4 (5.4)	t=0.127	478	0.899
	yes	28	95.3 (5.4)			
Diabetes	**no**	436	95.5 (5.3)	t=1.171	478	0.242
	yes	44	94.6 (5.8)			

SD = Standard deviation

Df = degrees of freedom

Shading denotes significance

Table 3.1.7: Mean CAMCOG scores by categorical independent variables for non-*APOE* ε4 individuals

The mean CAMCOG scores by *APOE* ε2 genotype, education level, parity, statin use, depression, hypertension, CVS event and presence of diabetes (type2) for the non-APOE ε4 group in the CAMCOG cohort. Education level was the only independent variable with significant differences of CAMCOG score between groups.

3.1.6.2 Multiple Linear Regression Analysis (non-APOE ε4)

Multiple linear regression (MLR) analysis was used to simultaneously examine the association between each independent variables and CAMCOG. Three model fitting strategies – stepwise forward, backward elimination and the full model approach - were undertaken so as to achieve a model of linear main effects by consensus (Table 3.1.8). For the stepwise forward method, independent variables were sequentially added to the model; the order based on the *P*-value derived from univariate analysis, with the lowest *P*-values entered first. Independent variables that were significant remained in the model, and those that were not were removed before the addition of the next independent variable. With the backward elimination model, all independent variables were included in the initial model and the independent variable with the highest *P* value was removed in sequence until only significant main effects remained. The full model involved forcing all of the independent variables into a model and observing the resultant *P* -values.

By consensus of the three model fitting strategies, age, education, LH and FSH were identified as significant main effects. Conversely, post-menopausal years, diabetes, Aβ40, *APOE* ε2, parity, hypertension, depression, CVS event, BMI and statin use were not found to be significant in relation to CAMCOG score (Table 3.1.8).

After establishing significant main effects, an investigation of interrelationships between these main effects on CAMCOG was conducted. Specifically, cross-products were computed from two-way combinations of the main effects Table 3.1.9. Only biologically meaningful cross-products were investigated. For this reason, the cross-product between education (3) and LH (edu (3)*LH) was not examined. Based on F-change value, both age*LH (P=0.015) and age*FSH (P=0.017) were found to be

significant interaction terms. Both were added to the model of main effects, and the final MLR model on CAMCOG for non-*APOE* ε4 individuals is shown in Table 3.1.10.

Overall Fit of the Model

The adjusted coefficient of multiple determination (adjusted R^2) was used as a measure of the fit of the model. Specifically, it is the variability of the dependent variable that is explained by the significant independent variables after taking into account the number of independent variables in the model. The adjusted R^2 for the CAMCOG model is 0.176. Therefore, 17.6% of the variation in CAMCOG scores is explained by age, LH, FSH and education.

Model Diagnostics

An examination of the residuals was undertaken to assess the assumptions of linearity and homoscedasticity (equal variance). The relatively random scatter of residuals shown in Figure 3.1.7 indicates that these two assumptions are not violated.

The assumption of normality was also assessed (Figures 3.1.8 and 3.1.9). The relatively normal distribution of the residuals indicates that the assumption of normality is acceptable. The fit of the regression standardised residuals around the line in Figure 3.1.9 supports the relative normality of the dependent variable, CAMCOG.

Variable		Univariate *P*-value	Full Model *P*-value	Stepwise Forward *P*-value	Backward Elimination *P*- value
Age		**<0.001**	**<0.001**	**<0.001**	**<0.001**
Education(3)	low	**<0.001**	-	-	-
	med	**-**	**<0.001**	**<0.001**	**<0.001**
	high	**-**	**<0.001**	**<0.001**	**<0.001**
LH (IU/l)		**0.001**	**0.001**	**<0.001**	**<0.001**
E2 (pmol/l)		**0.029**	0.256	-	-
Post-menopausal (age in years)		0.060	0.469	-	-
FSH (IU/l)		0.184	0.190	**0.050**	**0.050**
Diabetes(2)		0.242	0.931	-	-
AB40 (pgm/mol)		0.275	0.427	-	-
***APOE* ε2(2)**		0.317	0.516	-	-
Parity(4):	0-1	0.502	-	-	-
	2-3	-	0.410	-	-
	4-5	-	0.164	-	-
	6+		0.774	-	-
Hypertension(2)		0.509	0.624	-	-
Depression(2)		0.564	0.280	-	-
CVS event(2)		0.899	0.553	-	-
BMI		0.909	0.836	-	-
Statin(2)		0.999	0.746	-	-

Numbers in brackets represents number of levels

Shading denotes significance (P<0.05)

Table 3.1.8: MLR model-fitting strategies on CAMCOG for non-*APOE* ε4 individuals

MLR model-fitting strategies including full model, stepwise forward and backward elimination were used to determine the relationships between the independent variables and CAMCOG score. Age, education and LH were found to be significant variables on CAMCOG score for non-*APOE* ε4 subjects.

Main Effects				Cross-product	F Change	df	*P*-value
Age -	edu_3 -	LH -	FSH -	age*edu3_d1 age*edu3_d2	- 0.008	- 2,442	- 0.992 [a]
Age	edu_3	LH	FSH	age*LH	5.905	1,443	**0.015**
Age	edu_3	LH	FSH	age*FSH	5.747	1,443	**0.017**
Age	edu_3	LH	FSH	LH*FSH	1.395	1,443	0.238 [a]

[a] not examined due to the lack of biological relevance of these two main effects

df= degrees of freedom

Shading denotes significance

Table 3.1.9: Significance of interaction terms on the MLR model of main effects on CAMCOG of non-*APOE* ε4 individuals

The interaction terms, *age and LH* as well as *age and FSH* were the only significant combined variables.

Variable		Unstandardised β	Unstandardised std error	*P*-value
Constant		147.749	19.667	<0.001
Age		-0.661	0.245	0.007
Education(3:)	**low**	-	-	-
	med	2.038	0.520	<0.001
	high	3.895	0.696	<0.001
LH		0.814	0.295	0.006
FSH		-0.831	0.297	0.005
Age*LH		-0.011	0.004	0.004
Age*FSH		0.011	0.004	0.004

[a] R^2=0.189, adjusted R^2=0.176, $F_{7,442}$=14.687, *P*<0.001

Shading denotes significance

Table 3.1.10: Final MLR model on CAMCOG for non-*APOE* ε4 individuals[a]

Age, education level, LH, FSH and cross products for both gonadotropins and age were found to be significantly related to CAMCOG score for non- *APOE* ε4 subjects.

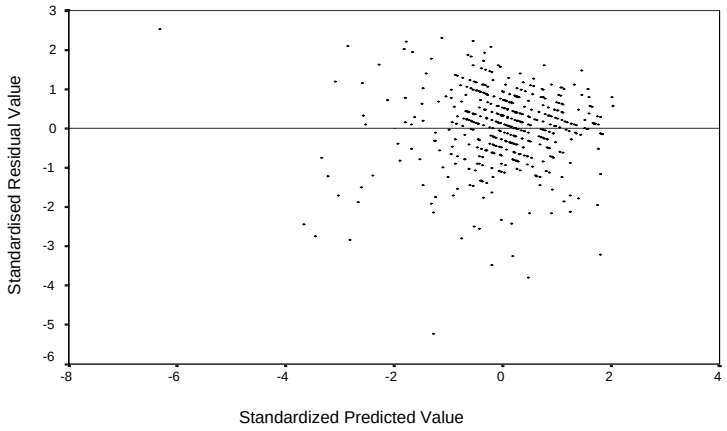


Figure 3.1.7: Scatterplot of standardised residuals vs. standardised predicted values

To assess linearity and homoscedasticity, residuals were investigated. The random nature of the residuals show that these two assumptions are not violated. Based on a standardised residual of either <3 or <-3, five cases were identified as outliers.

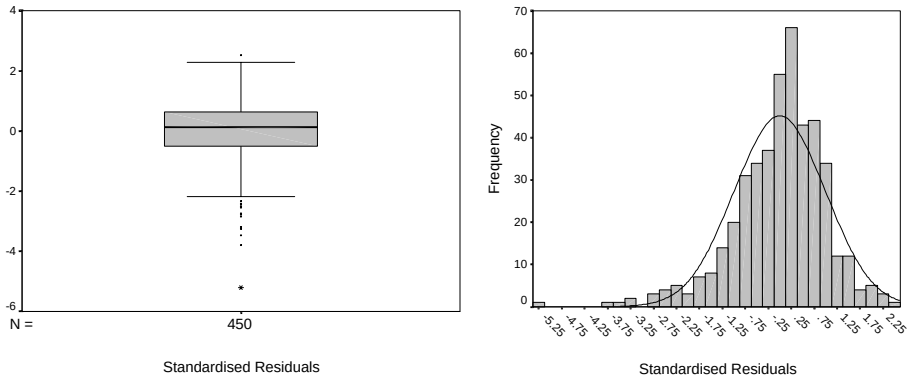


Figure 3.1.8: Histogram and boxplot of standardised residuals

The histogram and boxplot show that normality is acknowledged.

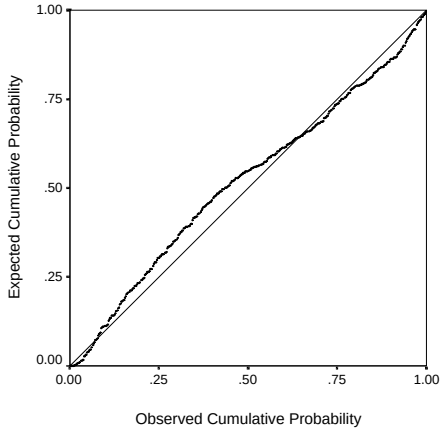


Figure 3.1.9: Normal P-P plot of regression standardised residuals

The fit of the regression standardised residuals around the line suggests normality of the dependent variable.

ID	Standardised Residual	CAMCOG	Age	Education	FSH (IU/l)	LH (IU/l)
266	-3.245	80	77	low	26.2	57.7
533	-3.189	84	78	high	20.9	40.2
1329	-3.793	78	76	low	36.0	44.7
2052	-3.474	78	78	low	63.0	18.1
2068	-5.21	67	82	low	57.7	54.6

Table 3.1.11: Case summary of outliers

Five cases identified as outliers. Subsequent removal of the outliers from the MLR model did not change the statistical significance of the main effects.

Based on a standardised residual of either ≥ 3 or ≤ -3, five cases were identified as outliers. Cases summaries of the significant independent variables for these five outliers are presented in Table 3.1.11. Each outlier was independently and simultaneously excluded from the data set and the final MLR model reproduced. On no occasion did the exclusion of any one of these outliers result in a change to the statistical significance of the main effects or interaction terms in the final MLR model on CAMCOG for non-*APOE* ε4 individuals.

As seen in Table 3.1.10, four variables were found to be significantly associated with CAMCOG: age, education, LH and FSH. CAMCOG scores increased with level of education. As compared to women with low education, those with medium and high levels of education had CAMCOG scores that were, on average, 2.0 and 3.9 points higher ($P<0.001$).

The significant age-dependent effect of LH and FSH on CAMCOG is graphically presented in Figures 3.1.10 and 3.1.11, respectively. In these figures, low, average and high values of LH, FSH and age represent the 10^{th}, 50^{th} and 90^{th} percentiles. Respectively, these values are 18.1, 33.6 and 68.1IU/l for LH, 25.6, 53.1 and 91.3IU/l for FSH and 77, 80 and 84 years for age. Estimates of CAMCOG scores are based on low levels of education (edu_3 = 0). As shown, CAMCOG scores decrease with increasing levels of LH. Conversely, CAMCOG scores increase with increasing levels of FSH. The age-dependent effect of both LH and FSH on CAMCOG indicates that the effect of each becomes more pronounced with age.

The age-dependent effect of LH on CAMCOG for non-*APOE* ε4 individuals*

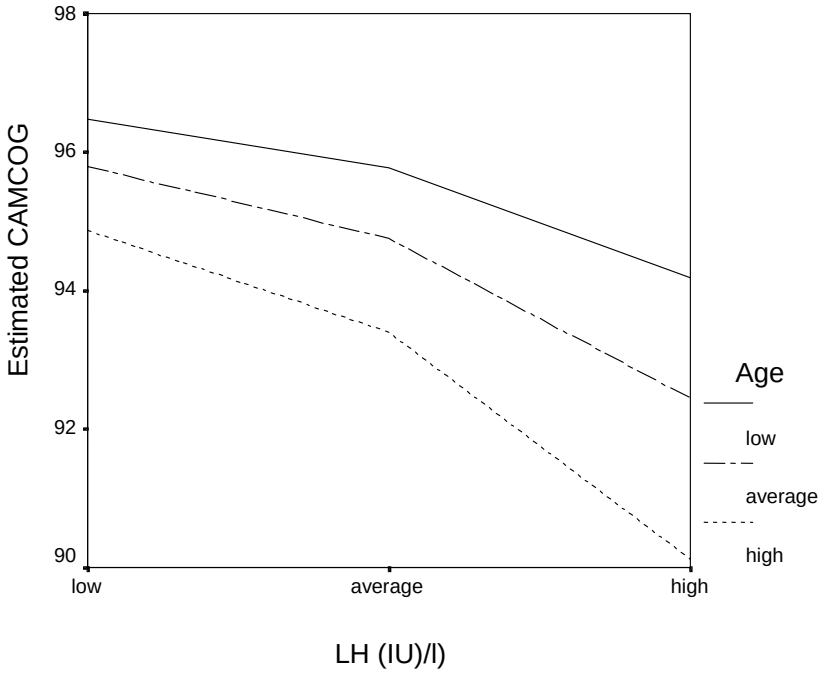


Figure 3.1.10: The age-dependent effect of LH on CAMCOG score for non-*APOE* ε4 individuals*

With increasing levels of plasma LH, estimated CAMCOG score declines. This effect is age-dependent, such that individuals in the highest age group tend to have lower CAMCOG scores dependent on LH levels. Conversely, subjects in the lowest age group (77 years old) tended to have higher estimated CAMCOG scores, again dependent on LH level. The estimated camcog score was calculated by using a regression equation composed of all main effects found in the MLR. For each LH level and age level the remaining significant effects were placed into the equation to determine estimated CAMCOG score.

*Assumes 10th, 50th and 90th percentiles for LH (18.1, 33.6 and 68.1IU/l) and age (77, 80 and 84 years)

The age-dependent effect of FSH on CAMCOG for non-*APOE* ε4 individuals#

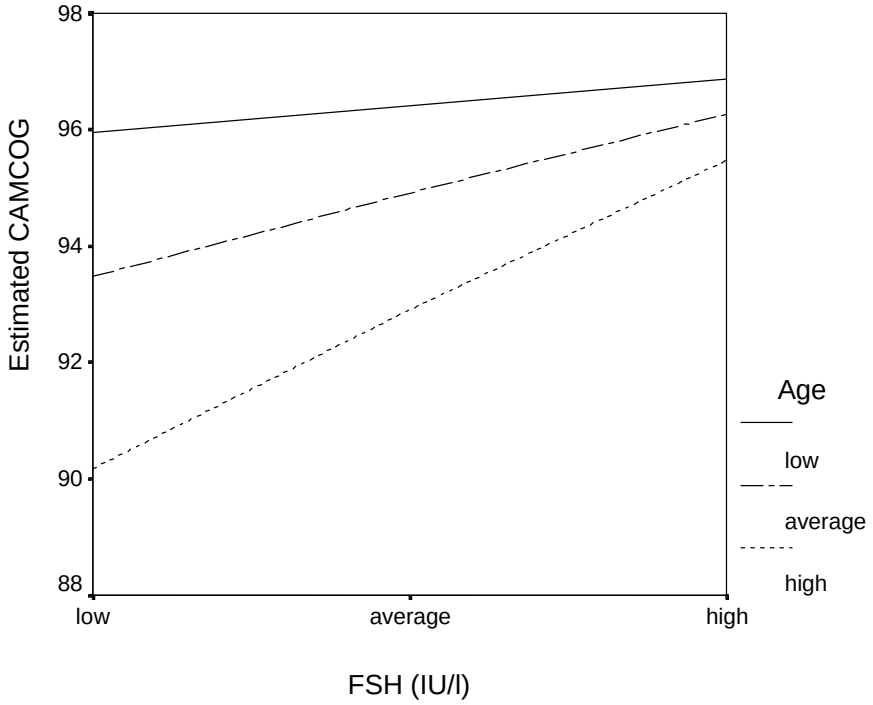


Figure 3.1.11: The age-dependent effect of FSH on CAMCOG for non-*APOE* ε4 individuals#

The levels of FSH in plasma were positively correlated to estimated CAMCOG score; again dependent on age. Subjects in the highest age group (84 years old), tended to have lower estimated CAMCOG score than individuals in the average and lowest age ranges.

The estimated camcog score was calculated by using a regression equation composed of all main effects found in the MLR.

Assumes 10th, 50th and 90th percentiles for FSH (5.6, 53.1 and 91.3IU/l) and age (77, 80 and 84 years).

It is equally important to note that among non-*APOE* ε4 women aged 75-87 years, all the other variables investigated here (but not included in the final MLR model), were not shown to be of statistical significance in relation to CAMCOG. These included E2, years since menopause, parity, Aβ40, BMI, statin use, depression, hypertension, CVS event and diabetes.

3.1.7 APOE ε4 Individuals

3.1.7.1 Univariate Analysis

Pearson's *r* and the age-adjusted *r* were used to assess linear relationships between the dependent variable, CAMCOG, and each of the independent variables with a continuous data format (Table 3.1.12). These linear relationships are graphically shown in scatter plots in Figure 3.1.12.

The univariate relationship between CAMCOG and each of the categorical independent variables was also examined (Table 3.1.13). The group of women with a high level of education had a significantly higher mean CAMCOG score than those in the low education group (*P*=0.038). Women with depression had, on average, significantly lower CAMCOG scores than those without depression (*P*=0.001). Statin users also had, on average, significantly lower CAMCOG scores than non-users (*P*=0.040). Parity, hypertension, CVS event and diabetes (type 2) were not found to be significantly related to CAMCOG score.

3.1.7.2 Multiple Linear Regression Analysis

Based on consensus of the three model-fitting strategies, FSH, Aβ40, education, depression and statin use were identified as significant main effects (Table 3.1.14). In light of the established importance of age and its *P*-value of borderline significance, age was also included as a main effect.

All biologically relevant cross-products between main effects were investigated for their potential significance as interaction terms. With an F-change value of 7.072 on 1 and 116 df, a significant interaction term between FSH and statin use (FSH*statin) was found (P=0.009). FSH*statin was added to the model of main effects, and the final MLR model on CAMCOG for APOE ε4 individuals is presented in Table 3.1.15.

Among the *APOE* ε4 individuals, multiple linear regression modelling revealed that six factors were significantly associated with CAMCOG: depression, education, parity, Aβ40, FSH and statin use (P<0.05). Age was included in the final MLR model because of its established significance in terms of cognition, which, in part, was confirmed here by the marginal significance of age on CAMCOG (P=0.069). Individuals with depression have, on average, a CAMCOG score that is 2.5 points lower than that of non-depressed individuals (P=0.038). A high level of education is associated with a CAMCOG score that is, on average, 4.8 points higher (P<0.001). As compared to women who have 0-1 offspring, those with parity of ≥2 have, on average, a lower CAMCOG score by 3.5 points (P=0.008). CAMCOG scores were shown to decrease with increasing levels of Aβ40, by an average of 0.3 points for every 10pg/mol increase in Aβ40 (P=0.005).

Both FSH and statin use were also identified as being significantly associated with CAMCOG, with the effect of FSH dependent on statin use (P=0.009). This interactive

effect of FSH and statin use on CAMCOG is shown in Figure 3.1.16. The low, average and high FSH values were derived from the 10th, 50th and 90th percentiles: 25.6, 53.1 and 91.3IU/l, respectively. Estimates of the CAMCOG scores are based on low/medium levels of education (edu_3=0), absence of depression (depression_2=0), 0-1 offspring (parity_2=0) and mean values of age (79.8 years) and Aβ40 (76.4pgm/mol). As shown, CAMCOG scores increase with increasing levels of FSH; an effect exacerbated among stain users (*P*=0.009). In essence, FSH has a more pronounced positive effect on CAMCOG among statin users than non-statin users.

Among *APOE* ε4 women aged 75-87 years, it is of relevance to highlight variables that were not shown to be of significance in relation to CAMCOG: years since menopause, LH, E2, BMI, diabetes, hypertension and CVS.

Model Diagnostics

The scatterplot of standardised residuals vs. standardised predicted values shown in Figure 3.1.13 indicates that the assumptions of linear and homoscedasticity are not in violation. Based on the distribution of standardised residuals (Figure 3.1.14) and the normal P-P plot of the regression standardised residuals (Figure 3.1.15), the assumption of normality is also acceptable.

The adjusted coefficient of multiple determination (adjusted R^2) is 0.291. This means that 29.1% of the variation in CAMCOG scores is explained by the significant factors in the final MLR: education, statin use, Aβ40, parity, depression and FSH.

Variable	n	*r*	*P*-value	*Age-adjusted r*	*P*-value
Age (years)	138	-0.086	0.316	-	-
Post-menopause(years)	132	-0.159	0.069	-0.159	0.070
LH (IU/l)	132	-0.123	0.159	-0.127	0.150
FSH (IU/l)	131	0.211	**0.016**	0.217	0.013
E2 (pmol/l)	138	-0.054	0.531	-0.072	0.400
Aβ40 (pg/mol)	131	-0.100	0.254	-0.105	0.235
BMI	133	0.021	0.813	0.022	0.804

Shading denotes significance ($P<0.05$)

Table 3.1.12: Linear relationship between CAMCOG and continuous independent variables for *APOE* ε4 individuals

Independent continuous variables age, post menopause years, LH, E2, Aβ40, and BMI were not shown to be significantly correlated with CAMCOG score. FSH levels showed a significant association with CAMCOG ($P=0.016$).

\

Variable	Levels	n	Mean (SD)	Test Statistic	df	*P*-value
Education:	**low**	78	94.5 (5.6)	F=3.362	2,134	**0.038**
	medium	43	95.4 (5.1)			
	high	16	98.2 (4.6)			
Parity:	**0-1**	16	97.6 (4.1)	F=1.914	2,135	0.151
	2-3	83	94.8 (5.8)			
	4+	39	95.0 (4.9)			
Statin	**no**	97	95.9 (4.6)	t=2.099	55.843	**0.040**
	yes	41	93.5 (6.8)			
Depression	**no**	115	95.8 (5.1)	t=3.412	136	**0.001**
	yes	23	91.8 (5.9)			
Hypertension	**no**	63	94.9 (5.9)	t=0.596	136	0.552
	yes	75	95.4 (5.0)			
CVS event	**no**	125	95.2 (5.6)	t=-0.040	136	0.968
	yes	13	95.2 (3.9)			
Diabetes	**no**	130	95.2 (5.5)	t=0.630	136	0.530
	yes	8	94.0 (4.2)			

SD = standard deviation

df = degrees of freedom

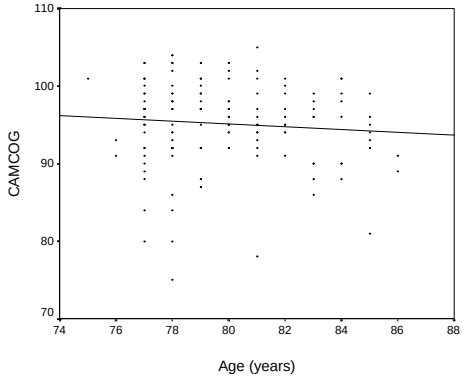
Shading denotes significance ($P<0.05$)

Table 3.1.13: Mean CAMCOG by categorical independent variables for *APOE* ε4 individuals

Only education, statin use and depression were found to have significantly different CAMCOG scores between groups for *APOE* ε4 subjects.

Independent variables and CAMCOG for *APO*ε4 individuals

a)



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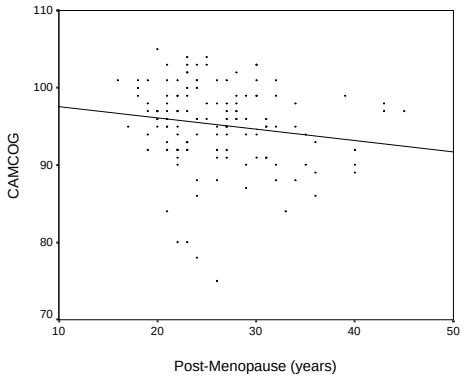

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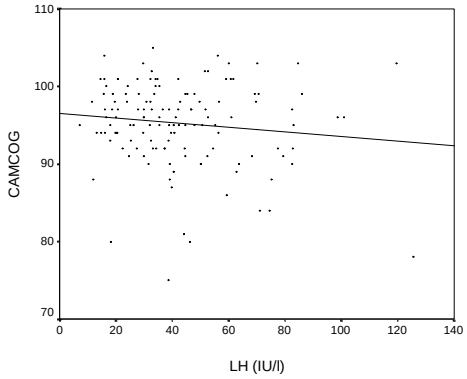

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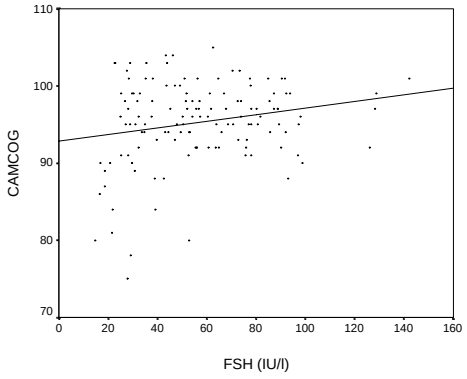


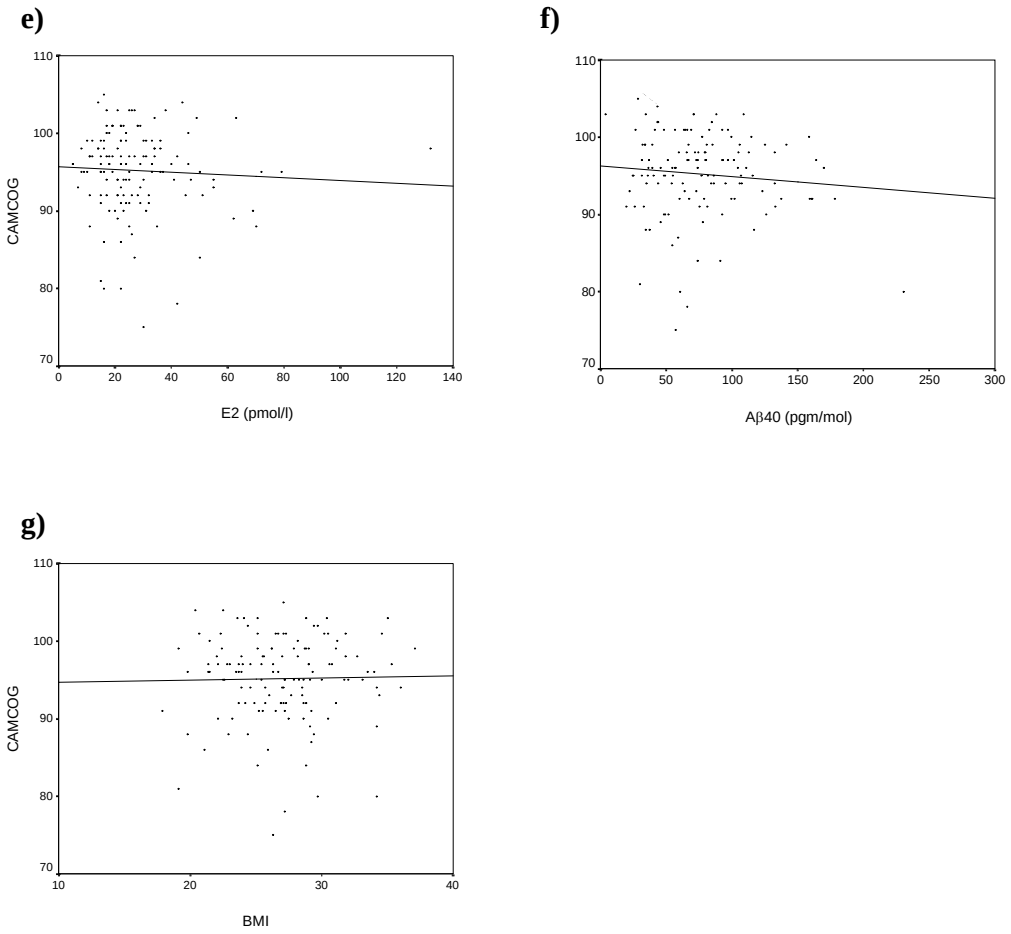


Figure 3.1.12: Scatterplots of independent variables and CAMCOG for *APOE* ε4 individuals

Scatterplots of age (a), post-menopause years (b), LH (c), FSH showing a positive correlation (d), E2 (e) , Aβ40 (f), and BMI (g) versus CAMCOG for *APOE* ε4 subjects.

Variable		Univariate *P*-value	Full Model *P*-value	Stepwise Forward *P*-value	Backward Elimination *P*-value
Depression(2)		**0.001**	**0.013**	**0.012**	**0.012**
FSH (IU/l)		**0.016**	**0.003**	**<0.001**	**<0.001**
Education(3):	**low**	**0.038**	**0.001**	**0.001[a]**	**0.001[a]**
	medium	**-**		-	-
	high	**-**		-	-
Statin(2)		**0.040**	0.174	**0.030**	**0.030**
Post-menopausal (age in years)		0.069	0.310	-	-
Parity(3)	**0-1**	0.151	**0.015**	**0.010[b]**	**0.010[b]**
	2-3	**-**		-	-
	4+	**-**		-	-
LH (IU/l)		0.159	0.073	**-**	**-**
Aβ40 (pg/mol)		0.254	**0.001**	**0.007**	**0.007**
Age (years)[c]		0.316	0.454	0.084	0.084
Diabetes(2)		0.530	0.429	-	-
E2 (pmol/l)		0.531	0.456	-	-
Hypertension(2)		0.552	0.053	-	-
BMI		0.813	0.753	-	-
CVS event(2)		0.968	0.448	-	-

[a] low/medium vs high [b] 0-1 vs 2+ offspring [c] age was included as a main effect

Table 3.1.14: MLR model-fitting strategies on CAMCOG for *APOE* ε4 individuals

MLR model-fitting strategies including full model, stepwise forward and backward elimination were used to determine the relationships between the independent variables and CAMCOG score. Depression, education, FSH, statin use, Aβ40 and parity were found to be significant variables on CAMCOG score for *APOE* ε4 subjects.

Variable	Unstandardised		
	β	std error	*P*-value
Constant	123.006	13.061	<0.001
Age (years)	-0.296	0.161	0.069
Education(2)[b]	4.776	1.323	<0.001
Statin(2)	-7.243	2.148	0.001
Aβ40 (pg/mol)	-0.032	0.011	0.005
Parity(2)[c]	-3.477	1.293	0.008
Depression(2)	-2.453	1.168	0.038
FSH (IU/l)	0.034	0.020	0.086
FSH*statin2	0.094	0.035	0.009

[a] R^2=0.337, adjusted R^2=0.291, $F_{8,116}$=7.368, P<0.001

[b] low/medium vs high [c] 0-1 vs ≥2 offspring

Table 3.1.15: Final MLR model on CAMCOG for *APOE* ε4 individuals[a]

The results indicate that education, Aβ40, parity and depression were significantly associated with CAMCOG score. Education (low/ medium (<15 years to 18 years) vs. high (19 years onwards), was found to have a positive relationship with CAMCOG score (P=<0.001). Aβ40 levels, parity and depression, were found to be negatively associated to CAMCOG score (P=0.005; P=0.008; P=0.038 respectively).

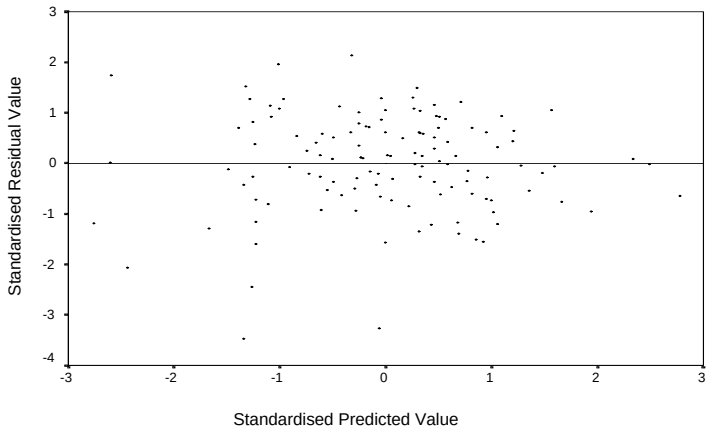


Figure 3.1.13: Scatterplot of standardised residuals vs. standardised predicted values (Based on a standardised residual of either <3 or <-3)

To assess linearity and homoscedasticity, residuals were investigated. The random nature of the residuals show that these two assumptions are not violated.

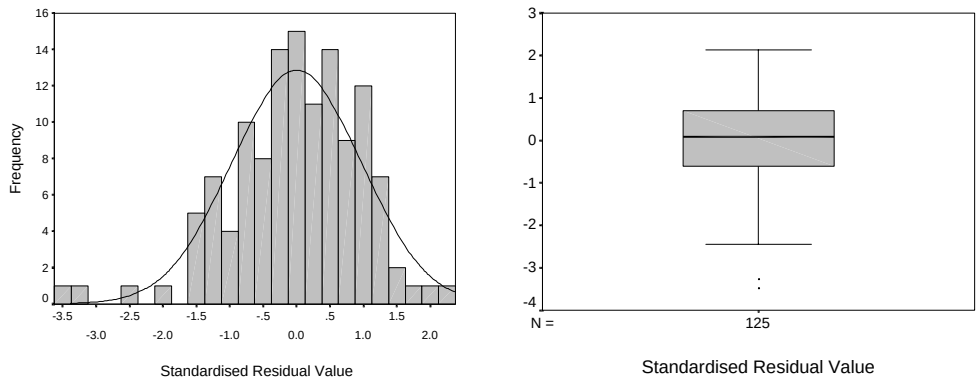


Figure 3.1.14: Histogram and boxplot of standardised residuals

The histogram and boxplot show that normality is acknowledged.

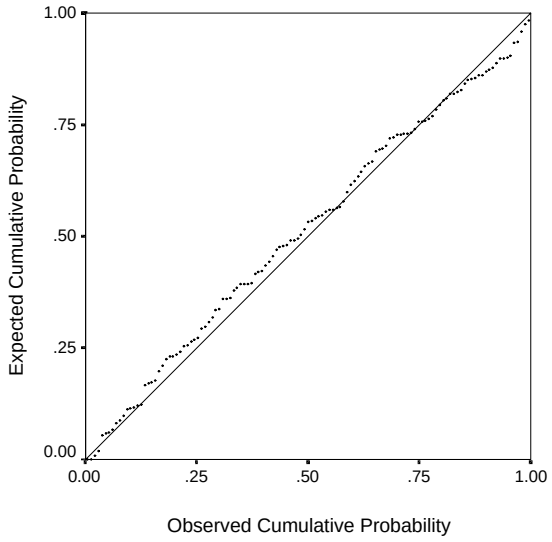


Figure 3.1.15: Normal P-P Plot of regression standardised residuals

The fit of the regression standardised residuals around the line suggests normality of the dependent variable.

The interactive effect of FSH and statin use on CAMCOG among *APOE* ε4 Individuals

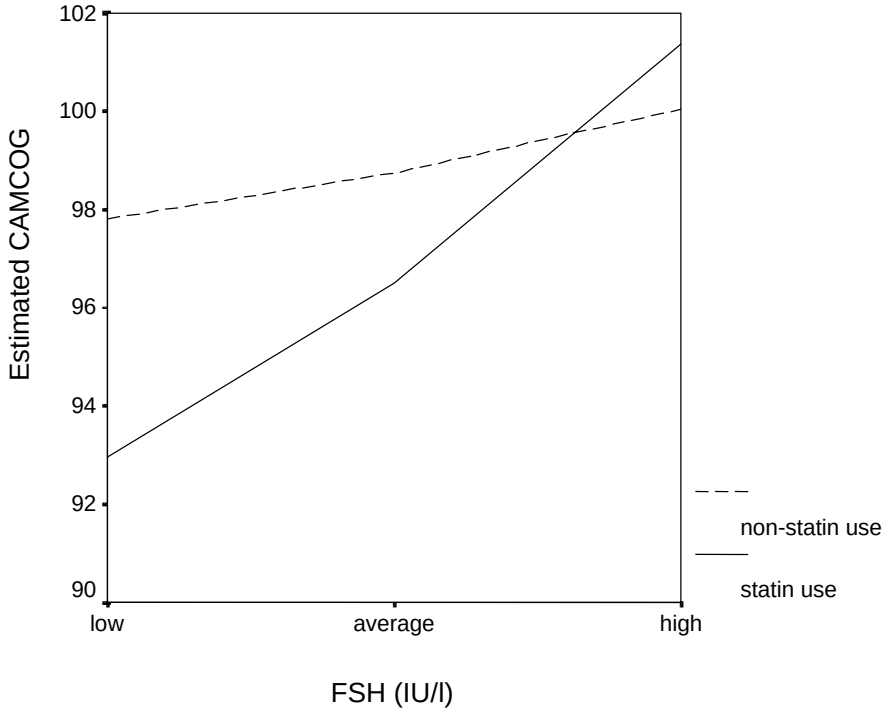


Figure 3.1.16: The interactive effect of FSH and statin use on CAMCOG among *APOE* ε4 Individuals *

The cross product between FSH and statin use was shown to be significantly associated with CAMCOG (*P*=0.009). As FSH levels increased, estimated CAMCOG scores were also found to increase dependent on use of statins. The effect of FSH on estimated CAMCOG was more pronounced in statin users.

The estimated camcog score was calculated by using a regression equation composed of all main effects found in the MLR.* Low, average and high FSH values were derived from the 10^{th}, 50^{th} and 90^{th} percentiles (25.6, 53.1 and 91.3IU/l, respectively. Estimates of the CAMCOG scores are based on low/medium levels of education (edu_3=0), absence of depression (depression_2=0), 0-1 offspring (parity_2=0) and mean values of age (79.8 years) and Aβ40 (76.4pgm/mol).

3.2 CAMCOG 12 Months

3.2.1 General Observations

After descriptive, univariate and multiple linear regression analysis no significant relationship between the main independent variable oestrogen (E2) and dependent variable CAMCOG 12 Months was found. Overall approximately 33 % of the follow-up subjects declined in CAMCOG score over the 12 month interval, whereas 57% improved. On average, mean CAMCOG scores improved by 1.2 points between baseline and follow-up CAMCOG assessments (P <0.001). A regression equation was created to explain the multiple effects of specific factors on cognition. It was revealed that CAMCOG baseline score had a significant positive association with estimated CAMCOG 12 Month results dependent on age (P<0.001). In addition, with increasing age estimated CAMCOG 12 month scores decreased and this effect was most pronounced on individuals with one or no offspring (P=0.009).

To reflect the overall change in CAMCOG over 12 months, the independent variable CAMCOG Change was created. The score at baseline was the only significant main factor that was associated with CAMCOG Change (P< 0.001). Interestingly, the subjects who did not return for their follow-up assessment had a higher prevalence of depression (16% vs. 7.9%), and were more likely to be *APOE* ε4 positive (27.6% vs. 20.1%), than participants who did return. Conversely, they also had a lower incidence of diabetes (Type 2) (4.1% vs. 10.3%).

3.2.2 Dependent Variable (Y), CAMCOG 12 Months

Of the 649 participants who completed the first CAMCOG test (CAMCOG Baseline) and were included in the former data analysis on CAMCOG, 455 (70.1%) undertook a second CAMCOG test approximately 12 months later (CAMCOG 12 Months). For this sample of 455 participants, descriptive statistics for CAMCOG Baseline and CAMCOG

12 Months are presented in Table 3.2.1, as are those for the computed variable, CAMCOG Difference, being the difference in CAMCOG score between Baseline and 12 Months. The mean score for CAMCOG Baseline is slightly higher for CAMCOG 12 Months (96.9) as opposed to baseline mean (95.7). The paired sample t-test revealed that the mean difference of –1.2 (95% confidence interval (CI) –1.6 to -0.8) between the population mean CAMCOG Baseline score and CAMCOG 12 Months score was statistically significant (t_{454}=-5.349; *P*<0.001).

The histogram and box plot of CAMCOG 12 Months is displayed in Figure 3.2.1 and the distribution is normal. Hence, the univariate analysis was considered for further investigation.

3.2.2.1 Univariate analysis

The relationship between the dependent variable, CAMCOG 12 Months and each of the independent variables was examined using univariate statistical techniques. Pearson's correlation coefficient (*r)* and the age-adjusted (*r*) were used to assess the linear relationship between each continuous variable and CAMCOG 12 Months. The independent variables CAMCOG baseline, age, FSH and post-menopausal years were found to be significantly associated with CAMCOG 12 Months (*P*= <0.001; *P*=<0.001; *P*=0.027; *P*=0.021 respectively).

In addition, mean CAMCOG 12 Month scores were found for each categorical independent variable. No significant difference between groups was found for the following categorical independent variables: *APOE* ε4, *APOE* ε2, parity, statin use, hypertension, cardio-vascular event (CVS) and diabetes (type 2). Mean CAMCOG 12 Months scores between low, medium and high education groups were found to be significantly different (*P*=<0.001). Mean CAMCOG scores were also found to be significantly different (*P*=0.027) between individuals with or without depression.

3.2.2.2 Multiple Linear Regression Analysis

Multiple linear regression (MLR**)** was used to simultaneously examine the association between each independent variable. Again, three model-fitting strategies were used; stepwise forward, backward elimination and the full model approach. By consensus of the three model-fitting strategies, CAMCOG baseline, age and parity were found to be significant main effects for CAMCOG 12 Months.

Two significant interaction terms were found, CAMCOG Baseline*age and parity*age, which means the effect of CAMCOG Baseline on CAMCOG 12 Months and parity on CAMCOG 12 Months is age-dependent. The effect of CAMCOG Baseline and age on CAMCOG 12 Months is graphically presented in Figure 3.2.2, which assumes parity of 0-1 offspring (base category) and based on 10^{th}, 50^{th} and 90^{th} percentiles for CAMCOG Baseline (89, 96 and 102) and age (77, 80 and 84 years). Likewise, the effect of parity and age on CAMCOG 12 Months is graphically shown in Figure 3.2.3. This relatively unexpected effect of parity on CAMCOG 12 Months may, in part, be attributable to the influential effect of only two cases, ID 375 and 2233. In effect, in the absence of either one of these cases the independent variable, parity, is not significant in relation to CAMCOG 12 Months.

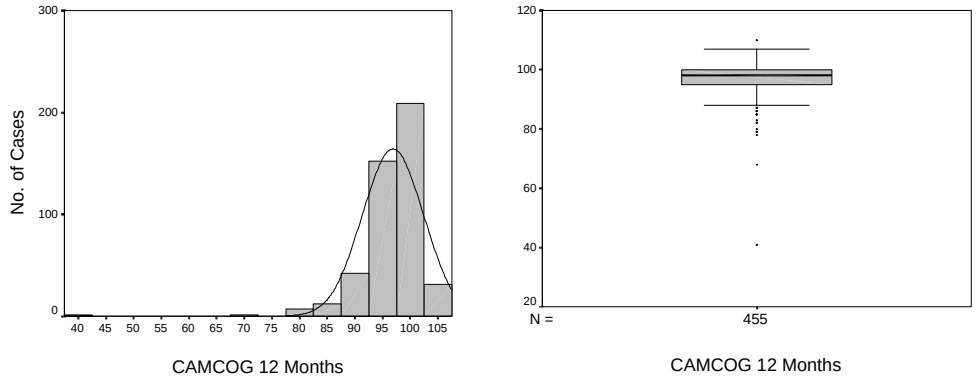
Overall Fit of the Model

The adjusted R^2 was 0.438, indicating that 43.8% of the variation in CAMCOG 12 Months scores is explained by CAMCOG Baseline, age and parity(2).

Model Diagnostics

The relatively random scatter of residuals shown in Figure 3.2.4 indicates that assumptions of linearity and homoscedasticity are met. Of note, four outliers with a standardised residual value of <-3 were identified from Figure 3.2.5. Case summaries of

the significant factors in the final MLR for these outliers are displayed in Table 3.2.6. With the exception of these four outliers, the assumption of normality is acceptable (Figure 3.2.6).



Total CAMCOG score = 106

Figure 3.2.1: Histogram and box plot of CAMCOG 12 Months.

The histogram and boxplot of the dependent variable CAMCOG 12 Months. Normality is acknowledged.

Variable	n	Min.	Max.	Mean	SD	Median	IQR
CAMCOG Baseline	455	67	106	95.7	5.4	96.0	6.0
CAMCOG 12 Months	455	41	106	96.9	5.5	98.0	5.0
CAMCOG Difference	455	-21	34	-1.2	4.7	-1.0	5.0

Table 3.2.1: Descriptive statistics for CAMCOG baseline and CAMCOG 12 Months.

Table showing the minimum, maximum, mean, standard deviation, median and inter-quartile range of the CAMCOG Baseline score, CAMCOG 12 Month score and CAMCOG Difference. The difference in mean scores between baseline and 12 Months is statistically significant ($P<0.001$).

Variable	n	*r*	*P* value	*Age-Adjusted r*	*P* value
CAMCOG Baseline	455	0.628	**<0.001**	0.614	<0.001
Age (years)	453	-0.231	**<0.001**	-	-
Aβ40 (pg/mol)	422	0.023	0.641	0.026	0.600
E2 (pmol/l)	453	-0.009	0.846	-0.017	0.723
LH (IU/l)	434	-0.055	0.254	-0.059	0.222
FSH (IU/l)	433	0.106	**0.027**	0.112	0.020
Menopause (years since)	431	-0.111	**0.021**	-0.024	0.613
BMI	435	-0.014	0.769	-0.028	0.563

Shading denotes significance ($P<0.05$)

Table 3.2.2: Linear relationship between CAMCOG 12 Months and continuous independent variables.

The linear correlations between CAMCOG 12 Months and continuous variables have revealed that only CAMCOG baseline score, age, FSH and menopause (years) were significant factors.

Variable	Levels	n	Mean (SD)	Test Statistic	df	*P*-value
***APOE* ε4**	**no**	346	97.0 (5.7)	t=0.758	431	0.449
	yes	87	96.5 (5.0)			
***APOE* ε2**	**no**	367	96.7 (5.7)	t=-1.059	431	0.290
	yes	66	97.5 (4.9)			
Education:	**low**	246	95.9 (6.1)	F=9.672	2,448	**<0.001**
	medium	141	98.1 (3.9)			
	high	64	98.2 (5.2)			
Parity:	**0-1**	69	96.3 (8.4)	F=0.482	2,451	0.618
	2-3	238	97.0 (4.7)			
	4+	147	96.9 (4.9)			
Statin	**no**	319	97.0 (5.5)	t=0.894	453	0.372
	yes	136	96.5 (5.4)			
Depression	**no**	419	97.0 (5.3)	t=2.214	453	**0.027**
	yes	36	94.9 (7.7)			
Hypertension	**no**	228	96.4 (6.2)	t=-1.865	453	0.063
	yes	227	97.3 (4.7)			
CVS event	**no**	425	96.8 (5.6)	t=-0.861	453	0.390
	yes	30	97.7 (4.1)			
Diabetes	**no**	408	97.1 (4.9)	t=1.443	49.095	0.155
	yes	47	95.1 (9.1)			

df= degrees of freedom

SD = standard deviation in brackets

Shading denotes significance ($P<0.05$)

Table 3.2.3: Mean CAMCOG 12 Months by categorical independent variables.

The mean values of the categorical variables by CAMCOG 12 Months score revealed significant differences between groups for education and depression.

Variable		Univariate *P*-value	Full model *P* value	Backward Elimination *P* value	Stepwise Forward *P* value
CAMCOG Baseline		**<0.001**	**<0.001**	**<0.001**	**<0.001**
Age (years)		**<0.001**	0.064	**0.001**	**0.001**
Education:	low	**<0.001**	-	-	-
	med	-	0.238		
	high	-	0.144		
Post-Menopause		**0.021**	0.466	-	-
FSH (IU/l)		**0.027**	0.774	-	-
Depression(2)		**0.027**	0.054	-	-
Hypertension(2)		0.063	0.103	-	-
Diabetes(2)		0.155	0.314	-	-
LH (IU/l)		0.254	0.449	-	-
***APOE* ε2**		0.290	0.119	-	-
Statin(2)		0.372	0.255	-	-
CVS event		0.390	0.102	-	-
***APOE* ε4**		0.449	0.603	-	-
Parity:	0-1	0.618	-	**0.030**[a]	**0.030**[a]
	2-3		**0.026**	-	-
	4+		0.123	-	-
Aβ40 (pg/mol)		0.641	0.590	-	-
BMI		0.769	0.146	-	-
E2 (pmol/l)		0.846	0.203	-	-

[a] 0-1 offspring (base category) vs ≥2 offspring

Table 3.2.4: MLR model- fitting strategies on CAMCOG 12 Months.

MLR model-fitting strategies including full model, stepwise forward and backward elimination were used to determine the relationships between the independent variables and CAMCOG 12 month score. CAMCOG baseline, age and parity were found to be significant variables on CAMCOG 12 Months score.

Variable	Unstandardised		
	β	std error	*P* value
Constant	512.679	101.929	<0.001
CAMCOG baseline	-3.803	1.053	<0.001
Age	-5.926	1.267	<0.001
Parity(2)[b]	-40.996	15.976	0.011
CAMCOG baseline*age	0.055	0.013	<0.001
Age*Parity(2)	0.524	0.198	0.009

[a] R^2=0.444, adjusted R^2=0.438, $F_{5,448}$=71.514, P<0.001

[b] 0-1 offspring (base category) vs ≥2 offspring

Table 3.2.5: Final MLR model on CAMCOG 12 Months[a]

The final MLR model revealed that the interaction terms between CAMCOG baseline and age as well as age and parity were significant factors on the CAMCOG 12 Months score.

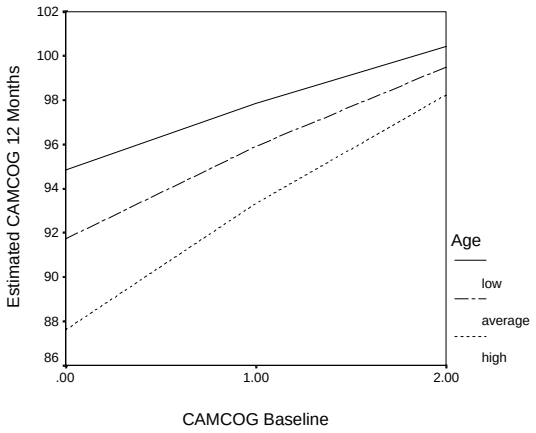


Figure 3.2.2: The effect of CAMCOG baseline and age on CAMCOG 12 Months*

* Assumes parity of 0-1 offspring (base category) and based on 10th, 50th and 90th percentiles for CAMCOG Baseline (89, 96 and 102) and Age (77, 80 and 84 years). A positive correlation exists between estimated CAMCOG 12 Months score and that of the CAMCOG Baseline score (P<0.001). This relationship is age dependent.

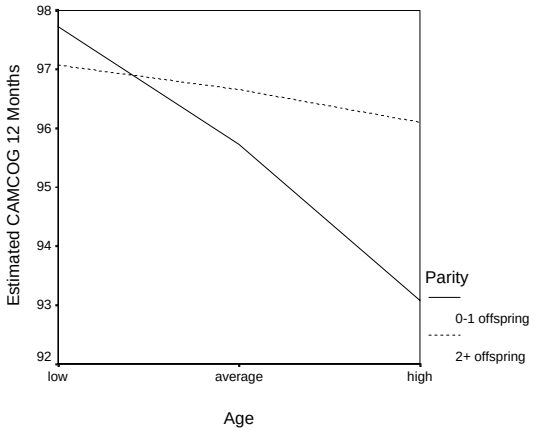


Figure 3.2.3: The effect of age and parity on CAMCOG 12 Months.
(Assumes 10th, 50th and 90th percentiles of age (77, 80 and 84 years))

Estimated CAMCOG 12 Month score decreased with increasing age. This parity dependent relationship is more pronounced in individuals with one or no births.

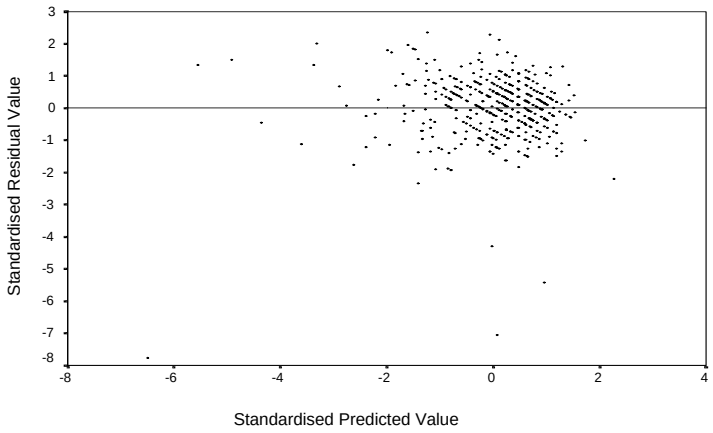


Figure 3.2.4: Scatterplot of standardised residuals vs. standardised predicted values.

To assess linearity and homoscedasticity, residuals were investigated. The random nature of the residuals show that these two assumptions are not violated. Based on a standardised residual of either <3 or <-3, four cases were identified as outliers.

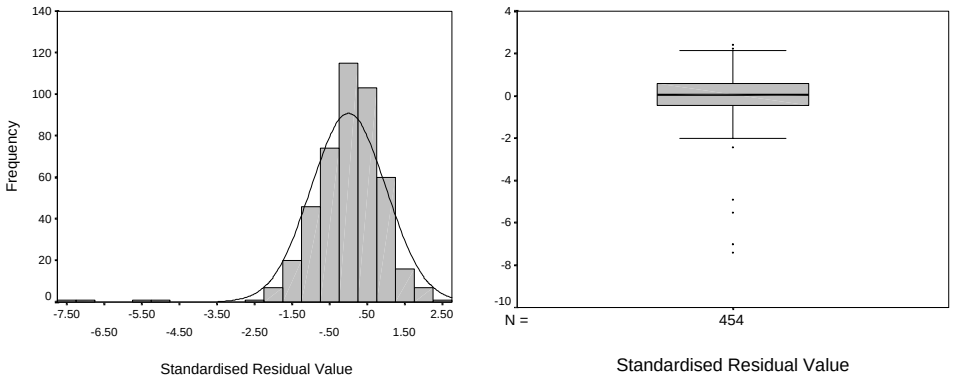


Figure 3.2.5: Histogram and boxplot of regression standardised residuals.

The histogram and boxplot show that normality is acknowledged.

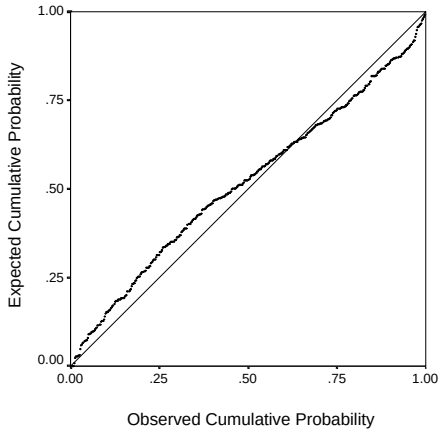


Figure 3.2.6: Normal P-P plot of regression standardised residuals.

The fit of the regression standardised residuals around the line suggests normality of the dependent variable.

ID	Standardised residual	CAMCOG 12 Months	CAMCOG Baseline	Age	Parity(2)
203	-5.422	78	102	79	0-1
375	-7.761	41	75	86	0-1
1192	-7.057	68	96	82	≥2
2233	-4.305	79	100	85	0-1

Table 3.2.6: Case summary of outlier

Four cases identified as outliers. Subsequent removal of cases ID 203 and ID 1192 from the MLR model did not change the statistical significance of the main effects. However removal of cases ID 375 and 2233 caused parity to become non-significant.

To ascertain the impact of each of the outliers detailed in Table 3.2.6, each was simultaneously excluded from the data set and the final MLR on CAMCOG 12 Months was reproduced. With the independent exclusion of ID 203 and 1192, the significance of the factors in the final model did not change, that is, converting from statistical significance to statistical non-significance. However, the independent exclusion of ID 375 and 2233 rendered parity non-significant, which demonstrates that these two cases had an influential effect on the significance of the independent variable, parity.

3.2.3 Dependent Variable (Y), CAMCOG Change

The difference between CAMCOG Baseline and CAMCOG 12 Months score was re-coded into a categorical variable and named CAMCOG Change. Out of the 455 individuals who undertook the follow-up CAMCOG test, 32.7% (n=149) declined in score from the original baseline CAMCOG result, 57.1% (n=260) improved and 10.1% (n=46) did not change after 12 months.

The association between CAMCOG Change and each categorical independent variable was investigated (Table 3.2.7). Education was the only variable to be significantly associated with CAMCOG Status (P=0.049), showing that individuals with a high level of education were less likely to experience a decline on the CAMCOG test 12 Months after baseline testing. All other categorical independent variables were not shown to be significantly associated with CAMCOG Change.

Mean differences between CAMCOG Change groups for each of the independent variables with a continuous format were examined (Table 3.2.8). The only variable to show significant mean differences between CAMCOG Change groups was CAMCOG Baseline (P<0.001). This suggests that the score received at baseline assessment could be used as an indication of future (12 Month) scores in cognition as measured by CAMCOG.

		Decline n (%)	No Change n (%)	Improvement n (%)	χ^2	df	*P* value
APOE ε4	**no**	202 (58.4)	37 (10.7)	107 (30.9)	2.855	2	0.240
	yes	45 (51.7)	7 (8.0)	35 (40.2)			
APOE ε**2**	**no**	208 (56.7)	39 (10.6)	120 (32.7)	0.577	2	0.750
	yes	39 (59.1)	5 (7.6)	22 (33.3)			
Parity	**0-1**	32 (46.4)	7 (10.1)	30 (43.5)	5.073	4	0.280
	2-3	143 (60.1)	22 (9.2)	73 (30.7)			
	4+	84 (57.1)	17 (11.6)	46 (31.30			
Education	**low**	149 (60.6)	23 (9.3)	74 (30.1)	9.518	4	**0.049**
	med	80 (56.7)	10 (7.1)	51 (36.2)			
	high	29 (45.3)	12 (18.8)	23 (35.9)			
Statin	**no**	180 (56.4)	32 (10.0)	107 (33.5)	0.308	2	0.857
	yes	80 (58.8)	14 (10.3)	42 (30.9)			
Depression	**no**	240 (57.3)	40 (9.5)	139 (33.2)	1.974	2	0.373
	yes	20 (55.6)	6 (16.7)	10 (27.8)			
Hypertension	**no**	126 (55.3)	22 (9.6)	80 (35.1)	1.143	2	0.565
	yes	134 (59.0)	24 (10.6)	69 (30.4)			
CVS	**no**	242 (56.9)	43 (10.1)	140 (32.9)	0.120	2	0.942
	yes	18 (60.0)	3 (10.0)	9 (30.0)			
Diabetes	**no**	231 (56.6)	41 (10.0)	136 (33.3)	0.619	2	0.734
	yes	29 (61.7)	5 (10.6)	13 (27.7)			

df = degrees of freedom

Shading denotes significance (*P*<0.05)

Table 3.2.7: Descriptive statistics of categorical independent variables by CAMCOG change.

The number and percentage of subjects who improved, declined or showed no improvement in the CAMCOG score over 12 months according to the categorical variables. Only education was shown to be significantly different between groups.

	Decline mean (SD)	No Change mean (SD)	Improvement mean (SD)	F value	df	*P* value
CAMCOG Baseline	93.9 (5.4)	97.4 (4.0)	98.3 (4.6)	38.822	2,452	**<0.001**
Age (years)	80.0 (2.6)	79.8 (2.2)	80.1 (2.7)	0.365	2,452	0.694
Aβ40 (pg/mol)	79.5 (43.1)	85.5 (45.0)	82.9 (39.7)	0.510	2,419	0.601
E2 (pmol/l)	30.2 (18.3)	28.2 (18.0)	27.2 (14.3)	1.521	2,450	0.220
LH (IU/l)	39.6 (22.2)	35.6 (17.8)	39.1 (21.1)	0.637	2,431	0.529
FSH (IU/l)	54.6 (22.0)	52.2 (23.0)	57.2 (26.0)	0.935	2,430	0.394
Menopause (years since)	26.6 (7.0)	26.9 (5.6)	26.1 (5.8)	0.389	2,428	0.678
BMI	27.1 (4.4)	26.6 (4.0)	26.9 (4.5)	0.234	2,432	0.791

[a] variables measured at baseline

Shading denotes significance ($P<0.05$)

Table 3.2.8: Descriptive statistics of continuous independent variables by CAMCOG Status.

The number and percentage of subjects who improved, declined or showed no improvement in CAMCOG score over 12 months according to the continuous variables. Only CAMCOG Baseline was shown to be significantly different between groups.

3.2.4 Participants who returned versus participants at baseline only

It was considered essential to investigate the characteristics of the participants who did not return for their follow-up CAMCOG assessment. From the 649 individuals who attended the baseline CAMCOG assessment, 194 subjects did not return for their follow-up. Table 3.2.9 shows the mean value of the continuous independent variables between participants who returned and those who only completed the baseline assessment. There were no statistically significant differences between participants and non-participants for each independent variable. In table 3.2.10, each categorical independent variable was investigated. Non-participants differed significantly from participants in terms of *APOE* ε4 status and the prevalence of depression and diabetes. In relation to *APOE* ε4 status, a significantly higher proportion of the non-participants were *APOE* ε4 positive, 27.6% vs. 20.1% (P=0.041). Non-participants had a higher prevalence of depression (16.0% vs. 7.9%, P=0.002) and a lower prevalence of diabetes (4.1% vs. 10.3%, P=0.009).

		n	Mean (SD)	t-statistic	df	*P* value
Age (years)	Participants returned	455	80.0 (2.6)	0.458	647	0.647
	Participants Baseline	194	80.1 (2.6)			
Menopause (years since)	Participants returned	431	26.5 (6.5)	1.018	612	0.309
	Participants Baseline	183	27.0 (6.1)			
FSH (IU/l)	Participants returned	433	55.2 (23.5)	-0.261	609	0.794
	Participants Baseline	178	54.7 (25.3)			
LH (IU/l)	Participants returned	434	39.0 (23.1)	0.998	613	0.319
	Participants Baseline	181	41.0 (21.4)			
E2 (pmol/l)	Participants returned	453	29.1 (17.1)	-0.094	643	0.925[b]
	Participants Baseline	192	28.0 (13.6)			
Aβ40 (pg/mol)	Participants returned	422	81.2 (42.2)	0.228	601	0.820
	Participants Baseline	181	82.1 (44.2)			
BMI	Participants returned	435	27.0 (4.4)	-0.551	617	0.582
	Participants Baseline	184	26.8 (4.0)			

[a] variables measured at baseline

[b] based on natural logarithm transformation of E2

df = degrees of freedom

Table 3.2.9: Participants returned versus participants at baseline only[a]; Continuous independent variables.

None of the continuous variables were found to be significantly different between participants returned and baseline participants.

		Participants returned n (%)	Participants at baseline only n (%)	χ^2	df	*P* value
***APOE* ε4**	**no**	346 (79.9%)	134 (72.4%)	4.176	1	**0.041**
	yes	87 (20.1%)	51 (27.6%)			
***APOE* ε2**	**no**	367 (84.8%)	156 (84.3%)	0.019	1	0.891
	yes	66 (15.2%)	29 (15.7%)			
Parity	**0-1**	69 (15.2%)	28 (14.4%)	1.373	2	0.503
	2-3	238 (52.4%)	94 (48.5%)			
	4+	147 (32.4%)	72 (37.1%)			
Education	**low**	246 (54.5%)	108 (56.0%)	0.117	2	0.943
	med	141 (31.1%)	58 (30.1%)			
	high	64 (14.2%)	27 (14.0%)			
Statin	**no**	319 (70.1%)	143 (73.7%)	0.860	1	0.354
	yes	136 (29.9%)	51 (26.3%)			
Depression	**no**	419 (92.1%)	163 (84.0%)	9.561	1	**0.002**
	yes	36 (7.9%)	31 (16.0%)			
Hypertension	**no**	228 (50.1%)	83 (42.8%)	2.925	1	0.087
	yes	227 (49.9%)	111 (57.2%)			
CVS	**no**	425 (93.4%)	181 (93.3%)	0.003	1	0.960
	yes	30 (6.6%)	13 (6.7%)			
Diabetes	**no**	408 (89.7%)	186 (95.9%)	6.153	1	**0.009**
	yes	47 (10.3%)	8 (4.1%)			

[a] variables measured at baseline

df = degrees of freedom

shading denotes significance

Table 3.2.10: Participants returned versus participants at baseline only[a]; Categorical independent variables.

Prevalence of diabetes and depression as well as *APOE* ε4 status were the only significant factors between participants who returned and participants tested at baseline only.

3.3 CVLT Baseline

3.3.1 General Observations

An analysis of the 'SUM1to5' parameter of the CVLT confirmed the role of age and education in cognition and memory. Multiple linear regression revealed that higher education was associated with higher learning rates over the 5 trials as measured by 'SUM1to 5' (P= 0.007). Increased age was related to lower scores on the parameter (P= 0.002). Also evident was a negative correlation between plasma levels of Aβ40 and 'SUM1to 5' score (P<0.001).

Analysis of the second parameter, the 'DISCRIM' measure, revealed five significant main factors. Age (P=0.001) and Aβ40 levels (P= 0.022) were again found to have negative correlations with discriminability. Education was discovered to be positively correlated with the 'DISCRIM' measure (P= 0.028). Interestingly, statin users were associated with higher scores on the 'DISCRIM' measure (P=0.021). Subjects with hypertension were associated with lower scores of discriminability (P=0.037). Analysis on the third and final parameter chosen from the CVLT, the 'FORGET' score, revealed no significant main factors on this dependent variable.

3.3.2 Descriptive Statistics

The CVLT, an assessment of verbal learning, can evaluate various components of cognition and memory. From the CVLT test format, 3 parameters were chosen to best assess early changes in cognition in elderly individuals. 'SUM1to5' parameter was calculated by the accumulative score of the first 5 trials in the CVLT. The 'DISCRIM' parameter was calculated by the addition of false positives and misses from the long delay recognition list over the total possibly correct. This was then converted to a percentage. The third dependent variable in the CVLT cohort was the 'FORGET' score and was defined as the difference between long term and short term free recall of list A.

These 3 parameters were used to investigate learning rates, delayed recognition and forgetting rates on newly learnt verbal material.

The CVLT baseline cohort consisted of 413 participants aged between 75 and 86. A set of independent variables, similar to the CAMCOG cohort, were investigated in the CVLT group. Of the 413 participants, a value for the variable, BMI, was missing for 120 subjects (29.1%) and a value for 'years since menopause' for 122 participants (29.5%) (Table 3.3.1). Consequently, these variables were not included in the multivariate data analysis.

Descriptive statistics for the independent variables at baseline are shown in Table 3.3.1. The 3 dependent variables 'SUM 1to5', 'DISCRIM' and the 'FORGET' parameters and their respective descriptive statistics are shown in Table 3.3.2.

Variable	n	Min.	Max.	Mean	SD	Median	IQR
Age (years)	413	75	86	80.2	2.7	80.0	4.0
E2 (pmol/l)	404	5	196	25.9	16.5	24.0	16.8
Aβ40 (pg/mol)	396	0.98	424.2	92.9	68.3	87.4	93.2
Menopause (years since)	291	12	53	27.4	6.8	26.0	8.0
BMI	293	17.4	41.8	27.1	4.4	26.6	5.8

SD = Standard Deviation

IQR = Inter-quartile range

Table 3.3.1: Descriptive statistics for independent variables

Minimum, maximum, mean, standard deviation, median and inter-quartile range for the independent variables of the CVLT cohort.

Variable	n	Min.	Max.	Mean	SD	Median	IQR
SUM1to5	413	17	74	42.0	11.1	41.0	15.0
DISCRIM	413	70.4	100.0	88.4	6.6	88.6	9.1
FORGET	413	-7	7	.56	1.92	1.00	3.00

SD = Standard Deviation

IQR = Inter-quartile range

Table 3.3.2: Descriptive statistics for dependent variables

Minimum, maximum, mean, standard deviation, median and inter-quartile range for the dependent variables SUM1to5, DISCRIM and FORGET.

3.3.3 Dependent Variable (Y), SUM1to5

The histogram and box plots of the 'Sum1to5' variable are shown in Figure 3.3.1. The data is defined as normal and allows for subsequent univariate and multiple linear regression analysis.

3.3.3.1 Univariate Analysis

The linear relationship between the dependent variable 'SUM 1to5' and the continuous independent variables are shown in Table 3.3.3. It is evident that both age (*P*=0.001) and the levels of Aβ40 (*P*=<0.001) were significantly associated with 'SUM1to5'. The levels of oestrogen were not found to be associated with the dependent variable. Table 3.3.4 show the linear relationships, with the categorical independent variables. *APOE* ε4, *APOE* ε2, parity, statin use, depression, CVS event and diabetes were not found to be significantly correlated to 'SUM1to5' variable. Only education (*P*=0.009) and hypertension (*P*=0.03) were found to be significant main factors affecting 'SUM 1to5' score.

3.3.3.2 Multiple Linear Regression Analysis (MLR)

Table 3.3.5 shows the multiple linear regression model fitting strategies for the SUM 1to5 variable. Using the 3 model fitting strategies as explained in Chapter 3.1 it was discovered that Aβ40 levels, age and education were significantly associated with 'SUM1to5'. Of note, however, when included in the final model *APOE* ε4 status and hypertension both had *P*-values of 0.069. Furthermore, when treated as main effects, no significant interactions terms were evident. As a consequence, *APOE* ε4 and hypertension were excluded from the final MLR on 'SUM1to5' (Table 3.3.6).

In the final multi-linear regression model, these 3 independent variables: Aβ40, age and education, were confirmed as significant main effects on 'SUM 1to5' (*P*=<0.001; *P*=

0.002; *P*= 0.007). This indicates that increasing levels of plasma Aβ (1-40) had a small but significant detrimental effect on learning rates over the 5 trials in the CVLT. Education had a protective effect, such that individuals with a high level of education tended to have higher learning rates in this assessment. In addition, age had a negative correlation with learning rates measured via 'SUM1to5'.

Model Diagnostics

The scatterplot of the regression residuals was used to assess the assumption of linearity and homoscedasticity (Figure 3.3.2). The relatively random scatter of points suggests that these two assumptions hold. The distribution of the standardised residuals is relatively normal, indicating that the assumption of normality is also met.

Only one individual had a standardised residual of either <3 or >3: ID 897, whose case summary of the significant main effects in the final MLR is presented in Table 3.3.7. This individual was excluded from the data set and the final MLR reproduced. There was no resultant change to the significance of the main effects in the final MLR model.

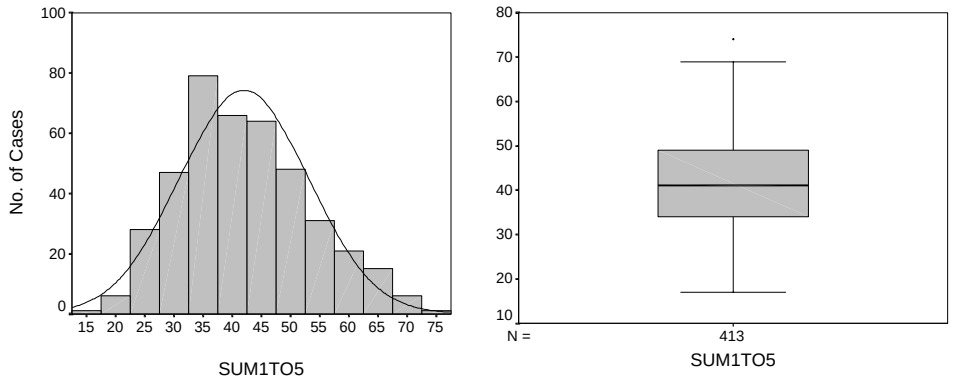


Figure 3.3.1: Histogram and boxplot of SUM1to5

SUM1to5 scores for 413 subjects showing a normal distribution.

Variable	n	*r*	*P* value	*age-adjusted r*	*P* value
age (years)	413	-0.163	**0.001**	-	-
E2 (pmol/l)	404	0.017	0.729	0.018	0.721
Aβ40 (pg/mol)	396	-0.236	**<0.001**	-0.233	<0.001

r = regression coefficient

Shading denotes significance ($P < 0.05$)

Table 3.3.3: Linear relationship between SUM1to5 and continuous independent variables

Age and Aβ40 levels were shown to have significant linear correlations with SUM1to5 score.

Variable		n	Mean (SD)	test statistic	df	*P*-value
***APOE* ε4**	**no**	315	42.5 (11.6)	t=1.645	168.9	0.102
	yes	85	40.6 (8.9)			
***APOE* ε2**	**no**	305	42.3 (11.1)	t=0.460	398	0.646
	yes	95	41.7 (11.0)			
education:	**low**	209	40.8 (10.3)	F=4.716	2,405	**0.009**
	medium	131	42.0 (12.0)			
	high	68	45.5 (10.8)			
parity:	**0-1**	64	40.6 (11.0)	F=2.075	3,409	0.103
	2-3	210	42.7 (11.0)			
	4-5	117	40.8 (10.9)			
	6+	22	46.1 (11.4)			
statin	**no**	298	42.2 (11.0)	t=0.623	411	0.543
	yes	115	41.5 (11.2)			
depression	**no**	381	42.0 (11.2)	t=-0.179	411	0.858
	yes	32	41.5 (11.2)			
hypertension	**no**	188	43.3 (11.5)	t=2.175	411	**0.030**
	yes	225	40.9 (10.6)			
CVS event	**no**	383	41.9 (11.0)	t=-0.561	411	0.575
	yes	30	43.1 (12.1)			
diabetes	**no**	381	41.9 (11.0)	t=-0.395	411	0.693
	yes	32	42.8 (11.5)			

SD = Standard Deviation in brackets

df = degrees of freedom

Shading denotes significance ($P < 0.05$)

Table 3.3.4: Mean SUM1to5 scores by categorical independent variables

Mean and standard deviation values are shown for each categorical variable. Only education and hypertension was found to be significantly different between each level.

Variable		univariate *P*-value	full model *P* value	backward elimination *P* value	stepwise forward *P* value
Aβ40 (pg/mol)		**<0.001**	**<0.001**	**<0.001**	**<0.001**
age		**0.001**	**0.002**	**0.002**	**0.002**
education(3)	low	**0.009**	**-**	**0.007[a]**	**0.007[a]**
	med	**-**	0.409		
	high	**-**	**0.006**		
hypertension(2)		**0.030**	0.123		
APOE ε4(2)		0.102	0.057		
parity(4):	0-1	0.103	-		
	2-3	-	0.257		
	4-5	-	0.661		
	6+	-	0.221		
statin(2)		0.543	0.423		
CVS event(2)		0.575	0.388		
APOE ε2(2)		0.646	0.642		
diabetes(2)		0.693	0.934		
E2 (pmol/l)		0.729	0.453		
depression(2)		0.858	0.774		

Shading denotes significance ($P < 0.05$)

[a] low/medium vs high

Table 3.3.5: MLR model-fitting strategies on SUM1to5.

MLR model-fitting strategies including full model, stepwise forward and backward elimination were used to determine the relationships between the independent variables and SUM1to5 score. Aβ40 levels, age and education were found to be significant variables on SUM1to5 score.

Variable	unstandardised		
	β	std error	*P* value
constant	95.663	16.092	<0.001
Aβ40	-0.036	0.008	<0.001
age	-0.636	0.201	0.002
education(2)[b]	3.824	1.416	0.007

a

R^2=0.096, adjusted R^2=0.089, $F_{3,388}$=13.683, *P*<0.001

b low/medium vs high

Shading denotes significance

Table 3.3.6: Final MLR model on SUM1to5[a]

Aβ40 levels, age and education were found to be significantly associated with SUM1to5 score in the CVLT cohort.

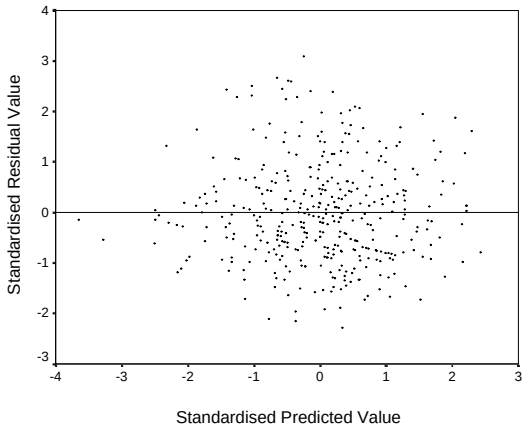


Figure 3.3.2: Scatterplot of regression standardised residuals vs standardised predicted values.

To assess linearity and homoscedasticity, residuals were investigated. Based on a standardised residual of either ≥ 3 or ≤ -3, one case was identified as an outlier.

ID	standardised residual	SUM1to5	Aβ40	age	education
897	3.097	74	135.4	78	low/medium

Table 3.3.7: Case summary of outlier.

Standardised >3 thus ID 897 was deemed an outlier. Therefore, ID 897 was excluded from the final MLR with no resultant change in significance for the main effects.

3.3.4 Dependent Variable (Y), DISCRIM

3.3.4.1 Univariate Analysis

The linear relationship between continuous independent variables, age, E2 and Aβ40 is shown in Table 3.3.8. Both age (*P*=0.002) and Aβ40 (*P*=0.019) levels were found to have negative correlations with the 'DISCRIM' variable. E2 was not a significant factor. The linear relationships between the categorical independent variables and 'DISCRIM' variable is shown in Table 3.3.9 and it is evident that only statin use was associated with 'DISCRIM' score. Statin users had a higher mean 'DISCRIM' score than individuals who did not use statin (*P*=0.026).

3.3.4.2 Multi-linear Regression Analysis

Based on the consensus of all three model fitting strategies (Table 3.3.10), age, Aβ40, statin, hypertension and education were all shown to be significant main effects (*P*<0.05). All biologically relevant cross-products of these main effects were examined, and none were found to be significant. The final MLR on 'DISCRIM' is shown in Table 3.3.11. An increase in age was shown to result in a decrease in 'DISCRIM' score (*P*=0.001). Increased levels of plasma Aβ40 also resulted in lower 'DISCRIM' scores (P=.0.022). Subjects with hypertension were associated with lower scores (*P*=0.037). In addition, higher education was associated with higher 'DISCRIM' scores (*P*=0.028).

Model Diagnostics

An examination of the assumptions of MLR was undertaken. The scatterplot of regression standardised residuals vs. standardised predicted values in Figure 3.3.4 suggests that the assumption of homoscedasticity is not without question, as the variance of the residuals varies with the predicted scores. The assumption of normality is met and based on the standardised residuals no outliers were identified.

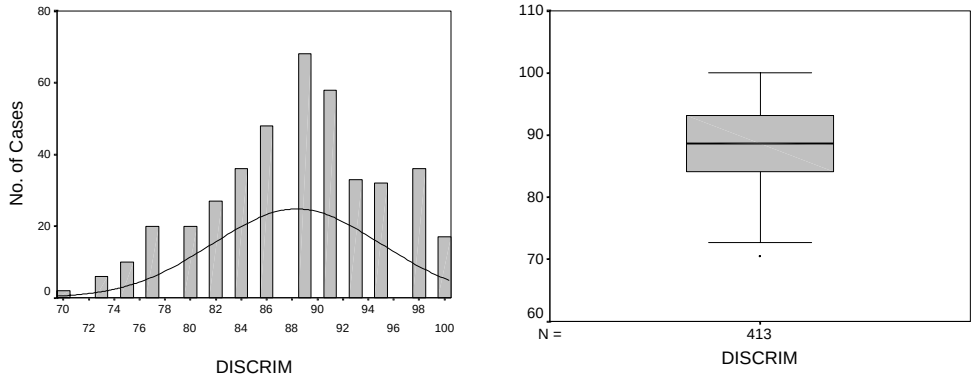


Figure 3.3.3: Histogram and boxplot of the DISCRIM variable

DISCRIM scores for 413 subjects of the CVLT cohort showing a normal distribution.

Variable	**n**	***r***	***P* value**	***age-adjusted r***	***P* value**
age (years)	413	-0.152	**0.002**	-	-
E2 (pmol/l)	404	-0.008	0.880	-0.007	0.882
Aβ40 (pg/mol)	396	-0.118	**0.019**	-0.113	0.025

r = regression coefficient

shading denotes significance (P<0.05)

Table 3.3.8: Linear relationship between DISCRIM and continuous independent variables

Age and Aβ40 levels were shown to have significant linear correlations with DISCRIM score.

Variable	levels	n	Mean (SD)	test statistic	df	*P*-value
***APOE* ε4**	**no**	315	88.3 (6.6)	t=-0.185	398	0.854
	yes	85				
***APOE* ε2**	**no**	305	88.5 (6.7)	t=0.945	398	0.345
	yes	95	87.8 (6.1)			
education:	**low**	209	88.0 (6.8)	F=2.519	2,405	0.082
	medium	131	88.0 (6.7)			
	high	68	90.0 (5.5)			
parity:	**0-1**	64	87.6 (6.7)	F=0.521	3,409	0.668
	2-3	210	88.4 (6.8)			
	4-5	117	88.8 (6.3)			
	6+	22	87.8 (7.3)			
statin	**no**	298	87.9 (6.7)	t=-2.227	411	**0.026**
	yes	115	89.5 (6.3)			
depression	**no**	381	88.2 (6.7)	t=-1.700	411	0.090
	yes	32	90.3 (5.7)			
hypertension	**no**	188	89.0 (6.4)	t=1.722	411	0.086
	yes	225	87.8 (6.8)			
CVS event	**no**	383	88.3 (6.6)	t=-0.431	411	0.667
	yes	30	88.9 (6.5)			
diabetes	**no**	381	88.2 (6.6)	t=-1.317	411	0.188
	yes	32	89.8 (6.4)			

SD = standard deviation

df = degrees of freedom

shading denotes significance (P<0.05)

Table 3.3.9: Mean DISCRIM scores by categorical independent variables.

Mean DISCRIM scores between groups for each categorical variable. Only statin use was found to be significantly different between groups in DISCRIM score.

Variable		univariate *P*-value	full model *P* value	backward elimination *P* value	stepwise forward *P* value
age		**0.002**	**0.003**	**0.001**	**0.001**
Aβ40 (pg/mol)		**0.019**	**0.021**	**0.022**	**0.022**
statin(2)		**0.026**	0.083	**0.021**	**0.021**
education(3)	low	0.082	**-**	**0.028**[a]	**0.028**[a]
	med	**-**	0.958	-	-
	high	**-**	**0.042**	-	-
hypertension(2)		0.086	0.053	**0.037**	**0.037**
depression(2)		0.090	0.086	-	-
diabetes(2)		0.188	0.459	-	-
***APOE ε2*(2)**		0.345	0.556	-	-
CVS event(2)		0.667	0.786	-	-
parity(4):	0-1	0.668	-	-	-
	2-3	-	0.196	-	-
	4-5	-	0.153	-	-
	6+	-	0.721	-	-
***APOE* ε4(2)**		0.345	0.677	-	-
E2 (pmol/l)		0.880	0.659	-	-

[a] low/medium vs high

shading denotes significance (P<0.05)

Table 3.3.10: MLR model-fitting strategies on DISCRIM.

MLR model-fitting strategies including full model, stepwise forward and backward elimination were used to determine the relationships between the independent variables and DISCRIM score. Age, Aβ40 levels, statin use and education were found to be significant variables on DISCRIM score.

Variable	unstandardised		
	β	std error	*P* value
constant	121.915	9.723	<0.001
Age (years)	-0.407	0.121	0.001
Aβ40	-0.011	0.005	0.022
statin(2)	1.710	0.735	0.021
hypertension(2)	-1.376	0.656	0.037
education(2)[b]	1.883	0.851	0.028

[a] R^2=0.080, adjusted R^2=0.068, $F_{5,386}$=6.729, P<0.001

[b] low/medium vs high

Table 3.3.11: Final MLR model on DISCRIM[a]

Age, Aβ40 levels, statin use, hypertension and education were revealed to be significantly associated with DISCRIM score.

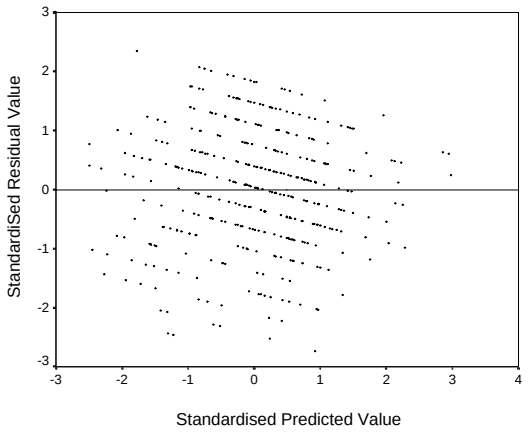


Figure 3.3.4: Scatterplot of regression standardised residuals vs. standardised predicted values

To assess linearity and homoscedasticity, residuals were investigated. The random nature of the residuals show that these two assumptions are not violated. Based on a standardised residual of either <3 or <-3, no cases were identified as outliers.

3.3.4.3 Univariate Analysis/ Multiple Linear Regression Analysis

The distribution of the dependent variable 'FORGET' is shown in figure 3.3.5. The linear relationships between the continuous independent variables, age, oestrogen, Aβ40 and the dependent variable 'FORGET' are shown in Table 3.3.12. No significant main effects on the 'FORGET' score were discovered. Table 3.3.13 illustrates the mean 'FORGET' scores between the categorical independent variables. Again, no significant main effects on 'FORGET' score were found. Multiple linear regression modelling revealed that none of the independent variables examined were of statistical significance in relation to 'FORGET' (Table 3.3.14).

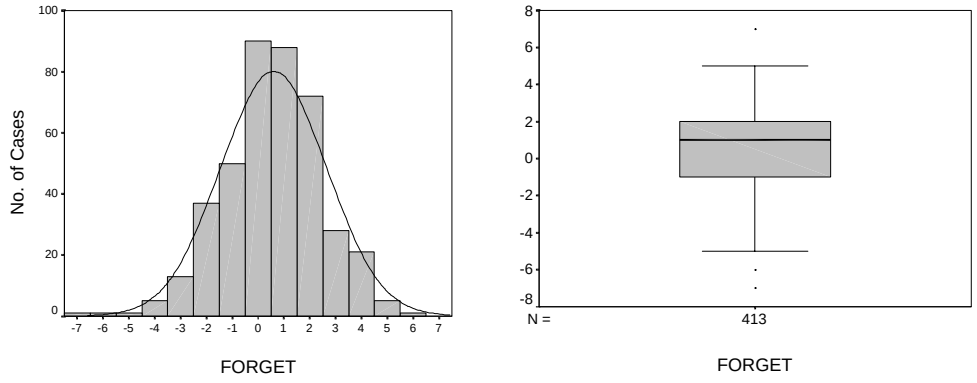


Figure 3.3.5: Histogram and boxplot of the FORGET dependent variable

FORGET scores for 413 subjects of the CVLT cohort showing a normal distribution

Variable	**n**	***r***	***P* value**	***age-adjusted r***	***P* value**
age (years)	413	0.028	0.575	-	-
E2 (pmol/l)	404	-0.013	0.802	-0.013	0.801
Aβ40 (pgm/mol)	396	0.039	0.436	0.038	0.446

r = regression coefficient

Table 3.3.12: Linear relationship between FORGET and continuous independent variables

No linear associations were discovered for the continuous independent variables.

Variable	levels	n	Mean (SD)	test statistic	df	*P*-value
***APOE* ε4**	**no**	315	0.54 (1.94)	t=-0.293	398	0.770
	yes	85	0.61 (1.88)			
***APOE* ε2**	**no**	305	0.52 (1.92)	t=-0.735	398	0.463
	yes	95	0.68 (1.93)			
education:	**low**	209	0.66 (2.02)	F=1.069	2,405	0.344
	medium	131	0.36 (1.84)			
	high	68	0.64 (1.78)			
parity:	**0-1**	64	0.31 (1.65)	F=0.525	3,409	0.665
	2-3	210	0.60 (1.97)			
	4-5	117	0.66 (1.99)			
	6+	22	0.41 (1.71)			
statin	**no**	298	0.49 (1.95)	t=-1.185	411	0.237
	yes	115	0.74 (1.83)			
depression	**no**	381	0.53 (1.92)	t=-1.039	411	0.287
	yes	32	0.91 (1.89)			
hypertension	**no**	188	0.45 (1.83)	t=-1.039	411	0.299
	yes	225	0.65 (1.98)			
CVS event	**no**	383	0.59 (1.89)	t=1.066	411	0.287
	yes	30	0.20 (2.19)			
diabetes	**no**	381	0.55 (1.93)	t=-0.202	411	0.840
	yes	32	0.63 (1.81)			

df = degrees of freedom

SD= standard deviation

Table 3.3.13: Mean FORGET scores by categorical independent variables

No significant associations were found for the categorical variables of the 'FORGET' score.

Variable		univariate *P*-value	full model *P* value	backward elimination *P* value	stepwise forward *P* value
statin(2)		0.237	0.266	-	-
depression(2)		0.287	0.318	-	-
CVS event(2)		0.287	0.498	-	-
hypertension		0.299	0.397	-	-
education(3):	low	0.344	-	-	-
	med	-	0.103	-	-
	high	-	0.998	-	-
Aβ40 (pg/mol)		0.436	0.443	-	-
***APOE* ε2**		0.463	0.393	-	-
age		0.575	0.596	-	-
parity(4):	0-1	0.665	-	-	-
	2-3	-	0.102	-	-
	4-5	-	0.263	-	-
	6+	-	0.885	-	-
***APOE* ε4**		0.770	0.929	-	-
E2 (pmol/l)		0.802	0.661	-	-
diabetes(2)		0.840	0.874	-	-

Table 3.3.14: MLR model-fitting strategies on FORGET.

No significant main factors were found to influence the dependent variable 'Forget' score during MLR analysis.

3.4 CVLT 12 MONTHS

3.4.1 General Observations

Multiple linear regression analysis of the 'SUM1to5 12 month' parameter revealed that only the baseline score of learning rate was a significant main factor in the final model ($P<0.001$). Interestingly, approximately 59% of the participants that returned for their follow-up assessment improved in their 'SUM1to5' score. Analysis of the 'DISCRIM 12 month' parameter again revealed that only the baseline result was significantly associated with the score received during the follow-up test ($P<0.001$). Approximately 53% of the returned subjects deteriorated in their ability for delayed recognition (discriminability) in the CVLT over the 12 months. None of the independent variables were found to be associated with the 'FORGET 12 Month' result. Around 49% of the returned subjects recorded a reduced 'FORGET' score when compared to baseline results (improved ability to remember).

An analysis performed on subjects who returned for their follow-up assessment as opposed to those that only participated in the baseline test of the CVLT found that BMI status and Aβ-40 levels were significantly different between groups. Interestingly, non-returnees had higher mean plasma levels of Aβ-40 than subjects who completed their follow-up test ($P = 0.01$). Non-returnees also had a higher BMI than individuals who returned 12 months later ($P=0.037$).

3.4.2 Descriptive Statistics

Of the 413 participants who completed the first CVLT test (CVLT Baseline) and were included in the former data analysis on CVLT, 212 (53.1%) undertook a second CVLT test approximately 12 months later (CVLT 12 Months). A similar set of independent variables were investigated to find any notable associations, as well as identify any changes in cognition over 12 months. The 3 parameters utilised from the CVLT baseline data were again employed for the follow-up assessment analysis.

3.4.3 Dependent Variable, SUM1to5 12 Months (Y): Learning Rates

Descriptive statistics for the 212 participants who completed the 'SUM1to5' test at both baseline (Sum1to5 Baseline) and 12 months (Sum1to5 12 Months) are detailed in Table 3.4.1. The paired sample t-test revealed that the mean difference of –3.9 (95% CI –2.3 to –5.4) between the population mean Sum1to5 score at baseline and that at 12 months was statistically significant (t_{211}=-4.972; P<0.001). In essence, mean scores for SUM1to5 improved significantly over the 12 month interval.

The distribution of 'SUM1to5 12 Months' (n=212) is displayed in Figure 3.4.1. The significance of the variables measured at baseline on 'SUM1to5 Months' will be examined using multiple linear regression analysis (MLR). For this purpose, the distribution of 'Sum1to5 12 Months' fulfils the assumption of normality.

3.4.3.1 Independent Variables for SUM 1to5 12 months

Descriptive statistics for the independent variables with a continuous data format are presented in Table 3.4.2.

3.4.3.2 Univariate Analysis

The linear relationships between the continuous independent variables and the independent variable 'SUM1to5 12 Months' has been shown in table 3.4.3. The baseline measure of 'SUM 1to5' and the number of years post-menopause are the only variables that are significantly associated with the dependent variable ($P < 0.001$ and $P = 0.035$ respectively). This indicates that the higher the baseline score for learning rates over the 5 trials the higher the 12 month score. It also suggests that as the number of post-menopausal years increase learning rates in the CVLT are correspondingly reduced. Table 3.4.4 illustrates the linear relationships between the chosen categorical variables and the dependent variable 'SUM1to5'. None of the variables were found to be significantly associated with 12 month learning rates in the CVLT.

Variable	n	Min.	Max.	Mean	SD	Median	IQR
Sum1to5 Baseline	212	17	74	42.6	11.0	42.0	14.8
Sum1to5 12 Months	212	16	80	46.5	11.6	46.0	15.0
Sum1to5 Difference	212	-32	24	-3.9	11.3	-4.0	16.0

SD = standard devaition

IQR = Inter-quartile range

Table 3.4.1: Descriptive statistics for SUM1to5 Baseline and 12 Months.

Table showing the minimum, maximum, mean, standard deviation, median and inter-quartile range of the SUM1to5 Baseline score, SUM1to5 12 Month score and SUM1to5 difference. The difference in mean scores between Baseline and 12 Months is statistically significant ($P<0.001$). Mean scores for 'SUM1to5' improved significantly over the 12 months.

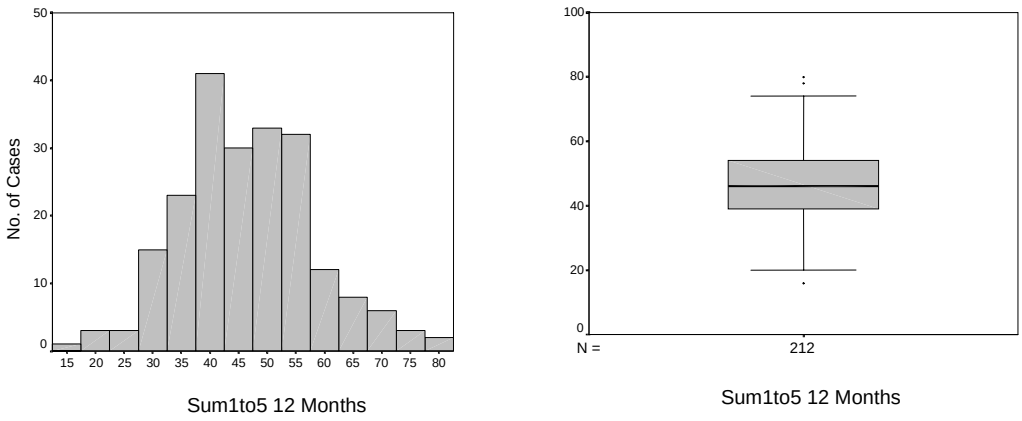


Figure 3.4.1: Histogram and boxplot of SUM 1to5 12 months.

The distribution of the 'SUM1to5 12 Months' score for the CVLT follow-up analysis. Normality is acknowledged.

Variable	n	Min.	Max.	Mean	SD	Median	IQR
Age (years)	212	75	86	80.0	2.7	79.0	4.0
E2 (pmol/l)	210	5.0	99.0	26.0	14.6	24.0	16.0
Aβ40 (pg/mol)	205	1.0	315.3	83.0	61.8	79.4	91.3
Menopause (years since)	152	12	53	27.0	6.6	25.0	8.0
BMI	153	18.1	39.8	27.7	4.5	26.9	5.8

[a] all independent variables were measured at baseline

SD = standard deviation

IQR = inter-quartile range

Table 3.4.2: Descriptive statistics for independent variables[a]

Minimum, maximum, mean, standard deviation, median and inter-quartile range of the independent variables for SUM1to5 12 months.

Variable	n	*r*	*P* value	*age-adjusted r*	*P* value
SUM1to5 Baseline	212	0.500	**<0.001**	0.498	**<0.001**
Age (years)	212	-0.055	0.427	-	-
Post-Menopause (years)	152	-0.193	**0.017**	-0.172	**0.035**
E2 (pmol/l)	210	0.040	0.568	0.042	0.550
Aβ40 (pg/mol)	205	-0.026	0.712	-0.028	0.695
BMI	153	-0.107	0.187	-0.125	0.126

Shading denotes significance

Table 3.4.3: Linear relationship between Sum1to5 12 Months and continuous independent variables.

The 'SUM1to5' Baseline score and Post-Menopause years were the only variables to be significantly associated with 'SUM1to5 12 Month' result.

Variable	Levels	n	Mean (SD)	Test Statistic	df	*P*-value
***APOE* ε4**	**no**	157	47.1 (11.9)	t=1.086	203	0.279
	yes	48	45.0 (10.5)			
***APOE* ε2**	**no**	159	46.5 (11.5)	t=-0.255	203	0.799
	yes	46	47.0 (12.0)			
Education:	**low**	108	46.2 (12.1)	F=0.071	2,206	0.931
	medium	70	46.9 (11.6)			
	high	31	46.5 (10.1)			
Parity:	**0-1**	31	44.3 (10.8)	F=0.679	2,209	0.508
	2-3	113	46.6 (11.9)			
	4+	68	47.2 (11.5)			
Statin	**no**	148	47.0 (11.3)	t=1.044	210	0.298
	yes	64	45.2 (12.3)			
Depression	**no**	197	46.5 (11.7)	t=0.028	210	0.978
	yes	15	46.4 (10.1)			
Hypertension	**no**	94	47.1 (11.2)	t=0.724	210	0.470
	yes	118	46.0 (11.9)			
CVS event	**no**	198	46.7 (11.7)	t=1.212	210	0.227
	yes	14	42.9 (8.4)			
Diabetes	**no**	191	46.3 (11.5)	t=-0.632	210	0.528
	yes	21	48.0 (12.5)			

SD = Standard Deviation in brackets

df = degrees of freedom

Table 3.4.4 : Mean Sum1to5 12 Months by categorical independent variables.

Mean and standard deviation values are shown for each categorical variable. No significant results were found.

3.4.3.3 Multiple Linear Regression, Sum1to5 12 Months (Y)

Multiple linear regression modelling was used to investigate the effect of independent variables, collected at Baseline, on 'SUM1to5 12 Months'. 'Sum1to5' Baseline was also included as an independent variable in these investigations, so as to control for the initial 'Sum1to5' score obtained at baseline. As detailed previously, three model fitting strategies were utilised (Table 3.4.5) to obtain a final model by consensus (Table 3.4.6). The independent variables, 'Post-menopause years' and 'BMI', were not examined in the regression analysis due to missing values for 28.3% and 27.8% of participants, respectively.

The results from Table 3.4.5 indicates that only the baseline score for 'SUM1to5' was found to be a significant factor influencing the 12 month assessment score and the final model is shown in Table 3.4.6 ($P<0.001$).

3.4.3.4 Dependent Variable (Y), Sum1to5 Change

'Sum1to5' Change was recoded into a categorical variable to reflect the overall difference in 'Sum1to5' Baseline and 'Sum1to5 12 Months'. After the 12 month interval a majority of the subjects who returned for their follow- up assessment improved in their learning rates (n=124, 58.5%). Approximately 38 % of subjects declined in learning rates after 12 months (n= 81) and about 3% stayed stable (n=7). Table 3.4.7 shows the descriptive statistics of the continuous independent variables by 'SUM 1to5' change. Only 'SUM1to5' baseline and Aβ40 levels were found to be significantly different between individuals who improved or stayed constant and those that had their 'SUM 1to 5' scores decline after the 12 month interval. Table 3.4.8 demonstrate the differences between improvers plus no change subjects and those that declined in 'SUM1to5' for each categorical variable. None were found to be significantly dissimilar.

3.4.4 Dependent Variable, DISCRIM 12 Months (Y)

Descriptive statistics for the 212 participants who completed the 'DISCRIM' test at both baseline (DISCRIM Baseline) and 12 months (DISCRIM 12 Months) are detailed in Table 3.4.9. The paired sample t-test revealed that the mean difference of 0.01 (95% CI –1.1 to 1.1) between the population mean 'DISCRIM' score at baseline and that at 12 months was statistically significant (t_{211}=-0.019; P=0.985). In essence mean scores of the 'DISCRIM' variable were identical from baseline to the follow-up CVLT assessment. The distribution of 'DISCRIM 12 Months' (n=212) is displayed in Figure 3.4.2. The significance of the variables measured at baseline on 'DISCRIM 12 Months' will be examined using multiple linear regression analysis. For this purpose, the distribution of 'DISCRIM 12 Months' fulfils the assumption of normality

3.4.4.1 Univariate Analysis

The linear relationship between the continuous independent variables and the dependent variable 'DISCRIM 12 months' is demonstrated in Table 3.4.10. Only 'DISCRIM' baseline score and Aβ40 levels were found to be significantly associated with 'DISCRIM 12 months' (P< 0.001 and P = 0.036 respectively). Again baseline scores accurately predicted how well an individual would perform for their follow-up assessment. Interestingly as Aβ40 levels increased 'DISCRIM' 12 month scores declined. Table 3.4.11 shows the linear relationships between categorical independent variables and 'DISCRIM 12 months'. No significant differences were found between the groups.

3.4.4.2 Multiple Linear Regression, DISCRIM 12 Months (Y)

Multiple linear regression modelling was used to investigate the effect of independent variables, collected at baseline, on 'DISCRIM 12 Months'. 'DISCRIM' Baseline was also included as an independent variable in these investigations, so as to control for the

initial ‘DISCRIM’ score obtained at baseline. The independent variables, ‘Post Menopause years’ and ‘BMI’, were not examined in the regression analysis due to missing values for 28.3% and 27.8% of participants, respectively. The final MLR on ‘DISCRIM 12 Months’ is presented in Table 3.4.12. As shown, only ‘DISCRIM’ Baseline was the only variable to be significant ($P<0.001$) in relation to ‘DISCRIM 12 Months’. The only factor that was significantly associated with discrimination rates of the CVLT at 12 months was the baseline discrimination rate.

Variable		Univariate *P*-value	Full Model *P* value	Backward Elmination *P* value	Stepwise Forward *P* value
Sum1to5 Baseline		**<0.001**	**<0.001**	**<0.001**	**<0.001**
CVS(2)		0.227	**0.031**	-	-
***APOE* ε4(2)**		0.279	0.766	-	-
Statin(2)		0.298	0.403	-	-
Age (years)		0.427	0.555	-	-
Hypertension(2)		0.470	0.503	-	-
Parity(3):	0-1	0.508	-	-	-
	2-3		0.950		
	4+		0.534		
Diabetes(2)		0.528	0.422	-	-
E2 (pmol/l)		0.568	0.828	-	-
Aβ40 (pg/mol)		0.695	0.214	-	-
***APOE* ε2(2)**		0.799	0.613	-	-
Education(3)	low	0.931	-	-	-
	med		0.711		
	high		0.356		
Depression(2)		0.978	0.843	-	-

Shading denotes significance ($P < 0.05$)

Table 3.4.5: MLR model-fitting strategies on Sum1to5 12 Months.

MLR model-fitting strategies including full model, stepwise forward and backward elimination were used to determine the relationships between the independent variables and SUM1to5 12 Months.

Variable	Unstandardised		*P* value
	β	std error	
Constant	23.949	2.779	<0.001
Sum1to5 Baseline	0.529	0.063	<0.001

[a] R^2=0.250,adjusted R^2=0.247, $F_{1,210}$=70.087, P<0.001

Table 3.4.6: Final MLR model on Sum1to5 12 Months[a]

Only SUM1to5 Baseline score was found to be significantly associated with the score at 12 months.

	Decline mean (SD)	No Change or Improvement mean (SD)	t-statistic	df	*P* value
Sum1to5 Baseline	48.1 (10.6)	39.2 (9.8)	6.216	210	**<0.001**
Age (years)	79.7 (2.6)	80.0 (2.8)	-0.790	210	0.430
Aβ40 (pg/mol)	66.5 (51.3)	93.4 (65.6)	-3.098	203	**0.002**
E2 (pmol/l)	25.7 (14.2)	26.2 (14.9)	-0.255	208	0.799
menopause (years since)	28.2 (7.0)	26.3 (6.3)	1.734	150	0.085
BMI	28.2 (4.5)	27.3 (4.4)	1.262	151	0.209

[a] variables measured at baseline

df = degrees of freedom

SD= standard deviation in brackets

Table 3.4.7: Descriptive statistics of continuous independent variables by Sum1to5 change.

The independent variables SUM1to5 Baseline and Aβ40 levels were found to be significantly different between those that declined and those who improved or did not change.

		Decline n (%)	No Change or Improvement n (%)	χ^2	df	*P* value
***APOE* ε4**	**no**	59 (37.6%)	98 (62.4%)	0.259	1	0.611
	yes	20 (41.7%)	28 (58.3%)			
***APOE* ε2**	**no**	62 (39.0%)	97 (61.0%)	0.063	1	0.803
	yes	12 (37.0%)	29 (63.0%)			
Parity	**0-1**	12 (38.7%)	19 (61.3%)	0.120	2	0.942
	2-3	42 (37.2%)	71 (62.8%)			
	4+	27 (39.7%)	41 (60.3%)			
Education	**low**	38 (35.2%)	70 (64.8%)	0.907	2	0.635
	med	29 (41.4%)	41 (58.6%)			
	high	13 (41.9%)	18 (58.1%)			
Statin	**no**	51 (34.5%)	97 (65.6%)	2.917	1	0.088
	yes	30 (46.9%)	34 (53.1%)			
Depression	**no**	75 (38.1%)	122 (61.9%)	0.022	1	0.882
	yes	6 (40.0%)	9 (60.0%)			
Hypertension	**no**	37 (39.4%)	57 (60.6%)	0.095	1	0.758
	yes	44 (37.3%)	74 (62.7%)			
CVS	**no**	73 (36.9%)	125 (63.1%)	2.276	1	0.131
	yes	8 (57.1%)	6 (42.9%)			
Diabetes	**no**	69 (36.1%)	122 (63.9%)	3.540	1	0.060
	yes	12 (57.1%)	9 (42.9%)			

df= degrees of freedom

Table 3.4.8: Descriptive statistics of categorical independent variables by Sum1to5 change.

No significant differences of the categorical variables between groups by SUM1to5 Change.

Variable	n	Min.	Max.	Mean	SD	Median	IQR
DISCRIM Baseline	212	70.4	100	88.9	6.5	88.6	9.1
DISCRIM 12 Months	212	61.4	100	88.9	7.2	88.6	11.4
DISCRIM Difference	212	-22.7	22.7	0.01	8.2	0.00	10.8

Table 3.4.9: Descriptive statistics for DISCRIM baseline and 12 months.

Table showing the minimum, maximum, mean, standard deviation, median and inter-quartile range of the DISCRIM Baseline score, DISCRIM 12 Month score and DISCRIM Difference.

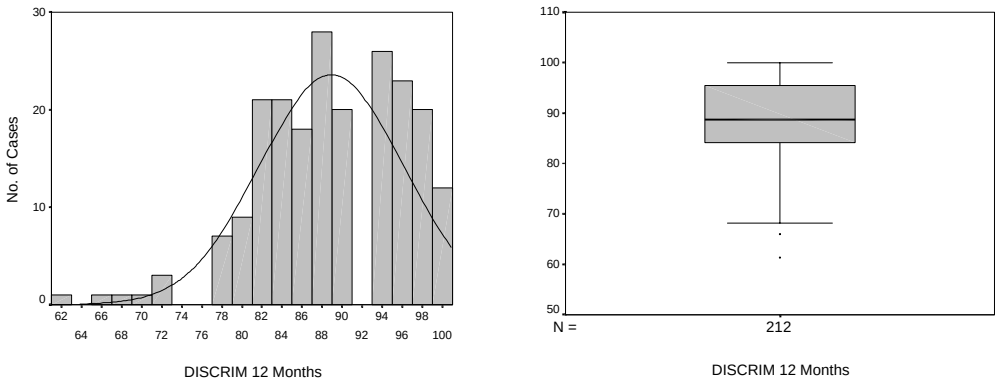


Figure 3.4.2: Histogram and boxplot of DISCRIM 12 months.

The distribution of the DISCRIM 12 Month score. Normality is acknowledged.

Variable	n	*r*	*P* value	*Age-Adjusted r*	*P* value
DISCRIM Baseline	212	0.291	**<0.001**	0.286	<**0.001**
Age (years)	212	-0.076	0.270	-	-
Post-Menopause (years)	152	-0.124	0.128	-0.097	0.235
E2 (pmol/l)	210	-0.030	0.666	-0.026	0.709
Aβ40 (pgm/mol)	205	-0.146	**0.036**	-0.149	**0.033**
BMI	153	-0.027	0.740	-0.045	0.584

Table 3.4.10: Linear relationships between DISCRIM 12 months and continuous independent variables.

Only the DISCRIM Baseline score and Aβ40 levels were found to be significantly associated with DISCRIM 12 Month result.

Variable	Levels	n	Mean (SD)	Test statistic	df	*P*-value
***APOE* ε4**	**no**	157	89.4 (7.1)	t=1.745	203	0.082
	yes	48	87.4 (7.2)			
***APOE* ε2**	**no**	159	88.9 (7.2)	t=-0.102	203	0.919
	yes	46	89.0 (7.4)			
Education:	**low**	108	89.1 (7.5)	F=0.457	2,206	0.634
	medium	70	88.3 (7.5)			
	high	31	89.7 (5.2)			
Parity:	**0-1**	31	86.6 (5.8)	F=2.000	2,209	0.138
	2-3	113	89.4 (7.1)			
	4+	68	89.1 (7.7)			
Statin	**no**	148	89.2 (6.5)	t=0.957	210	0.340
	yes	64	88.2 (8.6)			
Depression	**no**	197	88.9 (7.1)	t=-0.262	210	0.793
	yes	15	89.4 (8.4)			
Hypertension	**no**	94	89.5 (6.5)	t=1.099	210	0.273
	yes	118	88.4 (7.6)			
CVS event	**no**	198	88.7 (7.2)	t=-1.695	210	0.092
	yes	14	92.0 (5.8)			
Diabetes	**no**	191	89.1 (6.9)	t=0.852	210	0.395
	yes	21	87.7 (9.5)			

df= degrees of freedom

SD = standard deviation in brackets

Table 3.4.11: Mean DISCRIM 12 Months by categorical independent variables.

The mean values of the categorical variables by DISCRIM 12 Months score revealed no significant differences between groups.

Variable	Unstandardised		
	β	std error	*P* value
Constant	60.501	6.461	<0.001
DSCRIM Baseline	0.320	0.072	<0.001

[a] R^2=0.085, adjusted R^2=0.080, $F_{1,210}$=19.451, P<0.001

Shading denotes significance (P<0.05)

Table 3.4.12: Final MLR model on DISCRIM 12 Months[a]

The final MLR revealed that only DISCRIM Baseline score was a significant factor on DISCRIM 12 Month score.

3.4.5 Dependent Variable (Y), DISCRIM Change

DISCRIM Change was created to reflect the overall difference in DISCRIM Baseline and DISCRIM 12 Months. Interestingly, by a small majority, 53.3% of the subjects who took part in the follow-up CVLT test declined after 12 months (n=113). Approximately 45% (n=97) improved in their DISCRIM scores and another 0.9% stayed constant over the 12 month period (n=2). Table 3.4.12 for continuous variables confirms that only DISCRIM Baseline score had significantly different results for subjects who declined as opposed to those who improved or stayed constant. Individuals who improved tended to have higher baseline results of the DISCRIM (P <0.001). For the categorical independent variables, no significant differences were found between subjects who improved/stayed constant and participants who declined after 12 months.

3.4.6 Dependent Variable, FORGET 12 Months (Y)

Descriptive statistics for the 212 participants who completed the FORGET component of the CVLT test at both baseline (FORGET Baseline) and 12 Months (FORGET 12 Months) are detailed in Table 3.4.15. The paired sample t-test revealed that the mean difference of 0.40 (95% CI 0.03 to 0.76) between the population mean FORGET score at Baseline and that at 12 Months was statistically significant (t_{211}=-2.135; P=0.034). The results indicate that forgetting rates for the 12 month assessment were significantly lower than those at baseline.

The distribution of FORGET 12 Months (n=212) is displayed in Figure 3.4.3. The significance of the variables measured at baseline on FORGET 12 Months will be examined using multiple linear regression analysis. For this purpose, the distribution of FORGET 12 Months fulfils the assumption of normality.

3.4.6.1 Univariate Analysis

The linear relationships between the continuous independent variables and the FORGET 12 score is illustrated in Table 3.4.16. No significant associations were discovered. Table 3.4.17 shows the linear relationships between the categorical variables and the dependent variable FORGET 12 Months. Again, no significant correlations were found.

3.4.6.2 Multiple Linear Regression, FORGET 12 Months (Y)

Multiple linear regression modelling was used to investigate the effect of independent variables, collected at baseline, on FORGET 12 Months. FORGET Baseline was also included as an independent variable in these investigations, so as to control for the initial FORGET score obtained at baseline. The independent variables, Post Menopause years and BMI, were not examined in the regression analysis due to missing values for 28.3% and 27.8% of participants, respectively. None of the independent variables were shown to be of significance in relation to FORGET 12 Months and were subsequently not shown.

3.4.7 Dependent Variable (Y), FORGET Change.

FORGET Change was created to reflect the overall change in FORGET Baseline and FORGET 12 Months. Overall 48.6% of the 12 month CVLT participants had reduced forgetting rates (n=103) with about 14% with no change (n=30) and approximately 37% (n=79) that had higher forgetting scores.

3.4.8 Participants who returned versus participants at baseline only

Table 3.4.20 demonstrates the differences between follow-up participants and those that did not return for their 12 month CVLT visit. Non-participants differed significantly from participants in terms of BMI status and Aβ40 levels. For non-participants, the mean BMI was significantly higher (SD 4.5) vs. 26.6 (SD 4.4)(P=0.037), and the mean

Aβ40 level significantly higher, 103.5 pgm/mol (SD 73.4) vs. 83.0 pgm/mol (SD 68.1) (*P*=0.010). For the categorical independent variables, no significant differences were discovered between those that returned and those that only did baseline testing for CVLT (Table 3.4.21).

	Decline mean (SD)	No Change or Improvement mean (SD)	t-statistic	df	*P* value
DISCRIM Baseline	85.7 (6.8)	92.7 (5.5)	-8.115	210	**<0.001**
Age (years)	80.0 (2.8)	79.9 (2.6)	0.233	210	0.816
Aβ40 (pg/mol)	86.2 (61.5)	79.5 (62.2)	0.779	203	0.437
E2 (pmol/l)	26.1 (14.1)	26.0 (15.2)	0.045	208	0.964
Menopause (years since)	26.7 (6.9)	27.5 (6.3)	-0.739	150	0.461
BMI	27.7 (4.7)	27.6 (4.2)	0.111	151	0.912

[a] variables measured at baseline

df = degrees of freedom

shading denotes significance ($P<0.05$)

Table 3.4.13: Descriptive statistics of continuous independent variables by DISCRIM Change.

The mean and standard deviation of subjects who improved / showed no improvement or declined in the CAMCOG score over 12 months according to the continuous variables. Only DISCRIM Baseline was shown to be significantly different between groups.

		Decline n (%)	No Change or Improvement n (%)	χ^2	df	*P* value
***APOE* ε4**	**no**	79 (50.3%)	78 (49.7%)	2.191	1	0.139
	yes	30 (62.5%)	18 (37.5%)			
***APOE* ε2**	**no**	85 (53.5%)	74 (46.5%)	0.024	1	0.878
	yes	24 (52.2%)	22 (47.8%)			
Parity	**0-1**	20 (64.5%)	11 (35.5%)	3.303	2	0.192
	2-3	62 (54.9%)	51 (45.1%)			
	4+	31 (45.6%)	37 (54.4%)			
Education	**low**	58 (53.7%)	50 (46.3%)	0.382	2	0.826
	med	36 (51.4%)	34 (48.6%)			
	high	18 (58.1%)	13 (41.9%)			
Statin	**no**	76 (51.4%)	72 (48.6%)	0.749	1	0.387
	yes	37 (57.8%)	27 (42.2%)			
Depression	**no**	107 (54.3%)	90 (45.7%)	1.147	1	0.284
	yes	5 (40.0%)	9 (60.0%)			
Hypertension	**no**	51 (54.3%)	43 (45.7%)			
	yes	62 (52.5%)	56 (47.5%)	0.062	1	0.804
CVS	**no**	106 (53.5%)	92 (46.5%)	0.066	1	0.798
	yes	7 (50.0%)	7 (50.0%)			
Diabetes	**no**	99 (51.8%)	92 (48.2%)	1.673	1	0.196
	yes	14 (66.7%)	7 (33.3%)			

df = degrees of freedom

Table 3.4.14: Descriptive statistics of categorical independent variables by DISCRIM Change.

The number and percentage of subjects who improved, declined or showed no improvement in the DISCRIM score over 12 months according to the categorical variables. No significant differences were observed.

Variable	n	Min.	Max.	Mean	SD	Median	IQR
FORGET Baseline	212	-7	7	0.6	1.9	1.0	3.0
FORGET 12 Months	212	-7	6	0.1	1.8	0	2.0
FORGET Difference	212	-7	7	0.4	2.7	0	3.8

Table 3.4.15: Descriptive statistics for FORGET Baseline and 12 Months.

Table showing the minimum, maximum, mean, standard deviation, median and inter-quartile range of the FORGET Baseline score, FORGET 12 Month score and FORGET Difference. The mean difference of 0.40 was statistically significant (*P*= 0.034)

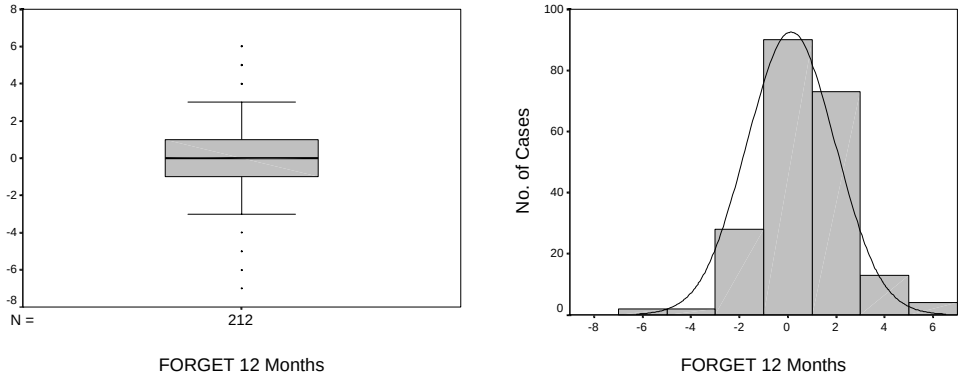


Figure 3.4.3: Histogram and boxplot of FORGET 12 Months.

The distribution of the FORGET 12 Month score is recognized as normal

Variable	n	*r*	*P* value	*Age-Adjusted r*	*P* value
FORGET Baseline	212	-0.068	0.327	-0.069	0.320
Age (years)	212	0.019	0.788	-	-
Post-Menopause (years)	152	-0.047	0.569	-0.070	0.393
E2 (pmol/l)	210	0.024	0.730	0.024	0.731
Aβ40 (pg/mol)	205	0.053	0.448	0.054	0.447
BMI	153	0.104	0.202	0.118	0.147

Table 3.4.16: Linear relationship between FORGET 12 months and continuous independent variables.

No linear relationships were found between the FORGET 12 Month score and the continuous variables.

Variable	Levels	n	Mean (SD)	Test statistic	df	*P*-value
***APOE* ε4**	**no**	157	0.17 (1.89)	t=1.037	203	0.301
	yes	48	-0.15 (1.58)			
***APOE* ε2**	**no**	159	0.08 (1.87)	t=-0.251	203	0.802
	yes	46	0.15 (1.67)			
Education:	**low**	108	0.28 (1.91)	F=1.068	2,206	0.345
	medium	70	-0.13 (1.68)			
	high	31	0.06 (1.79)			
Parity:	**0-1**	31	0.48 (1.57)	F=0.853	2,209	0.428
	2-3	113	0.01 (1.92)			
	4-5+	68	0.18 (1.76)			
Statin	**no**	148	0.20 (1.77)	t=0.774	210	0.440
	yes	64	-0.02 (1.95)			
Depression	**no**	197	0.13 (1.81)	t=-0.149	210	0.882
	yes	15	0.20 (2.08)			
Hypertension	**no**	94	0.05 (1.97)	t=-0.561	210	0.575
	yes	118	0.19 (1.71)			
CVS event	**no**	198	0.17 (1.72)	t=0.651	13.621	0.526
	yes	14	-0.36 (2.98)			
Diabetes	**no**	191	0.18 (1.76)	t=1.233	210	0.219
	yes	21	-0.33 (2.35)			

df= degrees of freedom

SD = standard deviation in brackets

Table 3.4.17: Mean FORGET 12 Months by categorical independent variables.

The mean values of the categorical variables by FORGET 12 Months score revealed no significant differences between groups.

	Decline mean (SD)	No Change or Improvement mean (SD)	t-statistic	df	*P* value
FORGET Baseline	1.8 (1.3)	-0.7 (1.4)	13.798	210	**<0.001**
Age (years)	79.8 (2.7)	80.0 (2.7)	-0.317	210	0.151
Aβ40 (pg/mol)	80.1 (64.0)	85.9 (59.7)	-0.673	203	0.501
E2 (pmol/l)	25.0 (14.0)	27.0 (15.1)	-1.021	208	0.308
Menopause (years since)	26.2 (7.2)	27.9 (5.9)	-1.529	150	0.128
BMI	27.0 (4.2)	28.3 (4.7)	-1.748	151	0.082

[a] variables measured at baseline

df= degrees of freedom

SD = standard deviation

Shading denotes significance ($P < 0.05$)

Table 3.4.18: Descriptive statistics of continuous independent variables by FORGET status.

The mean and standard deviation of subjects who improved / showed no improvement or declined in the CAMCOG score over 12 months according to the continuous variables. Only FORGET Baseline was shown to be significantly different between groups.

		Decline n (%)	No Change or Improvement n (%)	χ^2	df	*P* value
***APOE* ε4**	**no**	74 (47.1%)	83 (52.9%)	1.222	1	0.269
	yes	27 (56.3%)	21 (43.8%)			
***APOE* ε2**	**no**	78 (49.1%)	81 (50.9%)	0.013	1	0.910
	yes	23 (50.0%)	23 (50.0%)			
Parity	**0-1**	12 (38.7%)	19 (61.3%)	2.376	2	0.305
	2-3	60 (53.1%)	53 (46.9%)			
	4+	31 (45.6%)	37 (54.4%)			
Education	**low**	50 (46.3%)	58 (53.7%)	2.188	2	0.335
	med	34 (48.6%)	36 (51.4%)			
	high	19 (61.3%)	12 (38.7%)			
Statin	**no**	70 (47.3%)	78 (52.7%)	0.325	1	0.568
	yes	33 (51.6%)	31 (48.4%)			
Depression	**no**	94 (47.7%)	103 (52.3%)	0.842	1	0.359
	yes	9 (60.0%)	6 (40.0%)			
Hypertension	**no**	43 (45.7%)	51 (54.3%)	0.545	1	0.460
	yes	60 (50.8%)	55 (49.2%)			
CVS	**no**	96 (48.5%)	102 (51.5%)	0.012	1	0.913
	yes	7 (50.0%)	7 (50.0%)			
Diabetes	**no**	93 (48.7%)	98 (51.3%)	0.009	1	0.926
	yes	10 (47.6%)	11 (52.4%)			

df = degrees of freedom

Table 3.4.19: Descriptive statistics of categorical independent variables by FORGET status.

The number and percentage of subjects who improved, declined or showed no improvement in the FORGET score over 12 months according to the categorical variables. No significant differences were observed.

		n	Mean (SD)	t-statistic	df	*P* value
Age (years)	participants	212	79.9 (2.7)	-1.884	411	0.060
	non-participants	201	80.4 (2.6)			
Menopause (years since)	participants	152	27.0 (6.6)	-0.956	289	0.340
	non-participants	139	27.8 (7.1)			
E2 (pmol/l)	participants	210	26.0 (14.6)	0.936	402	0.350[b]
	non-participants	194	25.7 (18.3)			
Aβ40 (pg/mol)	participants	205	83.0 (61.8)	-2.575	394	**0.010** [b]
	non-participants	191	103.5 (73.4)			
BMI	participants	153	27.7 (4.5)	2.100	2.91	**0.037**
	non-participants	140	26.6 (4.4)			

[a] variables measured at baseline

[b] based on natural logarithm transformation of E2

df = degrees of freedom

shading denotes significance ($P < 0.05$)

Table 3.4.20: Returned participants versus participants at baseline only[a] Continuous independent variables: CVLT cohort.

Only Aβ40 levels and BMI were significantly different between returned participants and those who only completed baseline assessment. Non-returnees had a higher mean value of Aβ40.

		Participants n (%)	Non-Participants n (%)	χ^2	df	*P* value
***APOE* ε4**	**no**	157 (76.6%)	158 (81.0%)	1.177	1	0.278
	yes	48 (23.4%)	37 (19.0%)			
***APOE* ε2**	**no**	159 (77.6%)	146 (74.9%)	0.399	1	0.528
	yes	46 (22.4%)	49 (25.1%)			
Parity	**0-1**	31 (14.6%)	33 (16.4%)	1.054	2	0.590
	2-3	113 (53.3%)	97 (48.3%)			
	4+	68 (32.1%)	71 (35.3%)			
Education	**low**	108 (51.7%)	101 (50.8%)	1.138	2	0.566
	med	70 (33.5%)	61 (30.7%)			
	high	31 (14.8%)	37 (18.6%)			
Statin	**no**	148 (69.8%)	150 (74.6%)	1.191	1	0.275
	yes	64 (30.2%)	51 (25.4%)			
Depression	**no**	197 (92.9%)	184 (91.5%)	0.276	1	0.599
	yes	15 (7.1%)	17 (8.5%)			
Hypertension	**no**	94 (44.3%)	94 (46.8%)	0.245	1	0.621
	yes	118 (55.7%)	107 (53.2%)			
CVS	**no**	198 (93.4%)	185 (92.0%)	0.282	1	0.596
	yes	14 (6.6%)	16 (8.0%)			
Diabetes	**no**	191 (90.1%)	190 (94.5%)	2.837	1	0.092
	yes	21 (9.9%)	11 (5.5%)			

df = degrees of freedom

Table 3.4.21: Returned participants versus participants at baseline only[a]. Categorical variables: CVLT cohort.

Between the categorical variables, there was no significant difference between returnees and non-returnees.

Chapter 4 DISCUSSION

4.1 Endogenous Sex Hormones and Cognition

4.1.1 Endogenous Oestrogen

Tantalising epidemiological evidence suggests a role for hormone replacement therapy (HRT) in delaying and even preventing AD. However, within the past five years, HRT has seen a dramatic fall from grace, with two papers adding to the growing evidence that HRT is ineffective if begun after the age of 65 years. Even more damning, was the recent finding that HRT was actually associated with increased incidence of dementia (Rapp et al., 2003; Shumaker et al., 2003). It has led some investigators to suggest that the negative findings were due to the progesterone, and not the oestrogen in the HRT regime.

After menopause, levels of oestrogen are markedly reduced due to ovarian dysfunction, with the result that circulating levels of FSH and LH increase in an attempt to 'drive up' these reduced levels (Wide et al., 1973). Besides the ovary, low levels of endogenous oestrogen (in post-menopausal women) are also produced, either directly or indirectly, by other peripheral tissue which includes adipose tissue, muscle and the adrenal glands (Siiteri et al., 1973). In this study, the effect of endogenous oestrogen level on cognitive ability (as measured by CAMCOG and the CVLT) in post-menopausal women was examined at baseline, and again 12 months later. The current study provides support for the gonadotropins, FSH and LH on modulating cognition, but this effect was not observed with endogenous oestrogen.

Plasma endogenous oestrogen had no independent or combined main effect on CAMCOG scores for either APOE ε4 positive or negative individuals at baseline. Further analysis of the CVLT data showed no relationship between oestrogen

concentration and CVLT score. However, there is substantial evidence in the literature indicating that oestrogen has numerous effects on the brain; which include enhancing cognition (Asthana et al., 1999; Honjo et al., 1989; Verghese et al., 2000; Miller et al., 2002). Oestrogen may act through two different intra-cellular oestrogen receptors, ER-α and ER-β, that exist in cell nuclei of nerve cells found throughout the brain, including the hippocampus (a brain structure which is strongly implicated in age-related memory decline). Non-nuclear oestrogen receptors are also found within the brain in dendrites, pre-synaptic terminals and glial cells (McEwen & Alves, 1999). Oestrogen has been shown to have neuro-protective effects on the brain, especially in the hippocampus and forebrain areas that are vital to memory and learning, acting to maintain functionality and provide protection from neural damage caused by noxious agents (McEwen & Alves, 1999). Oestrogen may block the action of neurotoxic agents (Behl et al., 1995), influence activation of acetylcholine metabolism, reduce apoptosis and peroxidation (Honjo et al., 2001), and improve glucose utilisation (Craft et al., 1992; Manning et al., 1993). Oestrogen may also influence APP metabolism (Jaffe et al., 1994), and may positively interact with *APOE* (Srivastava et al., 1996; Tang et al., 1996).

There have also been numerous studies on the link between exogenous oestrogen treatments and AD, with conflicting results ranging from positive outcomes in a number of small case control studies, to two large double blind placebo control clinical trials which showed that HRT or oestrogen treatment was associated with an increased frequency of dementia (Rapp et al., 2003; Shumaker et al., (2003). It could be argued that a number of the previous studies possessed design flaws, which include the type of oestrogen administration, age of administration, comorbidity within the cohorts, and lack of adequate genotyping (*APOE*). These potential flaws may have masked the actual study findings.

In contrast with my findings that endogenous oestrogen levels do not influence cognition in post-menopausal women, others have reported that endogenous oestrogen does have an effect on cognition (Drake et al., 2000; Wolf & Kirschbaum, 2002; Senanarong et al., 2002). Drake et al., (2000) have shown that differing circulating oestrogen levels may have positive effects on certain aspects of cognition. Yaffe et al., (2000) have shown that elevated levels of endogenous oestrogen was associated with the prevention of cognitive decline in post-menopausal women, while Wolf & Kirschbaum (2002) have demonstrated specific gains in verbal memory and frontal lobe mediated functions as a result of higher oestrogen levels. Furthermore, Senanarong et al., (2002) reported that lower endogenous oestrogen levels correlated with poorer cognitive scores. However Drake et al., (2000) and Wolf & Kirschbaum (2002) relied on a small sample size of 37 and 38 women respectively and the Yaffe et al., (2000) report has utilised a comparatively crude measure of memory (modified version of the MMSE).

It is possible that lack of effect observed in my study may be due to the older age of the cohort (75 to 87 years), as compared to the relatively younger age group as employed by Yaffe et al., (2000). There is also some evidence, contrary to the above reports, that endogenous oestrogen levels may have little or no link to cognitive ability. Studies by Heijer et al., (2003). Barrett-Connor et al., (1999) and Yaffe et al., (1998), indicate that endogenous levels of oestrogen are not associated with enhanced memory or cognitive performance in older post-menopausal women, which is consistent with my findings in the current study.

A specific threshold for oestrogen may exist in relation to its influence on cognition. It is possible that clinically measurable effects of oestrogen may only occur at higher concentrations than those levels of the hormone that are naturally present in post-menopausal women. While endogenous oestrogen levels in my study were observed not

to be associated with improved verbal learning (as demonstrated on the CVLT), several studies using CVLT have found an improvement in performance after oestrogen replacement therapy (Philips & Sherwin, 1992; Jacobs et al., 1998; Kampen & Sherwin, 1994). Exogenous treatments of oestrogen may better match those levels found in pre-menopausal women, and therefore may be more likely to have an impact on memory and learning. However, some studies that have reported positive effects of oestrogen on cognition have used insensitive tools to measure cognition. For example, the study conducted by Senanarong et al., (2002) utilised the MMSE, which has inherent psychometric problems such as low ceiling effects and a lack of sensitivity to verbal learning and memory, which is an important aspect of cognition that is modulated by oestrogen. Interestingly, a study by de Jager et al., (2002) found that the CAMCOG was sensitive for detecting changes in working memory, language and processing speed, but not for assessing episodic and semantic memory components. However, since oestrogen primarily affects verbal components of learning, the CAMCOG may not pick up subtle differences in performance in this memory domain (Asthana et al., 2001; Davis, 2002; Fluck et al., 2002, Sherwin, 2003).

In the current study, increased endogenous oestrogen levels were also found not to be associated with improved verbal memory scores, as measured by the 'SUM1to5', 'DISCRIM' or 'FORGET' components of the CVLT. However, even these measures may be constrained by ceiling and floor effects. This notion is consistent with the observation that a number of healthy individuals were volunteers for this study. These volunteers may represent the upper echelon of high achievers and the CVLT may have missed their valuable contribution. Thus, these individuals need to be tested with a measure possessing a higher ceiling in order to obtain more meaningful results. Conversely, subjects who refused to complete the assessment may have influenced the final outcome as they were excluded for the analysis. Taken together, the ceiling and

floor effects observed with the CVLT may have masked any possible association between oestrogen and cognition.

The findings in this study do not show an association between endogenous oestrogen and cognitive decline. This finding is consistent with recent clinical trials in which oestrogen replacement did not protect against decline in memory, but in fact appeared to be associated with increased frequency of dementia (Rapp et al., 2003, Shumaker et al., 2003).While this current study does not support oestrogen's role in subserving cognition, it is nevertheless consistent with the major body of evidence indicating that menopause is associated with an increase in cognitive decline. Indeed, it is possible that other factors that are altered during menopause (such as the levels of LH and FSH), may explain the increased frequency of dementia and AD in post-menopausal women. However, oestrogen cannot be completely ruled out as a cognition-promoting agent based on the findings to date. All approaches have not yet been exhausted, particularly the notion that oestrogen is most effective when given near menopause, rather then two decades later as undertaken in the recent trials.

4.1.2 Luteinising hormone, follicle stimulating hormone and cognition in post-menopausal women

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) are part of the hypothalamic-pituitary-ovarian axis (HPO), which is responsible for governing the endocrine functions responsible for reproduction in women (see Figure 4.1). At menopause, oocyte depletion and aging of the ovary has profound effects on the female endocrine system. A few years prior to menopause, LH and FSH levels steadily rise and continue to increase well after the onset of menopause, but they gradually decrease with advancing age due to pituitary atrophy (Burger, 1999). In AD, the increase in LH after menopause is even greater, despite increasing age, as reported in small case control studies (Bowen et al., 2000; Short et al., 2001). A central goal in the present study was

to examine plasma LH and FSH, together with other relevant biological and genetic factors to elucidate their impact on cognition in post-menopausal women. The results indicate that dependent on age, LH was a significant factor in mediating variability in CAMCOG score in those women who were non-*APOE* ε4 individuals (P= 0.004). More specifically, the most pronounced effect of LH on CAMCOG scores was observed in individuals in the highest age group (i.e. ≥ 84 years of age, 90th percentile) who were *APOE* ε4 negative. Since LH levels do not generally rise in much older post-menopausal women, this age-related effect of increased LH may be attributed to greater sensitivity to LH in this high (84 years) age group. The results of the current study, which involved a large cohort of post-menopausal women, is consistent with earlier clinical studies (Bowen et al., 2000; Short et al., 2001), in so far as increased LH levels were associated with decreased levels of cognition. However, the current findings with FSH contrasted with these earlier reports, in that increased levels of FSH were associated with better cognitive performance. The current findings have potentially greater impact, as the cohort size was 651 (CAMCOG; baseline) compared with earlier studies with 284 subjects (Short et al., 2001) and 69 subjects respectively (Bowen et al., 2000). Furthermore, the current findings demonstrate for the first time that the impact of FSH levels is *APOE* genotype dependent. LH is associated primarily with reproduction, and its receptors were previously thought to be restricted to the gonads. However, it is now recognised that there are other sites that are influenced by LH, including: the retina of the eye (Thompson et al., 1998), urinary bladder (Zhou et al., 1999), breast (Tao et al., 1997), prostate (Reiter et al., 1995), and bone (Li et al., 2000). These links have been noted in mouse and human subjects. More importantly, LH, together with human chorionic gonadotropin (hCG) has receptors co-localised in the brain with the hippocampus being the richest source (Lei et al., 1993; Huang et al., 1995).

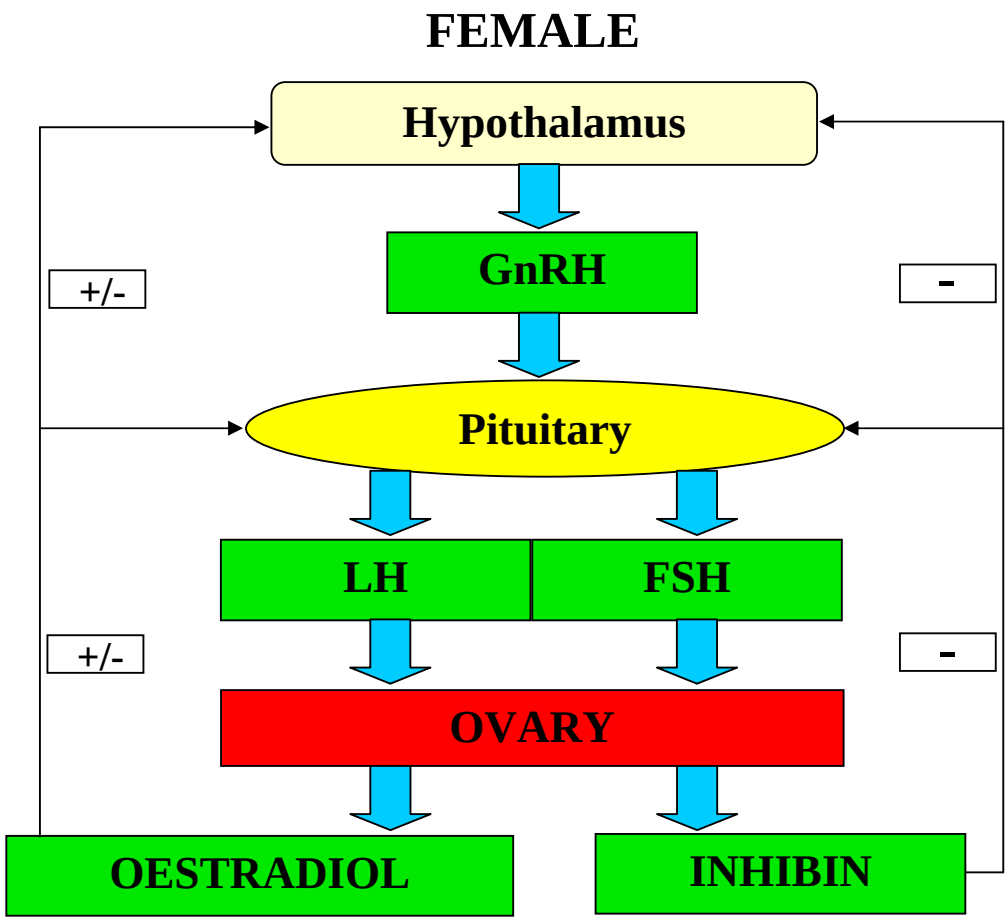


Figure 4.1: The HPO Axis in Reproductive Age Women.

The HPO axis is responsible for controlling the endocrine functions for reproduction in women. In a cycling women oestrogen has a feedback relationship with the gonadotropins LH and FSH which is, in turn, regulated by GnRH. Inhibins and activins also play a role in regulating FSH levels. Inhibins reduce the production of FSH in the anterior pituitary whereas the activins up-regulate FSH secretion. After menopause, the depleted ovaries have a diminished output of oestrogen, and are effectively resistant to LH/FSH levels resulting in an increase in gonadotropin levels. The axis is effectively released from the feedback loop.

Receptors for luteinizing hormone have been found in areas vulnerable to AD pathology, namely the hippocampus (Lei et al., 1993; al-Hader et al., 1997) and the neocortex of the parietal lobe (Bukovsky et al., 2003). These findings suggest that LH may have specific effects on brain function. It has been further reported that LH receptor (LHR) expression is related to flattened nuclei and spiny processes of neurons (Bukovsky et al., 2003). Findings suggest that microglia and grey matter neurons may be especially sensitive to changes in LH (Bukovsky et al., 2003). LH has also been found in the cytoplasm of pyramidal neurons (Bowen et al., 2002). Taken together, the findings of these studies appear consistent with the notion that increases in circulating LH levels may have a detrimental influence on cognitive functioning in post-menopausal women.

The majority of AD cases are sporadic late-onset (LOAD), in which numerous potentially influential factors are implicated, including the influence of the ε 4 allele which codes for a variant of the protein known as apolipoprotein E (ApoE). The *APOE* gene is polymorphic in nature (Utermann et al., 1980, 1982), with its ε4 allele over-represented in AD cases (Martins et al., 1995). It has a gene dosage effect (Frisoni et al., 1995), and its presence leads to an increased risk of AD with age (Farrer et al., 1997). The presence of this allele is also associated with acclerated age-related memory decline and an increased risk of AD (Dik et al., 2000; Deary et al., 2002).

The findings of the present study indicate there was no significant effect of LH on CAMCOG scores in ε4 positive individuals, though increased LH levels were associated with decreased levels of cognition in non-ε4 individuals. These combined findings may be attributed to the powerful influence of the ε4 genotype on AD pathology, which may be independent of the effect of LH. The impact of the *APOE*

genotype on cognitive decline may mask the contribution of LH on cognition. By contrast, the influence of LH was clearly apparent in the non-ε4 group.

The association between apoE levels and LH has been demonstrated previously (Zhang et al., 1998), where LH was shown directly to increase *APOE* levels. Theca and interstitial cells of the ovary were cultured and found to produce greater amounts of apoE, when LH levels were increased with a corresponding reduction in lyase expression and decreased androgen/oestrogen production. Interestingly, increased LH levels are also associated with a greater risk of AD. In light of the influence of LH on cognition in the present study, it can be argued that elevated levels of LH could affect ApoE production, which confers risk independently of APOE genotype, and thereby increase age-related cognitive decline and the risk of AD (Laws et al., 2003). This hypothesis needs further exploration.

The effect of LH on cognition may work through mechanisms other than direct effects on the brain. One plausible *modus operandi* for LH, with respect to neurocognitive functioning, is its biological association with melatonin (which has been linked to the brain and cognition). Melatonin is known to inhibit levels of LH by way of a decrease in the (Ca^{2+}) influx and in cAMP concentration (Viguie et al., 1995; Vanecek et al., 1998). Melatonin acts on GnRH to inhibit LH and FSH levels in the *pars distalis* region of the pituitary gland. Melatonin is involved in the diurnal light/dark cycle, which is mediated by the pineal gland, specifically the suprachiasmatic nucleus (SCN; Johnson et al., 2003). Deprivation in melatonin has been linked to sleeplessness and night restlessness.

AD patients are observed to have higher rates of sleeping disorders, and this has been attributed to impaired melatonin production. A study by Skene et al., (2003) found changes in the timing and amplitude of melatonin rhythm in elderly individuals and further reduced levels in AD patients, potentially caused by age-related changes in the

hypothalamic suprachiasmatic nuclei and pineal gland. Zhou et al., (2003) suggest that reduced CSF levels of melatonin may be a precursor to AD progression. Asayama et al., (2003) found that melatonin administration improved the amount of sleep in AD patients, as well as performance on cognitive and non-cognitive measures.

Additionally, melatonin has been found to modulate the metabolism of APP, reducing the secretion of soluble Aβ (Lahiri et al., 1999). Zatta et al., (2003) found that melatonin reduced the free radical formation produced from the interaction between transition metal ions (such as zinc and copper) and Aβ. The effect of melatonin on a transgenic mouse model of amyloidosis, resulted in inhibition of an expected elevation of Aβ, lowered abnormal nitration of proteins, and increased longevity of the mice (Matsubara et al., 2003). The proposed neuroprotective effect of melatonin may be mediated by suppression of LH levels via its action on GnRH. With aging, this feedback effect of melatonin is compromised, with the resultant increase in LH (which may be further exacerbated by the naturally occurring decrease in oestrogen in post-menopausal women).

Like LH, FSH is produced in the beta cells of the anterior pituitary gland and works in concert with it to control the release of oestrogen and progesterone. FSH levels remain elevated for many years in post-menopausal women. It is thought that this increase is mainly due to decreased levels of oestrogen and inhibin. This relationship with oestrogen prompted me to examine this role of FSH in cognition.

To date only a handful of studies have been undertaken to investigate the role of FSH on cognition. However these studies clearly indicate a positive correlation between increased FSH levels and cognition. More specifically, it was found that increased gonadotropin levels were associated with enhanced performance in fluency tasks in men (Gordon et al., 1986a). In another study (Gordon et al., 1986b), endogenous levels of

LH/FSH in young men and women were measured, and the authors found differential effects of the gonadotropins on specific aspects of cognition. Interestingly, the reverse was seen for increased FSH levels and visuo-spatial skills (Gordon et al., (1986a). In young women, increased levels of FSH were associated with higher word fluency (Gordon et al., 1986b). However, the effects of visuo-spatial skills were less pronounced, unlike the magnitude of the effect seen on men (Gordon et al., 1986a; Gordon et al., 1986b). In both of the above studies, LH was shown to exhibit similar effects as observed with FSH. However these two studies suffered from a deficiency where the experimental approach involving the administration of LHRH results in the simultaneous increase in both LH and FSH. Thus, distinguishing the specific actions of LH and FSH is difficult to ascertain. In the current study I show different effects between LH and FSH on cognition with FSH associated with higher cognition and the reverse observed with LH. This differential effect of these two hormones is possible in the human model studies here where physiological levels of hormones were assessed unlike the intervention studies referred to earlier. However, the current study employing the CAMCOG lacks the sensitivity to differentiate between word fluency and other cognitive parameters. Use of more sensitive instruments such as the CVLT (gonadotropins not measured in the CVLT cohort), may allow these cognitive features to be evaluated.

The link between activin and FSH is perhaps relevant to consider here, since they have the ability to stimulate FSH production (Burns & Matzuk, 2002). Inhibins, closely related to activins, have the opposite effect of suppressing production of FSH. Studies have shown that activin expression is not only associated with the gonads, but it is also found in the placenta, bone marrow and brain (Lai et al., 1997). Three main forms of activin exist: the homodimeric activin *A*, the homodimeric activin *B* and the heteromeric activin *AB*, as well as other less known activin chains that have been recently found (Yu

& Dolter, 1997). The role of activin and FSH is a largely unexplored area with regards to cognition and memory. It was recently discovered that activin *A* levels continually rise with age, with augmented levels in the last decades of life (Baccarelli et al., (2001). There is also a general rise in FSH after menopause. It is therefore possible that the post-menopausal rises in both FSH and activin levels are inter-related (Veldhuis et al., (1999).

Interestingly, activin has also been shown to affect neuronal viability independent of FSH. In *vitro* studies on cultured neurons, found that activin *A* increased survival rate, and may protect against neurotoxic damage (Krieglstein et al., 1995). A study conducted by Tretter et al., (1996), has indicated a strong up-regulation of Activin *A* after neuronal injury. Lai et al., (1997), suggest that after hypoxic or ischemic injury, activin A levels are increased in specific areas of the brain (including the hippocampus) and that release of activin A is a functionally active component of the neuronal response to brain injury. Taken together with the previously cited evidence, the question therefore remains open; whether the neurocognitive effects of FSH observed in this study represent independent effects of FSH, or whether they are linked to activin-related mechanisms.

4.2 Age-Related Factors and Cognition in Post-Menopausal Women

Numerous studies have found that increasing age is associated with cognitive decline, with epidemiological research also showing that AD incidence increases with age (Ott et al., 1995, Yoshitake et al., 1995). Currently, AD prevalence is estimated at 9% for individuals over 65, 34% for people over 85, and 43% for individuals over 95 (Muller-Spahn et al., 1999). Studies utilizing the CAMCOG and CVLT tests of cognition also demonstrate age-related decline in learning and memory (Huppert et al., 1995; Blessed et al., 1991). This finding has been confirmed in the present study, with age being a

significant factor accounting for variability in cognitive scores on both these tests of cognition (i.e. CAMCOG and CVLT).

In normal aging, different areas of the brain seem to be differentially affected, leading to characteristic performance in different domains of cognitive functioning. Research suggests that verbal skills, implicit learning and semantic memory are largely spared in normal aging processes, whereas episodic memory, attention, working memory and spatial learning seem to be most affected (Kausler, 1992). Age was found to be a significant negative factor accounting for variability in the CAMCOG score in the current study, indicating that in this group, age seems to have affected a broad range of cognitive domains. The age of subjects in the present study represents the later stages of life in these post-menopausal women, when the accumulated effects of environmental, lifestyle, genetic and comorbid factors have the most potential to affect cognitive performance. Interestingly, since changes were seen within the narrow age group, namely 75-87 years of age, this would indicate that this period reflects a greater rate of cognitive decline, which is consistent with the published epidemiological data.

The current results suggest that the majority of subjects probably possess varying degrees of age associated memory loss that may or may not represent the preliminary changes associated with AD. The hippocampus, a region of the brain which plays a major role in memory and learning, exhibits significant deficits in normal aging. More specifically, reports indicate that advancing age is associated with a decrease in neuronal numbers in the hippocampus (Issa et al., 1990), reduction in synaptic connections (Geinisman et al., 1995; Grady et al., 2003), and increased vulnerability to NFT (Raz et al., 1999). These changes are also known to occur in the hippocampus in AD, where they are more pronounced and these subjects usually present with a learning deficit. This may manifest as deficits on the CAMCOG and the CVLT. Additionally, the loss of dopamine receptors in the basal ganglia, the anterior cingulate and the pre-

frontal cortex, can indirectly account for a variety of age-related declines in cognition (Braver et al., 2001). Currently, there is no clear consensus regarding the distinction between neurocognitive loss due to old age, and those changes characterizing AD. Of course, these possibilities are not mutually exclusive. The group could comprise some individuals manifesting normal age-related changes in cognition, whereas other individuals in the group may be manifesting cognitive changes that are characteristic of early stage AD. Further work is needed to distinguish those cognitive changes that are early predictors of cognitive decline leading to dementia.

4.3 Education and Cognition in Post-Menopausal Women

Published research suggests an association exists between education and cognitive ability (Farmer et al., 1995; Elias et al., 1997; Cerhan et al., 1998; Fritsch et al., 2002). Higher levels of education, especially early in life were found to contribute to increased performance in measures of cognition (Deary et al., 2000). Individuals with higher education levels are more likely to participate in mentally stimulating activities, and this has been shown to influence later life cognitive decline (Wilson et al., 2002).

Research on AD prevalence in elderly populations has found that education is also a major controlling factor in determining risk of dementia. Low education levels are associated with increased prevalence of clinical AD or dementia. (Qiu et al., 2001; Ravaglia et al., 2002). Studies performed on twins in relation to education and future risk of dementia found a lower level of education in the twin who later became demented (Gatz et al., 2001).

Results from the current study lend credence to the published literature, with education found to be a major factor in determining cognition in both the CAMCOG cohort (non APOE- ε4 subjects P= 0.001) and the CVLT group (SUM1to5 score; P = 0.007). This has been confirmed by other studies on the CAMCOG assessment indicating that

education (Huppert et al., 1995) and the number of years of formal schooling (Blessed et al., 1991) is associated with CAMCOG score. There are about 100 billion neuronal cells in the adult brain (Goswami, 2004) each with complex and multiple connections to other neurons. Learning generally results in alterations in the connectivity of these communication conduits, strengthening certain connections and allowing for better storage and retrieval of information (Goswami, 2004). Thus, the level of education an individual receives during their lifetime can play a major role in memory and learning capabilities in later life.

Recently, a theory was proposed explaining this education effect; that higher education levels and certain occupation types increase the "cognitive reserve" of the brain, thus making it resistant to neuro-cognitive changes associated with age. This idea originated from the observation that neuronal damage in the brain sometimes did not correlate with the expression of clinical symptoms (Stern, 2002). "Cognitive reserve" may be seen as the ability of the brain to compensate for AD or dementia-like pathology. The brain may be able to optimize alternate neuronal networks and maintain a level of functioning close to normal (Stern, 2002). One study considered cognitive reserve by measuring total years of education, occupation and an estimation of pre-morbid intelligence, and compared this data with neuropsychological functioning in AD patients. Again, it was found that subjects with low cognitive reserve had poorer scores on the tests of memory, attention, executive functioning and visuospatial performance (Sanchez et al., 2002). These findings are consistant with results in the current study, suggesting that cognitive reserve may explain the significance of education in this cohort.

Individuals with lower education may not be accustomed to the techniques used for evaluating cognition or may be unable to deal with the test format and perform poorly. Schooling and tuition in the 1920's and 1930's had greater emphasis on verbatim learning (Staff et al., 2004) and participants who received little or no education in

middle and older age may not be as mentally prepared for assessments such as the CAMCOG and CVLT. Interestingly, even though the majority of individuals (53%) had low levels of education (<15 years: formal education ceased at <15 years), the strong effect of education and tuition is evident in the results of both cognitive assessments. Therefore, education may be a strong indicator of cognitive ability in this age group (75 to 87).

Studies have found that low education levels are also associated with a lower socioeconomic status, which has been suggested to be related to lower cognitive stimulation levels at home (Ardila et al., 2000). This may have a detrimental effect on the developing CNS system of a young child and cause future deficits in cognitive functioning (Alvarez et al., 1983). In fact, the early stages of development in children and young adults may be critical to the risk of future AD (De Ronchi et al., 1998). Collectively, early life factors such as socioeconomic status, childhood and adolescent life quality, environmental and genetic factors as well as education may be important in determining risk of later life dementia.

4.4 Statin Use and Cognition in Post-Menopausal Women.

The results from the final MLR model indicate that the combined effect of FSH and statins each had a significant main effect on CAMCOG scores for ε4 individuals. Furthermore, the positive effect of FSH on cognition score was exacerbated for statin users. The DISCRIM measure of the CVLT also indicated a significant statin use factor. Taken together, both the CAMCOG and CVLT instruments indicate that statin use has a significant positive influence on factors mediating cognition.

As mentioned in the introductory chapter of this thesis, statins are used as pharmaceutical agents that reduce the plasma levels of cholesterol by inhibiting the activity of the enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme. Accumulating evidence

suggests that the use of statins lowers the risk of dementia (Jick et al., 2000), and reduces the risk of ischemic stroke (White et al., 2000; Vaughan et al., 2003). The reduction of lipids and cholesterol has a direct benefit on reducing the risk of AD and dementia. Statins are also thought to possess pleiotropic (multiple) effects, with evidence that these effects are exercised on different tissues and metabolic pathways in other disease states (Waldman et al., 2003).

Statins may improve endothelial homeostasis by increasing the accessibility of nitric oxide (Vaughan, 2003); thereby promoting nitric oxide-dependent vasodilation which may facilitate chemical messaging between synapses, and could be essential in memory formation. Sterzer et al., (2001) examined this phenomenon in patients with subcortical small vessel disease, showing that short term use of pravastatin increased cerebral vasomotor reactivity; this effect may account for the numerous beneficial effects of statins.

More specifically, Fassbender et al., (2001) describe the anti-amyloidgenic effect of simvastatin (a type of statin). These authors found that simvastatin reduced Aβ40 and Aβ42 *in vivo* and *in vitro,* in cultures of hippocampal neurons and mixed cortical neurons. Statins may additionally modulate pre-cerebral atherothrombosis in the aorta and the carotid artery, hence affecting plaque disruption and artery-to-artery thromboembolism (Vaughan, 2003).

The findings of the studies reviewed in this section, together suggest that statins have a modulating effect on important factors that have been shown to affect memory and learning in subjects, and may be an important therapeutic agent in delaying the progression of AD pathology.

4.5 The Influence of Parity on Cognition

An interesting finding in the APOE ε4 positive group, was the significant main effect of parity on CAMCOG scores (p=0.008). Parity was stratified into two groups: a 'low/medium' parity group (defined as equalling zero to one births) and a 'high' parity group (defined as equalling more than two births). The current results suggest that with increasing number of births, subjects were more likely to record lower scores on the CAMCOG. The significance of this finding is far reaching, especially when considering the age of the subjects and the potentially long-lasting effects of pregnancy and motherhood on cognition in older age. These findings may be linked to the well known acute effects of pregnancy on memory also referred to as "maternal amnesia" (Brett & Baxendale, 2001), and/or with more permanent influences on brain functioning (due to the longer term effects of hormonal changes that characterise pregnancy). Of course, one should be mindful of the fact that these observations represent associations, and (as with any association) they therefore do not necessarily imply causal relationships. For example, there could be a causal factor which is itself linked to higher levels of pregnancy which is driving the association observed in the present study between parity and cognitive status. However, in support of my findings, a recent study reported that nulliparity with later age of menopause was associated with greater MMSE scores in post-menopausal women on oestrogen treatment (McLay et al., (2003).

Subjective accounts of the immediate effects of pregnancy on cognition in primiparous women indicate that the most common deficits involve lack of concentration, absent-mindedness and poorer memory performance (Parsons & Redman, 1991). However, these findings may also be attributed to the increased levels of both mental and physical stress associated with pregnancy and childbirth, as stress may be a significant factor in cognitive decline (Wijma et al., 1997). Brindle et al., (1991) argue that implicit memory difficulties may exist in pregnant women, especially in the second trimester. Verbal

learning impairment and further deficits in memory homologous to impaired hippocampal function have also been reported by Poser et al., (1986), Eidelman et al., (1993) and Keenan et al., (1998). However, these deficits are mostly transient in nature, though few cases of continued memory loss have been reported (Poser et al., 1986).

Keenan et al., (1998) propose that the deficits in memory performance may be attributed to the changes in hormones that are apparent in pregnancy and childbirth. In the latter stages of pregnancy, there are dramatically increased levels of oestrogen in an expectant mother and these high levels are dependent on the functional integrity of the placenta and rapidly diminish after the baby has been delivered (Willcox et al., 1985). As discussed previously, oestrogen has numerous beneficial effects on the brain, especially in the area of the hippocampus and other regions responsible for memory function. What may be important in this context is oestrogen's effect on augmenting synaptic excitability in the hippocampus (Wong & Moss 1992; Brett & Baxendale, 2001). Brett & Baxendale (2001), suggest that the apparent oestrogen-related increase in neuronal excitability may predispose to excitotoxity (negative reaction with certain receptors in the brain) and ultimately increased apoptosis. Brawer et al., (1993) and Desjardins et al., (1993) report that oestrodiol is selectively toxic to beta-endorphin neurons in the hypothalamic nucleus. Furthermore, it may be the longer term (i.e. 9 months during pregnancy) exposure to elevated levels of oestrogen that has subtle toxic effects on neurons in the brain.

Another factor that deserves attention is human cortical gonadotropin (hCG) which is notably elevated during the first trimester of pregnancy (Davies et al., 2003). Interestingly, hCG is a homologue of LH and binds to the β subunit of the LH receptor. hCG may affect the performance of the hippocampus (Lukacs et al., 1995) and mimic LH influence on cognition and memory.

Raised glucocorticoid levels due to increased stress may also contribute to cognitive decline later in life (Lee et al., 2002) and this could be an issue during pregnancy. Stress hormone levels markedly increase during foetal development and labour, and this may cause hippocampal cell loss (Brett & Baxendale, 2001). Varying progesterone levels during pregnancy and after birth may also be a factor, as this hormone is responsible for the inhibition of hippocampal excitability. In particular, progesterone levels drop considerably post-partum, making the hippocampus potentially vulnerable to the elevated levels of oestrogen and cortisol that are present at this time. Taken together, the cited literature identified a number of key factors that are associated with cognitive decline during pregnancy. These factors include both oestrogen and hCG. Further studies are required to elucidate whether any of these factors play a causal role, rather than being merely associated.

4.6 Plasma Levels of Aβ40 and Cognition in Post-Menopausal Women

As discussed previously (Chapter 1), extracellular neuronal deposition of beta-amyloid peptide (Aβ) in the brain and in cerebral blood vessels are major characteristics of AD (Selkoe, 1997). Research on familial forms of AD has shown that mutations in genes involved in Aβ production cause earlier onset of disease symptoms and associated pathology (Marechal et al., 2003). LOAD is linked to polymorphisms in the gene (APOE) that interacts with APP and Aβ and is a known risk factor for this later onset form of AD (Farrer et al., 1997). Aβ is a product of the amyloidogenic processing of APP and it is the accumulation of Aβ in the brain that is the primary influence in AD (Hardy & Selkoe, 2002). Overall, the published literature indicates that Aβ is a key factor in the pathogenesis of AD.

Increased levels of Aβ may have a detrimental impact on cognitive processing (Cummings et al., 1996; Yankner et al., 1996). Additionally, studies have specifically

linked altered levels of Aβ40 in plasma, serum or CSF with decreased cognition (Gillett et al., 2003; Mayeux et al., 2003). A study on male patients with dementia or subjective memory loss indicated that these individuals had increased levels of plasma Aβ-40 (Gillett et al., 2003). Aβ plasma levels have also been shown to rise before the onset of AD symptoms (Scheuner et al., 1996). Collectively, these reports suggest that circulating levels of Aβ may be an important indicator of underlying pathologies associated with cognitive decline.

The results from the current study support the published literature showing that cognitive scores are negatively correlated with levels of Aβ40. Higher levels of Aβ40 were associated with lower scores on learning capability as measured by SUM 1to 5. Interestingly it was also noted that non-returnees in the CVLT cohort had a higher mean level of Aβ than subjects who completed their follow-up assessment. High plasma levels of Aβ may indirectly compromise the ability to learn new verbal material. The centres of learning and memory in the brain, include the hippocampal region; this being a key area in the extracellular accumulation of Aβ peptide. Subjects with higher Aβ levels may be beginning to show the early symptoms of cognitive impairment that may later manifest into more substantial memory deficits. Additionally, circulating Aβ was found to damage vessels in the brain and periphery, and activate microglia and astrocyte production (Su et al., 1999). This was seen without aggregation or fibril formation that is associated with AD pathology, and suggests that circulating Aβ can have direct effects on functionality of the brain.

APOE ε4 individuals also show a similar trend of increased plasma Aβ correlating with lower scores in the CAMCOG cohort. Reports indicate that higher levels of Aβ are associated with *APOE* ε4 individuals (Nicoll et al., 1995). These increased levels of Aβ are thought to result from impaired clearance of the peptide in ε4 carriers (Holtzman et

al., 2001). *In vitro* studies show that *APOE* e4 subjects are associated with increased aggregation of Aβ protein into amyloidogenic fibrils (Ma et al., 1994). Additionally, *APOE* ε4 may also exert a reduced neuroprotective effect when compared to other *APOE* isoforms due to reduced binding to Aβ and cytoskeletal proteins (Mazur-Kolecka et al., 2002). Amyloidogenic pathology may be more influential in ε4 carriers having a greater impact on memory in even normal healthy subjects than those possessing non-*APOE* ε4 isoforms. The state of imbalance between the clearance and the production of Aβ may be the crucial factor in AD development (Hardy & Selkoe., 2002) and the published findings suggest those possessing the *APOE* ε4 allele may be less able to deal with removing and combating the harmful effects of this important factor in AD pathology.

4.7 Depression and Cognition

A number of studies have reported a link between depression and memory decline (Kral et al., 1983; Alexopoulos et al., 1993; Devanand et al., 1996) suggesting that the early clinical symptoms of AD and dementia sometimes mimics that of depression. Depressed elderly individuals usually exhibit some form of memory deficit and increase their risk of future development of AD (Alexopoulos et al., 1993). Elderly people have a greater risk of depressive disorders and symptoms. Those with even minor depression have reduced physical, social and occupational functioning, and worse self-perceived health than normal individuals (Wells et al., 1989; Judd & Akiskal, 2000; Judd et al., 2000).

In the present study, depression was found to be a factor in determining the variability of CAMCOG scores (P= 0.038) in *APOE* ε4 individuals at baseline. Lavretsky et al., (2003) reported that a greater number of depressive symptoms were found in *APOE* ε4 carries in their dataset. A study by Krishnan et al., (1996) suggests that subjects with late-onset (after age 60), depression were more likely to be *APOE* ε4 positive. This

suggestion is supported by the findings of the current study as seen in the CAMCOG cohort. The *APOE* ε4 allele may increase the vulnerability of the brain to certain insults, or may impair repair mechanisms (DeCarli et al., 1999). This may influence the incidence of age-related depressive symptomatology, as well as being a forerunner to AD.

Iidaka et al., (1996) states that sub-cortical vascular changes especially in the white and grey sub-cortical areas are associated with major depression in later life. The existing literature suggests that the symptoms of depression has quite specific effects on cognitive functioning. It is possible that a proportion of subjects in the present study manifesting lower cognitive scores had some form of depressive disorder instead of their cognitive decline being due to AD or age-related dementia. Depression was found to specifically affect verbal fluency (Boone et al., 1994) and verbal memory (Channon et al., 1993). Performance on the CVLT might therefore be expected to show a negative effect of depression. This was not revealed in the present study but may have been due to the smaller numbers in the CVLT dataset compared with the CAMCOG dataset.

4.8 Changes in Cognition in Post-Menopausal Women: A Longitudinal Study

Of the 649 participants who completed the first CAMCOG test, 455 returned approximately 12 months later to complete a follow-up CAMCOG test (CAMCOG 12 months). The paired sample t-test between the baseline CAMCOG score and the CAMCOG follow-up, showed that cognitive scores were significantly different (t_{211}=-4.972; P<0.0010) with a mean difference of -1.2 indicating that CAMCOG scores generally improved after the 12 months. Approximately 33 % of the subjects who completed the second test had a decline in scores. The findings that 57% of subjects showed significant improvement in cognition is contrary to many longitudinal studies

on memory that suggest that cognitive ability gradually diminishes with advancing age (Mitrushina & Satz, 1991; Lamar et al., 2003; Nilsson, 2003).

The Berlin Aging Study (Singer et al., 2003) reports that perceptual speed, memory and fluency declined with age. Nilsson et al., (2003) state that semantic memory, short-term memory, perceptual and procedural memory show a relatively steady performance level with increasing age, but episodic memory is distinctive in showing age related decline. The current study differs from the previous longitudinal investigations in that it is the only one that employs the CAMCOG for cognitive assessment. These studies focused on selected aspects of memory, whereas CAMCOG measure is a global assessment of cognition. Thus, it is possible that the CAMCOG better represents cognition and memory than other assessments for the age group tested in the current study. However, the study by de Jager et al., (2002) suggests that the CAMCOG may not be sensitive in picking up changes in episodic memory. This deficiency may have contributed to the different findings between the current study and the previous longitudinal investigations.

Another explanation for the improved cognition in the majority of participants in the CAMCOG follow-up study may be due to practice effects. The time period of 12 months between assessments may have contributed to the results seen in the majority of subjects, with this period being not long enough to eliminate practice effect on the CAMCOG. This is consistent with comments made by some subjects on follow-up, when they stated that they remembered some aspects of the assessment. Some participants remembered answers to key questions such as recalling their address, historical questions and 'abstract thought' questions. However, this initial drawback may be beneficial in distinguishing those individuals who are cognitively normal, from those that have actual memory impairment leading to cognitive decline.

It was noted that individuals who performed poorly at baseline exhibited either minor improvement or actually declined in performance on follow-up, perhaps foreshadowing further cognitive decline. One other factor that may explain differences in the CAMCOG study may be differences in educational levels in this cohort, than in other groups. The variable 'CAMCOG Change' was created to reveal the overall change in CAMCOG scores over the 12 months. Using this approach, education was found to be the only independent variable that had a significant affect on CAMCOG Status (P= 0.049). This confirms the role of education in cognition in this group of subjects, with higher levels of education giving some degree of protection against decline in memory. Education may therefore be the primary factor influencing cognition in this group.

Of the 212 subjects that volunteered for a second CVLT assessment (approximately 53%), 58% at follow-up showed improvement in their scores, while 38% exhibited a decline over 12 months, similar to the longitudinal trend seen in the CAMCOG subset. These findings are very similar to that observed by the CAMCOG, thus giving the latter studies more credence. Generally, the CVLT is considered quite robust against practice effect but the time period of 12 months between tests may not be long enough to withstand the effects of test- retest practice. This problem may have been prevented if the word lists were different at follow-up (using alternate word lists). While the current study clearly differed for both the CAMCOG and CVLT cohorts in showing that a majority of subjects exhibited an improvement in scores, there is still a significant number (>30% for both tests) that clearly demonstrated a decline in cognition. It could be argued that this study discriminated between the healthy cognitive members in this age group and those that were at high risk of developing dementia. In support of this notion Cooper et al., (2001), investigated AD patients and controls that were subjected to repeated administrations of a common test of memory within a short time period. These investigators found that the non-demented subjects were the only group to

demonstrate practice effects whereas the AD group showed no such practice effect over several repeated administrations of these verbal fluency tests. Further follow-up studies will determine the clinical significance of my current findings.

4.9 Mild Cognitive Impairment: A Post-Menopausal Prospective

One of the objectives of the current study was to investigate the factors that influence cognitive status in older, healthy, non-demented post-menopausal women. In an attempt to achieve this goal, a biochemical analysis of selected candidates was undertaken on plasma samples from study participants in order to characterize the biological features that may influence memory and cognition in this group. Two measures of cognition (i.e. CAMCOG and CVLT) were chosen to identify specific features underlying cognitive decline in this cohort. In investigating age-related deficits in neurocognitive status, it is necessary to investigate the diagnostic category of 'mild cognitive impairment' (MCI), and the functional transitions that allow us to differentiate between normal aging, MCI and AD. Arguably, the identification of MCI in the elderly may represent the earliest intervention time for possible therapeutic treatments before the expression of frank brain pathology and clinical symptoms associated with AD.

As stated in Chapter 1, mild cognitive decline (MCI) can be likened to the transition stage between healthy cognitive aging and dementia, i.e. it represents a prodromal phase of AD (DeCarli, 2003). Lopez (2003) states that up to 22% of individuals 75 years or older manifest some form of MCI. Usually, the symptoms present as memory impairments, but deficits in other realms of cognition are also apparent. Impairments in language, visuo-spatial awareness and attention can be features of MCI. Petersen et al., (1999) state that MCI is typically characterised by symptomatic and progressive memory impairment that can later lead to the development of AD. Importantly, there is an absence in MCI of the *bona fide* qualities of dementia, broadly defined by the

Diagnostic and Statistical Manual of Mental Disorders (3rd edition). Age-related impairments in memory may be defined more specifically as amnesiac MCI. Morris et al., (2001) have shown that amnesic MCI cases manifest neuropathology associated with AD, including increases in the density of neurofibrillary tangles and neuronal loss. Guillozet et al., (2003) showed that NFT increases occurring in MCI are pronounced in the medial temporal lobe (which is associated with memory performance). But, interestingly, MCI patients do not show a decrease in choline acetyl transferase activity, as AD patients do (Lopez et al., 2003). This may be a later feature of the disease.

MCI can be classified as an early stage of AD, with studies suggesting that deficits in several memory domains may predict future AD development (Blanchet et al., 2002). However, this decline may take many years to progress to clinically recognisable symptoms. Other sub-classifications have been suggested to further define MCI as a clinical condition, such as "MCI- multiple domains slightly impaired" and "MCI single non-memory domain" (Busse et al., 2003). Nevertheless, studies on AD patients show that deficits in both episodic and semantic memory (especially the former) are the earliest indications of the disease (Corey-Bloom et al., 1995). Rapid forgetting of newly learnt material and semantic deficits can be traced back to lesions in the entorhinal cortex, medial temporal structures and temporal neocortical areas (Braak & Braak, 1991).

de Jager et al., (2002) examined the sensitivity of a variety of psychometric tests for identifying MCI. Cognitively intact individuals, MCI diagnosed and possible AD or probable AD patients were given tests in memory, attention, executive function, speed and visuo-spatial skills. The specific measures of category fluency, episodic memory and executive functioning were proven to be sensitive in differentiating MCI patients from AD afflicted individuals. Furthermore, tests assessing new learning, delayed recall and attention/executive functioning (Arnaiz et al., 2003) as well as assessments of

verbal and visuo-spatial aspects of cognition (Bondi et al., 1999; Flicker et al., 1993; Almkvist et al., 1998) were proficient at identifying changes characteristic of MCI.

An overview of the literature on MCI and the memory domains typically affected in this condition indicates that the different component measures on the CVLT could be used to differentiate between MCI and normal elderly individuals. The three measures that were extracted from the CVLT (namely, SUM1to5, the Discriminability Index and the Forgetting Index) may therefore be effective in detecting the early cognitive changes occurring in MCI and AD. In particular, the Sum1to5 measure may be most effective, as it documents changes in verbal learning rates across all five acquisition trials. The current study identified 33% of subjects with cognitive decline after the 12 month follow-up assessment. A longer follow-up period is required to ascertain the sensitivity of the CVLT instrument in the detection of early stage dementia in this cohort.

The CAMCOG measure was used in the present study as part of an epidemiological investigation into the risk factors associated with cognitive decline. The CAMCOG may also pick up early changes associated with MCI and AD. As part of the "Odense Study", Lolk et al., (2000) reported that the CAMCOG may be effective at diagnosing dementia in its earliest stages. The authors found that determining an individualised cut-off score for CAMCOG resulted in a sensitivity of about 80% in picking the subtle changes associated with very early dementia (Cut-off score equalling 88/89).

Numerous studies have examined specific predictive factors in order to try to detect individuals who will later develop AD and dementia. Neuroimaging techniques have been demonstrated to be effective tools to assess disease progression. Magnetic resonance imaging (MRI) studies show that hippocampal atrophy assays are effective at showing age-related changes well before the symptoms of AD are apparent (Fox et al., 1996; Jack et al., 1997; Kaye et al., 1997). These measures, along with tests of

cognition, measures of cerebral blood flow, genetic makeup, cortical glucose metabolism and daily living measures (specifically, measures of basic physical functions as well as more complex instrumental activities) can be effective predictors of future cognitive decline (Tabert et al., 2002). In future studies, it would be advisable to obtain MRI (and possibly PET) scans of relevant brain areas, in addition to deriving biochemical markers, in order to provide a more comprehensive, multidimensional depiction of the factors influencing cognitive decline in the elderly.

4.10 Polycystic Ovary Syndrome (PCOS)

Polycystic ovary syndrome (PCOS) is a condition that causes irregular ovulation or anovulation in women (Polson et al., 1988; Goldzieher, 1981). It is usually accompanied by symptoms of androgen excess including hirsutism, obesity and acne. The ovaries of affected women are usually enlarged (Polson et al., 1988), oyster white in appearance and cyst like under the surface. Research has indicated that women with PCOS have an increased ratio of LH to FSH, and it is this excess of LH that is thought to cause the over-production of androgens in these women (Goldzieher, 1981). Studies in women with PCOS using regular blood tests and sensitive LH assays have shown that more than 75% of anovulatory women showed increased LH blood concentrations (Taylor et al., 1997). Characteristically, LH secretion is exhibited with increased amplitude and an accelerated pulsatile rate in PCOS women compared with non-PCOS women. This pattern of over-secretion of gonadotropins results from the increase in GnRH pulse activity, which promote impaired follicle development (Barontini et al., 2001). Existing literature suggests that PCOS clusters in families. Indeed, there is empirical evidence of a genetic component which appears to reflect an autosomal dominant heritability (Govind et al., 1999) or an oligogenic mode of inheritance in two key genes, CYP11a and the insulin gene VNTR locus (may be associated with AD) (Legro et al., 1998; Majores et al., 2002).

Of most relevance for the current study is the link between elevated LH levels and PCOS and the clinical symptoms that have been described in affected women. An established characteristic of PCOS is insulin resistance (Franks, 1995). IGF-1 (insulin-like growth factor) actively brings-about insulin resistance, and increases androgen production (Majores et al., 2002). IGF-1 also induces the expression of LH receptors and through increased LH production causes increased androgen secretion. Interestingly, patients with type 2 diabetes exhibiting insulin resistance are at higher risk of developing AD (Majores et al., 2002).

Another interesting parallel between AD and PCOS, is the fact that the ovary is compromised (for different reasons) resulting in reduced oestrogen levels. Like AD, PCOS subjects are exposed to both decreased oestrogen and increased LH; which together may be implicated in increased Aβ production. In addition Rasgon et al., (2003) report increased incidence of depression in women with PCOS, with many studies showing a link between insulin resistance and depression (Brunswick et al., 1988; Okamura et al., 1999; Chiba et al., 2000). One theory for the increased rates of AD and dementia in depressed patients may be related to elevated cortisol, which can have a detrimental effect on the hippocampus (Pomara et al 2003). Homologous to cortisol's effect on the hippocampus, LH as stated previously, may also have a detrimental influence on hippocampal functioning since elevated LH is characteristic of PCOS. Empirical studies into the relationship between PCOS and dementia need to be carried out to elucidate the possible association between the diagnostic features of this condition, and the incidence of AD and dementia.

4.11 Conclusions and Future Implications

Considering the plethora of literature surrounding oestrogen and memory in post-menopausal women, it is perhaps surprising to find there are no conclusive outcomes in

the published literature. There is substantial evidence from small case control studies, suggesting that use of exogenous oestrogen supplements in post-menopausal women is beneficial for cognitive functioning, and that the use of such treatment reduces the risk of future dementia and AD. Evidence also exists on possible neuroprotective effects of oestrogen, and oestrogen's ability to increase neurotransmitter supply in the brain. Nevertheless, large double-blinded, placebo controlled studies such as the Women's Health Initiative (WHI) investigation into oestrogen treatments and cognition (Rapp et al., 2003) and the recent clinical trial by Shumaker et al., (2003) have proven otherwise, finding little effect of the hormone on memory capacity.

Less evidence exists on the role of endogenous oestrogen in cognition which was the focus of this study. The outcome of the current study again puts oestrogen's role in cognition into question. In particular, the present study does not support the hypothesis that endogenous oestrogen levels are positively correlated with cognition, as measured by CAMCOG and CVLT. These two measures were used to assess specific characteristics of memory and cognition. Furthermore, the relatively large dataset obtained in the study has allowed for powerful statistical analysis techniques (such as multiple linear regression) to be applied. The reasons for this outcome have already been considered, but perhaps the level of oestrogen in these elderly women had little correlation with cognitive status at this stage of their lives. The amount of adipose tissue released oestrogen would also be compromised. It is possible that oestrogen levels at younger ages (in the earlier post-menopause years, particularly) would therefore be better predictors of future cognitive status, because differences between low risk and high risk individuals can be more discernable.

The improvement in the majority of individuals' test scores at 12 months for both the CAMCOG and CVLT test data was not anticipated. However, given that both of the above tests were undertaken over a relatively short time between test sessions, this

outcome is not surprising. An interesting future study in this participant sample may involve only analyzing those subjects who showed decline in cognitive performance across the course of testing. These individuals are most likely to suffer from some form of future clinical decline in memory, and may have biochemical profiles that may be more relevant and useful to study.

Investigating the role of FSH and LH in sub-serving cognition proved to be more informative in this study. High LH plasma levels were associated with lower levels of cognition in non-*APOE* ε4 individuals, whereas FSH had the opposite association with cognition, whereby increasing FSH levels were associated with higher scores of cognition. This finding could prompt a flurry of hypotheses regarding the role of the gonadotropins as potentially important biochemical markers of age-related cognitive status. Previous studies have linked the gonadotropins to AD, but there is limited evidence regarding their effects on memory or on brain regions associated with memory and cognitive functioning. The present finding suggests a differential role of the gonadotropins LH and FSH. Furthermore, it seems conceivable that elevated levels of FSH may exert its beneficial effects of oestrogen on cognition, via reduction of Aβ levels. Future studies are warranted to test this notion.

The dynamics of the HPO axis dictate that oestrogen and the gonadotropins comprise elements of a feedback system, but after menopause diminished oestrogen causes a rise in FSH and LH. The once established rules governing the reproductive cycle are broken at menopause. Individuals with higher levels of endogenous oestrogen after menopause and into older age may have lower levels of circulating gonadotropins, so that the potential effects of these hormones on cognitive functioning may not be apparent. This may not be the case with individuals possessing very low oestrogen levels, who

presumably manifest higher levels of the gonadotropins. This notion needs to be addressed by further investigation

Although the CAMCOG data is valuable and very informative, it would have been preferable if the CVLT data had been analyzed in the context of gonadotropins levels as well. Unfortunately, due to time and financial restraints, a measurement of plasma levels of FSH and LH was not performed on the CVLT cohort. These analyses would not only have substantiated the results obtained with the CAMCOG, but would also have provided more insight into how LH/FSH may influence verbal memory. A longitudinal study over 3 to 5 years into the influence of the gonadotropins and oestrogen on memory, taking into account other factors such as ApoE status, medical history and Aβ levels, may be the next natural progression for this program of research. Incorporating two or more additional cognitive measures into the study design may also prove useful.

The present study has confirmed the importance of factors such as age and education when investigating the relationship between biological markers and neurocognitive status. Similar to most other studies conducted in this area, age had a marked influence on cognition. Education was also a significant factor, with higher levels of education related to better cognitive scores. An individual's levels of Aβ40 did not prove to be an overwhelmingly strong predictor of cross-sectional cognitive status in this group, though an association was found in CAMCOG for *APOE* ε4 individuals and for two measures of the CVLT cohort. Subsequent analyses of Aβ40 over time may prove to be more informative. More importantly, Aβ42 analyses in the future may be more informative, since this form of Aβ is more tightly coupled to the pathogenesis of AD.

What are the main biochemical predictors of cognitive decline in post-menopausal women? This research study has considered a wide variety of biological and social

factors that may affect memory, and potentially assist in the diagnosis of dementia and AD. There may be a need in the future to routinely measure FSH and particularly LH levels in women - perhaps starting many years previous to menopause. Changes in the HPO axis may have effects much earlier in life, but only have clinical significance many years later. Measuring LH and oestrogen levels in plasma samples may therefore indicate future cognitive status. If future studies confirm the interesting findings reported in this study, the therapeutic significance of lowering LH levels will be self-evident.

Furthermore, it may be important to emphasize that education early and throughout life can possibly increase the 'cognitive reserve', at a functional level, and perhaps even the 'biological reserve', at the neuronal level, thereby decreasing the risk of future neurocognitive decline. Additional emerging evidence exists on the use of statins, which may be useful in reducing the risk of certain factors important in AD development. Considering the substantial evidence available, regular measurements of amyloid-beta (both Aβ-40 and Aβ-42) may also assist in future longer term diagnosis of AD. *APOE* genotype is a significant factor in the incidence of AD, and needs to be examined in conjunction with LH for predictive value.

It is apparent that the study of cognition and its decline with age and in diseases such as AD requires a multi-disciplinary approach. Biochemical, genetic, psychosocial and environmental factors all have a role in how the major neural regions and mechanisms underpinning memory and cognition hold up as the individual ages. To understand the complex relationships between these factors requires considerable laboratory resources, personal commitment and intellectual insight. In the present study, a large sample of older women were evaluated using two different measures of memory and a range of hypothesis-driven biological tests, In future, additional insight will be gained from

extended longitudinal investigations of this participant sample. More specifically, the rate of cognitive decline over time and the biological markers associated with this cognitive decline should be evaluated over several years of investigation to gain further insight into the mechanisms underlying neurocognitive deterioration in the ageing individual.

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