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Results from the Canadian Study of Health Aging

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CIRCULATING LEVELS OF BIOAVAILABLE TESTOSTERONE AND ESTRADIOL AS RISK FACTORS FOR DECLINE IN COGNITION. RESULTS FROM THE CANADIAN STUDY OF HEALTH AND AGING

by

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Thesis submitted to the Faculty of Graduate and Postdoctoral Studies in partial fulfillment of the requirements for the MSc degree in Epidemiology.

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ABSTRACT

Using the data from the 1991-2001 Canadian Study of Health and Aging, this study examined the association between sex hormone levels and cognitive decline. The objective was to reach a better understanding of the effect of sex steroid hormones on cognition in men and women over the age of 65. Initial hypotheses postulated the existence of a protective effect of circulating hormone levels on the risk of developing dementia. This would support the judicious use of hormone replacement therapy (testosterone and/or estradiol) in dementia; either delaying the onset of dementia or slowing its progress.

The Canadian Study of Health and Aging (CSHA) included 10,253 participants. The outcome of interest was the decline in cognitive function. This was defined as a change in cognitive status from normal or impaired cognition to dementia. Blood samples were collected in 1991, 1996 and 2001. Total testosterone, bioavailable testosterone, estradiol (E2) and sex hormone binding globulin (SHBG) levels were measured. The E2/SHBG ratio was calculated to estimate the active form of estradiol. Hormone levels from subjects with no decline in cognition over a five-year period (1991-1996 or 1996-2001) were compared to those with a significant decline in cognition. A total of 485 subjects (303 females and 182 males) were included in the analysis.

Statistical analyses were performed to determine the relationship between hormone levels and cognitive decline. Distribution of hormone levels for each diagnostic group was examined. Some diagnostic groups had very few subjects. Odds ratios described the association between hormones and diagnostic group. Odds ratios were estimated by logistic regression; confounders and effect modifiers included age, smoking and alcohol intake, body mass index, Geriatric Depression Scale and years of education. The mean age of the

population was 78.7 for men and 81.3 for women. Education level for men was 10.3 years and 9.8 years for women. The mean 3MS score was 84.5 for men and 86.1 for women. Levels of circulating hormones in men were for Total Testosterone (TT), 22.1 (± 9.1) nmol/l for Bioavailable testosterone (BT), 5.3 (± 2.3) nmol/l; Estradiol (E2), 173.9 (± 133.2) pmol/l; Sex Hormone Binding Globulin (SHBG) 55.4 (± 22.8) nmol/l; E2/SHBG, 3.6 (± 3.0). Respectively for women the results were TT, 2.8 (± 2.2) nmol/l; BT, 1.7(± 0.7) nmol/l; E2, 147.9 (± 196.6) pmol/l; SHBG, 77.4 (± 46.1) nmol/l; E2/SHBG, 2.2 (± 2.4).

The results from this study did not reveal any significant association between the circulating levels of total testosterone, bioavailable testosterone, estradiol or the E2/SHBG ratio and cognitive decline. In the logistic regression, the ORs were all very near 1 and not statistically significant. This led us to question the impact of the lack of sensitivity of assays for testosterone and estradiol because in an elderly population when the hormone levels are at their lowest. Also, the small sample size and heterogeneity in a number of diagnostic groups could account for the absence of statistical significance.

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INTRODUCTION

The last ten years have made apparent the growing burden of cognitive impairment in the Canadian aging population and thus taking its toll on the Canadian healthcare system and Canadian society. As an active family physician, it is a daily concern to deal with patients who can no longer remain safely in their environment because of slow cognitive decline, to support families who are overtired in caring for their aged parents and to care for the psychological stress of all those involved. Researchers, policy-makers and administrators alike are aware of the increasing problem. The suggestion that the level of sex hormones could be linked to a proportional loss of cognition is thus appealing for those looking at avenues to alleviate the burden of aging. Levels of sex hormones, we know, decrease with age and are part of the aging process. Similarly, cognition declines with age. This raises the question as to whether there exists a correlation between sex hormone levels and cognition. If such a correlation could be proven, new pathways to alleviate the burden of cognitive decline and dementia could be explored thus benefiting patients, caregivers and health professionals.

In November 2002, the author participated as physician in the phase 3 of the Canadian Study on Health and Aging (CSHA). This included the task of examining the participants of the Chicoutimi region in the province of Québec and that of attending the consensus meetings together with the nurse and the neuropsychologists. This allowed the author to become familiar with the methods of the study and elicited a number of questions with regards to aging. The issue of the relationship of sex hormones with aging was one of those, and is the subject of this work. Blood samples of the CSHA population had been obtained at different phases of the study, these could therefore be analyzed and the levels of

the sex hormones could be examined in relation to the cognitive level of the subjects. Funds ($56,000) to process the blood samples were obtained from Organon a pharmaceutical company specializing in contraceptives and hormonal therapy.

CHAPTER 1: BACKGROUND AND SIGNIFICANCE

BURDEN OF DEMENTIA

Epidemiological studies show that dementia is age related (Fratiglioni, 2000; Hendrie, 1998; Canadian Study of Health and Aging, 1994) and that the incidence of dementia increases exponentially to the age of 90 (Jorm, 1987). Along with this, figures reveal that our population is aging. Indeed, in July 2002, Statistics Canada reported findings from the 2001 census concerning the age of the Canadian population. It reports that 'the population's median age (37.6) has reached an all-time high and the change since 1991 represents the highest census-to-census increase registered in a century'. There was a 6% increase of the population aged between 65 and 69 between 1991 and 2001, reaching 1 million of individuals; the population aged between 70 and 79 reached nearly 2 million (an increase of 27%). Statistics Canada reported the highest increase in the oldest portion of the Canadian population (aged 80 and over). This portion of the Canadian population increased by 41% in the 10-year span between the two censuses. Altogether, the number of Canadians over 65 years has reached nearly 4 million; this represents 7.5% of the total population. In consequence, clearly, the prevalence of dementia will increase. Alzheimer's disease (AD) is the most frequent type of dementia. In 2001, an estimated 238 000 Canadians had AD and the number is expected to rise to 750 000 for 2031 (Canadian Study of Health and Aging, 1994).

Estimates have been made of the economic costs of Alzheimer's disease. A recent report commissioned by the Alzheimer's Association (Hebert, 2003) states that Alzheimer's disease is costing the American economy US $61 billion a year. For Canada, a similar analysis has estimated the cost to be of CAN $ 9 billion each year. Clearly, the cost of Alzheimer's disease does not derive only from the cost of the care to patients themselves. Therefore, the economic analysis looked at the issue with a societal perspective and included costs related to the caregivers, thus costs associated with absenteeism, loss in productivity, replacement cost of workers (chiefly among family or relatives acting as primary caregivers) and insurance fees. The personal toll on those caring for a loved one with AD is often undervalued; the burden inevitably carries over to the workplace and overall quality of life. The societal cost of aging, especially generated by degenerative diseases like dementia and AD, is surely underestimated. It is indeed difficult to produce an accurate estimate of the cost of diseases that affect very elderly people. Thus, any age-related disease becomes cause for concern for health policy-makers, health professionals and society, as the burden it represents will continue to rise.

The aging of the Canadian population is thus a growing societal concern. Because of this, the factors contributing to the degenerative processes of aging and the ways in which we can modify these factors to slow the aging process are of interest. The quest to identify factors associated with aging and those that contribute to cognitive decline has thus far yielded limited knowledge. Yet a number of biochemical changes accompany the aging process. One of these, and well known to the general public, is the deficiency in sex hormones with the onset of menopause in women and andropause in men. There is growing evidence that levels of circulating sex hormones influence the integrity of cognitive function. Hence, a better understanding of this association may indicate the feasibility of modifying the

incidence of cognitive decline with aging via pharmaceutically altering levels of sex steroid hormones.

We will first define cognition and review which sex hormones are relevant to the present study.

COGNITION

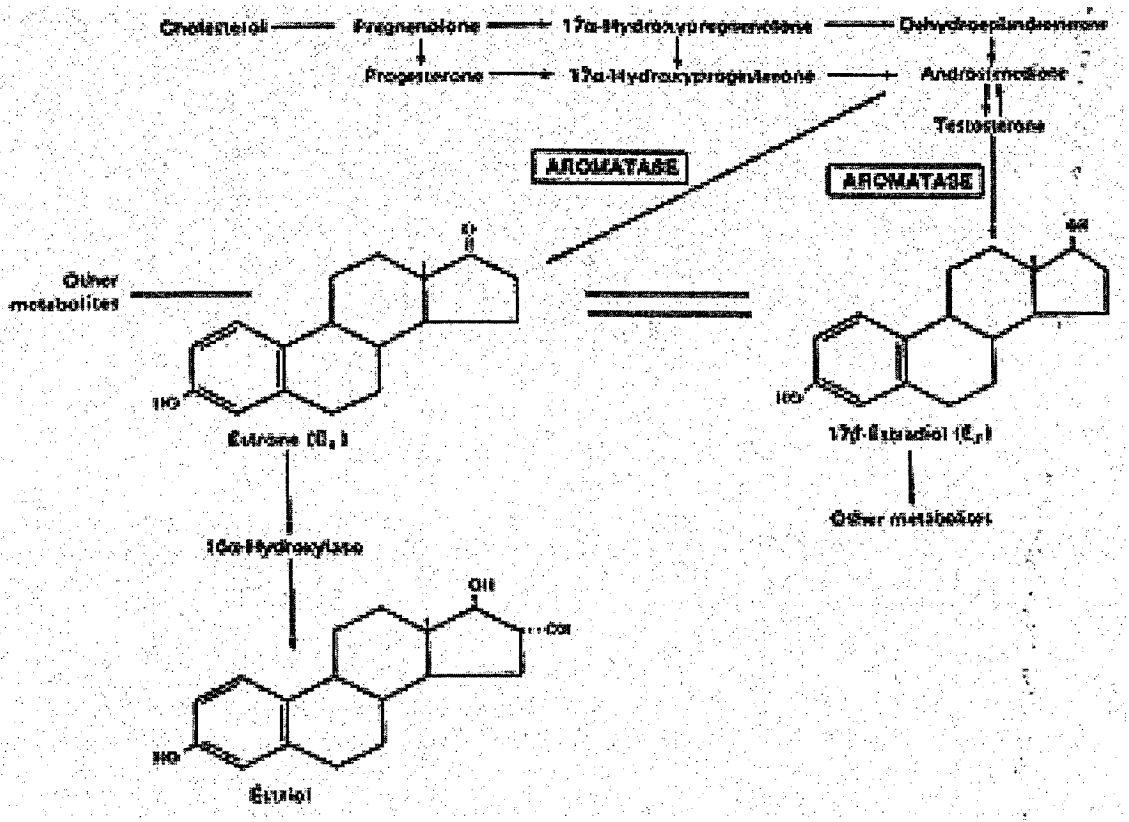
Cognition is a multidimensional entity and must be seen as such. Because of this, exploring cognition is a complex procedure and a number of different tests are often necessary to study the different facets of cognition. Cognition encompasses such functions as memory (verbal, visual and spatial), language (including verbal fluency), attention, learning, abstract thinking, problem solving, and pattern recognition. Such functions are easily tested by a number of validated tests but cognition also represents complex intellectual functions and psychomotor skills. Memory, which is only one component of cognitive processing, is in itself, composed of numerous processes. Research has shown that different anatomical sites of the brain are responsible for long-term and short-term memory. Halpern (1992) has determined that both sexes reveal differences in performance and abilities of different cognitive processes. In tasks of verbal skills and memory women tend to perform better. They perform also better than men in tasks of perceptual speed and accuracy as well as in fine motor skills. On the other hand, men have been shown to outperform women on tests of visuospatial working memory, and on mathematical and spatial ability. Researchers think that these differences, which correspond to differences in organizational and activational skills, are the result of hormone influence on the brain occurring early in life, during the foetal period. This information allows us to hypothesize that estrogens would thus have more effect on those cognitive tasks

identified by Halpern as female tasks, such as verbal skills, perceptual speed, memory and accuracy and fine motor skills and androgens would act more specifically on visual memory, spatial ability and mathematical ability. This could be mediated either because of a direct effect of estrogens on the brain, by a higher ratio of estrogens than androgens or by an increased number of receptors of estrogen receptors in specific areas of the brain.

SEX HORMONES

Testosterone is the principal male hormone and is synthesized in the testes; it also exists in small quantities in women. Estradiol (E2) is the most potent natural estrogen and circulates predominantly in a protein-bound form. The ovary is the main producer of estradiol in women. In men, the testes and androgen precursors are the principal source of estradiol as testosterone is, in itself, a precursor for the synthesis of estrogens in the testes, adrenal gland and fat tissue. In postmenopausal females, estradiol is the principal estrogenic hormone in circulation, and is in metabolic equilibrium with estrone (E1). The small amount of testosterone measured in female plasma results mainly from peripheral conversion of androstenedione (androgen produced by the adrenal gland) to testosterone. Under which form (protein bound or non-protein bound) the hormones circulate in the plasma is of importance as it determines the availability of the hormones to target cells.

Figure 1: Testosterone/estrogen metabolism



Serum testosterone as well as estradiol is bound to a specific plasma carrier protein, the sex-hormone binding globulin (SHBG) and to albumin. About two-thirds of the total testosterone is bound to protein. Free testosterone and free estradiol are the circulating fractions not bound to either SHBG or albumin. The bioavailable fractions of testosterone or of estradiol are the parts not bound to SHBG (i.e. the free plus the albumin-bound fractions). The bioavailable components appear to be the most influential in physiological changes as they permit better access to the target tissues. Therefore in this study, we will measure total testosterone, bioavailable testosterone, estradiol and SHBG in both men and women.

As cognitive decline and dementia represents a definite burden on families and society, researchers strive to understand factors that influence the mechanisms underlying

normal and pathological aging. It seems legitimate to ponder whether the changes in sex hormones at menopause and andropause affect the brain, cognition and the development of dementia. Recent advances, which we will review, have looked into the effects of sex hormones on cognition and dementia If, indeed, a significant role were to be identified, then prevention through hormone replacement therapy (HRT) could become an appealing approach to slow the degenerative process. However, this may not be risk-free: the recent results of the Women's Health Initiative (Writing Group for the Women's Health Initiative, July 2002) showed an increase by 26% of the relative risk in breast cancer in women taking HRT. A fuller understanding of the true effect of androgens in men and women on cognition becomes all the more pertinent and crucial.

We will now review the literature on the levels of estrogens and androgens in menopause and andropause and the effects of hormones on cognition and how hormonal change, through replacement therapy, can influence cognition.

CHAPTER 2: HORMONE LEVELS DURING MENOPAUSE AND ANDROPAUSE

HORMONE LEVELS DURING MENOPAUSE

A first step in the analysis of the role of sex steroid hormones is to determine if and how the blood levels of hormones vary once menopause commences. A number of authors have followed women in the years following menopause to record changes in hormone levels.

Chakravarti (1976) studied the concentrations of testosterone, estrone and estradiol in 60 normal postmenopausal women at different menopausal ages. Compared to the values of the early phase of the menstrual cycle, estradiol and estrone concentrations had decreased to about 20%, in the year following menopause. The levels remained low in the subsequent 10 years into menopause. The levels of testosterone decreased to 60% of their premenopausal values as early as 2 to 5 years after menopause.

Rannevik (1986) examined the testosterone levels of 30 healthy women at regular intervals before and during the menopause. He tested the women over a 7-year period starting 3 years before menopause. None of these women were on hormone replacement therapy. Testosterone levels decreased progressively. In the 6 months prior to the onset of menopause, levels had decreased by 18% and then by 30% , three years after menopause.

In a later study, Rannevik (1995) studied 160 women not receiving hormonal therapy around the time of onset of the menopause to study the changes between the pre and postmenopausal period. In the first year following menopause, blood samples were drawn

every 6 months and then blood was collected every year. Altogether, the women were followed for a minimum of 12 years. In the 6 months around menopause, there was a decrease in estrogen levels (more significant for estradiol than for estrone). During the next three years, the levels of estradiol and estrone declined moderately and in a parallel fashion. Serum levels of testosterone and SHBG decreased around menopause. At three years after menopause and over the following 5 years the levels of testosterone and SHBG had reached a steady state and were relatively constant.

Over a period of 10 years, Jiroutek (1998) measured the levels of steroid hormones in 32 menopausal women. The measurements were started in the first year of menopause and done thereafter at 4 to 6-month intervals. Jiroutek found that the levels of estradiol declined significantly, however he did not find any significant change in the concentrations of estrone. In the same 10-year period, the levels of testosterone had increased.

Overlie (1999) studied the hormone levels of 59 Norwegian women annually for 5 years in the transition period before the onset of menopause and after menopause. The women were not on hormone therapy. Hormone levels were measured every 12 months. There was a decrease in the levels of both estradiol and estrone in all women before menopause and in the two years following menopause. Testosterone started to decline in the three years preceding menopause.

Hankinson (1995) studied the reproducibility of plasma hormone levels in 79 postmenopausal women aged between 51 and 69 years of age, over a 2 to 3-year period. Estradiol, free estradiol, estrone, SHBG and testosterone were among the hormones tested. As there was little fluctuation in the levels of hormone after menopause, the authors stated that after 3 years of menopause, a single measurement was sufficient to determine the

average level of hormones in postmenopausal women (This is pertinent to our study and its study sample).

In these studies, women were followed up between 5 and 12 years into menopause. The results are consistent. Estradiol is the hormone that shows the most significant decrease after menopause. This decrease starts from 6 months to 2 years prior to menopause and continues up to 3 years into menopause, when the levels then reach a steady state. A similar trend occurs for testosterone, with a slow decline in the months prior to menopause until 2 to 3 years after menopause. Only Jiroutek reported a rise of testosterone prior to its decline but only 32 women were tested. As the median age for menopause is 50 years, we can assume that in a population over 65 years of age (such as that of CSHA), hormone levels will be in a physiological steady state and will not vary significantly except through exogenous influences.

HORMONE LEVELS DURING ANDROPAUSE

Androgens have also been investigated to understand the role they may play in the development of dementia in men. Androgen levels decline progressively with age in men, described as the climacteric; those symptoms of male menopause can be reversed with testosterone therapy. The question arises: Could this type of intervention also benefit cognitive processes? The terms andropause or male menopause, although commonly used, are inappropriate as the levels of sex hormones do not suddenly decrease in men such as occurs in women at menopause. Testosterone production and plasma concentrations decline in middle-aged and elderly men and the term age-related androgen deficiency appears more appropriate. Both cross-sectional and prospective studies (Nankin, 1986; Gray, 1991;

Deslypere, 1984; Vermeulen, 1985; Simon, 1992) show a decline that begins during middle age and then progresses in a linear fashion. Along with this decline in testosterone is an age-associated increase of the sex hormone binding globulin (SHBG) in the bloodstream, which binds more testosterone, thereby further reducing bioavailable testosterone: the circulating or biologically active portion of testosterone.

Concentrations of bioavailable testosterone decrease by "as much as 50% between the ages of 25 and 75 years at an approximate rate of 10% per decade" (Korenman, 1990). Thus, with respect to bioavailable testosterone, as many as 50% of men over the age of 50 are in a hypotestosteronemic state. Authors define hypotestosteronemia as testosterone levels below 11nmol/l.

In a study of 372 males aged from 20-85 Vermeulen (1999) found testosterone levels between 14-27 nmol/l in the younger population and levels of 10- 21 nmol/l in the elderly. Free testosterone showed a decrease of nearly 50% declining from 0.72-0.44 nmol/l to 0.39-0.22 nmol/l. Obesity was also associated with lower concentrations of bioavailable testosterone.

Maier (2001) measured a number of hormones in aging men. He found that levels of testosterone, free testosterone, DHEA, LH, and growth hormone decreased, whereas estradiol, FSH, LH and SHBG increased.

Thus andropause represents a slow decline of testosterone over the years reaching a steady state at or about 75 years of age. This state of hypogonadism leads to a decline of energy, sexual dysfunction, depression, poor concentration and memory as well as various metabolic alterations.

Tan (2001) surveyed 302 men. Most of the sample of men (71%) was over 60 years of age. In those patients who felt they had experienced andropause, 36% reported some form of memory loss. Retrospectively, 64% of the men surveyed, reported they could date their andropause between the ages of 50 and 70. As it is known that the aging process of andropause is characterized by a decline in testosterone levels, the authors speculate that testosterone may have a role in the memory loss reported by men at that period.

The purpose of our study was to further examine the association between cognitive function and circulating levels of sex hormones in men and women. For this, we measured the hormone levels from blood samples collected in the Canadian Study of Health and Aging (CSHA). In this age group of people over 65, levels of sex hormones are at their lowest levels and their measurement can be challenging; the following paragraphs will therefore review the accuracy of measuring bioavailable testosterone and its relevance as marker for cognitive decline.

ACCURACY OF SEX HORMONE LEVEL MEASUREMENT IN AN ELDERLY POPULATION

Testosterone, bioavailable testosterone and SHBG

Men with low testosterone concentrations are usually hypogonadal. As there are also variations in the transport protein, sex hormone binding globulin (SHBG), which directly influences concentrations of the active form of testosterone concentrations, confirmation of low testosterone with a measurement of free testosterone or bioavailable testosterone is recommended. SHBG is known to increase with age: it increases slowly throughout life until the mid-80s when levels are about two-fold higher than concentration at 20 years (Selby,

1990). Free and non-specifically bound plasma hormone levels therefore generally reflect the clinical situation more accurately than total plasma hormone levels. It is thus important to have reliable measures of these fractions. Free testosterone as well as the fraction of testosterone not precipitated by 50% ammonium sulfate (non SHBG-T), often referred to as bioavailable testosterone, appear to represent reliable indices of biologically active testosterone.

To test the validity of the different indices used for free or bioavailable testosterone Vermeulen (2001) compared those indices in a large number of sera with variable SHBG capacities (low in hirsute women, extremely high in hyperthyroidism). The indices he measured, included AFTC (apparent free testosterone concentration obtained by equilibrium dialysis), FT (free testosterone obtained by calculation from total testosterone and immunoassayed SHBG (iSHBG) concentrations), aFT (analog free testosterone, direct immunoassay of free testosterone with a labeled testosterone analog), FAI (free androgen index which is the ratio T/SHBG) and non-SHBG-testosterone levels. The study revealed that those indices could reliably be interchanged. Thus the value of free testosterone derived by calculation from testosterone and iSHBG and the non-specifically bound testosterone, calculated from free testosterone was a reliable index of bioavailable testosterone.

Ooi et al. (1997) measured the circadian variation in the levels of free testosterone, total testosterone in 27 healthy Caucasian males. The authors used the Diagnostic Products Company Coat-A-count to measure free testosterone. In their analysis there was no effect of age, smoking, physical activity or sleep duration on the concentrations of free testosterone. However, there were 33% of subjects who had a significant (more than 35%) intra-individual variability in their hormone levels. The authors therefore recommended that the time of

sampling should be specified and be taken into consideration when interpreting the results of such hormones.

Winters (1998) examined the relationship between total testosterone, free testosterone and bioavailable testosterone with SHBG. The Coat-A-Count assay, from Diagnostic Products was used for the analysis. The study included 29 men and 28 women. In men, total testosterone was strongly and positively correlated with SHBG ($r = 0.68$; $p<0.01$); but bioavailable testosterone was independent of SHBG ($r=0.02$). In women, the levels of total testosterone, free testosterone and bioavailable testosterone all tended to be lower with increasing levels of SHBG. Thus, in men, levels of free testosterone and total testosterone are determined in part by the plasma levels of SHBG. Accordingly, when using only this assay in men with low SHBG, there is a risk of misclassifying androgen deficiency. Because of the interrelationship of testosterone and SHBG, and the possible variations in SHBG due to different factors (hypertension, hyperinsulinemia, diabetes) it is always recommended to determine levels of SHBG when measuring only total testosterone. On the other hand, bioavailable testosterone may be used on its own, as it seems to be independent of the SHBG levels.

Another reason that could account for low free testosterone concentrations is the use of inappropriate reference intervals, Ooi (1998). Ooi, at the Ottawa Civic Hospital, found that the reference values quoted in commercial kits were too high for their clinical population. Over 50% of patients attending an impotence clinic had subnormal values when using the recommended values from the kits. In view of this observation, there maybe a rationale to establish in-house reference intervals. This was also the experience of two other Canadian centres: the Women's College in Toronto and the Jewish General Hospital in Montreal.

Reliability of assays

In older men and women, circulating levels of testosterone and estradiol are at their lowest and dealing with error of measurement is of concern for the laboratories. A number of studies have also addressed the issue of inter-laboratory reproducibility and concordance of results in serum assays of hormone levels.

McShane (1996) examined the reliability and validity of serum measurements. Variance components analyses and estimated coefficients of variation were computed. Coefficients of variation were less than 10% for testosterone and less than 15% for estradiol.

Hankinson (1994) tested the ability of four well-established endocrine laboratories to reproduce the measures of plasma levels of estrone, estradiol, free estradiol, SHBG, and testosterone in postmenopausal women. Within-person coefficient of variation was low (<15%), but the laboratory error could be as large as over 25% in the measurements of estrone and estradiol levels. The results in the levels of testosterone and SHBG were also variable. When carrying out assays for research purposes, the authors emphasise to take into account the laboratory performance when interpreting laboratory results of hormone analysis. In the Methods section we will describe the laboratory chosen for this study.

CHAPTER 3: MECHANISMS OF ACTION OF SEX HORMONES ON THE BRAIN

In order to describe the biological plausibility for the hypothesis that sex hormones act on different aspects of cognitive function, some of the mechanisms that are most relevant will be briefly reviewed.

ESTROGENS

Estrogen receptors were identified in the brain in the early 1980s and cells that contain receptors were identified and mapped (Attardi 1976, Ginsburg 1977). Nerve cells containing estrogen receptors were identified in the hippocampus, cerebral cortex, midbrain and brainstem (Pfaff, 1980). Animal studies on rats have shown the crucial role played by the brain region of the hippocampus, the plasticity of which is in part modulated and regulated by hormones. It therefore has significant relevance in the study on human cognitive processes and neurological disorders. Spatial and explicit memory functions are closely related to the plasticity and the connectivity of the hippocampus. (Eichenbaum, 1992). The plasticity of the brain, which implies morphological changes in the adult brain, is supported by findings that steroids alter and affect the structure of the brain. Indeed, the influence of sex steroids on the brain includes remodelling and formation of synapses, changes and remodelling of dendritic structure and neurogenesis (McEwen, 2001). All of these actions affect the basic structure of the brain. Estrogens have been found to activate specific intracellular receptors. There are two types of estrogen receptors: ER-α and ER -β (McEwen, 1999). These receptors can be

either in the nucleus, the cytoplasm or the plasma membrane. Activation of these receptors by estradiol will modulate transcription and protein synthesis, thus having an effect on the genome this is referred to as the genomic mechanism of the estrogens. The estrogens also have direct non-genomic mechanisms. These actions include actions on the cell membrane including regulation of neurotransmitter transporters. "The combination of genomic and non-genomic mechanisms endows estrogens with considerable diversity, range and power to regulate neural function" (Wong, 1996). Increased neuronal survival is achieved through non-genomic actions. These "estradiol-activated mechanisms increase cell survival and protect cortical neurons from excitotoxic cell death" (Honda 2000; Singer 1999; Singh 1999). This was also confirmed by Bi who has found that estradiol protects hippocampal neurons from excitotoxicity through different signalling pathways (Bi, 2000). Toran-Allerand (1996) has established a connection between estrogen levels and the signalling of growth factor in the brain. Interactions between neurotrophic growth factors and estradiol regulate the life of the target neurons from neurogenesis, to differentiation, to protection from apoptosis enhancing neuron survival. Growth factors and estrogens can act synergistically or by regulating one another. Female hormones are responsible for induction of synapses in the hypothalamus and hippocampus of female rats. Studies of this process have shown the interactions between steroid hormones and neural cells and between hormones and neurotransmitters (McEwen 1995; Chadwick 2000). In female rats, findings suggest that the effects of sex steroid hormones on the 5HT2 receptors and serotonin transporters in the brain may induce mood changes. The potential for estrogens to modulate neurotransmitter release or reuptake may have direct clinical applications (McEwen, 2001). Estrogens "regulate synaptogenesis in the CA1 region of the hippocampus", studies show that the "formation of new excitatory synapses is induced by estradiol" (McEwen, 1997). This role of estrogens in

the formation of new synapses in a region involved in the memory processes opens a number of potential pathways to modulate memory (Cholerton, 2002). The effects of estrogens on the brain also include the increase of cerebral blood flow, and protection against apoptosis (death cell). Estrogens reduce the actions of free radicals that cause cell damage, but have also direct anti-inflammatory actions and anti-oxydant properties. In its protective effect against Alzheimer's disease, estradiol alters the metabolism of the amyloid precursor protein (APP). This protein is involved in the pathophysiology of Alzheimer's disease. Estrogens seem to facilitate the breakdown of the β-amyloid precursor protein. Once broken down in smaller fragments, the APP is less likely to accumulate as β-amyloid. This is yet another mechanism that supports the potentially beneficial effects of estrogens in the post-menopausal brain. Baker has also shown that the hormone estradiol may also serve as an A beta lowering agent (Baker, 2003).

ANDROGENS

As sex steroid hormones, androgens have similar mechanisms of action on the brain to estrogens. The effects of androgens are also mediated through androgen-specific receptors and through estrogenic effects by the aromatisation of testosterone to estradiol. Androgen receptors are localized throughout the brain and more specifically in regions involved with learning and memory. Fink et al (1999) reported their research on castrated male rats. They showed that the action of testosterone could be mediated after its conversion to estrogen via aromatisation. But animal studies also suggest different pathways of actions. Testosterone can affect cognitive function through actions on neurotransmitters and receptors; it promotes the release of acetylcholine and up-regulates nicotinic receptors. Studies have also looked at the effect of testosterone on the APP (A beta precursor protein) and Tau protein that are involved in the pathophysiology of Alzheimer's disease. It has been shown that by giving

animals androgen supplements, deposition of the Tau protein can be affected. In rats, for example, studies on neurons found that "testosterone increases the secretion of non-amyloidogenic APP" (A-beta precursor protein) fragment and "decreases the secretion of A-beta peptides" (Gouras 2000). Ramsden (2003) found that "androgens act as endogenous modulators of the beta amyl protein" (A-beta). When gonadectomy was performed in male rats, there was a direct decrease in sex steroid hormones, and a significant increase in A-beta brain compared to the levels in normal rats. When these rats were supplemented with testosterone (5-alpha dihydrotestosterone, DHT), the effects were reversed. Therefore demonstrating that modulation of A-beta levels in the brain was under the direct influence of androgens, as DHT cannot be metabolized to estrogens. "These findings suggest that age-related androgen depletion may result in an accumulation of A-beta in the male brain and thereby act as a risk factor for the development of Alzheimer's disease" (Ramsden et al., 2003). Thus, this raises the possibility that also testosterone may have a protective effect if used in the treatment of Alzheimer's disease.

Androgens may also act through different pathways. The synthesis of estrogens in the brain from the in situ aromatisation of androgens is androgen-dependent. Animal studies have shown that aromatase activity in the brain is modulated by androgen through the androgen receptors (Connolly, 1990). Thus, estrogens may mediate some effects of androgens on the brain. Leranth (2003) demonstrated that, both in male rats and ovariectomized female rats, synaptic density in the CA1 area of the hippocampus resulted from the direct effects of testosterone on the hippocampal androgen receptors, and that these effects were not mediated through the intracerebral estrogen synthesis. This could indicate "that androgen treatment, in females may be as effective as estrogen replacement therapy in

reversing the decline in hippocampal CA1 spine synapses that follows loss of ovarian function." (Leranth et al., 2004).

Testosterone also inhibits the induction of Interleukin-6. Interleukin-6 is a cytokine that is part of the immunological reactions involved in the major pathways. In Alzheimer's disease, the brains of patients have elevated concentrations of this cytokine. The neurodegenerative plaques of Alzheimer's disease are the sites of an active inflammatory cascade of reactions in which Interleukin-6 is involved. "The fact that IL-6 is detectable in early stage diffuse plaques encourages the speculation that the acute-phase process is crucial in the pathogenesis of Alzheimer's disease" (McCarty, 1999). Thus, through its inhibitory action on IL-6, testosterone could have a repressive effect on plaque formation in Alzheimer's disease.

In summary, there seems to be scientific evidence of a protective role on the brain for both estrogens and androgens. This protective effect is regulated through synaptogenesis, reduction of apoptosis, modulation of neurotransmitters and receptors as well as specific actions in the pathways of APP, the protein responsible for the neuro-degenerative plaques found in Alzheimer's disease. The exact biochemical mechanisms need to be better defined, as does the clinical importance of the declining hormone levels with age and their subsequent effect on the decline in cognition. Chapter 4 will review observational studies and therapeutic hormone replacement trials that demonstrate the role and influence of sex steroid hormones on cerebral functions and cognition

CHAPTER 4: ESTROGENS AND COGNITION

CIRCULATING LEVELS OF ESTROGENS AND COGNITION

As early as 1986, reports linked cognitive function to the use of estrogen replacement therapy in post-menopausal women. Evidence of an effect still remains doubtful because of inconsistent reports, but high levels of estrogen have been associated with enhanced verbal fluency, articulation and memory.

In 1996, Tang found the relative risk of developing Alzheimer's disease decreases with estrogen and that estrogens delay the onset of the disease. The authors studied 1124 elderly women. These subjects were taking part in a longitudinal study on aging and health in a New York City community. Of the 968 women who had not used estrogens, 158 (16.3%) developed Alzheimer's disease, whereas of the 156 women who had taken estrogen after menopause, only 9 (5.8%) developed Alzheimer's disease, giving a relative risk of 0.40 (95% CI 0.22-0.85, $p < 0.01$). The results also showed that the onset of Alzheimer's disease occurred later in women who had taken estrogen than in those who had not. The authors estimated the annual incidence of Alzheimer's disease to be 8.4% in non-users and 2.7% in estrogen users. Thus, this study gave an indication that estrogens could delay the onset of Alzheimer's disease.

Paganini-Hill (1996) carried out a case-control study to assess the effects of different estrogen preparations, dosages and duration of hormone replacement therapy (HRT) on the risk of developing Alzheimer's disease in postmenopausal women. This case-control study was part of a cohort study of residents of Laguna Hills, California (1981-1995). There were

8877 women in the cohort. Through information collected from death certificates, of the 3760 women who died between 1981 and 1995, 248 were identified as having had Alzheimer's disease. The study found that in estrogen users, the risk of Alzheimer's disease was significantly lower than in nonusers (OR 0.65; 95% CI 0.49-0.88). The decrease in risk was proportional to the dosages and duration of the estrogen replacement therapy. The lowest observed risk was for long-term users who received high doses. For these women, the odds ratio of having Alzheimer's disease was 0.48 (95% CI 0.19-1.17). This suggested that estrogen replacement therapy could be useful for preventing the onset of Alzheimer disease in postmenopausal women.

Kawas (1997) studied the relationship of estrogen replacement therapy on Alzheimer's disease in the Baltimore Longitudinal Study of Aging (BLSA) that included 472 post or perimenopausal women. The women were followed up for 16 years and estrogen replacement therapy was documented at each visit. Analyses study compared users versus non-users, and the relative risk for Alzheimer's disease was 0.46 (95% CI, 0.21-0.99). Although the findings showed additional support for a protective effect of estrogen in Alzheimer's disease the authors cautioned on the necessity to carry out randomized clinical trials to confirm this association.

In 2000, Drake showed an association between circulating steroid hormones and cognition in 68 non-demented elderly women in a longitudinal study. After exclusion of subjects and refusals there were 39 final participants. They measured circulating levels of estradiol and found that subjects with high estradiol levels performed better on delayed-recall memory and retrieval efficiency tests, whereas when estradiol levels were low, the performance was better for immediate and delayed visual memory.

Also in 2000, Manly examined the relation between endogenous levels of estradiol and Alzheimer's disease in post-menopausal women. This was a case-control design with 143 participants: 50 women with Alzheimer's disease and 93 non-demented controls. The data were collected over a period of one year. The results suggested that patients with Alzheimer's disease were four to six times more likely to have low levels of estradiol than the controls after adjusting for age, years of education, presence of an APOE-4 allele, ethnicity and body mass index.

However Mulnard's study (2000) failed to show any beneficial effect of estrogen therapy once the disease is present. The study was a randomized, double blind, placebo-controlled trial carried out between 1995 and 1999. There were a total of 120 participants. The women had had a total hysterectomy and had mild to moderate Alzheimer's disease and Mini Mental State Examination scores between 12 and 28. Participants were randomized to receive either estrogen 0.625mg per day (n = 42), estrogen 1.25mg per day (n = 39) or placebo (n = 39). A total of 97 subjects completed the trial. The primary outcome was change on the Clinical Global Impression of Change (GCIC); secondary measures included cognitive domains such as memory, attention and language. Follow-up assessments were made at 2, 6, 12 and 15 months. For the GCIC score, the performance was worse in 80% of participants taking estrogens compared 74% of participants taking the placebo (p = 0.48). Secondary outcome measures also showed no significant differences. For the Clinical Dementia Rating Scale, the results suggested worsening among patients taking estrogen. The authors concluded that their study did not show any beneficial effect of estrogen replacement therapy. In their population, estrogen replacement therapy taken for a period of one year could not slow the progression of the degenerative process. Moreover, global, cognitive, or functional outcomes in women with mild to moderate Alzheimer's disease had not improved.

These results may suggest that once the degenerative process is initiated, estrogens may not be able to slow down or reverse the process.

ESTROGEN REPLACEMENT THERAPY

Effect of estrogen replacement therapy on circulating levels of androgens and estrogens

Women on hormone replacement therapy (HRT) usually receive oral conjugated equine estrogen, which is a mixture of different estrogens. This makes it difficult to measure precisely the estradiol levels in women on HRT, and the use of a high-performance radioimmunoassay to test for levels of estradiol, is highly recommended in such situations. Women on HRT achieve different levels of estradiol. The levels can be divided into low, middle or high estradiol level groups: low is typically defined as < 15 pg/ml, middle between 15 and 25 pg/ml and high >25 pg/ml (Yasui 2001).

Casson (1997) measured testosterone, estradiol and SHBG in 28 postmenopausal women on HRT. They received 2 mg/day of oral micronised estradiol. Estrogen replacement therapy raised mean serum estradiol from 8.7 pg/ml to 117 pg/ml. On the other hand, testosterone fell from 16.1μg/dl to 9.4 μg/dl, and SHBG increased by 160%. Because of the important increase of SHBG, they inferred that bioavailable testosterone must be profoundly reduced.

Gavaler (2002) examined estradiol concentrations in 309 postmenopausal multiracial women receiving hormone replacement therapy. The authors observed that only 51% of the women achieved therapeutic levels of at least 45 pg/ml. Different factors were deemed

responsible for women not achieving a therapeutic level. These factors included: 1) receiving HRT in a dosage lower or equal to 0.625mg daily, 2) being of Caucasian race and 3) low level of education (high school or lower). Thus, even if women report being on HRT, this does not mean that their level of estrogens is therapeutic or high. Conclusions can therefore not be derived by self-reporting of HRT but must be confirmed by serum level measurement.

These studies show that various factors can cause differences in hormone levels when women are on hormone replacement therapy. Interestingly, knowing that estrogen replacement therapy influences the levels of bioavailable testosterone through the increase of the SHBG raises the question as to whether any beneficial effect of increasing the levels of estradiol may be countered by significantly lowering bioavailable testosterone.

Effect of estrogens on cognition

Rice (2000) examined cognition in relation to the use and non-use of HRT in a cohort of the KAME Project. The KAME project is a longitudinal, population-based study. It was initially designed to establish what were the rates of dementia in older (over 65 years of age) Japanese Americans in the Seattle region, Washington. The objective was to determine whether there was an association between unopposed estrogen or combined estrogen-progestin use and the 2-year rate of cognitive change in this cohort. There were 837 women at baseline and at the 2-year follow-up. Cognitive performance was measured with the Cognitive Abilities Screening Instrument (CASI) (Teng, 1992). The results showed that there was some beneficial association for current estrogen-only users with the 2-year cognitive change. For the current estrogen-progestin users, the rate of cognitive change worsened.

In a small intervention trial, Asthana (2001) conducted a study on 20 postmenopausal women with AD. The patients were randomized to receive high doses of estradiol or placebo during 8 weeks. Subjects on estrogen therapy had improved performance on attention (Stroop Color Word Interference Test), verbal memory (Buschke Selective Reminding Test), visual memory (Figure Copy Memory), as well as improved performance of semantic memory (Boston Naming Test). Although this small study supported the evidence for a cognitive benefit of estrogen for women with AD, this is contrary to Mulnard's study who did not show any beneficial effects of giving hormone therapy to Alzheimer patients; larger studies and longer periods of treatment are definitely warranted.

Henderson (2003) looked at the relation between episodic verbal memory in women at menopause and estrogen exposure at midlife. There were 326 women aged between 52 and 63 years. These subjects were part of the Melbourne Women's Midlife Health Project. They were asked to complete a word list memory task: episodic verbal memory was assessed via recall of a word list. During the first 8 years of menopause, the results showed that episodic memory performance was stable. The results in performance were not associated with current or prior treatment with estrogen. The authors did not find any variation with the duration of exposure to hormone replacement therapy and memory performance was unrelated to the measured levels of estradiol. Parity, however, was positively associated with memory. The authors observed that women who began taking HRT during the menopausal period had better memory scores than women who began estrogen after their last menstrual period.

Animal studies have shown the effects of estrogen on memory. In rats that have had their ovaries surgically removed the effects are more evident when the interval between the ovariectomy and estrogen administration is relatively short. This indicates that there might be

a critical time at which replacement therapy needs to be instituted in order to influence memory. The following table summarizes the studies reviewed.

Table 1: Summary of the studies reviewed on estrogen and cognition.

YEAR	AUTHOR	SUBJECTS	INTERVENTION	OUTCOME	RESULTS
1996	Tang	Estrogen Users (156) vs Non-users (968)	Longitudinal study	AD	RR 0.40 of AD (95% CI 0.22-0.85) Age of onset was later in users Annual incidences 8.4% for non-users, 2.7% in users.
1996	Paganini-Hill	Identified 248 women with AD (death certificate) from 3780 who died Results from a cohort study (8877)	Case-Control	AD Users vs non users Long term /high dose users	OR 0.65 (95% CI 0.49-0.88) OR 0.48 (95% CI 0.19-1.17)
1997	Kawas	427 women	Longitudinal F-up 16 yrs	AD Users vs non users	OR 0.46 (95% CI 0.212-0.99)
2000	Manly	143 participants 93 N vs 50 AD	Cross-sectional Estradiol levels	AD High levels vs low levels	4-6 times higher risk of AD for low levels
2000	Mulnard	120 women with mild to moderate AD	RCT Estrogen 0.625 mg /estrogen 1.25mg /placebo	Clinical dementia rating scale	Worse in ERT
2000	KAME project	837 women	F-up 2 years ERT vs ERT+ progestin	Cognitive Abilities Screening Instrument CASI)	Moderate beneficial effects in ERT only users
2000	Drake	39 final participants	Estradiol levels High levels vs low levels	Neuro-psy Tests	Increased delayed-recall memory Increased retrieval efficiency Worse immediate and delayed visual memory
2001	Asthana	20 women with AD	RCT 8weeks High ERT vs placebo	Neuro-psy Tests	Improved attention Improved verbal memory Improved semantic memory
2003	Henderson	326 women 52-63 years-old	Follow-up 8 years	Episodic verbal memory	Stable over 8 years No association with HRT or estradiol levels Better memory scores if HRT started in perimenopaausal period.
1999	Barrett-Connor	393 women	Cross sectional Bioavailable Testosterone Bioavailable Estradiol	12 neuro-psych Tests	Testosterone improved performance on one test

Taken together, these studies support the existence of a positive association between levels of estrogens and cognition: high levels of estrogens may favourably influence cognitive performance. However, some studies show only a modest effect. Important methodological limitations need to be underlined. Some studies had low numbers of participants. Measurement of cognition was not always standardized, with some based on clinical examination and on neuropsychological testing, others were based on brief self-report scales such as the CASI, making it difficult to draw valid comparisons. Also, in order to measure the potential benefits of estrogen administration in dementia or in its prevention, it is necessary to know which route of administration is best (oral, transdermal or parenteral) or if different routes make a significant difference.

Moreover, it is not known whether there is a target range of circulating hormone levels that needs to be reached in order to have an effect, or whether there is a critical time at which HRT is to be instituted. Larger sample sizes and longer duration of treatment are also necessary before the therapeutic potential of estrogen replacement for women with or without dementia can be firmly established.

The Women's Health Initiative Study of Cognitive Aging (WHISCA) and the cognitive component of the Women's International Study of Long Duration Oestrogen after Menopause-Cognition (WISDOM-COG) (to be completed in 2005 and 2006, respectively) will provide information on the effects of HRT on cognitive aging. The Preventive Postmenopausal Memory Loss and Alzheimer's with Replacement Estrogen study (PREPARE), the Women's Health Initiative Memory Study (WHIMS) and the dementia component of WISDOM-COG (to be completed in 2003, 2005 and 2009, respectively) will

address the question of whether estrogens can delay or prevent dementia. These ongoing trials will also study the effects of estrogen with or without progesterone and the effects of HRT in women with natural versus surgical menopause on cognitive aging and risk of dementia.

CHAPTER 5: ANDROGENS AND COGNITION

CIRCULATING LEVELS OF ANDROGENS AND COGNITION

Androgens play an important role in the development of cognitive function. Various trials have attempted to determine if testosterone levels are associated with cognition. In humans, the evidence showing relationships between circulating levels of androgens and cognition have come from several sources. These either measure the levels of hormones and examine the relationship with various cognitive tests or study the effect of hormone replacement therapy in specific populations. Results vary according to whether cognition is analyzed as a global measure or whether different neuropsychological tests are used to measure specific cognitive processes.

Effects of testosterone on spatial and verbal skills.

Christiansen et al. (1987) investigated 117 healthy young men. They measured total testosterone levels, dihydrotestosterone and free testosterone in the serum and the saliva. They also measured performance on 5 spatial and 6 verbal tests. The authors found a linear relationship between performance and testosterone levels: a positive correlation for spatial ability but a negative correlation for verbal performance.

Gouchie & Kimura (1991) took salivary measures of testosterone in 42 males and 46 females. The participants were grouped according to their testosterone levels (high or low). Men in the lower testosterone groups performed better than other groups on spatial and mathematical ability. Women with high levels of testosterone performed better than those

with low levels of testosterone. The authors concluded that there exists a non-linear relationship between testosterone concentrations and spatial ability.

Hassler et al. (1992) measured the levels of testosterone and estradiol (E2) in 26 young males and 25 young females and tested them on 3 spatial tests, verbal fluency and musical ability tests. The authors did not find any significant relation between testosterone and estradiol levels with any of the cognitive or musical tests.

Neave (1999) focused on the performance of both heterosexual and homosexual males and females on four cognitive tasks and measured salivary free testosterone. He noted significant differences between and within sexes in spatial tasks, but no differences in verbal tasks. On the Mental Rotation Tests the results showed a curvilinear relationship. In high and low levels of testosterone there was poorer performance.

In these studies the findings suggests that spatial performance in particular may be influenced by fluctuations in androgen levels. Moderate levels of testosterone may suppress estrogen levels and thereby enhance spatial skills whereas with higher levels of testosterone there is enhanced estrogen production through aromatisation thus impairing spatial ability. Moreover in these studies, the participants were young healthy subjects and these conclusions may not apply to our population of interest, which is over 65.

Effects of testosterone on memory and cognition.

Barrett-Connor (1999) performed an exploratory analysis on the population-based cohort of the Rancho Bernardo Study (1972-1974). There were 547 community-dwelling men ranging between 59 and 89 yrs of age, none were using replacement therapy. Total and bioavailable testosterone were tested. Twelve neuropsychological instruments were administered. In age

and education-adjusted analyses, men with higher levels of bioavailable testosterone had better scores on the Blessed Information Memory Concentration test (BIMC), a standardized measure of mental control and verbal memory, and on the Buschke-Fuld Selective Reminding Test which assesses memory storage, retention and retrieval of spoken words with a verbal list learning task. High testosterone levels predicted better performance on several tests of cognitive function the BIMC test, Trail making tests, the World-spelt-backward component of the Mini Mental State Examination (MMSE). The authors also reported that in older men, low estradiol levels were associated with better performance on the two standard cognitive tests (MMSE and BIMC). High levels of bioavailable testosterone levels predicted better performance on tests of verbal memory and mental control. This longitudinal, population-based study supports an association between endogenous sex hormone levels and cognition in older men.

Yaffe (2002) measured sex hormone levels (total testosterone, bioavailable testosterone, total estradiol and bioavailable estradiol) in 310 men between 66 and 80 years of age. Cognitive function was measured using the MMSE, the Trails B, and Digit Symbol tests. The relation between total testosterone levels and cognitive function between was never consistent of any tendency. Men who had high levels of bioavailable testosterone were found to have better cognitive scores on all three cognitive tests ($p <= .001$). Estradiol levels were associated with worse cognitive scores on the Digit Symbol ($p < .001$) and Trail B ($p = .002$). There was no association found between the levels of bioavailable estradiol and cognitive function tests.

ANDROGEN REPLACEMENT THERAPY

Experimental studies of the effect of androgen replacement therapy on memory and cognition are another way to show the effects of circulating hormone levels on cognition. Studies of the effects of testosterone replacement therapy on cognition suggest that testosterone therapy may improve mood. The effects of exogenous testosterone on cognition are less clear. As we shall see, some studies indicate that the administration of testosterone to non-demented subjects is associated with improved visual-spatial functioning, but deterioration of verbal skills.

Janowsky (1994) reported an increase in spatial cognition but no change in other cognitive functions in healthy old men after 3 months of transdermal testosterone. The study involved healthy older men (over 65) who had no prior history of memory loss. They also found that memory improved in those men after a one month treatment with testosterone. The authors suggested that testosterone might enhance memory even in normal men.

Sih (1997) carried out a randomized controlled trial on 32 hypogonadal men. The participants were randomized to receive either testosterone (intramuscular injection of 200 mg of testosterone cypionate twice weekly for 1 year) or intramuscular injection of placebo. The subjects were tested for memory. The results did not show any significant effect on memory performance. There were no measures of spatial skills in this study.

Alexander (1998) also studied cognitive abilities in hypogonadal men. The authors compared 33 hypogonadal men on testosterone replacement therapy, 10 eugonadal men receiving testosterone, and 19 eugonadal men not receiving any testosterone. The subjects were tested on 4 tests of visuo-spatial ability, three verbal fluency tests, two tests measuring

perceptual speed and one of verbal memory. There were no differences on most cognitive measures including visuo-spatial ability according to different testosterone levels. However, The hypogonadal men, who were found to be impaired in their verbal fluency tests at baseline, improved their performance following testosterone treatment. The authors concluded that supplementation of testosterone improves verbal fluency in hypogonadal men and that estosterone may influence some aspects of cognitive function.

Wolf (2000) conducted a randomized clinical trial on 30 men aged over 65. They received a single injection of testosterone enanthate 250 mg. Spatial and verbal skills were measured before treatment and 5 days later. The results showed that verbal fluency was worse for the treatment group than the placebo group. Also, there was no effect on either verbal or spatial skills. Testosterone and estradiol levels were measured and were above the physiological range. The study was very short (5days) and the authors did acknowledge that although they only registered detrimental and null effects, beneficial effects could still occur but may need more time to develop.

Cherrier (2001) tested the relationship between exogenous testosterone and cognitive testing in healthy older men. This was a study of 25 men between the ages of 50 and 80 years in a randomized, double blind, placebo controlled study. The treatment arm received 100 mg of intramuscular testosterone enanthate weekly for 6 weeks. Cherrier measured the circulating levels of testosterone and estradiol. Total testosterone was raised by an average of 130% from baseline to week 3 and a further 116% by week 6 in the treatment group. Because of aromatisation of testosterone, estradiol levels also increased an average of 77% from baseline to week 3 and 73% by week 6 in the treatment group. Cognitive abilities were measured at baseline, week 3 and week 6. There was improved spatial memory (recall of a

walking route) spatial ability (block construction) and verbal memory (recall of a short story). No measures were taken after treatment; thus, there is no indication as to how long the effects were sustained, or whether delayed effects occurred. The authors could not conclude whether these improvements in cognition were due to increased levels of testosterone or to the increased levels of estradiol.

O'Connor (2001) injected 30 eugonadal men with 200 mg enanthate testosterone weekly and 7 hypogonadal men biweekly for 8 weeks. Neuropsychological assessments were administered at baseline, week 4 and week 8. There was decreased spatial ability and enhanced verbal fluency in the treatment group at 4 weeks. There were no significant changes on the other tests.

Tan (2003) conducted a pilot study on thirty-six male patients with a recently diagnosed with Alzheimer's disease. Total and bioavailable testosterone levels were measured. Ten patients (28 %) were biochemically hypogonadic. Of those, five were randomized to receive testosterone and five to receive a placebo. MMSE scores were measured at baseline. The Clock Drawing Test (CDT) was used to measure visual-spatial ability. In the treatment group, the men received 200 mg of intramuscular testosterone every two weeks for 12 months. Cognitive measures were done at 3, 6, 9 and 12 months. The mean MMSE improved from 19.4 to 23 ($p < 0.02$). Clock Drawing Test also improved from 2.2 to 3.2 ($p < 0.03$). This pilot study suggested that, in hypogonadal men, testosterone could improve cognition (including visual-spatial skills) in mild to moderate dementia.

The results of these six trials are summarized in Table 2.

Table 2: Summary of Testosterone therapy on cognition

Year	Author	Subjects	Intervention	Hormone Rx	Cognitive tests	Results
1997	Sih et al.	32 hypogonadal men	Rx gp vs placebo	Testosterone 200 mg IM bi weekly for 1 yr	Memory	No difference
1998	Alexander et al.	62 men	33 hypogonadal 10 eugonadal 19 eugonadal	Testosterone Rx Testosterone Rx No Rx	Visuospatial Perceptual speed Verbal fluency	No difference No difference Improved in hypogonadal men
2000	Wolf et al.	30 men over 65 yrs	Testing after 5 days Rx gp vs placebo	Testosterone 250 mg IM One single dose	Verbal fluency Verbal skills Spatial skills	Worse No difference No difference
2001	Cherrier et al.	25 men 50-80 yrs	RCT Double blind	Testosterone 100 mg IM weekly for 6 weeks	Spatial memory Spatial ability Verbal memory	Improved Improved Improved
2001	O'Connor et al.	30 men + --- 7 hypogonadal	Rx gp vs placebo	Testosterone 200 mg IM weekly for 8 weeks --- Bi-weekly for 8 weeks	Spatial ability Verbal fluency Other tests	Decreased Improved No difference
2003	Tan et al.	36 men with AD --- 10 were hypogonadal	--- 5 received Rx 5 placebo	Testosterone 200 mg Every 2 weeks for 9 months	MMSE CDT	Improved Improved

Rx: treatment, IM: intramuscular, Gp: group, MMSE: Mini Mental State Examination, CDT: Clock Drawing Test

The trials demonstrate positive effects of testosterone on cognition, but also negative effects on some functions. There is thus some support in the scientific literature for the possible effect of testosterone levels on cognition in the aging male, but it is unclear if this is the direct effect of testosterone or through its conversion to estradiol. It may be testosterone alone, but as Barrett-Connor suggests that there may also be deleterious effects from estradiol on the cognitive function of older men. Some authors suggest the cognitive dysfunction observed with aging may be caused by a decrease in receptors that accompanies aging.

TESTOSTERONE LEVELS IN WOMEN

The relationship of levels of circulating testosterone and cognition has also been studied in women. Barrett-Connor in the Rancho Bernardo population found that higher levels of total and bioavailable testosterone predicted better categorical performance on the Mini Mental State Examination in 393 community-dwelling women aged 55 to 89 years who were not on estrogen replacement therapy (1999)

Eva Hogervorst et al. (2001) compared Alzheimer's disease patients with controls. There were 39 men with Alzheimer's disease and 41 controls, and 44 women with Alzheimer's disease and 62 controls. She measured levels of total testosterone. Men with Alzheimer's disease had lower levels of total testosterone than men without AD, but there was no such association in women. Adjustments were made for age, body mass index, alcohol intake, smoking and past hysterectomy. The design of the study did not allow the author to determine whether the low testosterone levels in women was a cause or an effect of Alzheimer's disease. The authors recommended furthering this issue through prospective longitudinal studies.

Postma (2000) gave a single dose of testosterone to healthy young women and measured their subsequent performance on spatial memory. The results showed that delayed recall (3 min delay) improved with testosterone when compared to pre-treatment performances.

The replacement therapy trials we have reviewed suggest an effect of testosterone on cognition also in women; whether the effect is due to testosterone alone or to its aromatisation to estradiol is not known. Simpson (2002) suggests " circulating levels of

androgenic steroids may serve an important role in the maintenance of local estrogen synthesis for example in the brain where estrogen has a strong influence on cognitive function". Studies in this field have been hampered by the insensitivity of most assays for testosterone at the lower end of range for a normal reproductive female. Present recommendations seem to be to offer replacement testosterone in women with specific conditions. The dose of testosterone administered should result in circulating levels as close to physiological levels as possible to avoid side effects.

CHAPTER 6: STUDY METHODS

OBJECTIVE

The objective of the study is to determine if there is an association between the levels of hormones and cognitive decline, controlling for different confounding factors such as age, smoking, drinking, Body Mass Index, Education and a Geriatric Depression Score.

SOURCE OF DATA

The Canadian Study of Health and Aging (CSHA) is a major study on aging carried out in Canada. Because of the important source of information it represented and because of the way in which the diagnosis of dementia was carried out, we used the CSHA data to examine the relationship of endogenous sex hormones and cognition in a Canadian population. Therefore, in a first step, we will review the methods used in the CSHA and then describe the methods used in our analyses based on the CSHA.

The CSHA is a multi-center population-based study involving subjects aged 65 years and older (CSHA Working Group, 1994). It is a longitudinal study carried out over 10 years in three phases: CSHA-1 (1991), CSHA-2 (1996) and CSHA-3 (2001). The study included 10,263 randomly selected participants from communities across Canada. There were 9,008 participants from the community and 1,255 from institutions. In all, 18 study centers were involved, and samples were drawn from 36 cities in Canada and their surrounding rural areas. The target sample size for each of the five regions of Canada was 1800 people from the

community and 250 from institutions. The five regions of Canada were British Columbia, The Prairies including Alberta, Saskatchewan and Manitoba, Ontario, Quebec, and the Atlantic region including New-Brunswick, Nova Scotia, Prince Edward Island and Newfoundland

The community samples were selected from provincial health insurance databases in four of the five regions. The exception was Ontario, in which the sample was drawn from election and municipal records that are merged into the Enumeration Composite Record. Sample sizes for age and sex groups were chosen through an optimum allocation procedure. This allowed the investigators to adjust the sample sizes according to expected rates of dementia in relation to age and sex, so as to reduce variance estimates (Cochran, 1977). This approach resulted in deliberate over-sampling of patients aged 85 years and older as well as the 75–84 group compared with the 65–74 age group (CSHA Working Group, 1994). Where individuals who declined to participate or could not be contacted, people of the same sex, age group, and region were randomly selected from the sampling frame to serve as replacements..

The CSHA institutional sample in 15 of the 18 study centers was derived from a sampling frame that included nursing homes, chronic-care facilities, and collective dwellings such as convents. The institutions were stratified by size ($\leq$ 25 beds, 26-100 beds, and >100 beds). Three large, three medium and six small institutions were randomly selected for each study center. The availability of provincial insurance databases covering institutions allowed three of the 18 study centers to randomly select their institutional patients directly.

Data were collected through interviews, usually directly but occasionally through a proxy. When memory problems prevented patients from providing accurate historical

information on prior exposures, questionnaires were administered to a person who knew the subject well (typically, the spouse or another family member).

In the community sample, an interview questionnaire gathered basic social and demographic information and summarized health status, disability and healthy aging. It also collected information on smoking, drinking, education and medication use.

The interview also included a standardized cognitive screening test: the Modified Mini-Mental State Examination (3MS) (Teng & Chui, 1987). Teng and Chui have shown the 3MS to be a reliable and valid screening test in dementia. The 3MS has a broader range of scores (0 to 100) than the MMSE (0 to 30), which is a more widely used screening test. The 3MS with a cut score of 77/78 proved a reasonable screening test (96% sensitivity, 90 % specificity) (Bland, 2001). The score values decrease with age and increase with years of schooling (Bravo & Hébert, 1997). All participants in the community sample who screened positive on the 3MS (scores < 78) plus a random sample of those who screened negative were asked to consent to a clinical assessment and to give a blood sample for future analyses. All participants in institutions had a clinical examination.

CLINICAL DIAGNOSIS OF IMPAIRED COGNITION AND DEMENTIA

To reach a diagnosis, a nurse, a neuropsychologist and a physician independently performed clinical assessments; the same procedure was used at CSHA-1 and –2, and minor variants were introduced at CSHA-3. A registered nurse administered the 3MS, recorded vital signs and took a medical history. She also drew a blood sample from consenting participants. Blinded to the results of the previous tests, a psychometrician administered 12

neuropsychological tests to the participants deemed testable (3MS scores >50). The neuropsychological tests assessed memory (Buschke Cued recall, Weschler Memory Scale, Rey Auditory-Verbal learning, Benton Visual Retention), abstract thinking (WAIS-R similarities Test-short form), executive functioning (WAIS-R Digit Symbol, Sub-test Trail Making), judgment (WAIS-R Comprehension) and aphasia (Tokens Test, Word fluency, Boston Naming Test, Animal Naming). The results of the tests were later reviewed and interpreted by a neuropsychologist.

A physician carried out the physical and neurological examination, unaware of the results of the neuropsychological tests. Physician and neuropsychologist independently made their diagnosis (no cognitive impairment, cognitive impairment or dementia) using the Diagnostic and Statistical Manual for Mental Disorders criteria: third edition-revised (DSM-III-R) (American Psychiatric Association, 1987). The DSM-IV (American Psychiatric Association, 1994) criteria were also included at phases 2 and 3. Finally, nurse, physician and neuropsychologist gathered for a consensus conference during which each chart was reviewed and a final diagnosis was made. When a consensus was reached, the participants were classified into three broad categories of cognition: normal, cognitively impaired but not demented, or demented; finer sub-categories were then specified within each. The author carried out the clinical examinations and participated in the consensus conferences for patients in the Chicoutimi region, Québec, at phase 3 of the study.

DEFINITION OF COGNITIVE STATES IN THE CSHA

The present analyses of patterns of cognitive change over five years will include study participants who received two diagnostic assessments five years apart, and who also provided

blood samples at the time of the first of these two assessments. The CSHA diagnoses divided participants into three principal groups (with numerous sub-groups). The principal groups were Cognitively Normal (here-designated N), Demented (here D), and the intermediate group of Cognitively Impaired, but Not Demented (CIND).

Cognitively Normal (N): Subjects were considered cognitively normal if the clinical and neuropsychological assessments diagnosed normal cognition.

Cognitively impaired but not demented (CIND): The CSHA used a category (termed Cognitive Impairment No Dementia (CIND) to describe people who did not meet the DSM criteria for dementia and yet who were not normal either. CIND was defined clinically and supported by cognitive test scores. In phase 1 of CSHA, the diagnosis of CIND was one of exclusion (neither dementia nor normal cognition). Various categories of impairment identified in the clinical examination and in neuropsychological testing defined different subcategories of CIND. These included delirium, chronic alcohol and/or drug use, depression, psychiatric illness, and mental retardation. Age-associated memory impairment was a seventh category. The physician assigned some individuals to an eighth group, other cognitive impairment. In such cases, analysis of the physician's notes occasionally led to a more accurate classification. For CSHA-2 and 3, the CIND group was characterized more thoroughly, using a checklist with formal criteria. In particular, it distinguished between memory impairments and deficits in other aspects of cognition such as aphasia, construction deficits or apraxia.

Dementia (D): In the case of dementia, the participants were grouped into 13 possible sub-categories. At CSHA-1, in addition to the DSM-III-R, the NINDCDS-ADRDA criteria were used for Alzheimer's disease (McKhann, 1984). The ICD-10 criteria of the World Health

Organization (1992) were used to define vascular and other specific types of dementia. In the case of vascular dementia, Hachinski's Ischemic Score (Hachinski, 1975) was added to the diagnostic criteria. At CSHA-2 and CSHA-3, dementia and Alzheimer's disease were diagnosed using the DSMIII-R (for comparability with phase 1) and the DSM-IV criteria. Criteria for Lewy body dementia were taken from McKeith, 1996. The NINDS-AIREN criteria by Román (1993) were also used to diagnose vascular dementia and the criteria outlined by Rajput (1993) were used for the diagnosis of Parkinson's disease. The diagnostic features of fronto-temporal dementia were derived from the Lund and Manchester group's criteria (1994).

PARTICIPANTS

As the present study examines the relation between cognitive decline and hormone levels a first requirement was to have had a blood sample drawn during CSHA. As described, all participants in the community sample who screened positive on the 3MS (scores < 78) plus a random sample of those who screened negative were asked to consent to a clinical assessment and to give a blood sample for future analyses. All participants from the institutional sample received a clinical examination and were asked to give a blood sample.

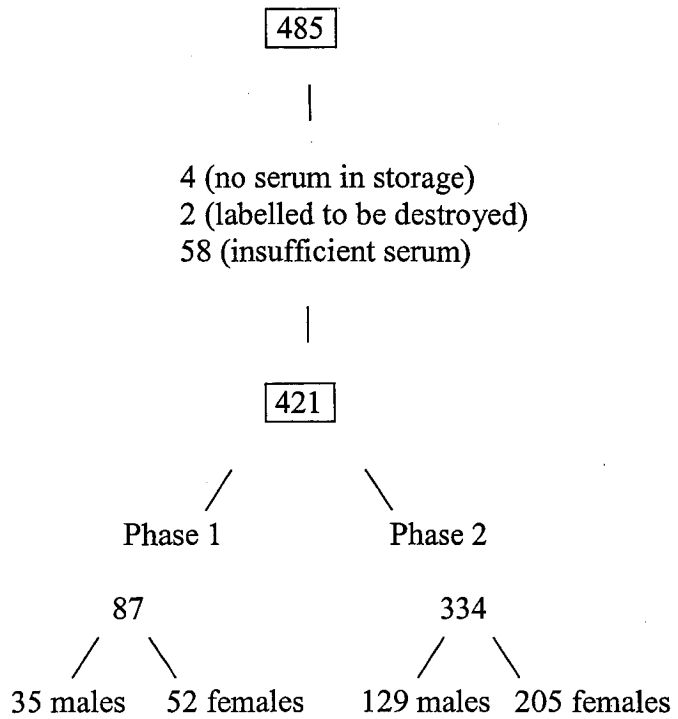
The eligible subjects for the present analysis were a subgroup of the non-demented participants at either the 1991 and 1996 CSHA waves, who underwent clinical examination and had a blood sample drawn, and who were followed up and had a repeat clinical examination five years subsequently, at phase 2 or 3 of the study. We excluded from the present analyses those who died or who were lost to follow-up. This was because at the time of assembling the data for the present study, the CSHA had just finalized the collection of

data in the phase 3 and the Coordinating Center had not completed the analysis of the deceased subjects. (The center has developed an algorithm that estimates the probability that a deceased person may have had dementia). Because of this lack of information, and because the majority of our samples originated from phase 2 of the CSHA, it was decided not to include the deceased subjects in our study.

This sample represented a total of 485 blood specimens. Of those, 4 had no serum in storage, 2 were labelled 'to be destroyed' and 58 had insufficient serum for the hormone analyses. The remaining 421 samples were analysed: 87 had been collected at phase 1 and 334 at phase 2. This represented 257 women and 164 men (Table 3). Participants provided a blood sample on a voluntary basis. In the first phase of the CSHA, financial and technical constraints limited the collection of blood samples; some centres collected no blood at all. This issue was addressed in phase 2 of the study and this resulted in more blood samples being sampled; this explains the greater number of participants from phase 2.

To increase the sample size and power for the analysis, the five-year assessments of cognition from 1991 to 1996 were combined with those from 1996 to 2001. No case was included twice, however. For the present analyses, all forms of dementia are grouped together. This was done to increase the sample size, while recognizing that thereby we may have introduced heterogeneity that would work against the value of the larger sample.

Table 3: Number of usable blood specimens for the eligible study population



All subjects, or their proxy when applicable, signed an informed consent permitting the use of their blood samples for further analysis. This was part of the CSHA protocol. The CSHA blood samples were stored at -20° C by the Laboratory of Health Canada in Winnipeg. All the samples were coded with an identification number, date of birth, sex and date of withdrawal. This is necessary to link samples to the CSHA database, and to allow the laboratory to proceed to technical and quality controls. No one has access to the personal identities of the people in the sample.

The CSHA Coordinating Centre gave authorization for access to the serum samples. We also obtained approval from the CSHA biomarkers' group and the Ethics Review Board of the Ottawa Hospital for the proposed analyses of the blood samples. Funding for the analysis and transfer of the blood samples to the laboratory of Cité Santé de Laval was

provided through a grant which the author obtained from Organon, a pharmaceutical company. Organon was in no way implicated in the study design or statistical analysis.

VARIABLES

Main Outcome

This analysis used the same cognitive states as outlined for the CSHA to categorize the subjects in three categories at each of the two time-periods: 1) normal (N), 2) cognitively impaired but not demented (CIND) and 3) demented (D). This study addresses the impact of hormonal levels on cognitive decline over a five-year period, based on a formal clinical examination, in people who were initially free of dementia. We decided to define cognitive decline in clinical terms, rather than in terms of scores on one or more of the neuropsychological tests. The reason for this choice is that ultimately the clinically important issue is to establish whether or not hormone levels protect against cognitive decline in a way that would be seen by a physician as being clinically important. It remains perfectly feasible to study whether hormone levels may be associated with particular aspects of cognition (and with changes in these). However, the option of studying the association of hormone levels to neuropsychological tests immediately raises the question of which test to consider. Twelve tests were used in the study, measuring different aspects of cognition; it would become necessary to establish some (perhaps arbitrary) decision as to which combination of tests should be considered, and how to weight each test in this combination. To resolve this dilemma, and as our prime concern is whether such associations can affect a patient at the global level, in clinically significant terms, it appeared simplest to rely on the

clinical diagnosis, which was based on the neuropsychological results and on a range of other pertinent information. Accordingly, the operational definition of decline is here based on the diagnostic groups used in the CSHA. Although the approach here is to treat cognition as a categorical variable, we fully recognize that cognitive ability, and cognitive decline, form continua, and that setting a threshold for what is considered a clinically significant decline is somewhat arbitrary. In consequence, there will be heterogeneity within each of the diagnostic groups created in this analysis, in the same way that classifying all people with dementia (or, indeed, all cognitively normal people) together will obscure possibly significant variation among them.

The importance of dementia focuses the analysis on studying the hormone profiles of people who declined from being cognitively normal to having a dementia. The main comparison group will include those who remained cognitively normal. Hence, we determined the decline in cognition by comparing the diagnosis at the beginning and end of the five-year period (from phase 1 to phase 2, or from phase 2 to phase 3 of the study) for each subject. We included those individuals who had normal cognition at the start of the five-year period. However, a decision had to be made over how to treat the cases of CIND. We rejected the idea of discarding them from the analysis completely, as this would leave only the extreme groups (normal and demented), over-simplifying reality and perhaps exaggerating the association between hormone levels and cognitive decline. Hence, those with CIND at the start of the five-year period were also included in the analyses.

This gives six possibilities: Those who were cognitively normal at the start of the five-year period could either stay normal (N-N) or become impaired (N-CIND) or become demented (N-D). For those subjects who were CIND at the start of the five-year period they

could either revert to normal cognition (CIND-N), stay impaired (CIND-CIND) or evolve towards dementia (CIND-D). Among these six groups, a decision had to be made over which to classify as having declined. Those who went from N to D illustrate clear decline, and form the main group of interest in the analysis, compared to those who remained N (N-N). The group of people who changed from CIND to dementia over five years can also reasonably be considered to have declined, as can those who progressed from N to CIND. However, we carefully considered the group who remained CIND. To classify these as non-declining appears legitimate, but ignores the fact that they are also cognitively impaired, and presumably must have declined from a prior normal state. It is possible that some may always have been cognitively impaired, but the neuropsychologists were supposed to adjust their interpretation of test results for estimates of prior cognitive ability, based on education, occupation and any history of mental retardation. Hence, concerns arise over whether this group should be merged with the N to N group: if hormone levels do lead to cognitive decline, perhaps they may have experienced mild levels of hormone deficit that led to CIND but were not sufficient to continue to dementia. But the fact that this group had not progressed to dementia over five years suggests that they may differ from the clearly declining group, the N-D group. There being no easy solution, we proceeded by including the CIND-CIND group with the non-decline group as being (if anything) conservative.

Independent Variables

The predictor variables studied in relation to the cognitive decline were the hormone levels, age, education, Body Mass Index, smoking, alcohol consumption, the 3MS score and the

score on the Geriatric Depression Scale. All measures of the independent variables were gathered from the data at the beginning of the five-year period.

Hormone levels. We measured the levels of total testosterone (TT), bioavailable testosterone (BT), estradiol (E2) and sex hormone binding globulin (SHBG) in both men and women. The levels were measured by radioimmunoassay with commercial kits, which require 0.5cc of serum per hormone studied. Dr Gilles Brisson a biochemist specialising in andropause and endocrinology, from the Cité de la Santé in Laval, Québec conducted and supervised the assays and the quality control. All assays were done on the Bayer ACS: 180 SE Automated Chemiluminescence System. Chemiluminescence is the generation of electromagnetic radiation as light by the release of energy from a chemical reaction. While the light can, in principle, be emitted in the ultraviolet, visible or infrared region, those emitting visible light are the most common. They are also the most interesting and useful. Chemiluminescence is used for in vitro diagnostics. The primary use of luminescence is in molecular biology applications for the detection of proteins and nucleic acids on gels, and in the visualization of expressed proteins in cells. The antibody-based system provides target specificity and versatility. One reason accounting for the growing popularity of chemiluminescent assays is their exquisite detection sensitivity. Measurement of light intensity is relatively simple, requiring only a photomultiplier or photodiode and the associated electronics to convert and record signals. The lack of inherent background and the ability to easily measure very low and very high light intensities with simple instrumentation provide a large potential dynamic range of measurement. With this technique, the coefficients of variation for Bioavailable Testosterone assays are 10% at 3nmol/L. For estradiol it is much less sensitive with a coefficient of variation of 20% at 100pmol/L. To process Bioavailable testosterone, it is first precipitated on ammonium sulphate.

Age was derived from the date of birth. Date of birth was confirmed from several sources: self-report, provincial health plan records and, the eventual death certificates.

Education was indicated by the number of years of education.

Body mass index (BMI) was calculated from the height and weight of each subject as the quotient of weight (kg) over height (m) ^{2}. BMI is a tool for indicating weight status in adults over 20 years old. BMI was classified into one of four categories: below 18.5,underweight; 18.5-24.9, normal; 25.0-29.9,overweight; 30.0 and above, obese (WHO, 1995). BMI correlates with body fat. The relation between fatness and BMI differs with age and gender. For example women are more likely to have a higher percent of body fat then men for the same BMI (Garrow, 1985). On average, older people may have more body fat than younger adults with the same BMI (Gallagher, 1996).

Smoking. To estimate smoking, the subjects were asked if they had ever smoked cigarettes, cigars, or pipes regularly; and if so, for how many years. Cigarette smoking was recorded as number of packs per day and years smoked; the cumulative exposure was computed as pack-years (*n* packs per day x *m* years of smoking). For those smoking pipe or cigars, a conversion to packs of cigarettes per day was made from the fact sheet of the American Lung Association, June 2002: it is estimated that 1-3 cigars per day is the equivalent to smoking 1 pack a day in cigarettes and 1-3 pipes are also equivalent to 1 pack a day. Some cases reported being smokers but the quantity was unknown either in the number of years, in pack of cigarettes smoked per day or both; in such cases, the missing value for pack-years was replaced by the mean of consumption in the "smoker" population.

Drinking. For alcohol consumption, participants were asked whether they had been regular drinkers of beer, wine or spirits, and if so, for how many years and whether they were still drinking. The questionnaire did not include questions concerning quantities (number of drinks or ounces of alcohol taken). In the need to combine the consumption from different types (beer, wine, spirits), conversion factors to pure alcohol were obtained from the World Health Organization website. The pure alcohol amount stated is 4.5 % for beer, 14% for wine and 42 % for spirits. The reported consumption was thus weighted accordingly. To estimate the alcohol consumption, we calculated alcohol-equivalent years, it being the product of *n* years of drinking times the % of pure amount of alcohol of drink reported. When there was missing information to estimate the alcohol-equivalent years, although the participant had reported being a drinker, the alcohol-equivalent years were estimated to be equal to the mean of the "drinker" group.

Geriatric Depression Scale (GDS). The short-form Geriatric Depression Scale (Yesavage, 1983) consisting of 15 items with a dichotomous scale (yes/no) was used to assess depressive symptoms. The GDS has been found to be a suitable screening test for depressive symptoms in the elderly. It is easy to administer and requires no psychiatric knowledge. The scoring intervals are as follows: 0 - 4: no depression; 5 - 10: mild depression; 11+: severe depression (McDowell & Newell, 1996).

Modified Mini Mental State Examination (3MS). The 3MS was used as a screening test to estimate cognition. The cut-off score being 78. There was impaired cognition with a score lower than 78. At this cutting point, the sensitivity of the screening test has been found to be between 87% and 96%, offering reassurance that there would be few false negatives, and the specificity at 89 to 90%. (Bland, 2001; McDowell et al, 1997).

STATISTICAL ANALYSIS

Our objective was to determine if there was an association between the levels of hormones and cognitive decline.

Male and female data were analyzed separately. All statistical analyses were performed on the SPSS software program Version 12.0 (Statistical Package for Social Sciences, SPSS Inc, Chicago, Illinois). Data were sub-grouped by cognitive decline. Frequency histograms were first plotted for each hormone to show the distribution of each hormone. The mean, standard deviation and 95% confidence intervals were calculated from the raw data (i.e., without making any modification to the data). Outliers were examined and the profile of each outlying case was reviewed.

To explore the association of hormone levels with dementia, logistic regression was used to compute the odds of developing cognitive decline within the different hormone levels. We adjusted these analyses for demographic characteristics and other confounding factors known to influence both hormone levels and cognition: age, education, body mass index, smoking, alcohol and the Geriatric Depression Scale.

In all the analyses, we explored the potential interrelationships between hormone levels and other potential risk factors and effect modifiers. Care was taken to base the exploratory analysis on biological evidence. There are several known risk factors for Alzheimer's disease, and these may interact with, or be independent of, hormone levels. Thus, age is strongly related to increasing risk of dementia, but levels of estrogens are also age-related, as the number of hormone receptors in the brain changes with age. Cigarette

smoking shows a complex relationship with dementia and also has an effect on serum concentrations of estrogens. We explored these factors to examine possible effect modification.

CHAPTER 7: RESULTS

SAMPLE CHARACTERISTICS

Our sub sample was compared to the samples of the CSHA study. Table 4 shows the age distribution in different samples of the CSHA study. Table 5 shows the sex distribution and education level of the present study compared with the CSHA study and the 1991 census. The age distribution of our sample is different from the total sample of the CSHA. Our sample shows an over sampling of the 75-84 population, as does also the samples from sub samples of phase 1 and 2 with clinical examinations or blood samples. The sex distribution and education levels are quite similar in comparison with the 1991 census and the CSHA sample.

Table 4: Comparison of the age distribution in the population of the CHSA study and the present study

		CHSA-1	CSHA-2	CSHA-1 Clinical examinations	CSHA-2 Blood samples	Present study
N		**10,623**	**6,925**	**2,914**	**1,307**	**421**
Age						
	65-74	38%	23%	19%	13%	19.5%
	75-84	36%	50%	46%	46%	56.8%
	85+	13%	27%	18%	41%	23.8%

Table 5: Sociodemographic characteristics of the study population compared to the CHSA-1 sample and the 1991 census

	1991	CSHA study	Present Study
Sex (Female)	58%	61 %	60 %
Education (mean years)	9.5	10.3	10.0

SUBJECT CHARACTERISTICS

The analysis was based on 421 subjects followed up over a five-year period. There were 164 males and 257 females. The baseline characteristics for the study sample are shown in Table 6. Because of different hormone profile between the sexes, these and all subsequent results are presented separately for men and women.

Table 6: Means, ranges and standard deviations in the baseline characteristics of the study population by sex.

		N	Mean	Minimum	Maximum	Std deviation
Men	Age	164	78.7	67	93	5.1
	BMI	158	25.5	14.8	39.1	3.8
	Education	162	10.3	0	22	4.8
	3MS	161	84.5	22	100	12.7
	GDS	157	2.1	0	14	2.8
Women	Age	257	81.3	66	98	6.6
	BMI	240	25.5	15.4	51.4	5.1
	Education	253	9.8	0	25	3.9
	3MS	257	86.1	45	100	10.4
	GDS	246	2.5	0	14	2.8

Age and education are expressed in years. BMI: Body Mass Index; GDS: Geriatric Depression Scale; 3MS: Modified Mini-Mental State Examination

The population is elderly, with a mean age of 78.7 years for men and of 81.3 years for women. The length of education was on average 10.3 years for men, and 9.8 years for women. The mean BMI was 25.5 in both men and women, which is just over the cut-off point for being overweight (BMI = 25). Assessing mood through the Geriatric Depression Scale revealed that the majority of this population showed few depressive symptoms. Indeed, the mean scores for the GDS are low, at 2.1 for men and 2.5 for women. These scores are within the range of what is considered a normal mood. At baseline, the measurement of cognition with the Modified Mini-Mental State Examination showed a mean score of 84.5 for men and 86.1 for women; the maximum score for a normal cognition is 100.

Results for smoking and drinking are shown in Tables 7 and 8. There was missing information for smoking in 11 men, and in 25 women. Tables 7 and 8 show that 75 % of men were, or had been, smokers. The mean consumption among smokers was 32 pack-years. In comparison, a third of women smoked or had smoked. Their mean cigarette consumption was 23.4 pack-years.

Table 7: Number and % of smokers and drinkers in the study population by sex.

		N	%
Men	Smoking	116	75.8
	Drinking	83	55.3
Women	Smoking	77	33.2
	Drinking	58	24.9

Table 8: Means, ranges and standard deviations of pack-years of cigarettes and alcohol equivalent-years in the study population by sex among drinkers and smokers.

		N	Mean	Minimum	Maximum	Std Deviation
Men	Smoking	116	32.0	1.5	90	18.9
	Drinking	83	259.3	5.0	677	182.0
Women	Smoking	76	23.4	1	93	19.5
	Drinking	58	216.5	2.0	700	187.7

Smoking is expressed in pack-years and drinking in alcohol equivalent-years

The cigarette consumption for men and women is shown in Figures 2 and 3.

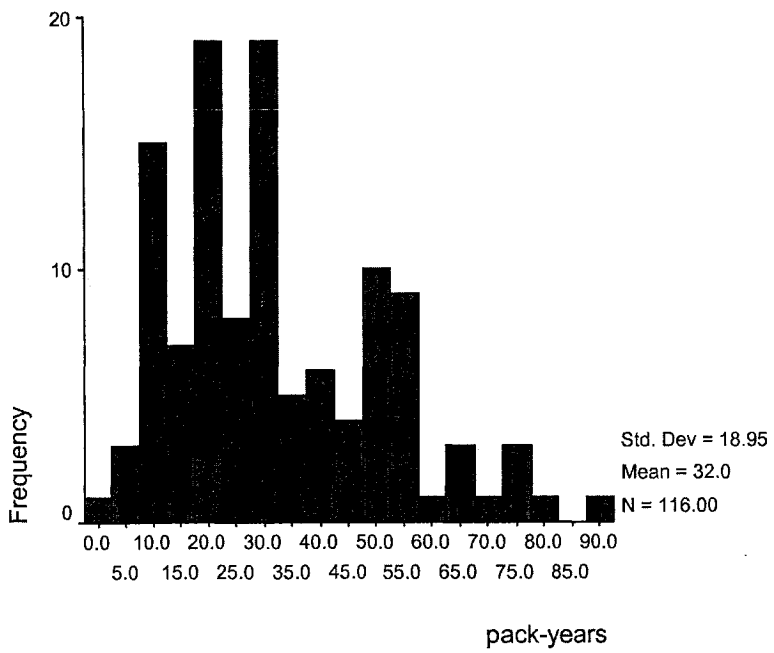


Figure 2: Cigarette consumption (pack-years) in male smokers

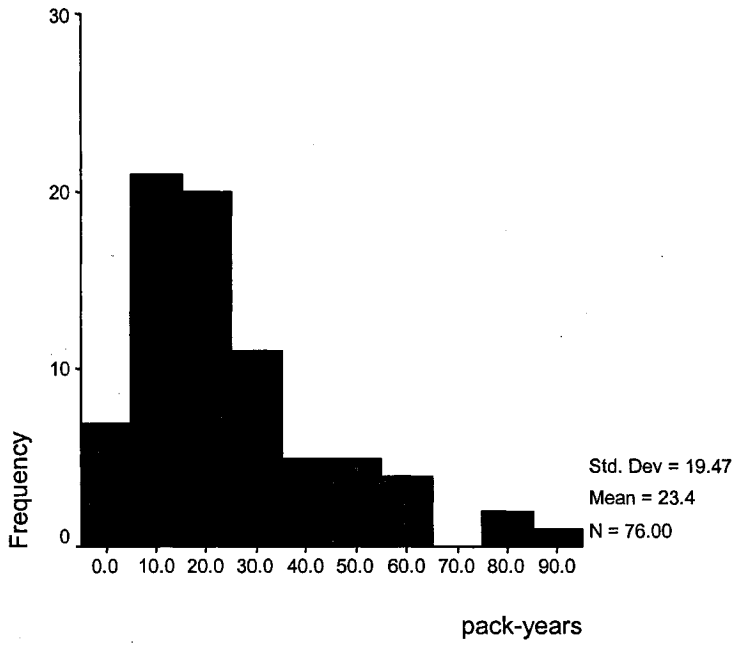


Figure 3: Cigarette consumption (pack-years) in female smokers

For alcohol consumption, there were 14 missing data for men and for 24 women. Results for alcohol consumption showed that more than half of men (55.3%) were drinkers whereas only a quarter (24.9%) of the women drank. The alcohol consumption for men was estimated at 259 alcohol equivalent-years and at 216 for women. (Figures 4 and 5). Although fewer women drank, their consumption of alcohol per capita was very similar.

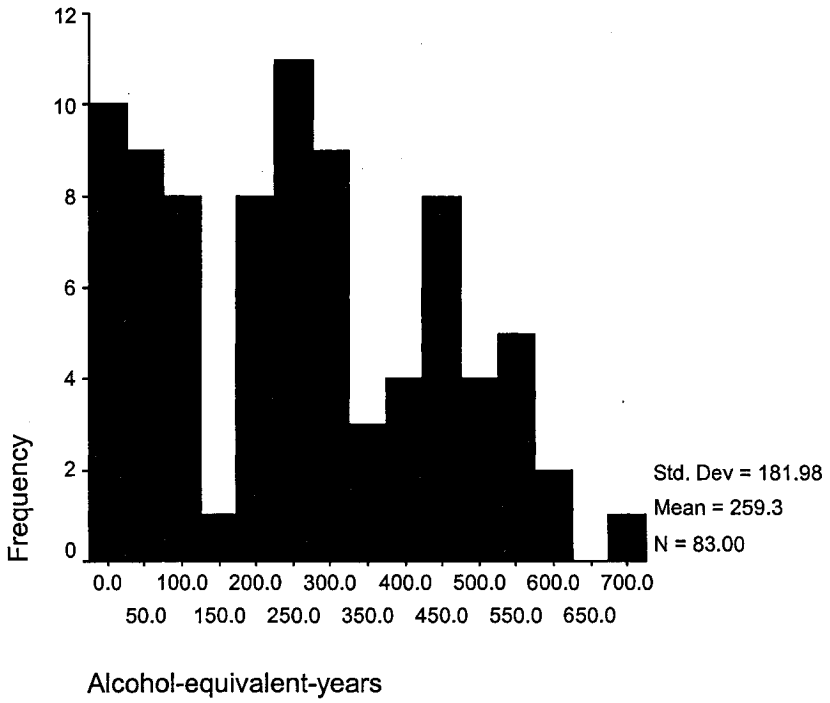


Figure 4: Alcohol consumption (alcohol equivalent-years) in male drinkers

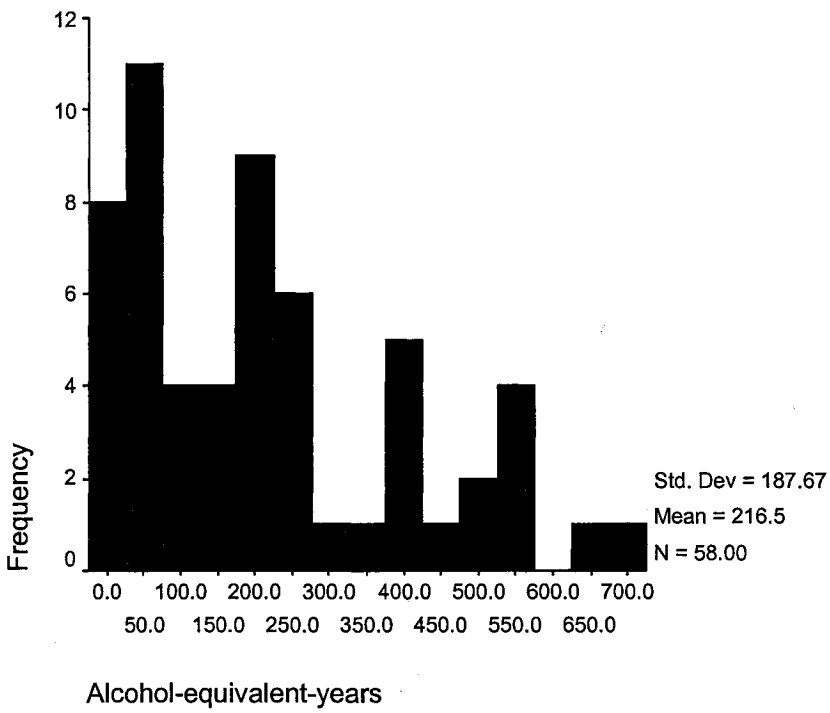


Figure 5: Alcohol consumption (alcohol equivalent-years) in female drinkers

COGNITIVE DECLINE GROUPS

Because our outcome of interest was decline in cognition, we classified the subjects according to the decline observed during the five-year period. This gave us six groups 1) N-N: consistently normal cognition; 2) CIND-CIND: staying cognitively impaired; 3) CIND-N: reverting from impaired cognition to normal cognition; 4) N-CIND: progressing from normal cognition to impaired but not demented; 5) N-D: from normal cognition to dementia and 6) CIND-D: from impaired cognition to dementia. Table 9 shows the number of participants in each group. The CIND-N category has very few subjects: 3 men and 3 women. The most plausible explanation was that the impaired cognition for this group was due to reversible factors such as depression, alcoholism or anxiety interfering with results of the neuropsychological tests.

Table 9: Number of men and women in each cognitive decline group.

	N-N	CIND-CIND	CIND-N	N-CIND	N-D	CIND-D	Total
Men	75	25	3	10	16	35	164
Women	121	32	3	14	26	61	257
Total	196	57	6	24	42	96	421

Table 10 and 11 show the baseline characteristics for each of the diagnostic group according to sex. In men and women, as expected, education is highest in the group that remained cognitively normal during the five-year period and the GDS score is lowest in this same group. The highest consumption of cigarettes and alcohol in men corresponds to the CIND-N group. In comparison, women in this category were non-smokers and non-drinkers

but their GDS scores were slightly higher (second highest after the CIND-CIND group); these results are plausible as they correspond to reversible factors we had mentioned earlier on.

Table 10: Means and standard deviations of the baseline characteristics in the six cognitive decline groups in men.

	N-N	CIND-CIND	CIND-N	N-CIND	N-D	CIND-D
N (%)	75 (45.7)	25 (15.2)	3 (1.8)	10 (6.1)	16 (9.8)	35 (21.3)
Age	77.7 ± 4.4	77.8 ± 5.4	78.7 ± 4.0	79.5 ± 4.9	82.8 ± 6.0	79.2 ± 5.5
Smoking	19.9 ± 18.4	29.9 ± 27.0	34.7 ± 17.5	24 ± 17.3	24.1 ± 17.0	29.6 ± 255
Drinking	141.0 ± 186.1	174.4 ±174.3	463.3 ± 119.3	157.2 ± 203.8	108.0 ± 173.3	117.5 ± 184.1
BMI	25.5 ± 3.4	26.3 ± 4.8	28.4 ± 2.4	24.1 ± 4.8	24.8 ± 3.2	25.3 ± 3.8
Education	12.2 ± 4.3	7.7 ± 4.9	10.7 ± 0.6	8.3 ± 4.7	9.6 ± 5.4	8.8 ± 4.2
3MS	92.3 ± 6.6	76.1 ± 12.3	91.3 ± 1.5	82.5 ± 6.2	83.5 ± 12.0	73.9 ± 14.0
GDS	1.1± 1.4	3.5 ± 3.4	1.0 ± 1.7	4.2 ± 3.6	2.8 ± 3.1	2.9 ± 3.6

Age and education are expressed in years. Smoking is expressed in pack-years and drinking in alcohol equivalent-years; N=Normal cognition; CIND=cognitively impaired not demented; D=dementia

Table 11 shows that, in women, the levels of both smoking and drinking were higher in the groups who declined in cognition. The depression score was highest in the N-CIND group and the CIND-CIND group. This observation may be clinically significant as dementia is often clinically preceded by a period of depressive state.

Table 11: Means and standard deviations of the baseline characteristics in the six cognitive decline groups in women.

	N-N	CIND-CIND	CIND-N	N-CIND	N-D	CIND-D
N (%)	121(47.1)	32 (12.5)	3 (1.2)	14 (5.5)	26 (10.1)	61 (24.6)
Age	79.2 ±5.7	83.4± 7.8	84.7± 8.0	79.4± 6.5	83.8± 6.0	83.5± 6.5
Smoking	6.8 1±15.4	5.1 ±12.9	0	15.1± 24.1	10.7± 16.5	7.6± 14.1
Drinking	51.8 ±131.0	38.5 ±104.5	0	55.0± 147.9	86.7 ±164.5	50.0± 127.6
BMI	25.8± 4.8	23.9± 4.4	27.3± 7.8	25.6 ±4.4	26.0± 7.1	25.3± 5.1
Education	11.0± 4.0	7.8± 2.6	7.3± 0.6	8.7± 4.0	9.6 ±4.8	9.0± 3.6
3MS	93.0± 6.0	78.4± 7.0	82.3± 7.1	82.6± 6.9	86.0 ±10.0	77.5± 10.5
GDS	1.5 ±1.9	3.7 ±3.2	3.3± 4.9	2.4± 1.9	2.8± 2.9	3.8±3.3

Age and education are expressed in years. Smoking is expressed in pack-years and drinking in alcohol equivalent-years. N=Normal cognition; CIND=cognitively impaired not demented; D=dementia

HORMONE LEVELS

The results of the hormone assays were data-entered and linked to the CSHA data set. Table 12 shows the mean values of the different sex hormone levels in the study population with the reference ranges. Reference ranges are hormone levels expected for normal healthy people. The references ranges were validated for males over 60 and postmenopausal females. There were no specific reference ranges for males or females over 75 years of age. The hormone level of 95% of normal healthy people will fall within this reference range. This also means however, that a few normal people (~5%) may have levels that fall outside the range. The reference ranges are very broad and the average levels of the different hormones in our study fell within the reference ranges for men. For women, bioavailable testosterone and estradiol were higher than the reference range. Bioavailable testosterone and estradiol in

women were respectively, 1.5 times and 1.2 times the upper limit of the reference range, keeping in mind that the references ranges available are for a younger population.

Table 12: Means and standard deviations for hormone levels in the study population and reference ranges for men and women.

	Men		Women	
	Mean (±SD)	Reference range	Mean (±SD)	Reference range
Testosterone (nmol/l)	22.1 (± 9.1)	12.1-24.8	2.8 (± 2.2)	0.8 –3.1
Bioavailable Testosterone (nmol/l)	5.3 (± 2.3)	4.5- 23.6	1.7(± 0.7)	0.05-1.2
Estradiol (pmol/l)	173.9 (± 133.2)	70-220	147.9 (± 196.6)	20-110
Sex Hormone Binding Globulin (nmol/l)	55.4 (± 22.8)	13-71	77.4 (± 46.1)	18-114
E2/SHBG	3.6 (± 3.0)	1.2-21	2.2 (± 2.4)	1.8-17

However, most of these results are in keeping with recent results reported in the literature. Women had lower values of estradiol compared with men. Estradiol levels are known to be as much as four-fold higher in older men when compared to women (Barrett-Connor, 1987). This is due to higher androgen levels, which result in increased peripheral conversion of androgens to estradiol, thus giving higher levels of circulating estradiol (Laughlin, 2000). For estradiol and SHBG, many of the standard deviations were high.

The distributions of the hormone levels for males and females are outlined in Figures 6 and 7. Note that the same scales could not be kept for the different hormones in men and women. The histograms show that the E2 distribution is clearly skewed to the right, the

distribution of SHBG is also slightly skewed in the same direction; more so for women than men. The median and mean values for these hormones were not similar, giving further evidence that the distribution was not normal. For SHBG, the mean, median and mode values, although not identical, were similar in men; this was not true for the SHBG distribution in women.

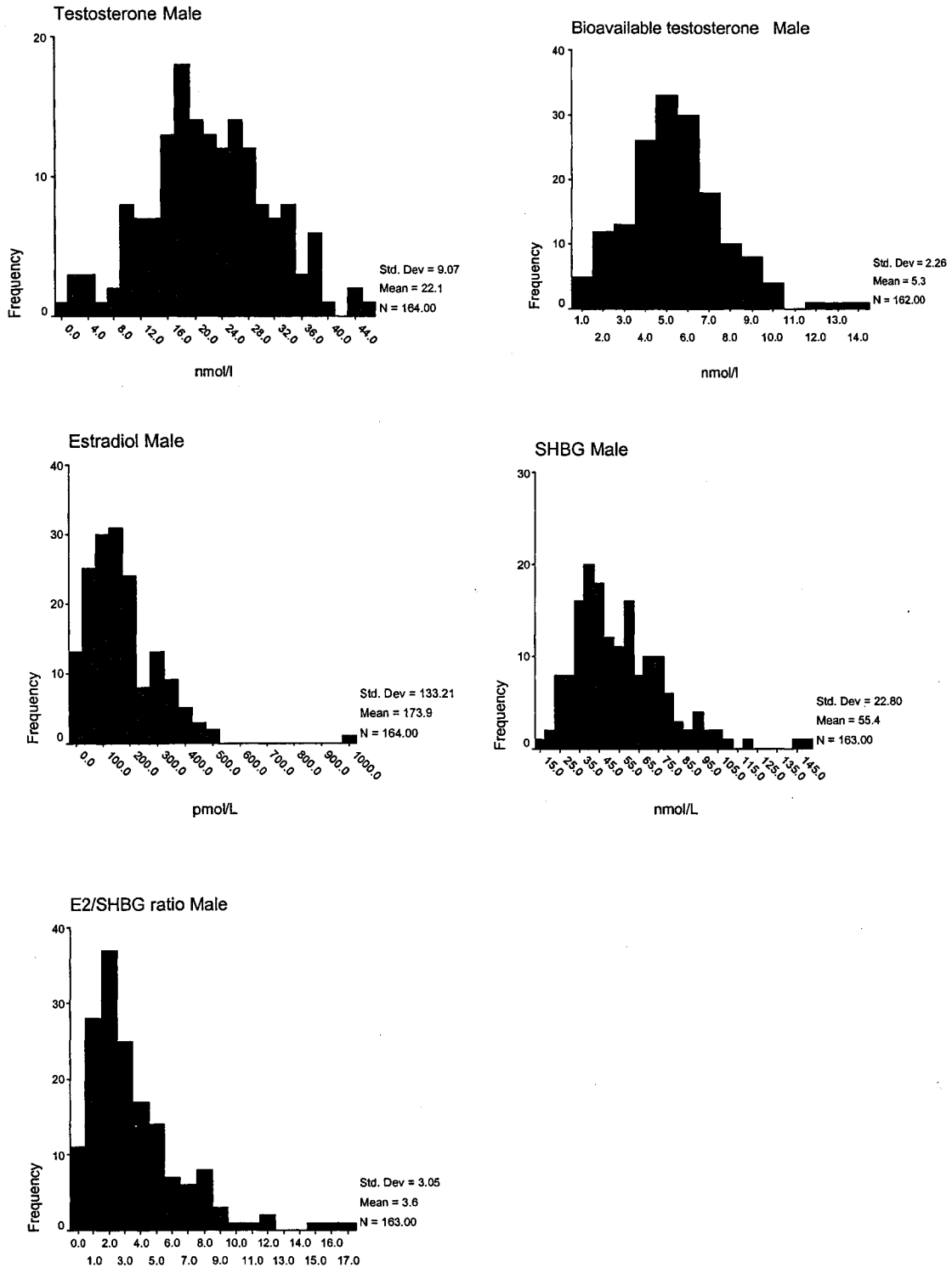


Figure 6: Hormone distributions of male study participants

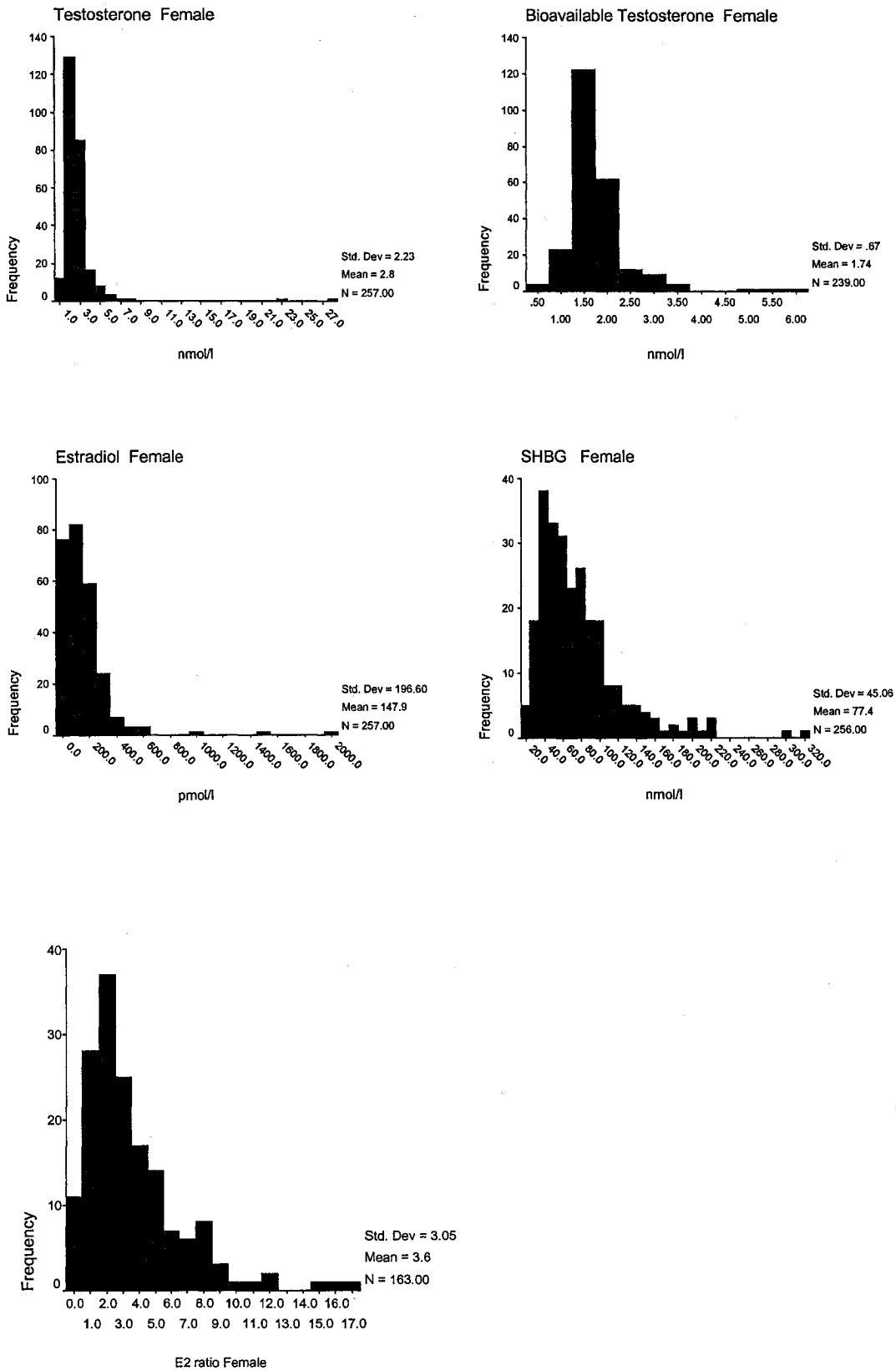


Figure 7: Hormone distributions of female study participants

The distributions showed a number of outliers, especially for estradiol and SHBG. These data were checked for proper entry and no discrepancies were found; we then examined the characteristics of the subjects with outlying values to search for a common factor or explanation. The characteristics of the apparent outliers for men and women are shown in Tables 13 and 14. For these cases, women on hormone therapy were identified. One of the male subjects with high levels of bioavailable testosterone and another with a high level of estradiol had a high score for GDS; for both cases, it is reasonable to assume that they were receiving anti-depressant therapy which could increase the hormone values.

Table 13: Description of outliers in men (outlying values are in bold).

	Cases					
	1	2	3	4	5	6
BT nmol/l	**12.3**	**12.5**	**13.5**	5.6	4.6	1.4
T nmol/l	31.1	21.4	37.5	36.9	18	3.1
E2 pmol/l	121	345.4	24.6	**1011.8**	144.7	9.6
SHBG nmol/l	61.1	35.9	88.3	83.0	**143.0**	**149.0**
E2 ratio	1.98	9.62	0.28	12.19	1.01	0.06
Age (yrs)	79	70	73	84	86	77
Smoking (pk-yrs)	30	50	0	NA	0	30
Drinking (ROH –yrs)	200	300	0	NA	0	0
BMI	18.8	32.1	20.8	NA	21.5	31.9
GDS	10	0	NA	10	0	2
Education (yrs)	6	3	0	4	8	4
3MS	80	78	NA	81	85	70
CD Group	N-C	N-N	C-D	C-C	C-D	C-D

BT=Bioavailable Testosterone; T=Testosterone; E2=Estradiol; SHBG=Sex Hormone Binding Globulin; pk-yrs=pack-years; ROH-yrs=alcohol-years; BMI=body mass index; GDS=Geriatric Depression Scale; 3MS= Modified Mini-Mental State Examination; CD group = cognitive decline group; N= normal; CIND=cognitively impaired not demented; D= demented

Table 14: Description of outliers in women (outlying values are in bold).

	Cases								
	1	2	3	4	5	6	7	8	9
BT nmol/l	**5.0**	**5.5**	**6.0**	3.6	2.1	1.3	1.4	1.4	1.5
T nmol/l	**27.6**	2.7	2.8	**23.0**	3.6	2.7	1.7	2.4	2.3
E2 pmol/l	81.1	87.9	39.5	69.2	**982.2**	**1523.4**	**2045.7**	**477.0**	**430.2**
SHBG nmol/l	80.2	64.1	75.5	29.3	134.0	98.3	97.2	**301.0**	**318.5**
E2 ratio	1.01	1.37	0.52	2.36	7.33	15.50	210	1.58	1.35
Age (yrs)	76	82	92	95	77	76	77	71	82
Smoking (pk-yrs)	10	0	0	0	0	0	0	10	0
Drinking (ROH-yrs)	0	0	0	0	0	0	0	0	0
BMI	21.8	24	28.5	26.3	20.9	25.7	20.1	22.6	23.9
GDS	0	6	2	7	1	3	0	0	0
Education (yrs)	13	8	12	Na	18	8	16	22	13
3MS	95	93	92	79	100	80	88	99	99
CD GROUP	N-N	N-D	N-D	C-C	N-N	N-C	N-C	N-N	N-N
E2 Rx	No	No	No	No	No	No	No	Yes	Yes

BT=Bioavailable Testosterone; T=Testosterone; E2=Estradiol; SHBG=Sex Hormone Binding Globulin; pk-yrs=pack-years; ROH-yrs=alcohol-years; BMI=body mass index; GDS=Geriatric Depression Scale; 3MS=Modified Mini-Mental State Examination; CD group=cognitive decline group; N=normal; C=cognitively impaired not demented; D= demented. E2Rx= estrogen therapy.

For women, cases 8 and 9 have high levels of both estradiol and SHBG, which are explained by the use of hormone therapy. For the other cases, there was no clear pattern of characteristics to suggest a plausible explanation for the existence of outliers.

Because of the outliers, we considered different approaches in the analysis. One approach was to log transform the data to obtain more normal distributions of the data. A second possibility was to give new values to the outliers. Rather than to replace the outliers with mean values, we considered giving them a value that would bring the outliers near the

values in the tail of the distribution. Figure 8 and 9 show the distributions of the hormones having given the outliers new values. Total and bioavailable testosterone have a near normal distribution. Estradiol and SHBG remain skewed, especially in the female population. Note that the scale has changed for some of the x axes. The third alternative was to exclude the outliers. This we considered to be the best option for two reasons: 1) the analysis of the characteristics of the outliers did not reveal a specific pattern, 2) the outliers represented less than 4% of the cases in both the male (3.9%) and female (3.5%) populations. In the logistic regression, we computed the univariate analysis of the hormones with and without the outliers from the raw data to assess the impact of these different approaches.

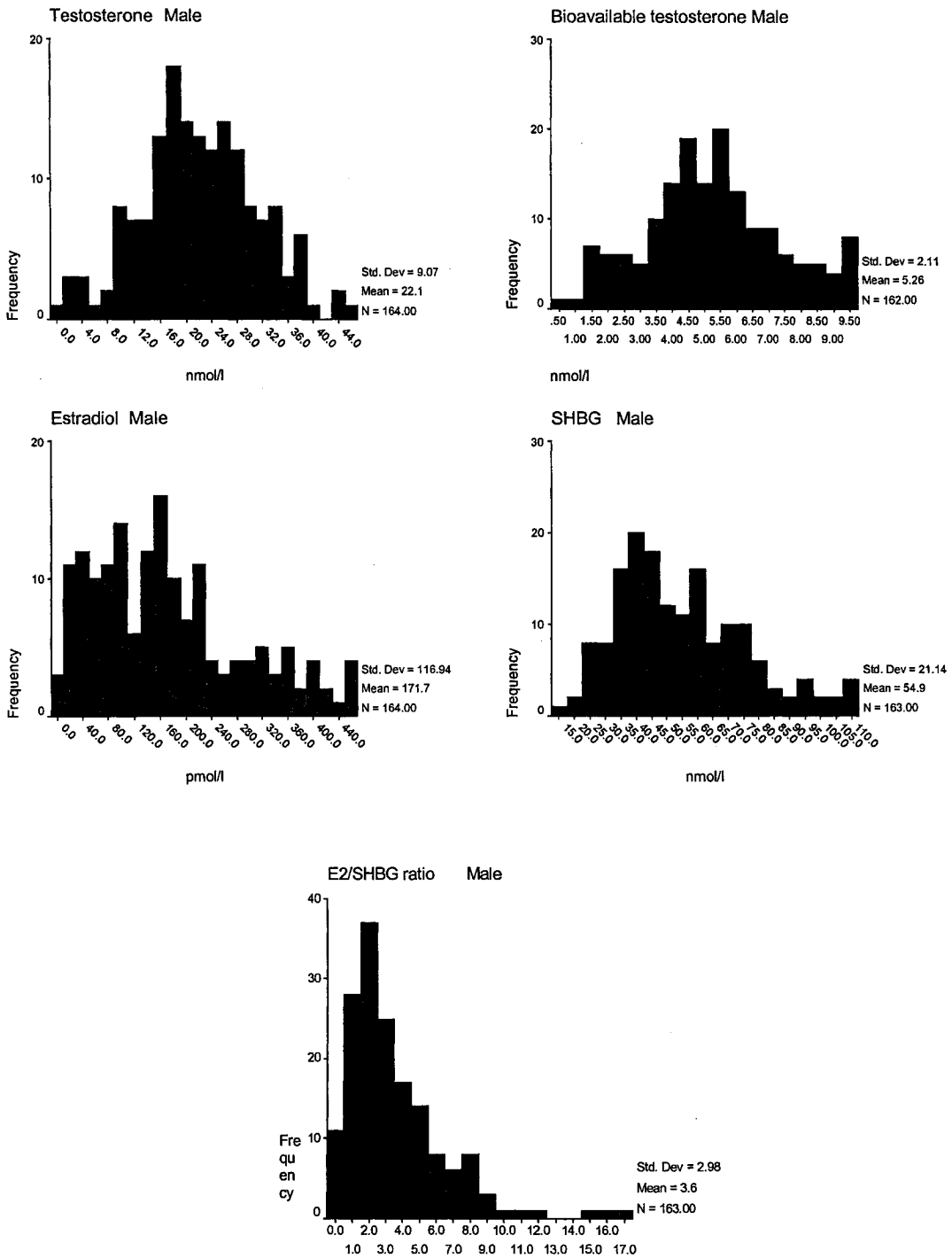


Figure 8: Distribution of hormone levels in males with new values for the outliers

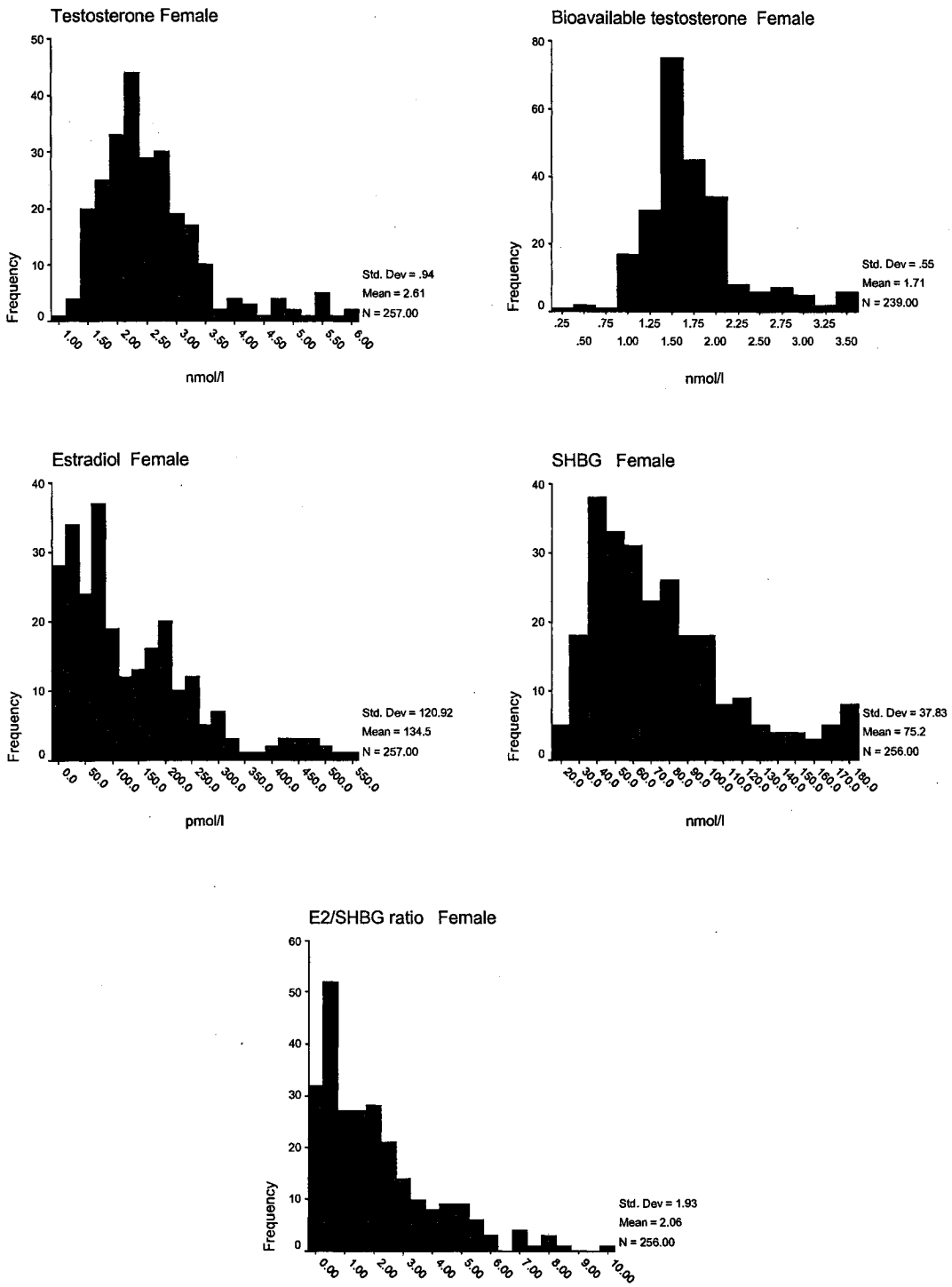


Figure 9: Distribution of hormone levels in females with new values for the outliers.

Our next step was to look at the mean hormone levels in each cognitive decline group. The results are shown in Tables 15 and 16. In males, the CIND-N group, while very small, stands out. The E2 has lower values than the other groups along with lower values for SHBG, and the CIND-N group also shows the lowest value for the E2/SHBG ratio. The men in this group were heavy drinkers, which could offer an explanation for the results observed. In women, the levels of E2 were lowest in the CIND-N group and the E2/SHBG ratio has the lowest value of all the cognitive decline groups.

Table 15: Means and standard deviations of the hormone levels in each group of cognitive decline in men.

	CD group					
	N-N	CIND-CIND	CIND-N	N-CIND	N-D	CIND-D
N (%)	75 (45.7)	25 (15.2)	3 (1.8)	10 (6.1)	16 (9.8)	35 (21.3)
Testosterone nmol/l	23.0 ± 8.9	22.3±10.2	19.2±1.6	22.1± 7.2	18.9±10.5	21.7±8.9
Bioavailable Testosterone nmol/l	5.5 ± 2.2	5.3 ±2.2	4.8±0.8	5.7±3.2	4.9±2.3	5.1±2.3
Estradiol pmol/l	171.9±110.9	199.8±218.7	30.7±28.9	129.5±80.2	218.2± 89.5	164.5±123.9
Sex Hormone Binding Globulin nmol/l	54.1±17.2	59.4±27.2	37.6±5.0	48.1±12.1	57.0 ±24.2	58.3±31.0
E2/SHBG	3.45 ± 2.5	4.1±4.8	0.87±0.9	2.6 ±1.2	4.5±2.5	3.6±3.3

CD group=cognitive decline group; N=normal; C=cognitively impaired not demented; D= demented

Table 15 shows that, if we exclude the CIND-D group, the lowest level of testosterone and bioavailable testosterone are in the normal to dementia group, whereas

estradiol and E2/SHBG have the highest levels for the N-D group. Suggesting maybe an inverse relationship between androgens and estrogens on cognition in men.

Table 16: Means and standard deviations of the hormone levels in each group of cognitive decline in women

	CD group					
	N-N	CIND-CIND	CIND-N	N-CIND	N-D	CIND-D
N (%)	121(47.1)	32 (12.5)	3 (1.2)	14 (5.5)	26 (10.1)	61 (24.6)
Testosterone nmol/l	2.7± 2.5	3.7±3.7	2.2±1.0	2.4±0.8	2.4±0.6	2.6±0.8
Bioavailable Testosterone nmol/l	1.7± 0.6	1.8±0.6	1.5±0.3	1.8±0.6	1.8± 0.9	1.7±0.7
Estradiol pmol/l	154.6±152.8	118.3±82.0	65.1±37.4	340.3±624.0	130.4±145.3	117.3±104.9
Sex Hormone Binding Globulin nmol/l	84.1± 53.1	72.8±40.4	76.6±41.2	55.3±21.9	77.4±39.8	71.5±32.9
E2/SHBG	2.0± 1.8	2.1±1.8	1.2±1.1	4.5±6.1	2.1±2.4	2.0±2.2

CD group=cognitive decline group; N=normal; C=cognitively impaired not demented; D= demented

Once again, when we examine the results in Table 16, if we exclude the CIND-N group, the lowest levels of Testosterone are in the N-CIND and N-D groups, bioavailable testosterone is very similar in all groups; Estradiol and E2/SHBG have the highest levels in the N-CIND group.

As the numbers in some cells were small, it was decided to form two groups: no decline and decline. Those in the first three groups (N-N, CIND-CIND and CIND-N) formed the no decline or reference group. The other three groups (N-CIND, N-D, and CIND-D) were combined into a group representing cognitive decline. Table 17 shows the number of subjects in the two groups by sex.

Table 17: Number of men and women in the no decline and decline groups.

	No decline in cognition	Decline in cognition	Total
Men	103	61	164
Women	156	101	257
Total	259	162	421

Tables 18 to 21 show the means and standard deviations of the different characteristics and hormone levels of the no-decline and decline groups for men and women separately. There was little difference in the hormone levels between the two groups.

Table 18: Means, Standard Deviation, Standard Error and 95%CI for all hormones in men

		N	Mean	Std. Deviation	Std. Error	95% C I for Mean Lower Bound	Upper Bound
Testosterone nmol/l	No Decline	103	22.75	9.06	.89	20.98	24.52
	Decline	61	21.04	9.07	1.16	18.72	23.36
Bioavailable Testosterone nmol/l	No Decline	101	5.42	2.15	.21	5.00	5.85
	Decline	61	5.14	2.44	.31	4.52	5.77
Estradiol pmol/l	No decline	103	174.56	144.77	14.26	146.27	202.86
	Decline	61	172.84	112.13	14.36	144.12	201.56
SHBG	No Decline	102	54.88	20.04	1.98	50.95	58.82

nmol/l	Decline	61	56.25	26.94	3.45	49.35	63.15
E2 Ratio	No Decline	102	3.52	3.15	.31	2.90	4.14
	Decline	61	3.69	2.88	.37	2.96	4.43

SHBG=Sex Hormone Binding Globulin; E2R= E2 ratio: E2/SHBG.

Table 19: Means, Standard Deviation, Standard Error and 95%CI for all hormones in women.

		N	Mean	Std. Deviation	Std. Error	95% C I for Mean	
						Lower Bound	Upper Bound
Testosterone nmol/l	No Decline	156	2.95	2.78	.22	2.51	3.39
	Decline	101	2.51	.78	.07	2.35	2.66
Bioavailable testosterone nmol/l	No Decline	145	1.72	.61	.05	1.62	1.82
	Decline	94	1.77	.76	.07	1.62	1.93
Estradiol pmol/l	No Decline	156	145.46	140.60	11.26	123.22	167.69
	Decline	101	151.59	261.31	26.00	100.00	203.17
SHBG nmol/l	No Decline	156	81.65	50.57	4.05	73.66	89.65
	Decline	100	70.76	33.94	3.39	64.02	77.49
E2/SHBG	No Decline	156	2.04	1.81	.145	1.75	2.33
	Decline	100	2.41	3.15	.31	1.78	3.03

SHBG=Sex Hormone Binding Globulin

In the other independent variables, once again, education was higher in the no decline group and the GDS score was lowest for the same group, and the confidence intervals do not show any overlap suggesting that these differences are significant.

Table 20: Means, Standard Deviation, Standard Error and 95%CI for the independent variables in men

		N	Mean	Std. Deviation	Std. Error	95% C I for Mean	
						Lower Bound	Upper Bound
Age	No Decline	103	77.77	4.59	.45	76.87	78.67
	Decline	61	80.21	5.68	.73	78.76	81.67
Smoking	No Decline	100	22.73	21.04	2.10	18.55	26.90
	Decline	53	27.22	22.11	3.04	21.12	33.31
Drinking	No Decline	99	158.86	188.82	18.98	121.20	196.52
	Decline	53	121.72	181.89	24.98	71.59	171.86
BMI	No Decline	100	25.77	3.76	.38	25.02	26.51
	Decline	58	24.97	3.79	.50	23.98	25.97
GDS	No Decline	101	1.68	2.26	.22	1.24	2.13
	Decline	56	3.12	3.46	.46	2.20	4.05
Education	No Decline	103	11.05	4.77	.47	10.12	11.98
	Decline	59	8.90	4.54	.59	7.72	10.08

Age and education are expressed in years; smoking in pack-years; and drinking in alcohol equivalent-years; BMI=body mass index; GDS=Geriatric Depression Scale.

Table 21: Means, Standard Deviation, Standard Error and 95%CI for the independent variables in women

		N	Mean	Std. Deviation	Std. Error	95% C I for Mean	
						Lower Bound	Upper Bound
Age	No Decline	156	80.17	6.46	.52	79.15	81.19
	Decline	101	82.99	6.48	.645	81.71	84.27
Smoking	No Decline	139	6.36	14.82	1.26	3.88	8.85
	Decline	93	9.59	16.63	1.72	6.17	13.02
Drinking	No Decline	143	48.39	125.31	10.48	27.68	69.11
	Decline	93	60.61	140.65	14.58	31.65	89.58
BMI	No Decline	148	25.50	4.79	.39	24.72	26.28
	Decline	92	25.50	5.54	.58	24.35	26.65
GDS	No Decline	154	1.96	2.48	.20	1.57	2.36
	Decline	92	3.33	3.10	.32	2.68	3.97
Education	No Decline	153	10.26	3.92	.32	9.64	10.89
	Decline	100	9.15	3.98	.40	8.36	9.94

Age and education are expressed in years; smoking in pack-years; and drinking in alcohol equivalent-years; BMI=body mass index; GDS=Geriatric Depression Scale.

LOGISTIC REGRESSION

We performed a logistic regression to examine at the association between cognitive decline and the different hormones while controlling for the effects of possible confounding factors.

We first carried out a univariate analysis of each variable in order to detect the most significant variables. For the baseline characteristics, the results are reported in Table 22. We see that age and the Geriatric Depression Score are positively and significantly related to cognitive decline in both men and women. Respectively OR 1.10 (p, <0.01) and OR 1.2(p, <0.01) for men and OR 1.07 (p, <0.01) and 1.09(p, <0.01) in women. Education is protective in men, OR 0.90 (p, <0.01) and in women, OR 0.93(p, 0.03)

Table 22: Odds ratios and 95% confidence intervals and *p*-values for cognitive decline in the univariate model with age, smoking, drinking, BMI, education and GDS.

Men

Model	Odds Ratio	95% CI	*P* (Wald)
Age	1.10	1.03 1.17	<0.01
Smoking	1.01	0.99 1.02	0.22
Drinking	0.99	0.99 1.00	0.24
BMI	0.94	0.86 1.03	0.20
Education	0.90	0.84 0.97	<0.01
GDS	1.20	1.06 1.36	<0.01

Women

Model	Odds Ratio	95% CI	*P* (Wald)
Age	1.07	1.03 1.11	<0.01
Smoking	1.01	0.99 1.02	0.13
Drinking	1.00	0.99 1.00	0.49
BMI	1.00	0.95 1.05	0.99
Education	0.93	0.8/7 0.99	0.03
GDS	1.19	1.08 1.31	<0.01

BMI= body mass index; GDS= geriatric depression scale

Raw data (i.e. no modification to the hormone values) were used in this analysis, but for the hormone variable, the analysis was repeated, first with, and then without, cases with outlying values.

Tables 23 and 24 show the results for men and women, reporting the analyses first with outliers on the hormone measures included, and then with them excluded. None of the hormones showed any significant relationship with the outcome. The odds ratio were all very

near 1, indicating that there is no statistical significant increase or decrease in the risk of having dementia. Moreover, none of the *p* values was significant and the confidence intervals all include 1, excluding any significance.

Table 23: Crude odds ratios and 95% confidence intervals for cognitive decline in the univariate model with, and without, the outliers in the male study population.

	Outliers included			Outliers excluded		
Model	Odds Ratio	95% CI	*P* (Wald)	Odds Ratio	95% CI	*P* (Wald)
Testosterone	0.95	0.95 1.01	0.30	0.98	0.94 1.01	0.24
Bioavailable Testosterone	0.95	0.82 1.09	0.44	0.90	0.76 1.05	0.16
Estradiol (E2)	1.00	0.99 1.00	0.94	1.00	0.99 1.00	0.73
Sex Hormone Binding Globulin (SHBG)	1.00	0.99 1.02	0.71	1.00	0.98 1.01	0.62
E2/SHBG	1.02	0.92 1.13	0.73	1.02	0.92 1.13	0.73

Table 24: Crude odds ratios and 95% confidence interval for cognitive decline in the univariate model in women with, and without, the outliers

	Outliers included			Outliers excluded		
Model	Odds Ratio	95% CI	*P* (Wald)	Odds Ratio	95% CI	*P* (Wald)
Testosterone	0.83	0.65 1.07	0.16	0.84	0.64 1.11	0.22
Bioavailable Testosterone	1.12	0.78 1.65	0.56	0.96	0.57 1.59	0.86
Estradiol (E2)	1.00	0.99 1.00	0.81	0.99	0.99 1.00	0.25
Sex Hormone Binding Globulin (SHBG)	0.99	0.98 1.00	0.06	0.99	0.98 1.00	0.13
E2/SHBG.	1.06	0.96 1.18	0.25	1.07	0.96 1.18	0.25

:

The inclusion or exclusion of outliers made little difference, the only change in the odds ratios being for bioavailable testosterone in women, which was 1.12 in the first analysis and 0.96 when the outliers were excluded, but this was statistically non significant. As excluding the outliers appeared to exert no difference, all further analyses included the outliers, and used the raw data for hormone values.

We next pursued several additional analyses to more closely examine the point estimates in the regression models. Using logistic regression and binary outcomes is useful because this analysis does not assume normal distributions of the independent variables. However, logistic regression does assume linearity of the log odds and the independent variables. We therefore tested for linearity of the point estimates. To check for linearity, the independent variables were first divided into quintiles and a beta value was estimated for

each range. The estimated coefficients were plotted versus the mid-point of the quintiles using 0 for the first quintile. On the graphs thus generated, there was linearity for bioavailable testosterone in males and the E2 Ratio in females. These are the two most biologically significant measures respectively for men and for women. The evidence of linearity was less apparent for the levels of Estradiol, Total Testosterone and SHBG. The SHBG result need not cause concern, as SHBG was measured solely to compute the E2/SHBG ratio, and there is no biological evidence that SHBG is associated with cognitive decline.

In a further step, we dichotomised the quintiles of hormone levels after the first level: 1 was coded for the first quintile and 2 for the remaining quintiles. This did not significantly reduce the deviance from linearity except for Estradiol in females; it increased the deviance for Testosterone and SHBG in females.

Table 25 shows the deviance from linearity results of a succession of tests for linearity. In the first model, the square of the hormone value was added to the model. The resulting model did not show any significant reduction of the deviance. In Table 25, "Linear" denotes that the hormone variable was regressed as a continuous variable; "Quintiles" indicates that the hormone variable was regressed using quintiles, treated as a continuous variable; "Dichotomous" indicates that the hormone was added to the model as a dichotomy, as previously described.

Table 25: Deviance values for four different coding of each hormone variable

TOTAL TESTOSTERONE

Males		**Females**	
Scale of hormone level	*Deviance*	*Scale of hormone level*	*Deviance*
Adding square of the hormone level to the model	215.202	Adding square of the hormone level to the model	339.345
Linear	215.103	Linear	340.915
Quintiles	215.960	Quintiles	342.906
Dichotomous	214.272	Dichotomous	344.206

BIOAVAILABLE TESTOSTERONE

Males		**Females**	
Scale of hormone level	*Deviance*	*Scale of hormone level*	*Deviance*
Adding square of the hormone level to the model	212.433	Adding square of the hormone level to the model	319.561
Linear	214.009	Linear	320.014
Quintiles	213.480	Quintiles	320.229
Dichotomous	212.572	Dichotomous	320.265

ESTRADIOL

Males		**Females**	
Scale of hormone level	*Deviance*	*Scale of hormone level*	*Deviance*
Adding square of the hormone level to the model	214.640	Adding square of the hormone level to the model	338.930
Linear	216.469	Linear	344.356
Quintiles	215.240	Quintiles	338.389
Dichotomous	215.382	Dichotomous	335.418

SHBG

Males		**Females**	
Scale of hormone level	*Deviance*	*Scale of hormone level*	*Deviance*
Adding square of the hormone level to the model	211.274	Adding square of the hormone level to the model	336.916
Linear	215.404	Linear	338.755
Quintiles	215.368	Quintiles	340.935
Dichotomous	215.541	Dichotomous	342.101

E2RATIO

Males		**Females**	
Scale of hormone level	*Deviance*	*Scale of hormone level*	*Deviance*
Adding square of the hormone level to the model	214.364	Adding square of the hormone level to the model	334.792
Linear	215.424	Linear	341.173
Quintiles	214.412	Quintiles	342.336
Dichotomous	215.382	Dichotomous	340.855

We also performed a Box-Tidwell transformation to the logistic regression to test for linearity of the logits. We regressed the logit of the independent variable (x) on both (x) and ln (x). The variable x ln (x) is added to the model containing x to assess departure from linearity where significance of the (x)*ln (x) suggests non-linearity, (Guerro and Johnson 1982, Hosmer and Lemeshow 1989). We did not find any evidence of non-linearity through this method.

From the comparison of the deviances in the different models and because of the result of the Box-Tidwell transformation, we pursued our analysis using the hormone variables as linear values treated continuously. However we did not include SHBG in the rest

of the analyses as there is no biological support of its association with cognition or decline in cognition and as the graph we computed of its beta values against the mid point of the quintiles was the least linear.

We next examined the impact of adding the variables age, smoking, drinking, education and the Geriatric depression score to the model. The change in deviance was assessed when those terms were added individually to the model. Age, education and the Geriatric Depression Score were found to be confounders as the change in deviance was statistically significant for the three variables. We therefore report the adjusted ratios. Tables 26-28 show adjusted odds ratios when successively adding the variables age, education and Geriatric Depression Score to the model. We report the results for androgens (testosterone and its active form BT) and estrogens (estradiol and the estimate of its active form E2/SHBG).

Table 26 shows that the odds ratio of total testosterone for cognitive decline when controlling for age (OR 0.98 0, *p*-value 0.19, for men and OR 0.81, *p*-value 0.10, for women) is not significant. For bioavailable testosterone (OR 0.98 for men and 1.11 for women) is also not significant with *p*-values respectively of 0.70 and 0.59. For estrogens results are similar. The odds ratio of Estradiol for cognitive decline when controlling for age (OR 1.00 for men and 1.00 for women) was not significant (*p*-values respectively of 0.93 and 0.43). For E2/SHBG, the odds ratio for cognitive decline when controlling for age (OR 1.05 for men and 1.10 for women) was also not significant (*p*-values respectively of 0.37 and 0.07).

Table 26: Coefficients from the logistic regression of hormone level for cognitive decline with age included in the model.

TOTAL TESTOSTERONE (TT)

		b	S.E.	*p*	OR	95% C.I. for OR	
						Lower	Upper
Males	TT	-.025	.019	.187	.976	.940	1.012
	Age	.099	.034	.003	1.104	1.033	1.179
Females	TT	-.216	.132	.102	.806	.622	1.044
	Age	.072	.021	.001	1.075	1.032	1.119

BIOAVAILABLE TESTOSTERONE (BT)

		b	S.E.	*p*	OR	95% C.I. for OR	
						Lower	Upper
Males	BT	-.029	.076	.706	.972	.837	1.128
	Age	.094	.034	.006	1.099	1.028	1.174
Females	BT	.107	.201	.595	1.113	.750	1.651
	Age	.060	.021	.004	1.062	1.020	1.106

ESTRADIOL (E2)

		b	S.E.	*p*	OR	95% C.I. for OR	
						Lower	Upper
Male	E2	.000	.001	.931	1.000	.997	1.002
	Age	.096	.033	.004	1.101	1.031	1.175
Female	E2	.001	.001	.429	1.001	.999	1.002
	Age	.069	.021	.001	1.072	1.029	1.116

E2/SHBG RATIO (E2RATIO)

		b	S.E.	*p*	OR	95% C.I for OR	
						Lower	Upper
Male	E2 Ratio	.050	.056	.375	1.051	.942	1.173
	Age	.101	.034	.003	1.106	1.035	1.182
Female	E2Ratio	.102	.056	.070	1.108	.992	1.237
	Age	.079	.021	.000	1.082	1.038	1.128

Table 27 reports the odds ratio for each hormone when controlling for education. The results show that the odds ratios for each hormone are close to one and not statistically significant. The results are: for total testosterone OR = 0.99 (*p* 0.52) for men, and OR 0.79, (*p* 0.10) for women; for bioavailable testosterone OR = 0.93 (*p* 0.31) for men, and OR 1.13 (*p* 0.54) for women; for estradiol, OR = 0.99 (*p* 0.43) for men and OR = 1.00 (*p* 0.80) for women. For the Estradiol/SHBG ratio the OR was 0.97 (*p* 0.57) for men and OR was 1.07 (*p* 0.21) for women.

Table 27: Coefficients from the logistic regression of hormone level for cognitive decline with education included in the model.

TOTAL TESTOSTERONE (TT)

		b	S.E.	*p*	OR	95% C.I. for OR	
						Lower	Upper
Male	TT	-.012	.019	.523	.988	.952	1.025
	Education	-.099	.037	.008	.906	.842	.975
Female	TT	-.234	.144	.103	.791	.597	1.048
	Education	-.083	.035	.018	.920	.859	.986

BIOAVAILABLE TESTOSTERONE (BT)

		b	S.E.	*p*	OR	95% C.I. for OR	
						Lower	Upper
Male	BT	-.076	.076	.314	.926	.798	1.075
	Education	-.110	.039	.004	.896	.831	.966
Female	BT	.122	.200	.541	1.130	.763	1.674
	Education	-.070	.036	.053	.932	.869	1.001

ESTRADIOL (E2)

		b	S.E.	*p*	OR	95% C.I. for OR	
						Lower	Upper
Male	E2	-.001	.001	.434	.999	.996	1.002
	Education	-.106	.038	.005	.899	.834	.969
Female	E2	.000	.001	.806	1.000	.999	1.001
	Education	-.074	.034	.030	.929	.868	.993

E2/SHBG RATIO (E2 RATIO)

		b	S.E.	*p*	OR	95.0% C.I. for OR	
						Lower	Upper
Male	E2 Ratio	-.032	.056	.568	.968	.868	1.081
	Education	-.107	.038	.005	.898	.833	.968
Female	E2 Ratio	.066	.053	.212	1.069	.963	1.186
	Education	-.070	.034	.041	.932	.871	.997

Table 28 reports the results of the logistic regression of each hormone when controlling for the Geriatric Depression Score. For total testosterone, the OR was 0.98 in men and 0.80 in women with, respectively, *p*-values of 0.19 and 0.11. For bioavailable testosterone, the OR was 0.89 in men and 1.08 in women with, respectively, *p*-values of 0.15 and 0.70. For estradiol, OR = 0.99 in men and 1.00 in women with, respectively, *p*-values of 0.59 and 0.89. For Estradiol/SHBG, the OR was 0.99 in men and 1.02 in women with, respectively, *p*-values of 0.90 and 0.68.

Table 28:Coefficients from the logistic regression of hormone level for cognitive decline with Geriatric Depression Score (GDS) included in the model.

TOTAL TESTOSTERONE (TT)

		b	S.E.	*p*	OR	95% C.I. for OR	
						Lower	Upper
Male	TT	-.025	.019	.195	.976	.940	1.013
	GDS	.185	.064	.004	1.204	1.063	1.363
Female	TT	-.228	.144	.113	.796	.601	1.055
	GDS	.189	.051	.000	1.209	1.093	1.337

BIOAVAILABLE TESTOSTERONE (BT)

		b	S.E.	*p*.	OR	95% C.I. for OR	
						Lower	Upper
Male	BT	-.117	.081	.149	.890	.759	1.043
	GDS	.189	.064	.003	1.208	1.066	1.369
Female	BT	.079	.204	.698	1.082	.726	1.613
	GDS	.167	.051	.001	1.182	1.070	1.305

ESTRADIOL (E2)

		b	S.E.	*p*	OR	95% C.I. for OR	
						Lower	Upper
Male	E2	-.001	.001	.591	.999	.997	1.002
	GDS	.189	.065	.004	1.208	1.063	1.371
Female	E2	.000	.001	.894	1.000	.999	1.001
	GDS	.176	.050	.000	1.192	1.081	1.315

E2/SHBG RATIO (E2 RATIO)

		b	S.E.	*p*	OR	95% C.I. for OR	
						Lower	Upper
Male	E2 Ratio	-.007	.060	.902	.993	.883	1.116
	GDS	.180	.064	.005	1.198	1.057	1.357
Female	E2 Ratio	.024	.057	.678	1.024	.916	1.144
	GDS	.173	.050	.001	1.188	1.078	1.310

The results shown in Tables 26-28 do not reveal any significant association of sex hormones with cognitive decline, even when controlling for confounding factors such as age, education and a depression score.

We then added the corresponding interaction term to each previous model to test for effect modification. Effect modification is judged to be present if the deviance of the model changes significantly when the interaction term is added. There was no evidence of effect modification for age, education or for the Geriatric Depression Score. In the model including bioavailable testosterone, age and the interaction term, the results showed a wide confidence interval. We therefore centered the value of bioavailable testosterone by subtracting the mean from the hormone value. This made very little change in the coefficients. As a further step we also centered the variable *age.* This resulted in more valid values. (Tables 29-31)

Table29:Coefficients of the model including Bioavailable Testosterone (BT), age and the interaction term BT*age

		b	S.E.	*p* (Wald)
Males	BT	.640	1.085	.348
	Age	.110	.081	1.869
	BT*Age	-.009	.014	.388
Females	BT	.966	2.598	.138
	Age	.111	.060	3.416
	BT*Age	-.010	.032	.099

Table 30: Coefficients of the model including Bioavailable Testosterone-centered (BT), age and the interaction term BT*age

		b	S.E.	*p* (Wald)
Males	Bt centered	.640	1.085	.348
	Age	.064	.033	3.843
	Bt centered* Age	-.009	.014	.388
Females	Bt centered	.966	2.598	.138
	Age	.094	.022	18.757
	Bt centered* Age	-.010	.032	.099

Table 31: Coefficients of the model including centered values of Bioavailable Testosterone-centered (BT), age and the interaction term BT*age

		b	S.E.	*p* (Wald)
Male	Bt centered	-.039	.077	.262
	Age centered	.093	.034	7.264
	Bt centered* Age centered	-.015	.015	1.014
Female	Bt centered	.130	.204	.408
	Age centered	.061	.021	8.646
	Bt centered* Age centered	-.015	.029	.264

To test for the significance of the interaction terms we looked at the change of deviance when the interaction was added, we found a significant change when we tested for the interaction of drinking on Bioavailable Testosterone for men. We therefore looked at the results in drinkers and non-drinkers separately. The results are presented in Table 32.

Table 32: Odds Ratio, *P* value for the Odds ratio from the regression of Bioavailable testosterone (BT) and cognitive decline in male and female drinkers and non drinkers

			b	*p* (Wald)	OR	95%C.I.for OR	
						Lower	Upper
Males	Drinkers	BT	-.319	.017	.727	.559	.946
	Non drinkers	BT	.126	.238	1.134	.920	1.399
Females	Drinkers	BT	.648	.262	1.911	.616	5.931
	Non drinkers	BT	.056	.794	1.058	.693	1.616

The results suggest that there is a protective effect of drinking in men the odds ratio is 0.727 with a significance of 0.02. The significance of which is unclear.

In conclusion, we have analysed the data from the CSHA and measured the hormone levels. We the regressed the hormone levels on cognitive decline as defined previously. We have found no significant relationship between levels of sex hormones and cognitive decline, whether these were examined separately or after controlling for confounders. We will therefore discuss the different limitations of the study and propose hypotheses to explain the absence of an association.

DISCUSSION

The last decade has seen the growing size of the aging population, and with it the growing burden of dementia. In dementia, the parallel is often drawn with osteoporosis. Osteoporosis is the loss of bone density occurring with age due to lower levels of testosterone and estradiol. The possibility that low levels of sex hormones, which is a natural process of aging, could be responsible for, or contribute to, cognitive decline is thus very appealing and has warranted a number of studies. Researchers have looked into the effects of sex hormones on the brain. The effects of testosterone and estradiol on the human brain have been established. Experimental studies have shown that sex hormones have a definite and specific action on the brain and the mechanisms of those actions are still being studied. However, the effects of circulating levels of sex hormones on the degenerative process of aging and cognitive decline are not yet well established. Clinical studies have reported diverse conclusions. Our study did not corroborate that levels of circulating sex hormones have any effect on cognition. This may be the result of different methodologies between studies or specific differences with the previous studies.

STRENGTHS OF THE STUDY

Several strengths of this study may be cited. First of all, the hypothesis relies on strong biological evidence; secondly, the clinical process to determine the diagnosis involved a team of health professionals, clear criteria and a consensus meeting. Unlike many of the previous

studies of hormones and cognition, this study used a representative population sample. This is both an advantage, but in some ways also a liability. A population sample increases external validity and permits inferences to the general population (for whom decisions such as whether or not to use hormone replacement therapy are relevant). By contrast, several of the existing studies have focused on selected populations such as women on hormone therapy, or people attending memory disorder clinics. A further advantage of a population sample is that all categories and severities of cognitive change are included, rather than merely people with severe dementia, and cognitively normal controls as in some of the existing case-control studies. The advantage is that if an association with hormone levels exists, it should be seen as a gradient across all levels of cognitive change, including the group with cognitive impairment but not dementia. A dose-response relationship with level of cognitive loss would support a conclusion of causality. However, the downside of this sample is that the groups studied are somewhat heterogeneous.

LIMITATIONS OF THE STUDY

A number of factors could explain why we have failed to find an association when perhaps there is one. Indeed, we know that the absence of evidence is not evidence for the absence of an association. We will first discuss the methodological issues and the limitations of the present study that offer possible explanations as to why we failed to find an association between cognition and the circulating levels of sex hormones. We will then review, in more detail, the characteristics of the studies that have measured serum levels of sex hormones and that have reported an association and, look how those studies differ or compare with this study.

A number of factors related to the design and methodology in this study could allow for a false negative. The most important limitation to our study was the small sample size but also the characteristics of the study population and the measurement of the assays may have brought limitations to the study and will be reviewed.

Sample

A major concern was the sample size in our study. Because of financial constraints, the number of blood analyses we could do was limited. We followed up only those patients who were found to be normal or cognitively impaired when examined at phase 1 or 2 of the study, and for whom we had a definite cognitive measure at the end of the 5 year period. Those who died or were lost to follow-up were not included (see p55). The need to analyse males and females separately yielded smaller sample subsets of 164 males and 257 females. They were then classified by change in cognition over time, producing 12 different groups. Some groups had very few participants, leading us to combine different cognitive decline groups. Although this gave 259 subjects in the no decline group and 162 in the decline group, as already mentioned, this created heterogeneous samples in the different categories. Our study was a secondary analysis of data and could not benefit from being designed specifically for the outlined objective. Another consequence of our small sample size is that we looked at dementia as a whole. The small sample size did not allow us to examine the data according to the specific types of dementia. The resulting cognitive decline groups were therefore heterogeneous in terms of underlying degenerative processes and pathophysiology. In the classification that we used we assume that hormones have the same effect on all types of dementia, which may well not be the case. This misclassification may have led to spurious conclusions, preventing us to identify a true difference. Indeed, this may have led to false

negative results by diluting possible effects in one or the other group and reduced our ability to detect an overall difference.

An elderly population

The design in the CSHA intentionally over-sampled the 75+ population. Our study population had a mean age of 78 at the time of blood sampling (beginning of the five-year follow-up periods). Selecting a very elderly population ensures that there will be a higher incidence of impaired cognition, which is age-related. This is probably beneficial as it is specifically this population at risk that we are interested in. However, on the negative side, age could be overriding whatever influence hormones may have on the brain at earlier ages. Hence, although sex hormones may have a protective effect on dementia, as is suggested by the biological pathways, the effect on cognition may be occurring at an earlier stage and at ages over 75, it may no longer be possible to detect a causal relationship. Carlson (2001) has suggested that in order for hormones to regulate or modify cognition, the timing and length of the exposure to sufficient levels of circulating hormones may be crucial; the period around menopause or andropause seems to be a critical time in establishing a protective effect against cognitive decline. Once an individual has reached 80, levels of circulating hormones, whether high or low, may be insufficient to prevent further loss of cognition as a result of the degenerative processes affecting the brain.

We may suggest several reasons why hormone levels at this time in life may have little influence on cognitive decline. There may be low levels of the active form of hormone as a result of an interaction with different chronic diseases and ensuing polypharmacy, all of which increase the levels of SHBG with a direct effect on the biologically active hormone.

Also, with age, affinity of the hormones for their specific receptors in the target organs may be decreasing, thus impeding the action of the hormones on the brain. Moreover, the receptors, which decrease in numbers with age, may themselves be deficient and less responsive to hormone stimulation. Age also affects the reliability of the hormone assays; the concentrations are at their lowest, so detectability may not be optimal. Therefore, not finding a causal relationship in this study of very elderly subjects does not exclude the existence of a prior causal influence of hormones on cognitive decline.

Blood samples and storage

In our study, the methods involving the blood samples and their storage could also have influenced the results. The samples we analysed had been in storage from 6 to 11 years before analysis. They had been stored at –20° C and had been used for various analyses. The samples were thawed to extract the necessary volume and then re-frozen for shipping. Some laboratories recommend colder temperatures for storage (- 70°C) to ensure better stability of the samples, although the Winnipeg laboratory strenuously denies this. As a result of the length of storage, or of possible cycles of thawing and re-freezing, the reliability of the assays could be questioned. Indeed, Scott (2002) who used samples that had been stored from 10 to 20 years, found a significant increase in testosterone levels with length of storage. The increase represented a date-related assay artefact. This might also be true for other sex hormones and explain the presence of our outliers. When we checked, a few of our outliers for estradiol came from samples taken at phase 1 of CSHA, more than ten years before the actual assays were done in the laboratory.

Another possible concern is the five-year time lag between the blood sampling and the outcome measurement. During this time, the concentration of hormones could have varied resulting in false negative findings. However, we have assumed the hormone levels were constant throughout that period as the studies we reviewed earlier in the literature concerning this issue did not report any change in hormone levels 3 years after menopause in women and, no significant change in men, after 65 years of age. We were, therefore, confident that had we tested the hormone levels more frequently over the five-year period this would not have been a significant addition to the final results and their interpretation.

We relied on the quality control testing done by the biochemist at the Cité de Santé Laval. The testing was performed before each processing and compared to the expected range. The observed-to-expected ranges were all reported to be satisfactory. Quality controls are done on a random sample of assays before each batch of vials are processed, but this does not exclude the possibility that outliers may be the result of purely technical issues, although our analysis with and without the outliers did not significantly alter the results. However, if indeed technical reasons were responsible for spurious measurement this also could explain a false negative result in our analysis.

Hormone levels and chronic diseases

The true validity and biological significance of hormone levels in such an elderly population where prevalence of chronic diseases is high, needs to be discussed. There is a high correlation between polypharmacy and chronic diseases, and both polypharmacy and chronic diseases have a direct and indirect influence on the levels of the sex hormones. This is especially true for our measurement the E2/SHBG ratio. In computing the E2/SHBG ratio,

any factors altering or influencing either of these two molecules may affect the interpretation of the findings.

1) SHBG fluctuates with different hormones: testosterone, insulin, estradiol and thyroid hormones. Serum SHBG concentrations are lower in obese subjects and increase with age, being higher in older women and men. Therefore, in overweight older patients with insulin-dependent diabetes or on hormone therapy, the subsequent interaction on SHBG will modify the active form of the hormones and thus modulate their effect on the brain.

2) Estradiol levels also vary with different factors. The concentration of estradiol in subjects over age 50 is dependent on the aromatisation of androgens in the peripheral tissues. Factors interfering with aromatase, the enzyme responsible for transforming androgens to estrogens will influence the levels of estradiol. The enzyme increases with age and with body fat, and our sample had a high mean BMI, at 25.5. However as our study measured the circulating levels of hormones, it is uncertain the results of the BMI would interfere with the results of estradiol levels. A number of medications (non-steroidal anti-inflammatories, cholesterol-lowering medications, anti-depressants, heart medications such as beta-blockers and calcium channel-blockers) increase the levels of aromatase and, thereby also increase circulating levels of estradiol. A high proportion of the elderly population make use of these medications. Excessive alcohol, through interference with the liver metabolism and the conversion of estradiol to inactive metabolites, results in decreased levels of estradiol.

As a number of different factors may influence both E2 and SHBG, this may increase the source of errors in the measurement and calculation of E2/SHBG, which is the ratio we

used to estimate the biological active form of estrogens. Therefore we may be underestimating the role of estrogens.

COMPARISON WITH PREVIOUS STUDIES

As we reviewed in the Chapter 2: Background and Significance, a number of studies have examined the association between sex hormones and cognition. However, most of those studies report the effects of an exogenous source of hormones either in small-randomized controlled trials with short periods of treatment and follow-up, or in longitudinal studies on the effects of hormone replacement therapy in women. Notwithstanding the fact that these studies have raised interest in the research and medical community, they differ substantially from our study, which directly measured the levels of circulating hormones (i.e. endogenous and exogenous) and measured cognition with a clinical and neurological examination as well as with neuropsychological tests at two points in time over a period of five years. In the attempt to highlight the differences between our study and those reviewed, we will examine further their methodology and concentrate on the studies that were based on direct measurement of the circulating hormone levels.

In the Rancho Bernardo longitudinal study Barrett-Connor (1999) measured total and bioavailable fractions of testosterone and estradiol, whereas we measured E2/SHBG as a surrogate for bioavailable estradiol. The 393 women she studied were not on hormone replacement therapy. Her study population was limited to ambulatory, middle to middle-upper class, and white subjects. Their mean age was 74 years, (range 55 and 89 years) which is younger than our population where women had a mean age of 81.3 years (range 66-98). The sera were analysed between 4 and 7 years after collection, which is similar to the time

lag in our study. The relationship examined was between hormone levels and the results of 12 neuropsychological tests. Testing of cognition was done only at one point in time. Smoking, alcohol use, body mass index and depressed mood were not associated with cognitive performance in linear regression models. Only age and education were associated with cognitive function, which was also our finding. Only total testosterone showed an association with one out of 12 tests. The population sampled in this study is different in age and background from our study. The outcome measured was performance on 12 different neuropsychological tests; there was no clinical diagnosis of cognition or dementia as in the CSHA. The cognitive battery of tests employed in Barrett-Connor's study differs from our clinical diagnosis of cognition, making direct comparisons between the two studies difficult.

Manly's study (2000) was cross-sectional and analysed the relationship of endogenous estrogen levels in post-menopausal women with Alzheimer's disease. Unlike our study, it was a case-control design comparing 93 non-demented controls and 50 women with dementia (Alzheimer's disease). Patients with Alzheimer's disease were found to have lower estradiol than did the controls. Because of the design, Manly could not conclude a causal effect. The possibility that the diseased brain slows down the regulation of estradiol production cannot be disregarded, nor the loss of weight or the loss in physical activity which comes with dementia which on their own could have a lowering effect on the levels of estradiol.

Tang, 1996 examined the relationship between sex hormones and dementia. This was an observational study that shed light on a probable association between sex hormone levels and dementia, but could not determine whether the low levels of hormones were a result from the disease itself, or whether they contribute to the neurodegenerative process.

Also in a cross-sectional study, Drake et al. (2000) reported that, in women, both estrogen and testosterone showed associations with cognitive performance. Among the hormones they measured, estradiol (total and bioavailable) and testosterone were included. They did not measure bioavailable testosterone. Again in this study, the sample size was small (39), the population studied was highly educated (14.3 years) in comparison with our study where the female population had a mean of 9.8 years of education. In both studies subjects were predominantly white. The median age of the participants was similar to ours: 78.8 years (range 65 to 90 years). The average time interval between the blood draw and the neuropsychological testing was 5 months (range 0-11), and assessment of cognition was measured essentially through 9 specific neuropsychological tests. Once again, they found two specific domains influenced by hormone levels, whereas the in two global measures of cognition they used (Mini-Mental State Examination and Blessed's modified Orientation Memory Concentration Test) they did not show any correlations with circulating levels of hormones. This emphasises that the conclusions one derives from an aggregate measure of cognition, as we did in CSHA, may differ from those obtained when measuring specific domains of cognition and render comparison inappropriate.

Of interest, the study performed by Scott (2002) in the Baltimore Longitudinal Study of Aging. Cognition was assessed at more than one point in time. He studied a population of 407 men aged from 50-90 years (mean age, 64 yrs). The duration of follow-up was 9.7 years and the mean level of education was high: 16.75 years. All men were non-demented. The men had a comprehensive medical examination, psychological and neurological evaluation every 2 years. Testosterone levels were measured at baseline and a free testosterone index (indicator of bioavailable testosterone) was calculated. Cognitive status was measured through neuropsychological testing at different points in time. A maximum of 14 tests were

administered. They found that a higher free index of testosterone was associated with better scores on specific domains of cognitive performance in older men. Compared to our study, the age span differs significantly in that the mean age was much lower than ours. The level of education is also higher in the Baltimore study (16.75 vs 10.7 in our study). The outcome measure was based on neuropsychological tests rather than a clinical status, and the hormone measured was an estimate of bioavailable testosterone through calculation of the free index of testosterone in contrast to a direct measurement. However, there was sequential neuropsychological testing and analysis of those cognitive tests for which there were sufficient data to allow for longitudinal analysis of cognitive decline revealed that both baseline and mean free testosterone index were associated with a reduced rate of decline in visual memory. This is a positive result but on only one cognitive test.

Two other studies measured testosterone and estradiol (total and bioavailable) in men. In a cross-sectional study of 310 men, Yaffe (2002) found an association of better performance on the neuropsychological tests when the levels of bioavailable testosterone were high. SHBG levels and total estradiol levels were negatively associated with cognition. Her study population was similar to ours, the mean age of the participants was 73 years and they were predominantly white and community-dwelling, but the men did not undergo a clinical assessment for cognitive impairment. Cognition was measured using three neuropsychological tests (MMSE, Trails B and Digit Symbol). It seems that hormone levels may be able to affect specific cognitive functions, but other antagonist or synergistic interferences may modulate the global effect on cognition.

Barrett-Connor and Goodman-Gruen (1999) found a negative association between estradiol and the MMSE and a protective effect from androgens on the tests of verbal

memory. This study included 547 community-dwelling men ranging between 59 and 89 years of age (mean age 70 yr), nearly ten years younger than our own, and there was only one measurement of cognition over time.

It is noteworthy that all these studies controlled for the same confounding factors: age, education, body mass index, smoking, alcohol use and a scale to assess mood that is similar to the one used in our study.

Finally, we cannot establish a comparison with the randomized, placebo controlled clinical trials that we reviewed and that are summarized in Tables 1 and 2. These are small interventional studies (25-62 participants) with no measurement of hormone levels. The participants received short courses of hormone therapy and were briefly followed-up. Because of these methodological issues, they cannot be compared to our study.

SUMMARY

In conclusion, the biological evidence of the action of sex hormone on the brain is well established and other facts are also well established: dementia is age-related, education is protective and there is most probably a correlation with depression. This study replicated the finding that age, education and depression have a clear association with dementia, but our study did not detect any significant association between hormone levels and overall cognitive decline. Adjustments for age, education, depression and drinking did not alter these negative results. In a number of studies, the age of the population differs from ours or the outcome measured is the performance on specific neuropsychological tests rather than a clinical status. The neuropsychological battery of tests used does not always overlap from one study to

another, rendering comparison difficult. One of the strengths of our study was the complex process set up to establish a precise clinical diagnosis. This makes this study closer to reality and easier to correlate the results to clinical practice.

The absence of finding an overall association between cognitive decline and circulating levels of sex hormones in the present study does not preclude the existence of an influence of hormones on different and specific domains of cognitive function. Our study population being older, the effect of low circulating hormone levels may have already taken its toll. Indeed, in the very old, certain factors may explain why hormone levels have no longer any relevant influence on cognition and in the absence of timely hormone replacement therapy the degenerative process will pursue. The outcome in this study was based on clinical diagnoses, which aggregated a number of cognitive functions, which also interact with one another. It appears that a complex pattern of relationships exists between sex steroid hormones levels and cognitive functioning. Thus, global and specific measures of cognition need to be performed in association with clinical diagnostic testing to enhance the picture. This is not to say that one measure need override another or is superior to another, but that clinically a global measure of dementia seems more useful to the clinician.

Our study showed neither a negative nor a positive effect from the hormone levels in a Canadian population over the age of 75. However, in view of the present body of evidence and because of the known actions of sex hormones on the brain, we still support the relevance of measuring circulating hormone levels in future studies exploring cognitive decline, albeit in younger populations. The objective would be to try and determine at what time of the life cycle the variation of hormone levels impact the most on cognition. Moreover, the outcome we measured was a global measure of cognition; further studies

could endeavour to precise more specifically what brain functions are most under the influence of hormone levels and which neuropsychological test is the most appropriate to detect any variation. It would be clinically useful to have the capacity to identify a sensitive and specific test as a screening tool.

Another issue that could be addressed would be to look at the rate of decline, and concentrate on the most biologically active forms of circulating hormones, that is bioavailable testosterone and bioavailable estradiol, with repeated measures of hormones and cognition over a significant period of time.

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