THE ASSOCIATION BETWEEN STEROID HORMONES AND COGNITIVE PERFORMANCE IN ADULTHOOD

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ABSTRACT - ENGLISH

The aim of this thesis was to investigate whether endogenous estrogen and testosterone were associated with cognitive performance in adulthood and whether hand preference affected the pattern of sex differences in cognitive performance. The analyses in Studies I-IV were based on data from the Betula project in Umeå, Sweden; a population-based longitudinal study on aging and memory. At the second follow-up, there were 3011 participants between 35 and 90 years of age, some entering the project for the first time and others visiting the second or third time.

In Study I, the association between endogenous testosterone and cognitive performance was investigated in men and women. We used (a) a reliable hormone assay, (b) a wide range of cognitive measures to discern regular trends in the data, and (c) participants of a wide age range to explore the hypothesized age-testosterone interaction. The results showed a positive association between testosterone and performance on episodic and spatial tasks for men that increased with age. In contrast, there was a negative trend between testosterone and cognitive performance in women. The results show that testosterone has a sex specific effect on cognition, with opposite directional effects observed for men and women.

The aim of Study II was to investigate whether there were sex differences in cognitive performance in non-right-handed individuals. Given that the bulk of individuals are right-handed, reports of sex differences are based on the majorities’ cognitive profile. Earlier studies have found an interaction between sex and hand preference in cognitive performance. Results from Study II revealed that there were sex differences in episodic memory, verbal fluency, and spatial ability among right-handed, but not among non-right-handed individuals. Non-right-handed men tended to perform better on verbal tasks and lower on the spatial task in contrast to right-handed men. Furthermore, non-right-handed women showed the reverse pattern, with lower verbal and higher spatial performance as compared to right-handed women. Tentatively, these data suggest atypical lateralization pattern for right-handed and non-right-handed individuals.

In Studies III and IV, the aim was to investigate whether the diminishing levels of estrogen in menopause were associated with cognitive decline. When cross-sectional data was investigated in Study III, no association between menopause phase and cognitive performance was found. In Study IV, longitudinal data was explored to investigate whether cognitive performance changed systematically for women passing through menopause, independent of age-related change in cognition. Results indicate that post-menopausal women show accelerated decline, or less gain, on tasks that measure spatial ability, verbal fluency, and episodic memory. The association between estrogen and accelerated rate of change was most pronounced in normal weight women. This association may reflect the fact that estrogen is largely produced in fat tissue post menopause, with women with higher body mass index (BMI) values having higher levels of estrogen.

This thesis shows that (a) there is a positive association between testosterone and cognitive performance in men, a relationship that increases with increasing age, (b) there are no sex differences in cognition in groups of non-right-handed individuals, (c) cognitive performance does not differ between groups of pre-, peri-, and postmenopausal women, but (d) following menopause, women reveal a higher rate of change for some cognitive measures independent of age-related cognitive change, an effect that is chiefly observed for women with normal BMI.
Syftet med denna avhandling var att undersöka huruvida endogent östrogen och testosteron är kopplade till kognitiv prestation i vuxen ålder och om handpreferens har en effekt på mönstret i kognitiv könsskillnader. Analyserna i Studie I-IV är baserade på data från Betulaprojektet i Umeå, Sverige; en populationsbaserad longitudinell studie rörande åldrande och minne. Vid den andra uppföljningen inbegrep studien 3011 individer mellan 35 och 90 år, några deltog för första gången och andra hade varit med vid tidigare teststillfällen.

I Studie I, undersökte associationen mellan endogent testosteron och kognitiv prestation hos män och kvinnor. I Studie I användes (a) en pålitlig hormoneanalys, (b) ett brett urval av kognitiva mått för att kunna skönja regelbundna trender i data, och (c) ett brett åldersspann så att eventuell interaktion mellan ålder och testosteron kunde undersökas. Resultaten visade ett positivt samband som tillhör med ålder mellan testosteron och prestation i episodiskt minne och spatial förmåga hos män. Hos kvinnor, däremot, fanns en negativ trend mellan testosteron och kognitiv prestation. Resultaten indikerar att testosteron har en könsspecifik effekt på kognition, med motsatt riktning för män och kvinnor.


I Studie III och IV var syftet att undersöka huruvida minskningen i östrogen i klimakteriet är associerad med kognitiv nedgång. När tvärsnittsdata undersöktes i Studie III kunde inget samband mellan klimakterifas och kognitiv prestation skönjas. I Studie IV undersökte vi longitudinalt huruvida kognitiv prestation förändras hos kvinnor som genomgår klimakteriet med hänsyn tagen till den åldersrelaterade förändringen i kognition. Resultaten visade att kvinnor efter klimakteriet försämras i högre takt, eller visar mindre förbättring, i uppgifter som mäter spatial förmåga, verbalt flöde och episodiskt minne. Sambandet mellan östrogen och snabbare förändringstakt finns framför allt hos normalviktiga kvinnor. Associationen speglar möjligen faktumet att östrogen huvudsakligen produceras i fettvävnad efter klimakteriet, där kvinnor med högre body mass index (BMI) också har högre nivåer av östrogen.

Avhandling visar att (a) det finns ett positivt samband mellan testosteron och kognitiv prestation hos män, ett samband som ökar med tilltagande ålder, (b) könsskillnader i kognition inte kan påvisas för icke-högerhänta individer, (c) den kognitiva prestationen inte skiljer sig åt mellan grupper av kvinnor före, i och efter klimakteriet, men (d) att kvinnor efter klimakteriet visar en ökad nedgång i vissa kognitiva uppgifter utöver åldersrelaterade kognitiva förändringar, en effekt som framför allt kan ses hos kvinnor med normalt BMI.
LIST OF PUBLICATIONS


IV Thilers P.P., MacDonald S.W., Nilsson L-G., Herlitz A. Accelerated postmenopausal cognitive decline is restricted to women with normal BMI: Longitudinal evidence from the Betula Project. Submitted
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>APOE4</td>
<td>Apolipoprotein E e4</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CAH</td>
<td>Congenital adrenal hyperplasia</td>
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<td>ER</td>
<td>Estrogen receptor</td>
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<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
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<td>FTI</td>
<td>Free testosterone index</td>
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<tr>
<td>HT</td>
<td>Hormone therapy</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>RH/LH</td>
<td>Right-handed/Left-handed</td>
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<td>Sry gene</td>
<td>Sex-determining region gene</td>
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<td>SHBG</td>
<td>Sex-hormone binding globulin</td>
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<tr>
<td>T1/T2/T3</td>
<td>Test wave 1/2/3</td>
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<tr>
<td>WAIS-R</td>
<td>Wechsler Adult Intelligence Scale-Revised</td>
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1 INTRODUCTION

In this thesis, the relationship between steroid hormones and cognition in adulthood and aging is investigated. As life expectancy increases, factors that may promote successful aging are highly relevant to most individuals. Both estrogen and testosterone have been suggested as factors associated with cognitive performance in aging. With changes in the hormonal environment as well as in cognitive performance across the adult life span, some suggest that there is more than a temporal relationship between these factors. Men show decreases in levels of free testosterone of around 50% between 30 and 80 years of age (Lamberts et al., 1997) while 10% of men over 50 years and 20% of men over 60 years have hypogonadal levels of serum testosterone (Harman et al., 2001). Moreover, with the prolonged life expectancy of today’s society, women live one third of their lives in near estrogen deprivation.

Gonadal hormones exert numerous effects on the nervous system that extend beyond their more obvious actions on the reproductive system. Additionally, both estrogen and testosterone differ quantitatively as well as qualitatively between the sexes, further suggesting that the organizational effects of gonadal hormones influence early sexual differentiation during pre- and postnatal development. However, when considering non-reproductive actions of circulating testosterone and/or estrogen, we need to consider brain structures other than the hypothalamus, such as the basal forebrain and hippocampus (McEwen and Alves, 1999).

There are behavioral sex differences in animals due to both estrogen and testosterone action in the hippocampus, where these influences seem to be sex specific. Gonadal hormones act on the dopaminergic, serotonergic, cholinergic, and noradrenergic transmitter systems that may contribute to differences between the sexes in affective states (Parker and Brotchie, 2004), movement disorders (Saunders-Pullman, 2003), aggression (Christiansen, 2001), and specific cognitive functions (Hampson, 1990; Sherwin, 1994). As we age, decreases are observed for hormonal levels as well as in cognitive performance (Genazzani et al., 2007; Tenover, 1997). Changes in estrogen in women and testosterone in men have been suggested to modulate cognitive decline in aging (Driscoll and Resnick, 2007; Simpkins and Singh, 2008).

The mechanisms for testosterone’s involvement in cognitive performance are less studied than those for estrogen. The effect of testosterone on behavioral performance in animals has, in contrast to estrogen, mostly focused on prenatal organizational effects, where studies on ovariectomized, testosterone injected, and early castrated animals are investigated in relation to spatial performance. However, there are also data supporting a neuroprotective role of testosterone where androgen treatment prevents excitotoxicity in hippocampal neurons, an area known to be involved in spatial abilities. Testosterone supplementation may also promote fiber sprouting after brain injuries (see Hogervorst et al, 2005 for review). By contrast, several possible mechanisms for estrogen’s effect on cognition have been proposed. Examples include modulation of acetylcholine (Gibbs et al., 1997), and promotion of nerve growth and reorganization of synapses (Gould et al., 1990). However, there is a large discrepancy between the body of research on humans that often indicates no or a small association between diminishing levels of estrogen in menopause and cognition (Lethaby et al., 2008; Luetters et al., 2007), and animal evidence indicating a neuroprotective effect of estrogen (McEwen, 2001).
Hormones and Cognition

In the following section, cognitive abilities will be described, as well as the influence of aging and sex on cognitive performance. Next, I will introduce key features of testosterone, handedness, and estrogen and their relationship to cognitive performance. In the second part of the thesis, I will briefly describe the Betula study followed by a summary of select findings. Further, I will summarize and discuss the main findings from Studies I-IV. The specific research aims for these studies were to: (a) investigate whether testosterone is associated with cognition in aging men and women and, if so, whether the pattern is similar for men and women; (b) explore differences in the magnitude of cognitive sex differences and hormone levels between right-handed (RH) and non-RH individuals; and (c) evaluate whether the diminishing levels of estrogen in and around menopause are associated with lower performance and/or an accelerated rate of cognitive decline.

1.1 COGNITION

The term cognition, from the Latin word cognoscere meaning “to know”, refers to how we encode, store, and retrieve various kinds of information. Cognition reflects our personal knowledge and skills, put to use when thinking, reasoning, solving problems, abstracting, memorizing, organizing, and planning different events.

1.1.1 Memory

Memory can be defined in many different ways but is most commonly divided into short-term/working memory and long-term memory. The working memory model was developed to address several shortcomings in theories of short-term memory (Baddeley and Hitch, 1974). Working memory is limited in capacity and deals with temporary storage of information, with information either briefly held at a conscious level (primary memory), or manipulated from directly accessible information (Baddeley, 1992). Information can be transferred to long-term memory as a result of encoding operations such as rehearsal and organization. Long-term memory encodes and stores information that is remembered for a significant period of time. Many theorists (e.g., Tulving, 1972) have separated long-term memory into two major categories, declarative and non-declarative memory, with further subdivisions within these categories (see Figure 1).

Given the focus of this thesis, I will review some, but not all, elements of declarative memory. Declarative, or explicit, memory concerns information that we are consciously aware of. Declarative memory is typically subdivided into its episodic and semantic forms. Episodic memory refers to our conscious, personal, and autobiographical recollection of specific events. For example, asking an individual to recall or recognize information encountered in an experiment is a common way of assessing episodic memory. Semantic memory, on the other hand, reflects our general knowledge, such as who the Swedish prime minister is, or how we know the difference between air and water. Semantic memory stores information without any reference to the temporal and spatial context present at the time of learning. Semantic memory is often referred to as our internal lexicon (Collins and Loftus, 1975), and is assumed to be organized in a hierarchical fashion including superordinate categories (vegetables), followed by more specific attributes (e.g. green), and then further down to lower-order categories (e.g. broccoli).
1.1.2 Verbal ability

Verbal ability is not a unitary construct, but is rather divided into several different subcategories including vocabulary, analogies, reading comprehension, and verbal fluency. The common factor for assessment of verbal abilities is that some aspect of language is needed to perform the task. Vocabulary involves semantic memory and is commonly assessed by finding synonyms to a specific word. In contrast, solving an analogy taxes reasoning ability and is assessed by asking the participant to solve a problem that maps on to the solution of a different problem. Verbal fluency, also called verbal production, is commonly assessed by requesting an individual to generate as many words as possible within a specific time frame (often 1 min). The words could start with a specific letter such as “F” (letter fluency), or belong to a certain taxonomic category such as “professions” (category fluency). Verbal fluency is considered to be partly a semantic memory task, as the fundamental component involves retrieving semantic information from long-term memory (Lezak, 1995). However, there is also evidence for an executive component in verbal fluency where frontal regions are activated during testing (Cabeza and Nyberg, 2000) and impaired performance can be observed after lesions in the frontal lobes (West, 1996). There are two fundamental aspects of language: a) comprehension and decoding of the input, and b) meaningful encoding and production of the output. Decoding refers to deriving meaning from the reference system being used (e.g., listening or reading), whereas encoding involves transforming thoughts into a form that can be expressed as linguistic output (e.g., writing or speech). Studies show that
people with high verbal abilities have the ability to rapidly decode the meaning of words and they also perform well on verbal working memory tasks (Daneman and Carpenter, 1980).

1.1.3 Visuospatial ability

Visuospatial ability is commonly defined as the ability to perceive the construction of an object in two or three dimensions, and can be divided into three sub-categories: spatial perception, spatial visualization, and mental rotations (Voyer et al., 1995). Spatial perception pertains to tests measuring the ability to determine a spatial relation regardless of distracting information. The Rod-and-Frame task is an example of a test that measures spatial perception. In this task, a line has to be positioned in the vertical or horizontal plane within a tilted frame (Witkin and Asch, 1948). Spatial visualization refers to the ability to manipulate complex spatial information when several steps are required to create the correct solution. For example, the Block design test from Wechsler Adult Intelligence Scale-Revised (WAIS-R; Wechsler, 1981) is frequently used to assess spatial visualization. In this test, participants are asked to reproduce a pattern using 12 three-dimensional blocks. Finally, mental rotations tax the ability to rapidly rotate a 2 or 3 dimensional figure in mind (Shepard and Metzler, 1971).

1.1.4 Aging and cognition

Age-related cognitive decline is observed for most cognitive functions, albeit to varying degrees. Performance on measures of episodic memory, semantic memory, and working memory have all been shown to decrease with age (Bäckman et al., 2001). However, in studies of otherwise healthy elderly persons, semantic memory is often found to remain relatively stable or even increase with age due to accumulated life experience/knowledge (Rönnlund et al., 2005). This pattern suggests that the organization and structure of the internal lexicon is relatively stable over the life span. Primary memory, as indexed for example by digit span forward from the WAIS-R, is another system that is little affected by the normal aging process (Wahlin et al., 1995). Thus, the ability to keep information in mind for a short period of time is well preserved in old age. By contrast, episodic memory and working memory exhibits pronounced impairment with increasing age, although great variability exists across individuals and tasks. Typically, the episodic memory deficit begins in middle to late adulthood, with a slow continuous progression (Bäckman et al., 2001). The same is true for working memory. The large decline in episodic memory with increasing age is often attributed to decline of other cognitive functions, such as working memory and perceptual speed (Hultsch et al., 1998).

Findings on verbal abilities have shown inconsistent results in the aging literature. Verbal fluency tasks typically reveal age-related performance deficits (Bowles and Salthouse, 2008; Henry and Phillips, 2006). By contrast, vocabulary measures often show age stability or even gains past age 60 (Hultsch et al., 1998; Schaie, 1996). These patterns may reflect the fact that the structure of the semantic network is largely unaffected in aging, although the ability to access lexical information rapidly declines (Bäckman et al., 2001).

Due to relatively recent techniques such as PET, MRI, and fMRI, it is now possible to investigate both structural and functional brain changes. For example, structural MRI studies suggest that cognitive deficits (e.g., memory) may reflect the loss of brain volume, particularly in the prefrontal cortex and hippocampus (Raz et al., 2004). Similarly, fMRI studies have shown that activation patterns during cognitive testing differ between younger
and older participants, where older participants tend to underrecruit select brain areas that facilitate successful performance used by younger subjects, and overrecruit areas not activated by the young (e.g. Cabeza, 2002). Whether such differential activation patterns for older adults reflect compensatory reorganization of function or lack of specificity of neural processing remains to be determined.

1.1.5 Sex differences

Sex differences in cognition are well established, with some abilities favoring men and others women. Sex differences are typically not observed for full-scale IQ tests, which are constructed to avoid differences between men and women (Herlitz and Yonker, 2002). However, sex differences are observed for specific IQ subtests. Broadly speaking, sex differences in cognitive performance favor women for measures of episodic memory (Herlitz et al., 1997; Lewin et al., 2001), and verbal fluency (Hyde and Linn, 1988). On the other hand, men commonly outperform women on a wide range of spatial abilities (Voyer et al., 1995). There are few studies of sex differences in semantic memory, and no reported systematic sex differences. Prior research suggests that the magnitude of cognitive sex differences is fairly stable over the life span (de Frias et al., 2006) and across cultures (Herlitz and Kabir, 2006).

1.1.5.1 Episodic Memory

Women tend to do better than men on episodic memory tests and this advantage is larger for recall ($d = 0.30$) than for recognition ($d = 0.12$; Herlitz et al., 1999). The difference between recall and recognition may be due to the fact that free recall requires higher verbal production skill; a task women typically excel on. Most episodic memory tasks showing a female superiority involve a verbal component, although sex differences are also present for non-verbal measures such as face recognition (Rehnman and Herlitz, 2007) and object location (Voyer et al., 2007). Some episodic memory tasks, requiring only visuospatial processing, have been found to favor men (Lewin et al., 2001), whereas episodic memory tasks without a verbal or spatial element yield either no differences or small differences favoring women (Herlitz et al., 1999). Performance differences in verbal episodic memory tasks are seen from the age of 5 years (Kramer et al., 1997) and persist to at least 80 years of age (de Frias et al., 2006). Figure 2 displays sex differences in episodic memory based on longitudinal data from the Betula study. Brain regions involved in episodic retrieval include prefrontal, medial-temporal, and posterior midline regions (Cabeza and Nyberg, 2000). Although only a few studies have been conducted investigating brain activation patterns using fMRI or PET during verbal episodic recall and recognition, it appears that the sexes activate similar regions during these tasks (Haut and Barch, 2006; Nyberg et al., 2000).

\[^1\] Cohen’s $d$ is a standardized measure of degree of disparity between two group means, and is calculated by subtracting one mean from the other and dividing the sum by the pooled standard deviation.
1.1.5.2 Verbal fluency
Women tend to do better than men on letter fluency ($d = 0.33$) although differences in category fluency are less well established (Kimura, 1999). As is true for episodic memory, men and women seem to activate similar brain regions during fluency tasks (Halari et al., 2006).

1.1.5.3 Visuospatial ability
There are prevailing sex differences in performance on visuospatial abilities in general. However, these differences vary in magnitude within spatial perception, spatial visualization, and mental rotation tasks. According to a meta analysis (Voyer et al., 1995), the effect sizes for spatial perception, spatial visualization, and mental rotations were 0.48, 0.23, and 0.66, respectively.
Sex differences in navigation (e.g., virtual maze learning) and geography (pointing out cities on a map) typically favor men, although, the performance differences become smaller or disappear if landmarks are present (Rizk-Jackson et al., 2006). Sex differences also exist when men and women give directions. Men tend to use Euclidean strategies (i.e., distance and directions) to a greater extent than women, and are also more accurate on these relational strategies compared to women. In contrast, women tend to use more topographic strategies (e.g., landmarks; Dabbs et al., 1998).

Figure 4. Sex differences in spatial ability (z-scores) across 3 different test occasions. Visuospatial ability is derived from the WAIS-R Block design task. The population-based sample comes from the Betula study and ranges in age from 35-80 years at T1, 35-85 years at T2 and 35-90 years at T3. For women, the mean T1 age = 55.2 years, T2 age = 61.2 years, and T3 age = 65.2 years. For men, the mean T1 age = 53.7 years, T2 age = 58.5 years, and T3 age = 62.9 years.

Sex differences in brain activation patterns have been observed during mental rotation tests. In addition to the right parietal activation in both sexes, women also tend to activate frontal regions to a greater extent, while men show an additional bilateral parietal activation (Butler et al., 2006; Gur et al., 2000; Thomsen et al., 2000; Weiss et al., 2003). When task difficulty is increased, women and men tend to show more circumscribed activation (Gur et al., 2000). The differences in activation patterns have been suggested to reflect a greater reliance on more effortful top-down processes by women, whereas men engage more in automatic bottom-up processing (Butler et al., 2006).

1.2 TESTOSTERONE

There are two major types of steroid hormones: androgens and estrogens. Women and men have both kinds of hormones even though testosterone, a type of androgen, is predominant in men and estrogen is predominant in women. Hormones and neurotransmitter systems are in many ways similar and highly dependent on each other, though there are some fundamental differences between these systems. First, hormones are released from the production site and travel in the bloodstream. In contrast, neurotransmitters travel in the axons of neurons. Second, steroid hormones are lipid soluble and therefore able to cross the cell membrane to act on specific receptor proteins, which in turn bind to DNA. The binding to DNA results in an increase, or decrease, in synthesis of specific proteins that can initiate a wide range of effects. Steroid receptors are sometimes referred to as transcription factors, meaning that they either increase or decrease the transcription of specific genes. These receptors are
predominantly found in the limbic and hypothalamic areas, but also in the neocortex and areas that regulate arousal and attention such as amygdala and the prefrontal cortex (de Castilhos et al., 2008; McEwen, 2005). In addition to neurochemical modulations, steroid hormones can also bring about structural changes in the brain such as increase the density of dendritic spines in the CA1 and CA3 areas in the hippocampus (Isgor and Sengelaub, 2003; Woolley and McEwen, 1994). In the subsequent section, research on testosterone of relevance to this thesis will be discussed.

Testosterone levels in men fluctuate during the day and over the year, being highest in the morning and in the autumn. Only about 2% of the testosterone in the body is free while the rest is bound with high affinity to sex hormone binding globulin (SHBG; 54%) and with low affinity to albumin (44%; Sodergard et al., 1982). After synthesis and release, testosterone is inactivated in the liver and excreted into the urine.

![Figure 5](image-url)

**Figure 5.** Mean levels and standard error bars of total testosterone (TT) and free testosterone (FT) in men and women over the life span. Data come from T3 in the Betula study.

Studies on aging indicate that total testosterone decreases relatively little over the life span in men (0.5-1.6 % per year from 40 years), whereas SHBG levels are increasing (about 1.3 % per year over the age of 40 years) resulting in lower levels of free testosterone with increased age (2-3 % per year; Allan and McLachlan, 2004; Feldman et al., 2002). The normal range of testosterone for adult men is between 10.4-34.7 nmol/l (Gruenewald and Matsumoto, 2003) and ranges from 1.04 – 2.43 nmol/l for women (Greenspan and Strewler, 1997). Due to women’s low levels of testosterone and little variability (see Figure 5), it is difficult to investigate the relationship between testosterone levels and behavior.

### 1.2.1 Organizational effects

Organizational effects of testosterone refer to the irreversible influence on brain organization that occurs predominantly before birth. Males and females begin the gestation period with identical gonads, but due to action of the Sry (sex-determining region Y) gene...
on the Y chromosome in the 6th week of gestation, primordial gonads develop into testis that, in the absence of the Sry gene would become ovaries. Both estrogen and testosterone become present in the prenatal environment of males and females, but in weeks 8 to 24, testosterone levels are significantly higher in the male fetus (Hines, 2006). These early differences in testosterone exposure have given rise to a hypothesis suggesting that not only physical attributes but also behavioral sex differences could be affected by early hormonal environment. Many attempts have been made to link early hormone environment to cognitive performance, but results have been inconsistent. Earlier studies have investigated organizational effects through (a) experimental animal studies, (b) measuring testosterone from amniotic fluid in the gestation period in healthy subjects in order to compare to later cognitive performance, and (c) by investigating cognitive performance in girls with congenital adrenal hyperplasia (CAH) to controls.

1.2.1.1 Animals
The most convincing evidence of an organizational effect of early hormonal exposure comes from animal models. Male rats learn complex mazes faster and make fewer errors in the learning process than do female rats (Williams et al., 1990), although, like in humans, the difference diminishes when landmarks are provided (Williams and Meck, 1991). Further, castrated rats exhibit impaired spatial abilities, whereas prenatal injections with testosterone enhance their performance (Naghdi et al., 2003). In addition to better spatial navigation performance, high vs. low testosterone rats have shown larger hippocampal volumes, an area known to be involved in spatial abilities. High-testosterone rats also exhibit longer CA3 dendrites and more expanded branching compared to low testosterone rats (i.e. castrated males and untreated female rats; Isgor and Sengelaub, 2003).

1.2.1.2 Humans
In humans, the evidence of a relationship between testosterone and cognitive performance is less straightforward. Some researchers have investigated the link between prenatal testosterone levels and spatial performance in childhood. However, these studies are few and inconsistent, revealing negative associations for girls (4 and 6 years of age; Finegan et al., 1992; Jacklin et al., 1988; Kerns and Berenbaum, 1991), a positive relationship for girls and the reverse for boys at 6 years of age (Grimshaw et al., 1995), or no association in boys at the age of 4 (Finegan et al., 1992).

Another way to study potential organizational effects of prenatal testosterone is to investigate females with CAH. This disease results in abnormally high prenatal levels of testosterone in girls, but once born, these girls are most often treated immediately and hormone levels return to normal. CAH is a rare disease (1:11500: Thilén and Larsson, 1990) and participants are therefore not easily recruited. There have been a few studies on spatial abilities in girls with CAH in which siblings and close relatives have served as controls. The findings are consistent and show that girls with CAH perform at a slightly higher level than their unaffected siblings on mental rotation tasks (Hampson et al., 1998; Hines et al., 2003; Resnick et al., 1986). For example, in one study (Resnick et al., 1986), females and males with CAH were compared with their unaffected relatives across three different tasks measuring visuospatial abilities (hidden patterns, card rotation, and mental rotations). Females with CAH outperformed their unaffected relatives on all three tests. There was no effect of CAH versus controls in men. Another study included girls and boys with CAH and sibling controls (Hampson et al., 1998). Participants were tested on a spatial relations test, where the children had to discriminate a target shape from among four alternatives that form a square when combined with the target figure (Thurstone and Thurstone, 1963). Findings revealed that CAH girls outperformed their unaffected siblings, whereas boys with the condition performed worse than controls. Yet another group reported
findings on targeting tasks (i.e. dart and ball throwing; Hines et al., 2003), with up to middle-aged females and males with CAH, and unaffected female relatives (mostly siblings). Results showed that unaffected males performed at higher levels than all other groups on various spatial abilities. Affected females, on the other hand, outperformed their sibling controls. However, some authors have suggested that testosterone only affect specific aspects of spatial abilities (Helleday et al., 1994; Hines et al., 2003). In summary, empirical evidence from studies on girls with CAH supports the claim that there is an organizational effect of prenatal testosterone on spatial performance in later life.

1.2.2 Activational effects

Activational effects refer to the effects when the hormone in question is present and altered in amount. Activational effects of testosterone in men have primarily been investigated with regard to cognitive performance after testosterone supplementation in hypogonadal, eugonadal, or more recently men with AD. One group has also conducted studies on transsexual individuals that receive hormone replacement in the form of estrogen or testosterone.

1.2.2.1 Clinical trials

Men with hypogonadalism suffer from low levels of testosterone (total testosterone < 300 ng/dl or free testosterone < 60 ng/dl), and often experience symptoms such as health problems, lower quality of life, decreased sex drive, reduced muscle mass and strength, and increased body fat (Miner et al., 2008). Hypogonadalism can also be accompanied by lower cognitive abilities (Beauchet, 2006). One clinical trial on testosterone supplementation in older men with hypogonadalism demonstrated improved performance on a spatial task after raising the testosterone levels to the normal range. Several randomized controlled studies have been conducted on healthy men. Results have shown improvements in spatial ability, verbal memory (Cherrier et al., 2001; Cherrier et al., 2003; Janowsky et al., 1994), and working memory (Janowsky et al., 2000) after testosterone administration. There are also findings reporting negative (Maki et al., 2007) or no effects of testosterone supplementation (Alexander et al., 1998; Emmelot-Vonk et al., 2008). However, it should be noted that in these later studies testosterone levels increased only marginally after treatment.

Other researchers have investigated different doses of testosterone supplementation (i.e. intra muscular) and found that a moderate (100 mg) dose of testosterone, rather than a low (50 mg) or high (300 mg) dose, resulted in increased performance on verbal and spatial memory (Cherrier et al., 2007). Particularly interesting findings indicate that men given testosterone in addition to an aromatase antagonist selectively show enhanced spatial performance, but not verbal memory performance (Cherrier et al., 2005a). In contrast, the group given only testosterone, which converts to estrogen in the brain through aromatization, shows increased performance on spatial ability as well as on verbal memory. Summarizing the clinical evidence, there are strong indications of an effect of testosterone on cognitive performance.

Studies conducted on transsexuals have yielded conflicting results. One study showed that female-to-male transsexuals improved on spatial abilities and performed worse on verbal production following testosterone treatment (Van Goozen et al., 1994). In a later study by the same group, these findings were replicated for spatial performance, but not for verbal production (Slabbekoorn et al., 1999). In yet another follow-up by this group, findings showed that right-handed (RH) homosexual transsexuals did not alter their cognitive performance following hormone treatment. In the two first studies, right- and left-handed
(LH) individuals were intermixed. The authors suggested that RH homosexual individuals may be less dependent on activational effects and more dependent on organizational effects of testosterone (van Goozen et al., 2002). This lack of consistency in results suggests that there are large individual differences in response to testosterone treatment within this group.

1.2.3 Endogenous testosterone and cognition

Numerous studies have been conducted on endogenous testosterone and cognition in older men (Barrett-Connor et al., 1999; Fonda et al., 2005; Hogervorst et al., 2004; Martin et al., 2007; Martin et al., 2008; Moffat et al., 2002; Muller et al., 2005; Wolf and Kirschbaum, 2002; Yaffe et al., 2002; Yonker et al., 2006). The results are inconsistent, as illustrated in Table 1. Discrepancies in results may reflect several factors, such as methodological differences between studies, and lack of solid longitudinal studies. In the following section, I will discuss methodological inconsistencies, review testosterone research in relation to global spatial abilities, verbal fluency, episodic memory, and semantic memory.

1.2.3.1 Methodological issues

Among potential methodological differences between studies (see Table 1), the hormone-sampling procedure employed represents a key factor underlying contradictory findings. First, some prior studies have measured total testosterone (Drake et al., 2000; Gordon and Lee, 1986; Hassler et al., 1992; Wolf and Kirschbaum, 2002) instead of bio-available testosterone (i.e., the non-specifically bound part of testosterone in the body; Barrett-Connor et al., 1999; Christiansen and Knussmann, 1987; Gouchie and Kimura, 1991; Hogervorst et al., 2004; Hooven et al., 2004; Moffat and Hampson, 1996a; Moffat et al., 2002; Postma et al., 1999; Silverman et al., 1999; Yaffe et al., 2002; Yonker et al., 2006). Bio-available testosterone, or free testosterone, provides a more accurate measure of the biologically active testosterone (Vermeulen et al., 1999). Studies that explore both free and total testosterone show no association between total testosterone and cognitive performance, yet a positive relationship between free testosterone and cognition (Hogervorst et al., 2004; Yaffe et al., 2002). Further, sometimes a free testosterone index (FTI) is used, which is calculated from the total testosterone value (Moffat et al., 2002). This index is highly correlated with the true value of free testosterone in normal samples with young participants. However, findings indicate that FTI does not give an accurate estimate of free testosterone, particularly not in elderly individuals (Vermeulen et al., 1999).

A second methodological issue relates to the use of saliva samples (Christiansen and Knussmann, 1987; Gouchie and Kimura, 1991; Hooven et al., 2004; Moffat and Hampson, 1996a; Silverman et al., 1999) instead of serum (Barrett-Connor et al., 1999; Christiansen and Knussmann, 1987; Fonda et al., 2005; Gordon and Lee, 1986; Hassler et al., 1992; Hogervorst et al., 2004; Martin et al., 2007; Martin et al., 2008; Moffat et al., 2002; Muller et al., 2005; Yaffe et al., 2002; Yonker et al., 2006) for analysis of free testosterone. Earlier findings indicate that this is problematic as discrepancies between plasma testosterone and saliva testosterone have been demonstrated (Selby et al., 1988). According to these findings, serum testosterone tends to give more reliable results (Rey et al., 1990), whereas free testosterone calculated from saliva is overestimated in women and underestimated in men (Rey et al., 1988).

Third, the direct radioimmunoassay kit (RIA) is a common, quick and convenient way of analyzing hormone levels. However, certain kits, particularly tests from Diagnostic Products Corporation (DPC) and Diagnostic Testing Laboratory (DSL) seem to yield unreliable
findings. These assays tend to underestimate levels of free testosterone in a nonlinear fashion (Gruschke and Kuhl, 2001; Vermeulen et al., 1999). In fact, results from cross-sectional studies on older men using these assays have shown reversed results (i.e., a negative association between testosterone and cognition in men; Martin et al., 2007; Yonker et al., 2006). More sensitive measures than direct RIA are crucial when estimating low plasma concentrations as seen in older men, women, and children (Miller et al., 2004; Padero et al., 2002).

Additional support for a positive association between testosterone and cognition come from studies showing that relatively low levels of testosterone are predictive of AD and found up to 10 years before diagnosis (Moffat et al., 2004). Several studies on men with AD have shown beneficial effects of testosterone supplementation on spatial abilities (Cherrier et al., 2005b; Lu et al., 2006; Tan and Pu, 2003). Cross sectional evidence indicate that healthy men with the genetic predisposition for AD, in the form of APOE ε4, have lower testosterone levels (Hogervorst et al., 2002). Further, these men have increased gonadotropin levels, which is related to an increase of plaques in the brain (Bowen et al., 2000). Findings have indicated an interaction between testosterone levels and APOE status in healthy participants where older men who are non-carriers of APOE ε4 and have high testosterone levels perform at a higher level on several cognitive tasks such as executive functions and attention. In contrast, men who are carriers of APOE ε4 show the opposite pattern where high testosterone men perform worse than low testosterone men (Burkhardt et al., 2006).

Summarizing Table 1, there are serious concerns with the selected hormone assays in the majority of studies, and sometimes insufficient information to determine the method used.
Table 1. Summary of methods and results in earlier studies on testosterone and cognition

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants*</th>
<th>Results</th>
<th>Hormone Analysis</th>
<th>Comment on hormone assay</th>
<th>General comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Younger adults</strong></td>
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</tr>
<tr>
<td></td>
<td>Men: Age = 24.1</td>
<td>VF: Non-significant</td>
<td></td>
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<tr>
<td></td>
<td>Age range = 20-30</td>
<td>VS: Positive on 4/6 tests.</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>VT: Negative on 2/6</td>
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<tr>
<td>Gordon et al. (1986)</td>
<td>Men: N = 32</td>
<td>Men</td>
<td>Serum sample. Analyzed by specific antibody method. No information on sensitivity or assay variability.</td>
<td>Total testosterone does not represent the active fraction of testosterone unbound from SHBG and albumin. However, other hormones were covaried, possibly making this less of an issue.</td>
<td>All subjects RH. Age and education not controlled for. The significant positive finding was seen in mental rotations.</td>
</tr>
<tr>
<td></td>
<td>Men: Age range = 18-35</td>
<td>VF: Non-significant</td>
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<tr>
<td></td>
<td></td>
<td>VS: Positive on 1/5 tests.</td>
<td></td>
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</tr>
<tr>
<td>Gouchie et al. (1991)</td>
<td>Men: N = 42</td>
<td>Men, VS: Non-significant</td>
<td>Saliva sample. In men, intra-assays correlated, r = .69 - .82 and in women, r = .64 - .73.</td>
<td>The RIA kit (DPC) considered unreliable according to earlier research (Rey et al., 1990; Rosner et al., 2007; Vermeulen et al., 1999) Free testosterone in saliva fluctuates to a much higher degree than serum samples (Rey et al., 1990).</td>
<td>Age and education not controlled for. High testosterone and low testosterone groups compared</td>
</tr>
<tr>
<td></td>
<td>Age range = 21.0</td>
<td>on 2/2 tests</td>
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<td></td>
<td>Age range = 18-27</td>
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<tr>
<td></td>
<td>Women: N = 46</td>
<td>Women, VS: Non-significant</td>
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<tr>
<td></td>
<td>Women: N = 46</td>
<td>on 2/2 tests</td>
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</tr>
<tr>
<td></td>
<td>Age range = 18-31</td>
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</tbody>
</table>
### Hormones and Cognition

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants*</th>
<th>Results</th>
<th>Hormone Analysis</th>
<th>Comment on hormone assay</th>
<th>General comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hassler et al. (1992)</td>
<td>Men: N = 26</td>
<td>Men: VS: Non-significant</td>
<td>Serum sample. Analyzed by Immucor Testosterone (125I) Assay. No information on sensitivity or assay variability.</td>
<td>Lacking information about the assay. Total testosterone does not represent the active fraction of testosterone unbound from SHBG and albumin.</td>
<td>Half of the subjects were right-handed and half were left-handed. Age in months correlated positively with testosterone levels; age was not covaried.</td>
</tr>
<tr>
<td></td>
<td>Women: N = 25</td>
<td>Women: VS: Non-significant</td>
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</tr>
<tr>
<td></td>
<td>M age = 18.8</td>
<td></td>
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<tr>
<td>Hooven et al. (2004)</td>
<td>Men: N = 27</td>
<td>Men: Positive</td>
<td>Saliva sample. Analyzed by a tritium-based RIA. Intra assay reliability was $r = 0.75 - 0.86$</td>
<td>Free testosterone in saliva fluctuates to a much higher degree than serum samples (Rey et al., 1990).</td>
<td>Within subject data. The spatial task was mental rotation.</td>
</tr>
<tr>
<td></td>
<td>M age = 23.0</td>
<td></td>
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<tr>
<td></td>
<td>(SD = 4.0)</td>
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<tr>
<td></td>
<td>Age range = 18-33</td>
<td></td>
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</tr>
<tr>
<td>Moffat et al. (1996)</td>
<td>Men: N = 40</td>
<td>Men: Negative</td>
<td>Saliva sample. An RIA, Coat-a-Count kit was modified to analyze saliva. Cross reactivity with DHT &gt; 5%. Intra-assay variation was 9.8% and sensitivity was 29.8 pmol/l in males and 14.9 pmol/l in women.</td>
<td>The RIA kit (DPC) considered unreliable according to earlier research (Rosner et al., 2007; Vermeulen et al., 1999). Free testosterone in saliva fluctuates to a much higher degree than serum samples (Rey et al., 1990).</td>
<td>Nineteen right-handed and 21 left-handed females and males. It was unclear if the effect was due to handedness and brain lateralization or testosterone levels.</td>
</tr>
<tr>
<td></td>
<td>M age = 21.8</td>
<td></td>
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<tr>
<td></td>
<td>(SD = 3.4)</td>
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<tr>
<td></td>
<td>Women: N = 40</td>
<td>Women: Positive</td>
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</tr>
<tr>
<td></td>
<td>M age = 23.0</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>(SD = 4.1)</td>
<td></td>
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</tr>
<tr>
<td>Silverman et al. (1999)</td>
<td>Men: N = 59</td>
<td>Men: Positive</td>
<td>Saliva sample. Analyzed at Salivary Radioimmunoassay Laboratory, University of Western Ontario. Inter assay variation was 9 - 18%.</td>
<td>Free testosterone in saliva fluctuates to a much higher degree than serum samples (Rey et al., 1990).</td>
<td>Higher testosterone is associated with better performance between subjects, but no effect was found within subject when investigating changes in testosterone levels. Age and education not covaried</td>
</tr>
<tr>
<td></td>
<td>M age = 22.4</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>(SD = 3.0)</td>
<td></td>
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</tbody>
</table>
### Study Participants* Results Hormone Analysis Comment on hormone assay General comment

#### Older adults

**Barrett-Connor et al. (1999)**

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants*</th>
<th>Results</th>
<th>Hormone Analysis</th>
<th>Comment on hormone assay</th>
<th>General comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td><strong>N = 547</strong></td>
<td>Serum sample. Analyzed by RIA. Testosterone unbound from SHBG. Sensitivity = 37 pg/ml, and the intra- and interassay variability of 3.7% and 5.2% respectively.</td>
<td><strong>VF (categories): Non-significant</strong></td>
<td>Blood samples and cognitive measures were taken several years apart.</td>
<td>Controlled for age and education.</td>
</tr>
<tr>
<td><strong>Age range = 59-89</strong></td>
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</tbody>
</table>

**Drake et al. (2000)**

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants*</th>
<th>Results</th>
<th>Hormone Analysis</th>
<th>Comment on hormone assay</th>
<th>General comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women</strong></td>
<td><strong>N = 39</strong></td>
<td>Serum sample. Analyzed by RIA. Sensitivity = 17.4 pmol/l. Intra- and interassay variability of 5 - 10% and 10 - 15% respectively.</td>
<td><strong>VS: Non-significant</strong></td>
<td>Blood samples and cognitive measures were taken 0 to 11 month apart. Total testosterone does not represent the active fraction of testosterone unbound from SHBG and albumin. Particularly problematic in an older sample.</td>
<td>Controlled for age and education.</td>
</tr>
<tr>
<td><strong>Age range = 65-90</strong></td>
<td></td>
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</tr>
</tbody>
</table>

**Fonda et al. (2005)**

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants*</th>
<th>Results</th>
<th>Hormone Analysis</th>
<th>Comment on hormone assay</th>
<th>General comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td><strong>N = 981</strong></td>
<td>Serum sample. Analyzed by a RIA kit and centrifugal ultrafiltration. Intra- and interassay variability is 5.1% and 8.9% respectively.</td>
<td><strong>VS: Non-significant</strong></td>
<td>The RIA kit (DPC) considered unreliable according to earlier research (Rosner et al., 2007; Vermeulen et al., 1999).</td>
<td>Population-based cohort study. Controlled for age. Authors used log transformations to correct for skewness.</td>
</tr>
<tr>
<td><strong>Age range = 48-80</strong></td>
<td></td>
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</tbody>
</table>

**Hogervorst et al. (2004)**

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants*</th>
<th>Results</th>
<th>Hormone Analysis</th>
<th>Comment on hormone assay</th>
<th>General comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td><strong>N = 79</strong></td>
<td>Serum sample. Analyzed by a competitive enzyme immunoassay. Sensitivity was 0.17 nmol/l with an intra- and interassay variability of 1 - 8% and 2 - 5%.</td>
<td><strong>VS: Positive</strong></td>
<td>Total testosterone does not represent the active fraction of testosterone unbound from SHBG and albumin. However authors adjust for SHBG levels, possibly making this less of an issue</td>
<td>Significant results were obtained when covarying SHBG. Age was covaried.</td>
</tr>
<tr>
<td><strong>Age range = 61-91</strong></td>
<td></td>
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<tr>
<td><strong>Women</strong></td>
<td><strong>N = 66</strong></td>
<td>Serum sample. Analyzed by competitive enzyme immunoassay. Sensitivity was 0.17 nmol/l with an intra- and interassay variability of 1 - 8% and 2 - 5%.</td>
<td><strong>VS: Non-significant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age range = 61-91</strong></td>
<td></td>
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</table>
# Hormones and Cognition

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants*</th>
<th>Results</th>
<th>Hormone Analysis</th>
<th>Comment on hormone assay</th>
<th>General comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Martin et al (2007)</td>
<td>Men: N = 1046</td>
<td>Men EM: Negative interaction with age</td>
<td>Serum sample. Analyzed by a RIA kit and chemiluminescent immunoassay IMMULITE 2000. Calculated FT (Ly and Handelsman, 2005) Sensitivity: 10.7 nmol/l. Intra assay variability = 9.3%.</td>
<td>The RIA kit (DPC) considered unreliable according to earlier research (Rosner et al., 2007; Vermeulen et al., 1999)</td>
<td>Population sample may be atypical; 8.41 % had anxiety disorder, average BMI was 28.9, 9.18 % reported insomnia and the mean alcohol intake per day was 19.9 g. Adjusted for multiple covariates.</td>
</tr>
<tr>
<td>Moffat et al. (2002)</td>
<td>Men N = 407</td>
<td>Men VS: Positive</td>
<td>Serum sample. Analyzed by RIA kit. Sensitivity: 0.42 nmol/l. Intra- and inter assay variability = 3.3 - 4.8% and 5.7 - 6.4%, respectively. FTI was used.</td>
<td>The RIA kit (DSL) used has been criticized for not being linear compared to an equilibrium dialysis. (Rosner, 2001)</td>
<td>Longitudinal data. Age and education were covaried. FTI is suitable in normal samples with no abnormal values. Low testosterone men were hypogonadal.</td>
</tr>
<tr>
<td>Muller et al. (2005)</td>
<td>Men N = 400</td>
<td>Men VF: Non-significant</td>
<td>Serum sample. Analyzed with RIA (Dr Pratt AZG 3290) Limit of detection was 0.24 nmol/l. Inter assay variation was 5.4% - 8.6%. Free testosterone was calculated without including albumin binding.</td>
<td></td>
<td>Results indicate that testosterone was associated with cognition in old age.</td>
</tr>
<tr>
<td>Study</td>
<td>Participants*</td>
<td>Results</td>
<td>Hormone Analysis</td>
<td>Comment on hormone assay</td>
<td>General comment</td>
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</tr>
</tbody>
</table>
| *Wolf et al.* (2002) | **Men:** N = 30  
Age = 69.0 (SD = 1.3)  | Men: Negative  
VS: Non-significant  
EM: Non-significant  | Serum sample. Analyzed by a commercial RIA with an intra- and inter-assay variability < 10%.  | Total testosterone does not represent the active fraction of testosterone unbound from SHBG and albumin, particularly in an older sample.  | The task used to measure visuospatial ability does not yield sex differences (rotation of letters). Covaried age and years of education.  |
|               | **Women:** N = 38  
Age = 68.0 (SD = 1.0)  | Women: Non-significant  
VS: Non-significant  
EM: Positive  |                                                                                               |                                                                                           |                                                                                  |
| *Taffe et al.* (2002) | **Men:** N = 310  
Age = 73.0 (SD = 6.8)  | Men: GC: positive  | Serum sample. Analyzed by RIA. Intra- and interassay were 4.9% and 6.6%. Bioavailable testosterone was determined by separation of SHBG, albumin and free steroid (Mayes and Nugent, 1968).  |                                                                                           | Part of a cohort study. MMSE, Trails B and Digit Symbol were the cognitive tests used. Age and education were covaried.  |
| *Tonker et al.* (2006) | **Men:** N = 450  
Age = high T: 54.3 (SD = 12.7), low T: 54.4 (SD = 12.8)  
Age range = 35-80  | Men: VF: Non-significant  
VS: Negative  
EM: Non-significant  
SM: Non-significant  | Serum sample. Free testosterone was analyzed with an RIA kit. Intra- and interassay was < 10%.  | The RIA kit (DPC) considered unreliable according to earlier research (Rosner et al., 2007; Vermeulen et al., 1999)  | Analyzed as high/low testosterone groups. Age and education were covaried.  |

**Note:** GC = Global cognition, VF = Verbal fluency, VS = Visuospatial ability, EM = Episodic Memory, and SM = Semantic Memory. FTI = free testosterone index. * = Number of participants, age mean and standard deviations, and age range are reported when data is available.
1.2.3.2 Cognitive performance

Studies investigating endogenous testosterone levels and general cognition in healthy aging men show positive (Barrett-Connor et al., 1999; Moffat et al., 2002; Muller et al., 2005; Yaffe et al., 2002) or no association (Fonda et al., 2005; Wolf and Kirschbaum, 2002; Yonker et al., 2006). Although inconsistencies in results clearly exist, high testosterone in older men is predominantly associated with better cognitive ability (see Table 1).

The strongest evidence of an association between testosterone and cognition is found for spatial abilities, particularly mental rotations, which also yield the largest male advantage among spatial measures. This is in line with the suggestion that prenatal testosterone not only determines physiological attributes, but is also involved in certain cognitive sex differences. If the different gestation environments predetermine sex differences in cognition, then we might expect to see larger effects of hormones in cognitive tasks showing larger sex differences.

As alluded to, evidence of an association between testosterone and spatial performance stems largely from animal research (Naghdi et al., 2003; Williams and Meck, 1991), but is also supported in clinical trials on humans (Cherrier et al., 2001; Cherrier et al., 2007; Hogervorst et al., 2005). However, the results from investigations focusing on spatial ability and endogenous testosterone are inconsistent. As can be seen in Table 1, studies investigating the relationship between testosterone and spatial ability in older men have reported positive (Hogervorst et al., 2004; Moffat et al., 2002), negative (Yonker et al., 2006), and no associations (Fonda et al., 2005; Martin et al., 2008; Wolf and Kirschbaum, 2002). In young individuals, the results also show positive (Christiansen and Knussmann, 1987; Gordon and Lee, 1986; Hooven et al., 2004; Silverman et al., 1999), negative (Moffat and Hampson, 1996a), and no associations (Christiansen and Knussmann, 1987; Gordon and Lee, 1986; Gouchie and Kimura, 1991; Hassler et al., 1992). Further, in older men, there is a lack of association between testosterone and verbal fluency (Barrett-Connor et al., 1999; Moffat et al., 2002; Muller et al., 2005; Wolf and Kirschbaum, 2002; Yonker et al., 2006), episodic memory (Hogervorst et al., 2004; Martin et al., 2007; Moffat et al., 2002; Wolf and Kirschbaum, 2002; Yonker et al., 2006) and semantic memory (Hogervorst et al., 2004; Martin et al., 2007; Yonker et al., 2006). As noted, these discrepancies may reflect differences across studies in hormone assays, sample selection, cognitive task, and age ranges, making it hard to draw firm conclusions about the association between testosterone levels and cognitive abilities. Nonetheless, there is physiological evidence strongly suggesting a neuroprotective role of testosterone on the brain, and similarly, findings from clinical trials indicate an association between testosterone and cognitive performance. Therefore, well-conducted studies on the relationship between testosterone and cognitive performance are warranted.

1.3 HANDEDNESS

The prevalence of left handedness is 6-16 % in the general population (Hardyck and Petrinovich, 1977). This percentage range has been stable for the past 100 years (Corballis, 1989). More males than females are reported to be left-handed (LH). The difference is around 25%, although it varies depending on the way in which handedness
Introduction

is assessed (Papadatou-Pastou et al., 2008; Sommer et al., 2008). Ambidexterity refers to the ability to shift writing hand interchangeably (about 3:1000), and is much more unusual than left-handedness or mixed handedness (Annett, 1998). Preferably, handedness should be treated as a continuous variable rather than as a dichotomy, and therefore measures of grip strength or pegboards can be included to complement handedness questionnaires (Annett, 2002).

1.3.1 Theories of handedness

1.3.1.1 Testosterone

Several different theories exist on how prenatal testosterone levels might affect handedness and cerebral language dominance (Geschwind and Galaburda, 1985a, 1985b, 1985c; Witelson, 1991). One theory assumes that high levels of prenatal testosterone levels predispose individuals for left-handedness and reduce language dominance by promoting the development of certain regions in the right hemisphere and inhibiting the same regions in the left hemisphere (Geschwind and Galaburda, 1985a, 1985b, 1985c). However, based on recent reports on handedness-related differences in corpus callosum and a higher incidence of left handedness in homosexuals (McCormick et al., 1990), other theories suggest that low testosterone levels within each sex during gestation lead to a higher incidence of left-handedness (Witelson, 1991). In line with this assumption, a study examining amniotic fluids in week 16 of gestation found that girls with higher prenatal testosterone were more frequently RH and more language lateralized (Grimshaw et al., 1993). However, as only a small number of participants were LH in Grimshaw’s study, it was difficult to explore systematic differences between LH and RH persons. At present, there is not much empirical evidence for any of the theories on handedness and prenatal levels of testosterone, one reason being that such research is difficult to conduct for both practical and ethical reasons.

Other studies have aimed at investigating the association between adult testosterone levels and handedness. Results from this line of research indicate that RH males and, to a lesser degree, RH females have higher levels of circulating testosterone levels than their LH counterparts (Moffat and Hampson, 1996b; Tan, 1991).

1.3.1.2 Genetics

There have been several suggestions that observed sex differences in the prevalence of hand dominance may reflect genetic factors. The right-shift theory (Annett, 1985), the modifier-gene theory (McMagnus and Bryden, 1992), and the recessive model (Jones and Martin, 2000) are examples of such theories. These theories differ in their proposed mechanisms. Annett, for example, postulates a gene for cerebral dominance that increases the probability of right-handedness. The right-shift theory holds that a gene works by impairing the growth of the right hemisphere in early life, incidentally weakening the left hand, and focusing language and speech functions to the left side. A sex difference in handedness is not necessarily predicted by this theory. On the other hand, the modifier-gene theory proposes a sex-linked recessive modifier-gene on the X-chromosome that suppresses the gene responsible for right-handedness. They further suggest that because men only have one copy of the genes on the X-chromosome, they
are more likely to express left-handedness. Finally, Jones and Martin hypothesize that the gene for left-handedness is recessive and located on the X-chromosome. This is in line with findings showing that sons of LH mothers, but not fathers, have a higher incidence of left handedness (McKeever, 2000).

1.3.2 Cognitive sex differences and hand preference

Levy (1969, 1971) developed several theories directed at finding an explanation for sex differences in cognition. She stated that women’s more flexible language representation enhances their verbal ability, exerts a deteriorating effect on spatial ability, and that verbal activation interferes with spatial performance. Further, strength of handedness has been studied as a moderating variable for brain lateralization, where left handedness has been associated with greater right hemispheric language dominance (Isaacs et al., 2006; Knecht et al., 2000), while others suggest a more complex relationship moderated further by familial sinistrality (Burnett et al., 1982), and yet others imply no relationship (Hecaen et al., 1981).

As alluded to, women tend to outperform men on episodic memory (Herlitz and Rehnman, 2008) and verbal fluency tasks (Hyde and Linn, 1988), whereas men excel on most spatial tasks (Voyer et al., 1995). Although the underlying mechanisms for these differences are not fully understood, previous fMRI work has suggested that women, although left dominant, show more bilateral activation during verbal tasks (Harrington and Farias, 2008) and are more right lateralized during spatial tasks (Voyer, 1996). Men show the opposite pattern, with a more bilateral activation pattern than women during spatial testing, and greater lateralization than women during verbal testing. These results support Levy’s theories suggesting that the ability bilaterally represented (e.g. verbal ability) leaves less space for a different ability to develop (e.g. spatial ability). However, other authors claim that sex differences in lateralization do not exist (Sommer et al., 2004).

A few studies have investigated the association between handedness and sex differences in cognition (Gordon and Kravetz, 1991; Gunstad et al., 2007; Moffat and Hampson, 1996a; Harshman et al., 1983; Snyder and Harris, 1996). However, for the purpose of this thesis, I will only review studies conducted to investigate spatial or verbal abilities, both tasks that typically exhibit sex differences in performance. Findings indicate an interaction between handedness and sex on tests of spatial performance where RH men outperform LH men and LH women do better than RH women (Gordon and Kravetz, 1991; Harshman et al., 1983; Snyder and Harris, 1996). The opposite pattern is seen for verbal abilities, where RH women do better than LH women and LH men outperform RH men (Gordon and Kravetz, 1991; Harshman et al., 1983; but see also Moffat and Hampson, 1996a). Some findings indicate that this sex by handedness interaction is specific to individuals with high reasoning ability, whereas the results are reversed for low performing individuals (Harshman et al., 1983). Taken together, these results may indicate different activation patterns or strategies for RH and LH individuals where LH men show a cognitive pattern closer to women’s cognitive pattern, and LH women perform closer to men’s cognitive pattern. Whether the conventional cognitive sex differences are found between LH men and women remains an open question.
1.4 ESTROGENS

There are three main classes of naturally occurring estrogens in women; estradiol, estriol, and estrone. Estradiol is the most potent estrogen in humans with effects on sexual behavior (Filiz Çayan, 2008), mood (Douma et al., 2005), bone structure (Uusi-Rasi et al., 2003), and brain organization (Schwarz and McCarthy, 2008). As is the case for testosterone, only a small portion (1.8%) of estradiol is unbound and free to circulate in the blood. The normal range of estradiol levels in premenopausal women after menarche is between 73-367 pmol/l in the early follicular phases and between 367-1285 pmol/l in the preovulatory and luteal phases (Greenspan and Strewler, 1997). In postmenopausal women, the range decreases to 37-110 pmol/l, being lower than the normal range in adult men (73-184 pmol/l). Most estradiol is produced in the ovaries (in the testis in men), although some is produced in the adrenal cortex, or converted by aromatization from testosterone. Estriol on the other hand, is mainly present during pregnancy and is produced in the placenta. Estriol has no relevance to the current research and will therefore not be further discussed. Estrone on the other hand, is important in postmenopausal women, as it is the most common estrogen after menopause. Further, levels of estrone have been shown to correspond well with women’s estradiol level (Cauley et al., 1991). Postmenopausal women’s estradiol levels are sometimes difficult to assess, whereas estrone is quantifiable. Post menopause, estrone is mainly produced in adipose tissue, and thus, women with higher BMI scores exhibit higher levels of estrogen (Lebrun et al., 2005).

In the forthcoming sections, I discuss estrogen action in the brain as well as various ways of studying effects of estrogen on cognition in adult women.

1.4.1 In the brain

Estrogen can affect the CNS in numerous ways, including binding to estrogen receptors. Estrogen is known to exert protective effects on neuronal cells, although the underlying mechanisms in humans are not fully understood. Estrogens act via two types of intracellular receptors, ERα and ERβ. ERα is predominantly found in the pituitary, kidney and adrenal gland, while ERβ is expressed in the brain, ovary, and uterus (Kuiper et al., 1998). ERβ in the brain is distributed in the hippocampus, cerebellum, neocortex, and hypothalamic nuclei. Structural and functional imaging techniques have confirmed that estrogen modulates glucose metabolism, cerebral blood flow, and various neurotransmitter systems (Eberling et al., 2000; Gur et al., 1999; Murphy et al., 1993). There are several possible mechanisms for estrogen’s effect on cognition. One such mechanism is through modulation of acetylcholine where estradiol-treated oophorectimized rats show higher release of this transmitter in certain brain areas and an increase in survival of cholinergic neurons (Gibbs et al., 1997). Estrogen-treated rats perform better on behavioral tasks than controls, and performance is related to higher choline uptake as well as increased levels of choline acetyltransferase in hippocampus and cortex. Further, estrogen may affect cognitive performance by promoting nerve growth and reorganization of synapses. For example, estrogen stimulates axonal sprouting and dendritic spine formation in the CA1 region of hippocampus in adult rats (Gould et al., 1990). Early neuronal loss in the CA1 region is associated with age-related cognitive dysfunction. Estrogen also modulates the expression of the APOE
gene in rats, a gene that has been firmly linked to Alzheimer’s disease (AD; Farrer et al., 1997).

1.4.2 Cognition

1.4.2.1 Menstrual cycle
There are three major ways of investigating activational effects of estrogens in humans. One is by examining cognitive changes over the menstrual cycle. Several studies on cognition and estrogen fluctuations during the menstrual cycle have been conducted, indicating a negative association between spatial ability and estrogen, with high estrogen levels in the luteal phase being associated with poorer performance on spatial ability (Hausmann et al., 2000; Maki et al., 2002; McCormick and Teillon, 2001; Moody, 1997; Silverman and Phillips, 1993; but see Gordon and Lee, 1993). In summary, results on estrogen fluctuations across the menstrual cycle indicate a negative association between spatial ability and estrogen levels and no association with category fluency in fertile women.

1.4.2.2 Hormone therapy
A second approach to studying estrogen’s effect on cognition is by examining women who use hormone treatment (HT). Past studies indicate that HT protects against cognitive decline (LeBlanc et al., 2001) and possibly also against degenerative diseases (Yaffe et al., 1998). In contrast, a recent review (Lethaby et al., 2008), including only double-blind randomized studies, concluded that there was no evidence that HT reduces age-related cognitive decline or improves memory in elderly postmenopausal women with cognitive impairment. However, it remains to be determined whether HT has an effect on subgroups of women, for example women who have recently entered menopause or women with menopausal symptoms, as suggested in a different review (LeBlanc et al., 2001). The inconsistency in the literature suggests considerable individual differences, as well as modifying variables in the estrogen-cognition relationship. In general, interpretation of results from research on HT and cognition is difficult, because of differences among studies in type and length of treatment, demographic characteristics, and age at treatment.

1.4.2.3 Natural menopause
Cognitive performance as a function of estrogen can also be studied in conjunction with menopause. A few studies have investigated the association between estrogen levels and cognitive function in naturally occurring menopause, both cross-sectionally (Elsabagh et al., 2007; Fuh et al., 2003; Halbreich et al., 1995; Herlitz et al., 2007; Kok et al., 2006; Luetters et al., 2007) and longitudinally (Fuh et al., 2006; Meyer et al., 2003). An overview of these studies is provided in Table 2. Results from cross-sectional studies do not suggest a relationship between menopause status and cognitive performance, with executive functioning as a possible exception (Elsabagh et al., 2007; Halbreich et al., 1995). However, it should be noted that there are methodological problems in the latter study (see Table 2).

Only one longitudinal study has been conducted on menopause status and cognitive performance relevant to the present thesis (Fuh et al., 2006). This study showed a negative relationship between menopause status and verbal fluency, in contrast to the absence of effect on the same task in a previous cross-sectional study (Fuh et al., 2003). This discrepancy in results suggests important differences between studies investigating cross-sectional differences and studies investigating change in cognitive
performance in and around menopause. While cross-sectional studies are confounded by age and cohort differences, longitudinal studies allow an individual to serve as one’s own baseline. Hence, the results indicated that there might be a relationship between changes in estrogen levels and cognitive performance within subjects. However, there is no evidence that high or low estrogen between individuals predict cognitive performance.

In conclusion, there is a paucity of studies on cognitive changes during the menopause transition. The available studies show not only inconsistent results, but also differences in methods and designs, samples, and cognitive measures. In light of this, there is a clear need for longitudinal studies on cognition in menopause, with particular emphasis on women in both the early and late stages of this process.
Table 2. Summary of studies and results for cognitive tasks as a function of menopause status. [-] = Negative Association, [0] = No Association.

<table>
<thead>
<tr>
<th>Cross Sectional Studies</th>
<th>Sample*</th>
<th>Spatial ability</th>
<th>Verbal Production</th>
<th>Episodic memory</th>
<th>Executive functioning</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elsabagh et al (2007)</td>
<td>N = 189</td>
<td>1 task: Category fluency Result: 0</td>
<td>4 tasks: Word recall Result: 0</td>
<td>2 tasks: Mental flexibility and planning Result: -2/2</td>
<td>Groups divided into early and late post menopause. Adjusted for IQ, age, degree of sleepiness</td>
<td></td>
</tr>
<tr>
<td>Fuh et al. (2003)</td>
<td>N = 1193</td>
<td>1 task: Verbal fluency Result: 0</td>
<td>1 task: Visual recognition Result: 0</td>
<td></td>
<td>Compared groups of pre-, peri-, and postmenopausal women. Other tasks: working memory (0), speed (0), learning tests (0). Adjusted for age and education.</td>
<td></td>
</tr>
<tr>
<td>Halbreich et al. (1995)</td>
<td>N = 24 premenopausal, 33 postmenopausal Age range = pre: 21-47, post: 41-59</td>
<td>1 task: Gestalt completion. Result: 0</td>
<td>1 task: word recall Result: 0</td>
<td>1 task: driving simulation. Result: -</td>
<td>Other tasks: working memory (0), speed (0), reaction time (-1/2). Results are derived from comparing age slopes for young and old women.</td>
<td></td>
</tr>
<tr>
<td>Kok et al. (2006)</td>
<td>N = 702</td>
<td>M age = 53 years</td>
<td>1 task: Word recall. Result: 0</td>
<td></td>
<td>Prospective cohort study. Other tasks: reading ability (0) and search speed (+). Controlled for cognitive scores at age 43 and education.</td>
<td></td>
</tr>
<tr>
<td>Luetters et al. (2007)</td>
<td>N = 1657</td>
<td>M age = 49.7 (SD = 2.6) Age range = 42-52</td>
<td>1 task: Story recall (immediate and delayed). Result: 0</td>
<td></td>
<td>Part of a longitudinal cohort study. Other tasks: working memory (0) and speed (0). Adjusted for age, education, ethnicity, symptoms, self-reported health, and BMI.</td>
<td></td>
</tr>
</tbody>
</table>
## Introduction

<table>
<thead>
<tr>
<th>Longitudinal studies</th>
<th>Sample*</th>
<th>Spatial ability</th>
<th>Verbal Production</th>
<th>Episodic memory</th>
<th>Executive functioning</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuh et al. (2006)</td>
<td>N = 495</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Other tasks: Working memory. 18-month follow-up. 114 progressed to perimenopause. Adjusted for age, education and baseline score</td>
</tr>
<tr>
<td></td>
<td>M age = 44.7 (SD = 2.3) and 47.1 (SD = 3.0)</td>
<td></td>
<td>1 task: Category fluency</td>
<td>2 tasks: Delayed word recall and visual recognition</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Results: -</td>
<td>Results: 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Note:</td>
<td>perimenopausal women do not gain as much as premenopausal women.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meyer et al. (2003)</td>
<td>N = 803</td>
<td>Age range = 42–52</td>
<td>0</td>
<td>-</td>
<td></td>
<td>Other tests: working memory (0) and perceptual speed (-) where postmenopausal women decreased in performance more than other groups. No HT in at least 3 months. Mean follow-up duration = 2.1 years. Mean number of follow-ups = 2.3 Adjusted for baseline age, education, family income, ethnicity and self-reported health</td>
</tr>
<tr>
<td></td>
<td>M age = Pre: 45.6 (SD = 2.8); early peri: 47.1 (SD = 2.8); late peri: 49.9 (SD = 2.9); post: 50.2 (SD = 3.9)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Note:** * = Number of participants, age means and standard deviations, and age range are reported when data are available. † = The negative association indicates that performance decreases as a function of the menopausal transition.
2 AIMS OF THE DISSERTATION

The primary objectives of this dissertation are to investigate the relationship between steroid hormones and cognition in adulthood and aging, and more specifically to investigate whether:

1. testosterone is associated with cognition in aging men and women and, if so, whether the pattern is similar across sex;

2. the magnitude of sex differences in cognition are similar in right- and left-handed individuals; and

3. the diminishing levels of estrogen in and around menopause are associated with lower performance and an accelerated rate of decline in cognitive performance
3  METHODS

3.1 BETULA

All studies in the current thesis are based on data collected in the Betula study in Umeå, Sweden. The Betula study is a prospective cohort study involving participants ranging in age between 35 and 80 years in Sample 1 (S1) at test wave 1 (T1). At T1 (S1) there were 100 persons in each age cohort (i.e., 35, 40, 50...). At T2 participants from S1 were between 40 and 85 years (13% dropout) and at T3 participants from S1 were 45 to 90 years of age (16% dropout). At T2 two new samples were introduced, S2 and S3 with close to 200 new participants in each cohort. Participants from S2 ranged in age from 35 to 80 years at T2, and at T3 these participants were between 40 and 90 years of age with a 15% dropout. Participants from S3 ranged in age between 40 and 85 years at T2 and between 45 and 90 years at T3 (14% dropout). The new sample at T3 (S4) consisted of an additional 50 persons in each cohort with an age range between 35 and 90 years. See Figure 6 for details on sample selection.

Figure 6. The Betula study design

Data collection for T1 took place from 1988-1990, for T2 from 1993-1995, and for T3 from 1998-2000. The data used in Studies I-III stem from T3 where 2556 participants volunteered to give blood for assessment of hormone levels. Study IV is based on longitudinal data deriving from the three test waves (T1-T3). The main objectives of
the Betula study are to investigate the progression of health and memory in aging, and to investigate aspects of dementia diseases in the population, such as prevalence, risk factors, and pre-clinical signs of dementia. The protocol includes medical assessment, blood sampling, activities of daily living, symptoms of depression, social status, critical life events, and an extensive cognitive battery. The majority of participants were tested on two occasions at each test wave; the first occasion included a medical assessment and the second was focused on cognitive testing. Individuals with developmental disorders, diagnosed dementia, visual or auditory handicap, or individuals with a native language other than Swedish were excluded from participation in the study.

3.2 COGNITIVE ASSESSMENT

In the Betula study, participants are assessed on a large number of cognitive tasks (see Table 3; Nilsson et al., 2004; Nilsson et al., 1997). In the following section, the cognitive tasks used in Studies I-IV will be described.

Spatial abilities. The block design task from the WAIS-R (Wechsler, 1981) was used to assess spatial ability. Participants are required to place red and white blocks so that they form the pattern shown to them. The score was based on the number of completed designs and time to completion. Maximum score was 51.

Semantic memory. A word comprehension task with 30 target words was used to assess semantic memory. The task was to choose the synonym to the target word among 5 alternatives. Seven minutes were allowed for task completion.

Verbal fluency. In the verbal fluency task, participants were asked to generate as many words as possible beginning with the letter “A”. In a second fluency task, participants were asked to generate five-letter words that start with “M”. One minute was allowed for each task.

Episodic memory. Eleven measures of episodic memory were used. These were either combined to form composites, or investigated separately.

In the subject-performed task (SPT) and the verbal task (VT; Nilsson et al., 1997), participants were presented with two lists of 16 verb-noun sentences, each denoting a simple action (e.g., lift the cup). The nouns belonged to four semantic categories. For SPTs, participants were requested to enact each sentence at encoding, using the specified object. The VT list was studied without enactment. Participants were first asked to freely recall the sentences, and then cued recall was assessed with the semantic category names serving as cues. Finally, in recognition, participants were presented with the target nouns randomly interspersed with distractor nouns, for yes-no recognition judgments. Hits minus false alarm scores were computed in recognition.

In the four conditions of word recall with or without divided attention, participants were given a list of 12 unrelated words that they were asked to immediately recall. There were four conditions: full attention, divided attention (card sorting) at encoding, divided attention at retrieval, and divided attention at both encoding and retrieval.

In the study phase of face recognition, participants viewed 16 pictures of children’s faces (8 sec/face) and they were asked to remember the faces for later recognition. In the recognition phase, participants were presented with pictures that they studied
intermixed with distractor faces and were required to indicate whether or not they had seen the face in the earlier study phase.

The last episodic memory measure, memory of activities, was administered at the end of the testing session in which participants were asked to incidentally recall all tasks that they had performed during the test session.

Table 3. Cognitive testing at T3 in Betula

<table>
<thead>
<tr>
<th>Cognitive Domain</th>
<th>Tasks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prospective memory</strong></td>
<td>With or without cues</td>
</tr>
<tr>
<td><strong>Spatial Ability</strong></td>
<td><em>Spatial perception</em></td>
</tr>
<tr>
<td></td>
<td>Block design</td>
</tr>
<tr>
<td><strong>Semantic memory</strong></td>
<td><em>Word comprehension</em></td>
</tr>
<tr>
<td><strong>Verbal Fluency</strong></td>
<td><em>Word fluency: initial letter A</em></td>
</tr>
<tr>
<td></td>
<td><em>Word fluency: initial letter M for five-letter words</em></td>
</tr>
<tr>
<td></td>
<td>*Word fluency: initial letter B for names of professions</td>
</tr>
<tr>
<td></td>
<td>*Word fluency: initial letter S for five-letter names of animals</td>
</tr>
<tr>
<td><strong>Episodic memory</strong></td>
<td><em>Free Recall</em></td>
</tr>
<tr>
<td></td>
<td>Subject performed task (SPT)*</td>
</tr>
<tr>
<td></td>
<td>Verbal task (VT)*</td>
</tr>
<tr>
<td></td>
<td><em>Word recall (12 words)</em></td>
</tr>
<tr>
<td></td>
<td><em>Word recall (12 words) with card sorting during encoding</em></td>
</tr>
<tr>
<td></td>
<td><em>Word recall (12 words) with card sorting during retrieval</em></td>
</tr>
<tr>
<td></td>
<td><em>Word recall (12 words) with card sorting during encoding and retrieval</em></td>
</tr>
<tr>
<td></td>
<td>Memory of activities from the test session</td>
</tr>
<tr>
<td><strong>Cued Recall</strong></td>
<td>*Cued recall of nouns from SPT sentences</td>
</tr>
<tr>
<td></td>
<td>*Cued recall of nouns from VT sentences</td>
</tr>
<tr>
<td><strong>Source recall</strong></td>
<td>*Source recall of nouns from SPT and VT sentences</td>
</tr>
<tr>
<td><strong>Recognition</strong></td>
<td><em>Face recognition (free-choice)</em></td>
</tr>
<tr>
<td></td>
<td>*Name recognition (forced-choice)</td>
</tr>
<tr>
<td></td>
<td>*Recognition of nouns from SPT sentences</td>
</tr>
<tr>
<td></td>
<td>*Recognition recall of nouns from VT sentences</td>
</tr>
<tr>
<td><strong>Priming</strong></td>
<td>*Stem completion (Implicit memory)</td>
</tr>
<tr>
<td><strong>Global cognition</strong></td>
<td><em>Mini-Mental State Examination</em></td>
</tr>
</tbody>
</table>

*Note: * = Tasks used in Studies I-IV
3.3 HORMONAL ANALYSES

Serum blood was drawn immediately after cognitive testing and was frozen at - 80°C. Analyses were carried out at the Clinical Chemistry Laboratory, Karolinska Hospital in Stockholm, Sweden.

Testosterone, SHBG, and Albumin. For analyses of testosterone, an AutoDELFIA (Wallac Oy, Turku, Finland) testosterone assay was used. Auto DELFIA is a solid phase fluoroimmunoassay based on competition between europium-labeled testosterone and sample testosterone for polyclonal anti-testosterone antibodies. The intra- and inter-assay variation is 7.05 % and 7.15 %, respectively, and the assay has a sensitivity of 0.3 nmol/l. The measurement interval for total testosterone is 0.02-50 nmol/l. The assay requires only one incubation step and is automated. To obtain SHBG values, a 1235 AutoDELFIA automatic immunoassay system was used with an intra-assay variation of 3.3-3.9 %, an inter-assay variation of 2.3-3.0 %, and a sensitivity of 0.5 nmol/l. An AutoDELFIA was used to obtain albumin levels. The intra- and inter-assay variability for albumin was 3.22-4.40 % and 4.60- 4.99 %, respectively, with a sensitivity of 0.2 nmol/l. A formula was utilized to compute free testosterone values, using binding constants for albumin and SHBG (Vermeulen et al., 1999).

Estradiol. A solid phase fluoroimmunoassay (AutoDELFIA Estradiol assay: Wallac Oy, Turku, Finland) was used to measure levels of estradiol. The assay was based on competition between europium-labeled estradiol and sample estradiol for polyclonal anti-estrogen antibodies. The intra-assay variation was 3.6 % and the inter assay variation was 4%. The sensitivity of the assay was < 10.6 pmol/l.

3.4 BACKGROUND VARIABLES

A number of background variables were used in Studies I-IV, including age, years of education, number of medications affecting cognitive abilities (i.e. opioids, long-term benzodiazepines, benzodiazepines, anticholinergic drugs, and corticoids), Mini-Mental State Examination (MMSE; Folstein et al., 1975), stress level (Levenstein et al., 1993), depressive symptoms (Radloff, 1997), self reported health, BMI, smoking, alcohol consumption, menopausal symptoms, sedimentation, and diastolic blood pressure. Most variables were used for purposes of sample description, but were also used as covariates in some studies.

3.5 ETHICAL CONSIDERATIONS

The Betula study was approved by the regional ethical review board in Umeå, Sweden, and conducted according to the Helsinki declaration. All subjects gave written and informed consent to participate.
4 RESULTS

4.1 STUDY I

Earlier results on testosterone and cognition have been inconsistent. Study I sought to address earlier shortcomings and investigate the relationship between testosterone levels and cognitive performance in aging. Participants were drawn from T3 of the Betula study (i.e., data were cross-sectional). There were 1107 men ($M_{\text{age}} = 62.2, SD = 13.5$) and 1276 women ($M_{\text{age}} = 63.8, SD = 13.6$) between 35 and 90 years of age. Out of 3040 participants at T3, 2556 volunteered to give blood and 2383 remained after excluding individuals with MMSE scores $< 25$, that is, individuals with presumed cognitive impairment (O'Connor et al., 1989).

For the main analyses in Study I, hierarchical regressions were used to investigate the relationship between free testosterone levels and cognitive performance in men and women separately. Analyses adjusted for age, years of education, and number of medications (with a possible effect on cognitive performance). Six measures of cognitive performance (visuospatial ability, semantic memory, verbal fluency, episodic recall, episodic recall with divided attention, and episodic recognition) were examined in relation to free testosterone and age.

**Main Results:** The results indicate that men with lower levels of testosterone generally exhibited poorer cognitive performance. The interaction between testosterone level and age point to an increasing cognition-testosterone relationship with increasing age. That is, older men with lower testosterone levels perform increasingly worse on block design (spatial ability), as well as on all episodic tasks (see Figure 7A-D). Each figure represents cognitive performance (Y axis) as a function of age (X axis) and level of free testosterone. For women, the opposite pattern is seen, with higher levels of cognitive performance linked to lower testosterone levels, although the effect is reliable only for verbal fluency. There are no interactions between age and free testosterone for women.
4.2 STUDY II

Women typically outperform men on episodic memory and verbal fluency tasks, whereas men tend to excel on visuospatial tasks. As the vast majority of individuals are RH, sex differences in the cognitive literature reflect laterality-specific patterns for RH individuals. In Study II, we examined the magnitude of cognitive sex differences as a function of hand dominance in samples of RH and non-RH individuals. Earlier findings have shown a handedness by sex interaction for certain cognitive domains, but here we wanted to examine the magnitude of sex differences in RH and non-RH individuals. Included in the current study were 95 RH women ($M_{age} = 60.7$, $SD = 12.7$), 97 RH
Results

men (M age = 59.7, SD = 12.1), 70 non-RH women (M age = 60.8, SD = 14.0), and 85 non-RH men (M age = 58.9, SD = 12.7). In Study II, analyses of covariance (ANCOVAs) were computed with the cognitive measures (i.e., visuospatial ability, verbal fluency, episodic memory, and semantic memory) as dependent variables, and with handedness (RH or non-RH) and sex (men or women) as independent variables. Age was covaried in all analyses.

Main Results: There were no significant differences between the groups with respect to age, education, use of medications, or MMSE scores. However, there was a significant difference between testosterone levels in RH and non-RH men, p = 0.056, with RH men having higher levels. The results show that the non-RH men performed better on verbal fluency and worse on the spatial task relative to RH men. However, the effect was significant only for the spatial task. The opposite pattern was seen for women, where non-RH women performed at a higher level on the spatial task and at a lower level on verbal fluency, although differences fail to surpass conventional alpha levels. Importantly, and contrary to patterns observed for RH individuals, there were no sex differences in cognitive performance in the non-RH group (see Figure 8). Confirming earlier findings, RH men have higher testosterone levels then non-RH men. No handedness-related difference in testosterone levels was observed for the women.

Figure 8. Sex differences in cognitive performance as a function of hand preference.

4.3 STUDY III

Although there have been multiple studies on hormone replacement therapy and cognition after menopause, few studies have investigated cognitive performance during the natural course of menopause. In Study III, we explored cross-sectional data on
cognitive performance in 242 women who were pre- (M age = 46.6, SD = 2.6), peri- (M age = 51.6, SD = 3.1), or postmenopausal (M age = 53.8, SD = 2.5) at the time of assessment. There were significant differences in age and years of education between groups and such variables were therefore adjusted for in the analyses.

Multivariate analyses of covariance (MANCOVAs) and ANCOVAs were used in Study III to investigate differences in cognitive performance (episodic memory, semantic memory, verbal fluency, visuospatial ability, and face recognition) among women in different phases of menopause (i.e. pre, peri or post menopause). The main analyses included women in three different age cohorts (45, 50, and 55 years). In order to control for age confounds and cohort differences, we analyzed each age group separately (i.e. 45 year olds that were pre, peri, or post menopause). Age confounds represent a critical issue in cross-sectional studies on this topic, as both estrogen levels and cognition decrease with increasing age.

**Main Results:** There was no effect of menopause status on cognition. These results were strengthened by the lack of association between menopause status and cognitive performance computed within each age group (i.e., 45, 50, 55 year olds). Unfortunately, the sample sizes were small in the latter analyses making it hard to draw firm conclusions. Nonetheless, after investigating group differences, there was no evidence of lower cognitive performance being associated with diminishing estrogen levels in menopause.

### 4.4 STUDY IV

Study IV was conducted to address limitations in Study III. The design and longitudinal data for Study IV permitted exploration of the actual rate of change in cognitive performance, as women transitioned from pre to post menopause. No previous study has investigated changes in multiple cognitive domains during both early and late stages of menopause.

In Study IV, longitudinal data were analyzed using multilevel models (Bryk and Raudenbush, 1992), investigating differences in rate of cognitive change due to chronological age (pre menopause) compared to rate of change post menopause. This statistical approach permits simultaneous assessment of within-person change and between-person differences in within-person change. There are several advantages of multilevel modeling in comparison to conventional repeated-measures ANOVA. First, one can have missing data at certain time points without employing listwise deletion (i.e. excluding data from all time points for a case that is missing only one occasion of measurement). This is particularly important as individuals with incomplete data may be different from those with complete data (e.g. having poorer health). Also, multilevel modeling does not require equal spacing between time points, which is rare in longitudinal studies.

In Study IV, we addressed previous limitations by including a continuum of cognitive measures, collecting data at three time points (up to 10 years), sampling women in earlier as well as later stages of the menopausal transition (40-65 years of age), and by
assessing potential health moderators of rate of change, including: sedimentation, diastolic blood pressure, and self-reported health status. Deviance scores for nested models were compared in order to assess whether model fit was significantly improved by the addition of each predictor (Singer and Willet, 2003). Variables that did not improve model fit were omitted from further consideration. In addition, as elevated BMI in postmenopausal women is related to higher levels of estrogen (Cauley et al., 1989), analyses investigating the potential moderating effect of BMI on postmenopausal trajectories were included. However, estrogen level was not entered in the model, as measures were only available from the last test wave.

To facilitate comparison between cognitive tasks, all measures were standardized ($M = 50, SD = 10$), using the baseline mean and standard deviation as the reference for normalization. We investigated change in menopausal transition for 10 cognitive outcomes (1 visuospatial task, 1 semantic task, 1 verbal fluency task, and 7 episodic tasks) in 193 women between 40 and 65 years of age who were postmenopausal during the last test wave. As all episodic memory tasks were considered to assess the same underlying cognitive function, a composite episodic memory score was created from these 7 measures. Body Mass Index (BMI) was entered in the model as a moderator. For all participants, chronological age was computed in years and months. Years post menopause was computed as years and months between last menstruation and time of testing for only those participants defined as postmenopausal. Women who did not have menses for at least one year prior to testing were considered postmenopausal.

**Main Results:** None of the health indicators improved model fit and were therefore dropped from the analyses. An effect of menopausal status on cognition was found, with normal weight (based on BMI) postmenopausal women showing more pronounced decline over time than premenopausal women for both visuospatial ability and episodic memory (see Figures 9A and 9B). In addition, regardless of weight, all women showed an increase in rate of change post menopause for verbal fluency (see Figure 10). The so-called inflection point referred to in the figures represents the difference in rate of change between normal aging (prior to inflection) and as a function of menopause (following inflection). Hence, the inflection point separates rate of change pre and post menopause. In figure 9A, for example, the black line illustrates how a prototypical normal weight woman, at the average age of menopause onset in this sample (49.1 years) and with an average amount of education (10.3 years), would decrease in cognitive performance both prior to and following menopause onset of 49.1 years. The gray line represents cognitive change pre vs. post menopause for an average woman in the high BMI group.
Figure 9A and 9B. The slopes before the inflection point (the average age of menopause onset = 49.1 years) represents rate of change in performance as a function of chronological age for A) spatial ability and B) episodic memory. The slopes following the inflection point represent change in performance post-menopause over and above age-related change. Change in cognitive performance is plotted for both normal and overweight women. The model is adjusted for age and education.

Figure 10. The slope before the inflection point (the average age of menopause onset = 49.1 years) represents age-related change in performance on verbal fluency. The slope after the inflection point represents change in performance post menopause that is independent of age-related change. The model is adjusted for age and education.
5 DISCUSSION

5.1 TESTOSTERONE AND COGNITION

In Study I, the main goal was to explore the association between free testosterone and cognitive performance in adulthood and old age. The secondary aim was to investigate whether the relationship was similar in men and women.

Previous studies have been inconclusive. Reasons contributing to discrepancies in results may be age differences in samples analyzed, but also the inappropriate use of hormone measures and analyses. As Table 1 indicates, 13 out of 18 studies used suboptimal methods for hormone analyses.

Consistent with some earlier findings (Hogervorst et al., 2004; Moffat et al., 2002; Muller et al., 2005; Yaffe et al., 2002), the results from Study I show an overall positive association in men between free testosterone and cognitive performance, an association that increases with age. Specifically, an association between free testosterone and visuospatial ability and episodic memory was observed from 65-70 years of age. The fact that the association increased with age may reflect several factors. One such explanation being that when cognitive resources are limited, as indicated by lower cognitive performance with increasing age (see Figures 7A-7D), individual differences in biological variables, such as testosterone, become increasingly important (Lindenberger et al., in press). In addition, the variation in both testosterone levels and cognitive performance is greater in older participants, making it easier to detect potential differences.

If levels of sex hormones are associated with cognitive performance, we may expect to see larger effects of hormones in tasks with more pronounced sex differences. In accordance with this, we find the largest association between testosterone and spatial ability, and no association between testosterone and semantic memory in men. Furthermore, we found that older men with relatively high levels of testosterone performed better on verbal episodic memory tasks, as compared with men with relatively low levels. Speculatively, this may reflect a conversion of testosterone to estrogen in the brain (Cherrier et al., 2001; Cherrier et al., 2005a).

Support for a relationship between testosterone and cognitive performance from clinical trials shows that testosterone supplement improve spatial ability in men (Cherrier et al., 2001; Janowsky et al., 1994). Hypogonadal young (Hier and Crowley, 1982) and old (Moffat et al., 2002) men tend to do worse on spatial tasks and improve in performance after testosterone treatment (Zitzmann et al., 2001). Furthermore, a previous study on testosterone supplementation and cognition indicate that the increase in testosterone levels need to be of a specific level in order to yield cognitive performance increments (Cherrier et al., 2007) suggesting a threshold for the testosterone-cognition association. This may be one of the reasons behind the small, and sometimes lack of, association between endogenous testosterone and cognition when investigating cognition in men who have normal levels of testosterone.
In women, free testosterone was negatively associated with verbal fluency and there was a similar, albeit non-reliable, trend for word comprehension. In contrast to the positive associations observed for men, associations between free testosterone and cognitive performance for women were all negative, with no interactions observed between age and free testosterone. The preponderance of studies for women indicate that free testosterone shares little or no association with cognitive performance (Drake et al., 2000; Gouchie and Kimura, 1991; Hassler et al., 1992; Hogervorst et al., 2004; Moffat and Hampson, 1996a). When effects are found in women, they are often negative in contrast to the positive associations observed for men, which supports the claim that free testosterone serves a sex specific function. However, this conclusion should be drawn with caution, as clinical trials have reported positive results of testosterone supplementation on spatial performance in women as well (Aleman et al., 2001).

The main findings in Study I support a sex specific pattern in the relationship between testosterone and cognitive performance. Men show a positive association while women show an overall negative trend between testosterone level and cognitive performance. In addition, men exhibit a greater relationship with increased age, a fact that may reflect greater inter individual variation in testosterone and cognitive resources. Additionally, we also found higher associations in tasks eliciting sex differences in performance in contrast to null results for tasks that do not tend to show these differences in performance. Earlier clinical findings, in addition to our reliable but small associations seen mainly in older men, suggest a threshold effect where the link between testosterone and cognitive performance becomes significant.

5.2 HANDEDNESS AND SEX DIFFERENCES IN COGNITION

The aim of Study II was to investigate sex differences in groups of RH and non-RH individuals. Differences in testosterone levels between handedness groups were also explored.

The main results from Study II are in line with earlier findings (Gordon and Kravetz, 1991; Harshman et al., 1983) and indicate that sex differences in cognitive performance are restricted to groups of RH individuals. In comparison to RH men, non-RH men exhibit patterns of cognitive performance more similar to women, with lower performance on spatial tasks and higher performance on verbal fluency and episodic memory tasks. Similarly, relative to RH women, non-RH women revealed a pattern similar to that observed for men, with better spatial abilities, but poorer verbal fluency and episodic memory performance (see Figure 8). Also, the current study is consistent with earlier work where non-RH men are shown to have lower circulating levels of free testosterone (Moffat and Hampson, 1996b). However, there were no differences in testosterone levels in non-RH vs. RH women.

Levy (1969) proposed that the superior performance by women in verbal abilities reflect women’s more bilateral representation during verbal performance, whereas their lower spatial ability is a result of interference from the bilateral verbal representation. Support for Levy’s theory is found in a meta-analysis indicating a more bilateral
involvement in verbal performance for women (Voyer, 1996). As non-RH individuals have been suggested to exhibit an atypical symmetry pattern (Kolb and Whishaw, 2003), less sex typical performance should be expected in this group, which is what we and others have found (Gordon and Kravetz, 1991; Harshman et al., 1983). In addition, we extend earlier findings by showing that there are no cognitive sex differences in groups of non-RH individuals. Nonetheless, it should be noted that in this and in previous studies on this topic (Gordon and Kravetz, 1991; Hampson and Moffat, 1994; Harshman et al., 1983) there is a lack of imaging data directly confirming an atypical symmetry pattern in the group of non-RH individuals, which gives reason to be cautious about the mechanisms underlying the behavioral pattern.

Others suggest that the handedness by sex interaction in cognition is due to different levels of testosterone among RH and non-RH individuals (Harshman et al., 1983; Moffat and Hampson, 1996b). In line with earlier work, the current study showed that RH males had higher levels of testosterone than non-RH males. In RH- and non-RH women, there were no such differences. The results from the present study partly support the notion that circulating testosterone is involved in cognitive differences between handedness groups. In Study I for example, we showed that older men with lower levels of testosterone performed worse on all cognitive tasks. If endogenous testosterone was involved in the cognitive differences between handedness groups, we would expect non-RH men to perform worse on all cognitive tasks. Counter to this expectation, the spatial task was the only task in which non-RH men performed worse than RH men; instead they tended to perform at a higher level on tasks of verbal fluency and episodic memory. This pattern of data is consistent with findings from other studies on cognition between sex and handedness groups (Gordon and Kravetz, 1991; Harshman et al., 1983). Although our data do not fully support the notion that endogenous levels of testosterone are driving the differences in cognitive performance patterns among RH and non-RH men and women, it is still possible that prenatal testosterone levels are involved in early brain organization, determining handedness and cognitive sex differences.

5.3 ESTROGEN AND COGNITION

The aim of Study III and IV was to explore whether diminishing estrogen levels in and around menopause were associated with poorer cognitive performance, independent of the cognitive changes occurring with increasing age. In Study IV, we also examined whether BMI status moderated the relationship between menopause status and rate of change in cognitive performance. The main findings in Study III and IV are contradictory, with Study III indicating no effect of menopause status on cognitive performance, and Study IV indicating a slight negative effect of menopause status in normal weight women.

In line with previous cross-sectional reports (Fuh et al., 2003; Luetters et al., 2007), Study III found no link between estrogen and cognition nor between menopause status and cognitive performance. However, due to the limitations of cross-sectional data, this non-significant finding does not necessarily imply the absence of an association between menopause status and cognition. Instead, high or low estrogen levels may be
within an individual reference frame and not directly comparable between participants. This concern can be addressed in longitudinal studies, in which between-person differences in within-person change is investigated. Second, there is the age confound, making it next to impossible to tease apart the influence of age and menopause status using cross-sectional data. We attempted to minimize the age confound in Study III by comparing women within different age groups. However, with the small effects of menopause status on cognitive change observed in Study IV, it is reasonable to conclude that we lacked statistical power to detect potential effects.

In Study IV, postmenopausal women exhibited faster decline in select cognitive tasks than premenopausal women. These effects were independent of age. The results also showed that the higher rate of change in postmenopausal women was present only for women within the normal BMI range (with the exception of verbal fluency). A possible explanation for this finding is that estrogen action helps to buffer against age-related cognitive decline in overweight women, who have higher estrogen levels post menopause (Saunders-Pullman, 2003).

There are certain practice effects associated with longitudinal studies (Rönnlund et al., 2005). In Study IV, there are clear gains in performance on the fluency task (see Figure 10) and a possible underestimate of the true decline on all tasks. An explanation for the higher practice effect in verbal fluency compared to the other tasks may be that the same letter was used on each test occasion whereas the content of the episodic word lists were different on all follow-ups.

A key question concerns how one should interpret the discrepancies between the two studies. I believe that the inconsistency is partly due to differences between cross-sectional and longitudinal studies, where cross-sectional studies are unable to detect change in cognitive performance within individuals. The discrepancy in results underscores the problem of teasing apart the effects of menopause from age-related cognitive decline in cross-sectional studies. Another reason for the inconsistencies is that rate of change may only be accelerated post menopause in specific subgroups of women (Yaffe et al., 1998), such as in normal-weight women. Another question relates to the discrepancy between animal data and research in humans on estrogen and cognition. The key issue is that there are fundamental differences in how we go about conducting research on estrogen in animals and humans. In animals, we do not typically investigate natural differences in estrogen levels and behavior, but instead experimentally deprive animals and compare them to animals injected with high levels of estrogen. Such studies cannot be conducted in humans, and it is therefore an open question whether estrogen deprivation or high-dose supplementation would affect human cognition (a question that probably would be associated with higher risks than benefits).

In summary, our results indicate that the effect of estrogen on human cognition is small and most likely only present in subgroups of women. Moreover, the effects seem to be restricted to analyses examining within-person changes of estrogen.
5.4 LIMITATIONS

Most scientific studies suffer from some limitations and those emanating from the Betula project are no exception. Most limitations stem from the fact that this data set is not exclusively focused on hormones in aging; instead, the hormonal interest emerged after the first test wave. As a consequence, we neither have longitudinal data on hormones, nor specific information on the phases of the menstrual cycle. In addition, the follow-up intervals are far apart (5 years), an obvious concern when attempting to pinpoint critical periods of hormone action, such as menopause. With these long follow-ups, we are lacking information as to when the rate of cognitive decline specifically changes, and it is difficult to draw conclusions about whether the specific event actually triggered the change. In exploring handedness, we lacked measures making it possible to treat this variable as a continuum, and we also lacked direct measures of lateralization. Despite these limitations, I consider myself lucky to have had the opportunity to work with such an extensive longitudinal data set. Indeed, the Betula study has numerous strengths including extensive information on health and disease factors, many cognitive measures, various hormone levels on over 2000 individuals, and much more.

5.5 FUTURE STUDIES

To date, there are very few longitudinal data sets examining testosterone and cognition. It would be of particular interest to follow adolescents before, during, and after puberty to explore cognitive sex differences as a function of hormonal development; and to investigate longitudinal changes in hormones and cognition in a middle-aged population. The latter study would enable the exploration of change patterns in cognitive performance in individuals that become hypogonadal. Such a study would also enable the investigation of men that later develop AD, the potential change in testosterone that precedes this, and the interaction with APOE ε4. Important in this field of research is that the study is specifically designed to investigate hormones and cognition, in contrast to most available projects that typically were initiated for other purposes.

Along these lines, it would be wonderful to see a longitudinal study with women, designed to investigate specific events around menopause, with short follow-ups and longitudinal data on estrogen in addition to estrone levels. Here, it would be of considerable importance to have yearly follow-ups with access to serum blood at each time point. Further, different subgroups (i.e. specific symptoms, previous disease, self-reported memory complaints, burn-out symptoms) of women should be explored, as well as social, and personality variables. I also believe that executive tasks tapping frontal ability in relation to menopause need further attention, as several recent studies have suggested such a relationship (Elsabagh et al., 2007; Joffe et al., 2006).

Finally, it would be interesting to explore hand preference and sex differences using fMRI in order to determine whether the behavioral data maps on to BOLD activation patterns. Specifically, investigating activation patterns in non-RH individuals to explore whether non-RH vs. RH men show a more bilateral activation pattern during verbal activation, and whether non-RH women show less bilateral activation in contrast to RH
women. Such data would give additional support to the theory indicating that interference is an important reason for sex difference in cognitive performance (Levy, 1969, 1971)
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7 REFERENCES


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References


Haut, K.M., Barch, D.M. 2006. Sex influences on material-sensitive functional lateralization in working and episodic memory: Men and women are not all that different. NeuroImage, 32, 411-422.


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