Endocrine and diagnostic aspects of cognitive impairment

with special reference to Alzheimer's disease

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"El haber positivo de un sabio hállase formado por el conjunto de los hechos originales que aporta. Las hipótesis pasan, pero los hechos quedan. Las teorías nos abandonan, los hechos nos defienden."

Santiago Ramón y Cajal (1852-1934)

"A scholar's positive contribution is measured by the sum of the original data that he contributes. Hypotheses come and go but data remain. Theories desert us, while data defend us."

Excerpt from
Reglas y Consejos sobre
Investigación Científica:
Los Tónicos de la Voluntad
Fourth edition 1916

For an aging population

CONTENTS

ABSTRACT

Hormones like insulin-like growth factor-I (IGF-I), thyroid hormones, and sex steroids decrease with normal aging. It is, however, unclear whether low hormone levels are related to age-related conditions such as Alzheimer's disease (AD) or whether hormone levels are associated with markers of aging like leukocyte telomere length (LTL). The aims of this thesis were to validate cerebrospinal fluid (CSF) biomarkers in AD and to investigate whether hormonal aberrations might contribute to reduced cognitive function.

Consecutive patients undergoing primary evaluation of cognitive impairment (n=60) and healthy controls (n=20) were included. The patients had AD dementia or mild cognitive impairment (MCI) that was later diagnosed as AD dementia upon follow-up (n=32), stable MCI (SMCI, n=13), or other dementias (n=15). The same physician examined all subjects. Serum and CSF samples were collected and LTL was analyzed using quantitative PCR technique.

In *Paper I*, the core AD biomarkers in CSF (amyloid β [$A\beta]_{1-42}$, total-tau [T-tau], and phosphorylated tau protein [P-tau]) demonstrated a very high ability to diagnose AD compared to combined groups of controls and SMCI (area under the receiver operating characteristic curve [AUROC]=0.97 [95% CI 0.93–1.00, $P<0.0001$]). The addition of other biomarkers only marginally increased the diagnostic accuracy. In *Paper II*, serum IGF-I was higher in patients with AD or other dementias compared to healthy controls ($P=0.01$ and $P<0.05$, respectively), whereas CSF IGF-I remained unchanged. In *Paper III*, AD patients showed marginally increased serum thyroid-stimulating hormone (TSH). CSF total thyroxine (T_4) level was lower both in patients with AD and other dementias compared to controls (both $P=0.001$). In *Paper IV*, both male and female patients showed increased serum concentrations of estrone (E_1) and estrone sulfate (E_1S) compared to controls of similar gender, but serum levels of other precursor sex steroids and cortisol were increased only in female patients. In *Paper V*, SMCI patients showed reduced LTL compared to AD patients (p=0.02) and controls (p=0.008).

In conclusion, the CSF biomarkers $A\beta_{1-42}$, T-tau, and P-tau were highly accurate to diagnose AD in a well-defined study population. There were multiple alterations in hormonal levels in AD. There might be reduced passage of IGF-I and thyroxine through the blood brain barrier and aberrations in sex steroids and cortisol were more apparent in female patients. Low LTL might indicate more marked biological aging in SMCI patients whereas low LTL does not appear to be a risk factor for conversion to AD.

Keywords: hormones, aging, cerebrospinal, Alzheimer

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LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following publications, which in the text will be referred to by their Roman numerals. All reprints in the publication list were made with permission from the publishers.

I. *Cerebrospinal fluid biomarkers for Alzheimer's disease – diagnostic performance in a homogeneous mono-center population.*
Johansson P[§], Mattsson N[§], Hansson O, Wallin A, Johansson JO, Andreasson U, Zetterberg H, Blennow K, Svensson J.
[§]Contributed equally.
J Alzheimers Dis. 2011; 24(3): 537-546.

II. *Serum but not cerebrospinal fluid levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 (IGFBP-3) are increased in Alzheimer's disease.*
Johansson P, Åberg D, Johansson J-O, Mattsson N, Hansson O, Ahrén B, Isgaard J, Åberg ND, Blennow K, Zetterberg H, Wallin A, Svensson J.
Psychoneuroendocrinology. 2013; Epub ahead of print.

III. *Reduced cerebrospinal fluid level of thyroxine in patients with Alzheimer's disease.*
Johansson P, Almqvist EG, Johansson J, Mattsson N, Hansson O, Wallin A, Blennow K, Zetterberg H, Svensson J.
Psychoneuroendocrinology. 2012; Epub ahead of print.

IV. *Mild dementia is associated with increased adrenal secretion of cortisol and precursor sex steroids in women.*
Johansson P, Johansson JO, Labrie F, Mattsson N, Hansson O, Blennow K, Zetterberg H, Wallin A, Ohlsson C, Svensson J.
Clin Endocrinol (Oxf) 2011; 75(3):301-308.

V. *Leukocyte telomere length (LTL) is reduced in stable mild cognitive impairment but low LTL is not associated with conversion to Alzheimer's disease: a pilot study.*
Movérare-Skrtic S[§], Johansson P[§], Mattsson N, Hansson O, Wallin A, Johansson JO, Zetterberg H, Blennow K, Svensson J.
[§]Contributed equally.
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BRIEF DEFINITIONS

Biomarkers	Measurable and quantifiable biological parameters, which serve as indices for physiology-related assessment.
Cognition	A process of perceiving, learning or handling information. It comprises memory, defined as a lasting representation reflected in thought, experience, or behavior. Learning is the acquisition of such representations.
Dementia	An acquired clinical syndrome with cognitive impairment leading to functional decline. Memory deficit and involvement of at least one other cognitive domain are mandatory for diagnosis. Etiology may differ, but organic brain injury is implied.
Hormones	Chemical substances secreted by various endocrine glands into the blood and transported to the target tissue, exerting a specific regulatory effect on the activity; e.g. IGF-I/GH-axis, thyroid, gonadal/sex and glucocorticoid/cortisol.

ABBREVIATIONS AND ACRONYMS

11β-HSD1	11β-hydroxysteroid dehydrogenase type 1
Aβ	β-amyloid
ACTH	adrenocorticotropic hormone
AD	Alzheimer's disease
ADGT	glucuronide derivatives of androgens
ADT	androsterone
APOE	apolipoprotein E
APP	amyloid precursor protein
AUROC	area under the receiver operating characteristic
BACE1	β-site amyloid precursor protein–cleaving enzyme
BBB	blood-brain barrier
BMI	body mass index
CBD	corticobasal degeneration
CNS	central nervous system
CRH	corticotropin-releasing hormone
CS	Cushing syndrome
CSF	cerebrospinal fluid
CV	coefficient of variance
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DHT	dihydrotestosterone
DLB	dementia with Lewy bodies
DSM-IV	Diagnostic & Statistical Manual of Mental Disorders, 4th Ed.
E1	estrone
E1S	estrone sulfate
E2	17β-estradiol
E3	estriol
ELISA	enzyme-linked immunosorbent assay
FTD	frontotemporal dementia
GC	glucocorticoid
GC-MS	gas chromatography/mass spectrometry
GH	growth hormone
GHD	growth hormone deficiency
ICD	International classification of disease
IGF-I	insulin-like growth factor I

IGFBP	IGF-binding protein
IRMA	immunoradiometric assay
LC-MS	liquid chromatography/mass spectroscopy
LTL	leukocyte telomere length
MCI	mild cognitive impairment
MCT8	monocarboxylate transporter 8
MMSE	mini-mental state examination
NFT	neurofibrillary tangle
P-tau	phosphorylated tau
PCR	polymerase chain reaction
PDD	Parkinson's disease with dementia
RIA	radioimmunoassay
SMCI	stable mild cognitive impairment
SNP	single nucleotide polymorphism
T	either testosterone or telomere
T_3	3,3',5-triiodo-thyronine
T_4	thyroxine (3,5,3',5'-tetraiodothyronine)
TPO-Ab	thyroid peroxidase antibody
TRH	thyrotropin releasing hormone
TSH	thyroid stimulating hormone
T-tau	total tau
UGT	uridine glucuronosyl transferase
VAD	vascular dementia

INTRODUCTION

Cognitive impairment and dementia

Prevalence

Age is the strongest individual risk factor for dementia [1]. Estimates suggest that the global prevalence of dementia exceeds 25 million directly afflicted individuals [1-3]. The World Alzheimer Report 2009 included a comprehensive prevalence study with an estimate of 35.6 million cases. These numbers will, due to extended life expectancy, rapidly increase and are expected to reach 63 million by 2030 [3]. The age-specific prevalence of dementia varies to a relatively small extent between world regions, and a majority of patients will live in less-developed countries. In both developed and developing nations, dementia has an increasingly large societal impact, being a major cause of disability and institutionalization in older people. In addition to the human strain, as dementia affects also the patients' relatives and caregivers, the aggregated costs of dementia are already substantial and the costs will increase further. In a systematic review 2008 from The Swedish Council on Health Technology Assessment, SBU, the national magnitude of dementia was estimated to be more than 140 000 patients [4]. The majority of the demented patients is suffering from Alzheimer's disease (AD) and related conditions [2,5,6].

Brief nosological remarks

During recent decades, the differentiation of normal cognitive aging from mild dementia has sharpened considerably [7]. Furthermore, the nosology of dementia has evolved from mere distinctions between senility, degenerative dementia, and vascular insults. The concept of dementia and its classification has developed on the basis of accumulating evidence from clinicopathological entities and presumed etiological factors. Post-mortem neuropathological examination is widely regarded the gold standard for the definitive diagnosis of AD [8]. Therefore, there is an obvious need for tools that enhance early-stage diagnostic accuracy in the clinical setting. Due to ample inter-individual differences in premorbid conditions and clinical manifestations, diagnostic procedure is not seldom complicated [9,10]. Intra-individual differences in cognitive impairment is important to ascertain, making anamnestic

information and repeated cognitive testing essential for diagnosis [7]. Because potentially reversible conditions may mimic dementia, an early and broad medical evaluation is crucial [11-13]. For example can normal pressure hydrocephalus (NPH) [14], be managed by surgical intervention with ventriculoperitoneal shunt insertion [15].

Mild Cognitive Impairment

Subjective memory complaint in the elderly occurs frequently, even in the absence of clinical pathology, and people search for medical expertise at this early stage. Therefore, predicting who is at greater risk for developing dementia poses a challenge for health care systems. Various terms have been employed to characterize the cognitive decline associated with aging, including benign senescent forgetfulness, age-associated memory impairment, and age-associated cognitive decline [16-18]. Mild cognitive impairment (MCI), a term proposed in the 1980s, and propagated by the Mayo Clinic [19,20] is intended to represent an intermediate stage between normal aging and the development of pathologic aging and dementia [17]. Identifying conditions between normal aging and dementia is important for the prevention of dementia[21], because interventions targeting neurodegenerative processes likely are most effective in early-stage disease rather than manifest dementia. The MCI concept is hindered by its etiological heterogeneity, and could compromise the AD diagnosis as MCI may represent the earliest symptomatic stage of AD [22]. However, a set of brief cognitive tests (e.g. the mini-mental state examination (MMSE) [23] are proven to be useful in predicting AD in MCI [24].

Clinical diagnosis of Alzheimer's disease

More than 50 % of the patients with dementia likely suffer from AD and related disorders [1]. The International Classification of Diseases, Tenth Revision, (ICD-10) bases the presumptive clinical diagnosis of AD on the following criteria: (i) insidious onset with slow deterioration, (ii) absence of indication of other systemic or brain disease that can induce dementia, and (iii) absence of apoplectic onset or focal neurological signs early in the disease [25]; see Appendix I. Later the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) work group for standardization of clinical criteria for diagnosis of AD [26] recommended the terms *possible AD* and *probable AD*. The criteria for probable AD include

- dementia established by clinical examination documented e.g. by MMSE [23], and confirmed by other neuropsychological

testing
- deficits in two additional areas of cognition
- progressive deterioration of memory and other cognitive functions
- no disturbance of consciousness, onset between the ages of 40 and 90, most often after 65
- absence of systemic disorders or other brain disease that might account for the progressive deficits (Appendix II).

The above criteria [26] were revised for research purposes in 2007 [27] and radically rewritten in 2011 [28-30]. In parallel, the American Psychiatric Association (APA) has developed the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria [31,32], which have been harmonized with the ICD-10 [33].

Neuropathological diagnosis of Alzheimer's disease

The unequivocal diagnosis of AD is based on a combination of appropriate clinical data and neuropathological examination [34,35]. The two major histopathological hallmarks of AD are intracellular neurofibrillary tangles (NFT) and extracellular amyloid plaques [36]. Clinicopathological correlative studies have shown that both lesions, if present in sufficient numbers, particularly in the neocortex, are considered the best morphological signposts for AD. Defining criteria for the morphologic diagnosis of AD is difficult due to the phenotypic heterogeneity of the disease and overlap of AD morphology with that observed in non-demented elderly individuals. This gray zone between normal to pathologic aging and pathognomonic AD is an important diagnostic problem. The accuracy of currently used clinical diagnostic methods, clinical and neuropathological data have been examined in several studies [37,38].

The clinical presentation of AD is highly dependent on the localization of the brain lesions. The hypotrophy may be diffuse, but is characteristically more severe in the entorhinal cortex and the rest of the hippocampal region, which plays an important role in formation of memory.

Amyloid cascade theory

Two decades ago, it was first hypothesized that the neurodegeneration in AD might be caused by deposition of amyloid beta-peptide (Aβ) in the brain tissue plaques [39,40]. According to this amyloid hypothesis, accumulation of Aβ in the brain is the primary driver of the AD pathogenesis. The rest of the disease process, including the formation of NFTs containing tau protein, may

result from an imbalance between Aβ production and Aβ clearance [41]. The 42 amino acid residues-long isoform β-amyloid$_{1-42}$ (Aβ$_{1-42}$) initiates a cascade of pathological events in AD, ultimately resulting in synaptic dysfunction, neuronal loss and brain atrophy [39,40]. Aβ$_{1-42}$ results from orchestrated β-secretase and γ-secretase cleavages of the large transmembranous amyloid precursor protein (APP). Through variations in the γ-secretase cleavage site, APP processing may also yield peptides with other C-terminal amino acids, (e.g. Aβ$_{x-38}$ and Aβ$_{x-40}$). Processing of APP also produces the N-terminal soluble fragments sAPP-α and sAPP-β [42]. Support for the amyloid cascade theory comes from the finding that transgenic mice expressing a mutant form of the human APP gene, develop fibrillar amyloid plaques and Alzheimer's-like brain pathology with spatial learning deficits [43-45].

Neurofibrillary tangles

NFT are aggregates of the microtubule-associated protein tau, which have become phosphorylated and accumulated intracellularly [46]. When this occurs, the microtubules disintegrate [47], resulting in malfunctions in the biochemical communication and later in apoptosis [48]. Hence, AD may also be considered a tauopathy [49]. The tau hypothesis denotes the idea that the tau protein abnormalities could initiate the disease cascade.

Cerebrospinal fluid biomarkers for Alzheimer's disease

In AD, analyses of AD biomarkers in the cerebrospinal fluid (CSF) can monitor altered brain metabolism. Several studies have shown increased levels of total-tau (T-tau) and phosphorylated tau protein (P-tau) in the CSF in AD patients, reflecting axonal degeneration and increased tau phosphorylation, respectively [50]. In contrast, Aβ$_{1-42}$ decreases in CSF, likely secondary to peptide sequestration within plaques (Fig. 1) [50,51].

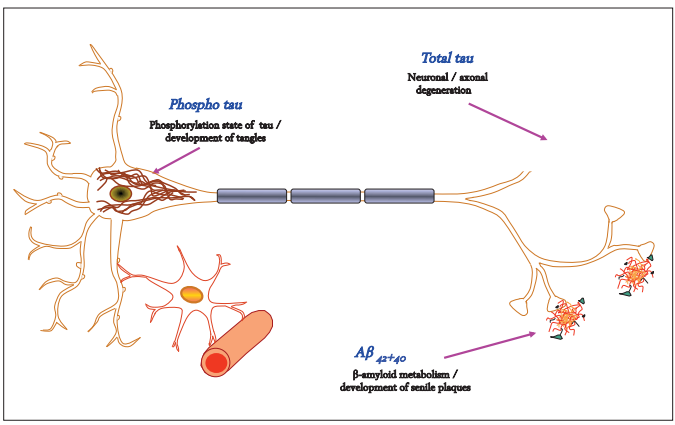

Fig. 1

Hallmarks of AD-pathology and biomarkers, by courtesy of Kaj Blennow

Used in combination, $A\beta_{1-42}$ and the tau proteins provide a diagnostic sensitivity and specificity of about 90% for clinically diagnosed AD versus controls [52]. These biomarkers are stable over time during disease progression and they are useful in the early stages of AD that precede established clinical dementia [53-56]. However, biomarkers have shown limited efficacy in diagnosing AD in heterogeneous study populations [57,58]. Moreover, candidate biomarkers like $A\beta_{x-38}$ are not routinely used.

Other forms of dementia disorders

Classification systems cannot fully account for the heterogeneity of AD and its clinical and pathologic overlap with other dementing disorders. However, in addition to AD, several other cognitive disorders with different etiologies and clinical presentations have been defined. Vascular dementia (VAD) which includes both macrovascular and subcortical small-vessel disease [59,60], is regarded as the second most prevalent dementia form amongst the elderly Europeans and North Americans. The concept of pure VAD has been challenged[61]. Although patients with VAD exhibit severe cognitive dysfunction, its predominant symptom may be reduced executive function rather than major memory loss. Therefore, some patients may not meet the necessary criterion for dementia. A broader term *Vascular Cognitive Disorder* [62,63], represents a global diagnostic category for vascular-based cognitive impairment, ranging from vascular cognitive impairment [64] to VAD.

Furthermore, it is possible to divide several other cognitive disorders on various grounds (e.g. anatomical, pathological, neurochemical or genetical). Synucleinopathies denotes a group of neurodegenerative disorders characterized by fibrillary aggregates of alpha-synuclein protein in the cytoplasm of selective populations of neurons and glia. These disorders include Parkinson's disease (PD), dementia with Lewy bodies (DLB), pure autonomic failure, and multiple system atrophy (MSA). Clinically, synucleinopathic disorders are characterized by chronic and progressive decline in motor, cognitive, behavioral, and autonomic functions, depending on the distribution of the lesions. Clinical diagnostic criteria have been developed for PD with dementia (PDD) [65] and DLB [66]. However, progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), two other diseases with to some extent similar clinical phenotypes, belong to the *taupathies*. The pathological hallmark of this group of neurodegenerative diseases is the presence of tau-positive intraneuronal filamentous inclusions, with or without additional inclusions in glial cells. Frontotemporal dementia (FTD) also shares these features [67] and for this entity clinical and pathological criteria consensus criteria have been established [68,69].

Hormones, aging and the brain

Age-related changes in hormonal concentrations

During normal aging, changes occur in the production and metabolism of various hormones, which potentially have clinical consequences. The levels of several endocrine systems show decreasing concentrations of circulating hormones [70] and some studies suggest that low hormone levels might associate with cognitive impairment in elderly individuals not afflicted by dementia [71-73]. A major question is whether age-related hormonal changes are physiologic events or pathological processes that could result in cognitive dysfunction and other adverse clinical phenotypes. Aging in humans alters almost all pituitary hormones. However, the aging-related effects are to some extent obscured by confounding factors such as gender, body composition, stress, comorbidity, use of medication, physical frailty, caloric intake, immune status, level of exercise, and neurocognitive decline [74].

The growth hormone (GH)/insulin-like growth factor I (IGF-I) axis declines with age [75,76]. At the age of 70 years, GH levels are estimated to be 20% of the levels seen at the age of 30 years [75]. Furthermore, in men the concentration of serum testosterone (T) decreases gradually with increasing age. In women, estradiol (E2) secretion is dramatically reduced at menopause [70] while compensatory increased levels of luteinizing hormone, follicle-stimulating hormone and sex hormone-binding globulin are seen. In both genders, dihydroepiandrosterone (DHEA) and its sulphate ester (DHEAS) gradually decrease with age [70]. In addition to the term menopause (E2), somatopause (GH/IGF-I), andropause (T), and adrenopause (DHEA) have been proposed but remain debatable [77-80].

Circulating triiodothyronine (T_3) might be reduced in normal aging due to alterations in thyroid-stimulating hormone (TSH) level and reduced peripheral conversion of thyroxine (T_4) to T_3, whereas there is no major change in T_4 levels [70]. In terms of the hypothalamic-pituitary-adrenal (HPA) axis, there is modest change in serum levels of cortisol and the ratio between cortisol and DHEAS are generally higher in older compared to younger subjects [81-87].

Classical hormonal syndromes and aging

Increased total body fat and reduced muscle mass are observed in hypopituitary patients with severe GH deficiency (GHD), which associates with low concentrations of circulating IGF-I [88]. Adult GHD also associates with reduced quality of life [89], decreased bone mass [90], reduced insulin sensitivity [91], impaired cardiovascular risk factors [92], and increased cardiovascular mortality [93,94]. Additionally, a later meta-analysis showed that reductions in most cognitive domains partly normalized in GHD adults following GH replacement therapy [95]. At the time when the studies included in this thesis were planned, it was proposed from our center that the clinical characteristics of adult GHD resemble normal aging [75,88,96].

Adult hypopituitarism links not only with low GH/IGF-I activity, but also with deficiencies of other hormones such as thyroid hormones, sex hormones, and adrenocorticotropic hormone (ACTH)/cortisol. At least in terms of thyroid and sex hormones, deficiencies could be risk factors for cognitive dysfunction. Both hypo- and hyperthyroidism are potentially reversible causes of cognitive impairment [97,98]. Furthermore, hypothyroidism and hypogonadism are accompanied by disturbances of body composition, alterations of cardiovascular risk factors, and wellbeing and mood disorders [99].

Cortisol deficiency is potentially life threatening, especially if the hypocortisolism results from non-productive adrenal glands (primary hypocortisolism or Mb Addison) [100]. However, high cortisol levels have been linked to a phenotype that resembles normal aging. In Cushing syndrome (CS) excess cortisol associates with impaired body composition (i.e. increased trunk fat mass), osteoporosis, muscle weakness, impaired cardiovascular risk factors, and mood disturbances [101]. Therefore, CS links with several symptoms resembling those seen in normal aging. Moreover, even in remission of the disease, patients with CS display impaired cognitive functions [102]. Hippocampal atrophy is also frequently seen in CS [103].

Hormones and the brain

Circulating levels of several hormones decline with increasing age and classic hormone deficiencies of hormones produce phenotypes that resemble normal aging. There is some additional support that hormones participate importantly in adult cognitive function or dysfunction. First, receptors for most hormones are widely distributed in the brain [104-108]. Second, several hormones participate

in the development of the central nervous system (CNS) [109-111]. In humans, IGF-I gene mutations associate with mental retardation [112] and congenital hypothyroidism might result in reduced cognitive performance later in life [113-115]. Third, several studies have shown inverse relations between low circulating hormonal levels (e.g. IGF-I, thyroid hormones, estradiol, and testosterone) and quality of life or measures of cognitive function in healthy individuals [89,116-120]. In healthy elderly subjects studied over time, elevated plasma cortisol associated with deficits in hippocampus-dependent memory tasks and loss of hippocampal volume [107,121-123]. Finally, although not fully supporting the theory that low hormonal concentrations are important for biological endpoints in healthy adults, treatment with GH and/or T improved body composition [124]. However, the effect of such treatment on functional endpoints (e.g. muscle strength) was small and side effects were common [125]. The extent to which T might be beneficial in reducing distress and improving well-being [126-128] remains unclear.

IGF-I/insulin and adult cognition

After we initiated the studies presented in this thesis, further evidence documented the importance of IGF-I for adult cognition. In experimental animals, circulating IGF-I regulates the density of blood vessels in the adult brain [129], mediates the exercise-induced increase in new neurons in the adult hippocampus [130], and associates with spatial learning and memory [131]. IGF-binding proteins (IGFBPs) closely regulate the bioavailability of IGF-I [111]. In the circulation, the quantitatively most important is IGFBP-3 [132]. IGFBP-3 could inhibit some of the neuroprotective actions of IGF-I by sequestering IGF-I from its receptor, thereby reducing cell proliferation and inducing apoptosis [132].

Brain IGF-I derive from the passage of IGF-I across the blood-brain barrier (BBB) [133] and from local production of IGF-I in the CNS [134,135]. IGF-I participates in Aβ processing in the brain, and systemic treatment with IGF-I reduced the Aβ burden in Tg2576 mice overexpressing a mutant form of human APP (APP695 KM670/671NL) [136]. At the level of the BBB, the choroid plexus endocytic receptor megalin may mediate IGF-I-induced clearance of Aβ and may also be involved in IGF-I transport into the brain [137]. In addition to the transport of IGF-I across the BBB involving megalin, also known as low-density lipoprotein receptor related protein 2 (LRP2), an earlier study demonstrated that, in response to neuronal activity, IGFBP-3 can bind to the LRP1 receptor in the choroid plexus, thereby inducing transcytosis of IGF-I through the BBB [138]. However, the role of IGF-I in the development and progression of AD is complex and not fully understood. In a mouse

model of AD, heterozygous inactivation of the IGF-I receptor reduced behavioral impairment [139]. This protection correlated with Aβ aggregation into packed, ordered plaques, possibly reducing the effects of the toxic soluble Aβ oligomers [139]. In patients with AD, several studies have reported conflicting results with both reduced [140-142] and increased [143-145] circulating concentrations of IGF-I. In CSF, IGF-I levels were unchanged [143] or increased [145] in AD patients.

In mammals, insulin can partially activate the IGF-I receptor [131]. Both insulin receptor and IGF-I receptor signaling regulate vital growth, survival and metabolic functions in the brain [146,147]. One study observed resistance to insulin and IGF-I signaling in the brains of AD patients without diabetes [148], and neurons that are resistant to insulin receptor/IGF-I receptor signaling might degenerate [147,149]. Some epidemiological studies pointed to a link between hyperinsulinemia/type 2 diabetes mellitus and increased risk of AD [150,151]. However, other studies failed to confirm plasma insulin as an independent risk factor for the development of dementia [152].

Thyroid hormones and adult cognition

Cognitive performance is dependent on adequate glucose supply to the brain and thyroid hormones could be one of the regulators of brain glucose metabolism [153]. Findings from our center already some decades ago suggested that AD patients could have a higher prevalence of hypothyreosis compared to an age-matched healthy population [154]. More recent cross-sectional studies reported that either increased or decreased circulating TSH levels within the normal range related to poor cognitive performance[73,155]. Increased serum total T3 level associated with a neuropsychological profile typical of prodromal AD in patients with MCI compared to healthy controls [156]. In several studies, subclinical hyperthyroidism associated with increased risk of dementia [157-161], while the results of some studies suggested that subclinical hypothyroidism might be a risk factor for the development of dementia [158,162,163]. However, some studies could not demonstrate an association between AD and thyroid disease [164,165]. Furthermore, both subclinical and clinical hypothyroidism impair cardiovascular risk factors [166], and increased serum levels of TSH have been observed in VAD [167].

Because incidence of thyroid autoantibodies may be increased among familial AD kindred's, AD could associate with autoimmune thyroid disease [168]. Furthermore, Alzheimer-related neurodegeneration may lead to reduced secretion of hypothalamic thyrotropin-releasing hormone (TRH) or

alterations in pituitary responsiveness to TRH that manifest as reduced levels of thyroid hormones [169]. The hippocampus of post-mortem AD brains show TRH depletion, which could worsen AD pathology by enhancing phosphorylation of tau proteins mediated by changes in phosphokinases activity [170]. Moreover, hypothyroidism in rodents is associated with increased expression of APP genes [171], suggesting that low levels of thyroid hormones in the CNS may increase APP expression, possibly resulting in increased accumulation of Aβ in brain tissue [171].

In a study that examined the influence of five single nucleotide polymorphisms (SNPs) of the thyroid hormone receptor alpha gene, the genetic variability related to the risk of AD [172]. Furthermore, experimental studies have shown that thyroid hormones participate importantly in the development and maintenance of the basal forebrain cholinergic neurons typically involved in AD [173].

Sex hormones and adult cognition

In addition to sex steroid secretion from the gonads, the adrenal glands secrete DHEA and DHEAS [174]. Thus, the adrenal glands are the major source of circulating sex steroids in postmenopausal women. Inactive DHEAS can be converted to DHEA in peripheral tissues and then processed intracellularly to active androgens or estrogens. Local regulation of the synthesis and degradation of androgens permit peripheral target tissues to adjust the formation and metabolism of sex steroids according to local needs [175]. Several studies in healthy subjects showed inverse associations between circulating sex steroid levels and cognitive function [176,177]. However, the Women's Health Initiative Memory Study challenged the concept that low E_2 levels participate in an age-associated decrease in women's cognitive function and indicated that estrogen-based replacement therapy might induce a small increase in the risk of MCI and AD [178-181].

Because previous studies have shown conflicting results [174,177,182], there is currently no clear consensus in terms of circulating levels of sex steroids in patients with AD or other forms of dementia. In the AD brain, increased production of DHEA correlates with DHEA levels in the CSF [183]. However, the regulation of DHEA metabolism in the CNS is complex. An earlier study reported that lower levels of DHEAS and unchanged levels of metabolites 7α-hydroxy-DHEA, 7β-hydroxy-DHEA, and 16α-hydroxy-DHEA accompanied increased DHEA values in the CSF of patients with AD or VAD [184].

Glucocorticoid hormones and adult cognition

Glucocorticoid hormones (GCs) exert numerous and potent effects on the CNS, including on learning and memory [185]. In stressful situations, cognition is affected by catecholamines and GCs. Short term exposure of the latter could be beneficial e.g. by modulating synaptic plasticity. However, prolonged exposure to excess GCs could have detrimental effects on neurons. Cognitive impairments involving declarative memory induced by GCs and stress are probably mediated by changes in the hippocampus [101]. This region has a high concentration of glucocorticoid receptors (GRs) and might be more vulnerable to aging and to long-term stress than other parts of CNS [186]. Stress-related steroids could affect the hippocampus in at least three ways: first, by reducing the excitability of some hippocampal neurons; second, by inhibiting the genesis of new neurons in the dentate gyrus; third, by causing hypotrophy of dendrites in pyramidal cells of the CA3 region [186]. Humans who had experienced severe, long-lasting traumatic stress showed atrophy of the hippocampus more than of other parts of the brain [187]. Cognitive dysfunction in stress-related exhaustion may be linked to distinct personality traits, low quality of life, and a decreased ACTH response to corticotropin-releasing hormone (CRH) [188].

Telomere length and Alzheimer's disease

Age is the strongest individual risk factor for AD, but the mechanistic relation between AD and aging is unclear. Telomeres protect chromosomes from illegitimate recombination and degradation [189,190]. Telomere length decreases with the increasing number of cell divisions [191]. Telomerase can preserve telomere length by adding tandem repeats de novo at chromosome ends [192]. However, telomerase activity is too low to enable full maintenance of telomere length, because telomere length decreases in human cells (e.g. peripheral leukocytes) with increasing donor age [193,194]. Therefore, the shortening of telomeres that accompanies increasing age may act as a mitotic clock and a biomarker of aging that determines the number of divisions a cell can undergo [191].

In peripheral leukocytes, short telomeres associate with reduced survival [195] and increased risk of age-associated phenotypes [196-201]. Some studies have observed an association between low leukocyte telomere length (LTL) and age-related cognitive decline in elderly individuals [200,202], but this association has been relatively small or absent in other studies [203-205]. One study reported a

modest association between decreasing telomere length and increased cognitive decline [206].

Previous studies have reported conflicting results whether low LTL is a risk factor for the development of dementia. In Down syndrome, reduced telomere length associated with MCI and dementia status [207-209]. In patients with AD, telomere length associated with disease status in one study [210] and mortality in another study [211]. In a cross-sectional study, the odds ratio for VAD was two-fold lower in individuals with long telomeres, and increased to more than three-fold in patients with short telomeres [212]. Furthermore, in non-demented stroke survivors, telomere length predicted post-stroke cognitive decline [213]. However, in three relatively large studies of very old subjects, telomere length did not predict dementia [214,215] or progression of MCI to dementia [216].

PATIENTS AND METHODS

Ethical considerations

We obtained oral and written informed consent from all participants. The ethical committee of University of Gothenburg approved all study procedures in *Papers I-V*.

Study participants

The study population in *Papers I-V* consisted of 60 community-dwelling Caucasian patients (30 men and 30 women) admitted for evaluation of cognitive impairment by their general practitioner to the Memory Clinic at Skaraborg Hospital in Falköping, Sweden. Prior to referral for specialist medical examination and conforming to local guidelines, all patients had undergone a routine medical examination to establish cognitive impairment and exclude secondary forms of dementia. A single specialized physician (the respondent) recruited all participants consecutively during 2000-2008. Aside from referral to Falköping Hospital for evaluation of suspected dementia, inclusion criteria comprised age (65-80 years), body mass index (BMI) (20-26 kg/m^2), and waist:hip ratio (0.65-0.90 in women and 0.70-0.95 in men). Exclusion criteria comprised serum creatinine > 175 mmol/L, diabetes mellitus, previous myocardial infarction, malignancy including brain tumor, subdural hematoma, ongoing alcohol abuse, medication with cortisone, and previous or present medication with acetylcholine esterase inhibitors. The study also included 20 age-matched healthy controls (10 men and 10 women) recruited contemporaneously from the same geographical area among spouses of the included patients or by advertisements in local newspapers. The control subjects had no subjective symptoms of cognitive dysfunction. The patients and the controls were matched groupwise in terms of age, gender, BMI, and waist:hip ratio (Table 1 in *Paper I*).

In *Paper III*, individuals receiving thyroxine therapy were excluded. Therefore, 59 patients (29 men and 30 women) and 19 controls (9 men and 10 women) were included in this study. In *Paper IV*, the study excluded patients and controls medicated with estradiol (E2), selective estrogen receptor modulators (SERMs), estriol (E3), or plant extracts with sex steroid activity.

Therefore, 50 patients (26 men and 24 women; D group) and 18 controls (9 men and 9 women; C group) were included in that analysis.

The strength of the mono-center studies presented in this thesis (*Papers I-V*) is that they were highly controlled regarding diagnostic and analytical procedures. All patients and controls were Caucasians and they were matched in factors that potentially could influence hormonal levels. No patient had ever received treatment with an acetylcholinesterase inhibitor and none of the participants received medication containing GCs. However, there were limitations. Because the design was cross-sectional, we could not follow our subjects longitudinally, except diagnostically for MCI patients. The study population was relatively small and the in- and exclusion criteria were relatively strict, i.e. frail patients were not included.

Diagnostic criteria

An independent specialized physician (O.H.) assessed all diagnoses in *Papers I-V*. The presence or absence of dementia was diagnosed according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (DSM-IV) [31]. Patients with dementia were classified as having AD [26] or VAD according to the requirements by *National Institute of Neurological Disorders and Stroke* in collaboration with *Association Internationale pour la Recherche et l'Enseignement en Neurosciences* (NINDS-AIREN) [59] or the guidelines by Erkinjuntti et al. for subcortical VAD [60]. FTD was diagnosed according to criteria by Neary and coworkers [69]. The consensus criteria by McKeith and coworkers [66] were applied when diagnosing DLB. The diagnosis of PDD was used as described by Emre and coworkers [65].

MCI was diagnosed in patients with cognitive impairment that did not fulfill the criteria for dementia[217]. MCI patients were followed at least annually for a median of 3 years (range, 1-7 years) to evaluate whether they later developed dementia. Table 1 in *Paper I* shows the causes of the cognitive impairment in the included patients. At primary evaluation, brain imaging revealed that 6 of the 24 AD patients showed signs of additional vascular pathology, but these patients did not differ from the remaining AD patients regarding CSF levels of AD biomarkers $A\beta_{1-42}$, T-tau or P-tau. During follow-up visits, 13 MCI patients remained in SMCI. Other patients progressed to dementia and were diagnosed with AD (n = 7), VAD (n = 3), or FTD (n = 1). CSF levels of $A\beta_{1-42}$, T-tau, or P-tau in MCI patients diagnosed with AD on follow-up visits did not differ in compared to those in patients with established AD at baseline.

Cognitive and physical examination

Together with careful clinical history, functional assessment and brain imaging, physical, neurological, and psychiatric examinations were performed at baseline. Cognitive testing was performed using MMSE [23], clock drawing-test (CDT) [218], cube-test [219], and counting [220]. Depressive symptoms were assessed by the 20-item Geriatric Depression Scale (GDS-20), a self-reported questionnaire developed to detect depressive symptoms in older populations [221]. The results of all these tests are given in *Paper I* whereas only MMSE score is presented in *Papers II-V*.

All study procedures were performed similarly in both patients and controls. On the morning of test day, all participants were fasting. Their body weight, height, and waist:hip ratio were determined; blood samples drawn, and lumbar puncture performed.

Body weight was measured to the nearest 0.1 kg, and body height was measured barefoot to the nearest 0.01 m. BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured in the standing position, using a flexible plastic tape placed midway between the lower rib margin and the iliac crest, and hip girth was measured at the widest part of the hip.

Clinical chemistry

Laboratory tests included routine blood analyses as well as analysis of apolipoprotein E (APOE) [222] genotype and albumin ratio (CSF albumin [mg/L]/ serum albumin [g/L]). All CSF samples were collected by lumbar puncture in the L3/L4 or L4/L5 interspace at the standardized time point 8:30 a.m.-9:00 a.m. The first 12 mL of CSF was collected in a polypropylene tube and immediately transported to the local laboratory for centrifugation at 2.000 g and +4˚C for 10 min. The supernatant was pipetted off, gently mixed to avoid possible gradient effects, and aliquoted in polypropylene tubes that were stored at -80°C pending biochemical analyses, without being thawed and refrozen.

Blood samples were drawn subsequent to the lumbar puncture and serum was prepared by centrifugation after coagulation at room temperature for 15–30 min, then aliquoted and stored in cryotubes at -80°C pending biochemical analyses, without being thawed and refrozen.

All biochemical analyses were performed at one occasion by experienced laboratory technicians, using the same batch of reagents. All analysts were blinded to the clinical diagnoses and other clinical information. CSF biomarkers were measured at the Clinical Neurochemistry Laboratory in Mölndal, Sweden. The results are presented in *Paper I* and have been employed in the correlation analyses presented in *Papers II, III* and *V*.

CSF markers of amyloid metabolism

In AD, $A\beta_{1-42}$ levels in CSF typically decrease, likely secondary to peptide sequestration within plaques [223]. CSF $A\beta_{1-42}$ levels were determined using INNOTEST® ELISA assay technology (Innogenetics, Ghent, Belgium) [224]. CSF sAβPPα and sAβPPβ levels were determined using the MSD® sAβPPα/sAβPPβ Multiplex Assay, as described by the manufacturer (Meso Scale Discovery, Gaithersburg, MD, USA) [225]. This assay employs the 6E10 antibody to capture sAβPPα and a neoepitope-specific antibody to capture sAβPPβ. Both isoforms are detected by SULFO-TAG™-labeled anti-AβPP antibody p2-1. CSF $A\beta_{x-38}$, $A\beta_{x-40}$ and $A\beta_{x-42}$ were measured using the MSD® Human/Rodent (4G8) Abeta Triplex Assay as described by the manufacturer (Meso Scale Discovery). This assay employs C-terminal-specific antibodies to specifically capture $A\beta_{x-38}$, $A\beta_{x-40}$ and $A\beta_{x-42}$. All isoforms were detected by SULFO-TAG™-labeled 4G8 detection antibody.

CSF markers of neural cell damage

CSF levels of T-tau and tau phosphorylated at threonine 181 (P-tau181) typically increase in AD [223]. These axonal damage markers were measured using INNOTEST® ELISA assays [46,226].

IGF-I, insulin and albumin ratio

In *Paper II*, we measured IGF-I, IGFBP-3 at the Department of Clinical Chemistry at Sahlgrenska University Hospital, Göteborg, Sweden, and insulin in serum and CSF at Lund University Hospital. Regarding CSF values, a weakness is that hormonal levels in CSF may not truly reflect those in the brain due to potential differences in permeability between the blood-CSF barrier and the BBB in AD patients or that IGF-I could be sequestered locally in the microenvironment.

IGF-I in serum and CSF were measured using ELISA (Mediagnost, Tübingen, Germany) with inter- and intra-assay coefficients of variance (CVs) less than 6.8 and 6.7%, respectively. IGFBP-3 in serum and CSF were measured using

ELISA (Mediagnost) with inter- and intra-assay CVs less than 6.3 % and 4.5 %, respectively. Measurable values of IGF-I and IGFBP-3 in CSF were obtained by not diluting the samples as normally performed for serum samples. The limits of quantification of the analytes measured in CSF were 0.01 ng/mL for IGF-I and 0.02 ng/mL for IGFBP-3.

Insulin was analyzed with an ultrasensitive sandwich immunoassay ELISA technique using double monoclonal antibodies against insulin (Mercodia, Uppsala, Sweden). This assay is specific for insulin and does not cross-react with intact proinsulin or des-31,32-proinsulin. The intra- and inter-assay CV is <3%.

Albumin levels were measured by immunonephelometry on a Beckman Immage Immunochemistry system (Beckman Instruments, Beckman Coulter, Brea, CA, USA). The albumin ratio was calculated as CSF albumin (mg/L) / serum albumin (g/L) and used as a measure of the BBB function [227].

Thyroid hormones

TSH, free T_4, total T_4, free T_3 and total T_3 in serum and CSF were analyzed in *Paper III* using electrochemiluminescent immunoassays (Roche Diagnostics, Mannheim, Germany) with CVs ≤ 2%, 10%, 5%, 10%, and 8%, respectively. Thyroid peroxidase antibody (TPO-Ab) concentrations were determined with the BRAHMS luminometric test anti-TPO (Henning, Berlin, Germany). Transthyretin was measured with kinetic nephelometry on an IMMAGE 800 analyzer (Beckman Coulter, Fullerton, CA, USA). The limits of quantification of the analytes measured in CSF were 0.01 mIU/L for TSH, 0.90 nmol/L for total T_4, 0.30 nmol/L for total T_3, and 0.02 g/L for transthyretin.

Sex steroids

The glucuronide derivative of androsterone (ADT) and the 3-glucuronidated (3G) and 17-glucuronidated (17G) form of 3α-diol-glucuronides can be measured using a liquid chromatographic/mass spectroscopic (LC-MS/MS) method [175,228-232]. Three enzymes (i.e. uridine glucuronosyl transferases 2 B7 [UGT 2 B7], UGT 2 B15, and UGT 2 B17) glucuronidate all androgens and their metabolites in the peripheral tissues in humans [175]. The combined measure of the glucuronide derivatives of androgens (ADTG + 3G + 17G) can be used as a marker of the total pool of androgens in both women and men [228-231]. For estrogens, a reliable parameter of total estrogen secretion and processing (comparable to the glucuronides identified for androgens) remains

undetermined. The inter-relations between the sex steroids measured in this study are given in Fig. 1 in *Paper IV*.

In *Paper IV*, levels of sex steroids were measured using either gas chromatography-mass spectrometry (GC-MS) or LC-MS technique. Earlier results suggest that the results of measurements of total and free T attained with GC-MS correlate better with biological phenotypes than measurements using a conventional RIA [231,232].

GC-MS [228,231-233] was used to measure DHEA (limit of detection, 0.20 ng/mL), 5-diol (limit of detection, 0.10 ng/mL), 4-dione (limit of detection, 0.05 ng/ml), T (limit of detection, 0.05 ng/ml), dihydrotestosterone (DHT) (limit of detection, 0.02 ng/mL), E1 (limit of detection, 5.00 pg/mL), and E2 (limit of detection, 1.00 pg/mL). GC-MS used a 50% phenyl-methylpolysiloxane capillary column with helium as carrier gas. The analytes and internal standard were detected using a HP5973 quadrupole mass spectrometer equipped with a chemical ionization source.

DHEAS (limit of detection, 0.075 µg/ml), ADTG (limit of detection, 2.00 ng/ml), 3G (limit of detection, 0.50 ng/ml), 17G (limit of detection, 0.50 ng/ml), and E1S (limit of detection, 0.075 ng/ml) were analyzed using a LC-MS/MS method [228,231-233]. This system uses a 4-mm particle size Synergy Hydro-RP column at a flow rate of 1.0 mL/min and the analytes are detected using a Sciex API3000 triple quadrupole mass spectrometer equipped with TurboIonSpray. The retention times of ADTG, 3G, and 17G correspond to those of the synthetic standards; the multiple reaction monitoring used to detect the analytes further ensure the specificity of the method. The highly specific GC-MS and LC-MS/MS techniques avoid the cross-reactivity seen using immunoassays [228,231-233].

SHBG was measured using an immunoradiometric assay (IRMA; Orion Diagnostics, Esboo, Finland; intra-assay CV, 3%; inter-assay CV, 7%). CSF samples were not diluted as normally performed for serum samples (the dilution buffer contains no substances that affect the test performance according to the manufacturer), thereby having measurable SHBG values using IRMA (Orion Diagnostics).

Analysis of leukocyte telomere length

In *Paper V*, genomic DNA was extracted from frozen whole blood using the GenoM-48 robot and the MagAttract DNA Blood Mini M48 Kit (Qiagen, Valencia, CA) and stored at -80°C for 4 months pending analysis. LTL was

determined using the quantitative polymerase chain reaction (Q-PCR) method described by Cawthon [234]. LTL measurement by the Q-PCR assay determined the relative ratio of telomere (T) repeat copy number to a single copy gene (S) copy number (T/S ratio), as previously described [235]. This ratio is proportional to the average telomere length. 36B4 encoding acidic ribosomal phosphoprotein P0 was used as the single copy gene. Each sample was run in duplicate or triplicate. Two master mixes of PCR reagents were prepared, one with telomere primer pairs [236].

The final concentration of the primers was 0.32 pmol/µL. An aliquot of 5 ng (2 µL) template DNA was added containing 5 µl SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). The final volume of each reaction was 10 µL.

The DNA quantity standards were serial dilutions of a reference DNA sample (a mixture of several DNAs) to produce six final concentrations measuring 0.031 – 1.0 ng/µL. On each plate, a standard curve and a negative control (water) were included. PCR was done on a 384-well ABI 7900HT TaqMan platform (Applied Biosystems). The thermal cycling profile and the generation of standard and dissociation curves were performed according to procedures described previously [235]. The CV was 10.7%.

RESULTS

The whole study population is described in *Patients and Methods*. Results of the cognitive testing are presented in Table 2 in *Paper I*. Regarding hereditary factors, AD patients showed a higher frequency of the APOE ε4 allele than the other groups (Table 3 in *Paper I*). There were no group differences for history of arterial hypertension, cardiac insufficiency, angina pectoris, atrial fibrillation, or hyperlipidemia (data not shown). Understandably, previous strokes were more common in patients with other dementias [n=4 (27%)] than in controls [n=0(0%); p=0.026]. Years of education were lower in patients with other dementias (median 7; range 6–13 years) than in controls (9; 7–14 years; $p<0.05$), but otherwise there was no significant difference between groups (education level in AD group: 7, 6–15 years; stable MCI group: 9, 6–14 years). No investigated CSF biomarker correlated with age or albumin ratio in the whole population or in any study group ($P>0.05$).

Alzheimer's disease biomarkers in cerebrospinal fluid

The univariate analysis in *Paper I* showed lower $A\beta_{1-42}$ levels and higher T-tau and P-tau levels in AD patients compared to controls and SMCI patients ($P<0.001$). In addition, patients with other dementias had lower P-tau levels than controls. For the additional investigated amyloid biomarkers, AD patients had lower $A\beta_{x-42}$ than controls ($P<0.001$) and SMCI patients ($P=0.007$) but $A\beta_{x-38}$, $A\beta_{x-40}$, sAPP-α, or sAPP-β levels did not differ between groups. Patients with other dementias had lower $A\beta_{x-38}$, $A\beta_{x-40}$, sAPP-α and sAPP-β than most other groups. Finally, AD patients had higher $A\beta_{x-38}/A\beta_{x-42}$ and $A\beta_{x-40}/A\beta_{x-42}$ ratios than all other groups (Table 4 in *Paper I*) [237].

In *Paper I*, controls and SMCI patients were merged into one group in multivariate analysis. Although SMCI patients had lower $A\beta_{1-42}$ than controls in the univariate analysis, the group merging was supported by a multivariate discriminate analysis where controls and SMCI patients were not separated using the combination of all investigated CSF biomarkers. AD patients were compared to the merged control-SMCI group and to patients with other dementias, using multivariate discriminant analysis (Figure 2 in *Paper I*). The corresponding receiver operation characteristics (ROC) analysis is shown in Figure 3 in *Paper I*. AD patients were significantly distinguishable ($P<0.0001$)

from all other groups, using the classical panel of $A\beta_{1-42}$, T-tau and P-tau, as well as the extended optimal panel, which included all additional amyloid related biomarkers.

IGF-I, IGFBP-3, and insulin

In *Paper II*, levels of IGF-I, IGFBP-3, or insulin in serum or CSF did not correlate with the CSF/serum albumin ratio [238]. Serum IGF-I was increased in AD patients and in patients with other dementias compared to healthy controls (P=0.01 and P<0.05, respectively). Serum IGFBP-3 concentration was higher in AD and SMCI patients compared to controls (P=0.01 and P<0.05, respectively). The serum IGF-I/IGFBP-3 ratio, which is a measure of bioactive IGF-I, and serum insulin level, were similar in all study groups. CSF levels of IGF-I and IGFBP-3 as well as the IGF-I/IGFBP-3 ratio in CSF did not differ between groups. CSF insulin level was similar in all study groups (*Paper II*).

The CSF/serum IGF-I ratio was lower in AD patients and patients with other dementias compared to healthy controls (Fig. 2A) (P=0.02 and P=0.002, respectively, *Paper II*) and in patients with other dementias compared to SMCI patients (P=0.04). CSF/serum levels of IGFBP-3 were lower in patients with AD, other dementias, or SMCI compared to controls (Fig. 2B) (P=0.001, P=0.01, and P=0.002, respectively), whereas the CSF/serum insulin ratio was similar in all study groups (*Paper II*).

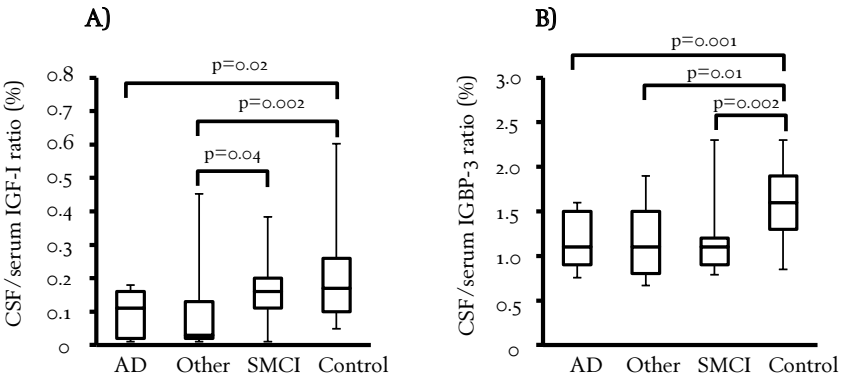

Fig. 2

CSF/serum ratios of A) IGF-I and B) IGFBP-3 in the study population of patients with AD (n=32), other dementias (n=15), SMCI (n=13), and healthy controls (n=20). Values in the box plots are given as medians (horizontal lines), 25th-75th percentiles (boxes), and ranges (whiskers). Between-group differences were assessed using the Kruskal-Wallis test for multiple variables, followed by the Mann-Whitney U test for pair-wise comparisons.

Correlation analyses performed in *Paper II* sought to determine whether the IGF-I system associates with AD biomarkers and MMSE score. Only variables that significantly differed between the study groups were included in the correlation analyses. In the total study population (n=80), serum levels of IGF-I and IGFBP-3 both correlated negatively with CSF $A\beta_{1-42}$ level (r=-0.29, *P*=0.01 and r=-0.27, *P*=0.02, respectively). The CSF/serum IGFBP-3 ratio correlated positively with CSF $A\beta_{1-42}$ level (r=0.32, *P*=0.004). In the AD group (n=32), the CSF/serum IGF-I ratio correlated positively with CSF P-tau level (r=0.42, *P*=0.02) (*Paper II*). Serum or CSF values of IGF-I, IGFBP-3 or insulin did not correlate with MMSE score in the total study population or in the AD group.

Thyroid hormones

In *Paper III,* two individuals receiving levothyroxine were excluded. Serum TSH concentration was higher in AD patients compared to patients with other dementias (*P*<0.05) and SMCI patients (*P*<0.01), but no significant difference was observed compared to healthy controls (P=0.09). Serum total and free T_4 and T_3 levels as well as serum total T_4/T_3 and free T_4/T_3 ratios were similar in all study groups. Serum transthyretin levels were higher in AD and SMCI patients compared to healthy controls (*P*<0.01 and *P*<0.05, respectively) (*Paper III)* [239].

Serum TPO-Ab level did not differ between groups (*Paper III*). Five AD patients (3 AD and 2 MCI-AD) and 2 SMCI patients had serum TPO-Ab levels above the upper reference range ≥ 60 kIU/L. After excluding patients with elevated serum TPO-Ab level, the serum TSH level remained elevated in AD patients compared to patients with other dementias and SMCI patients (both *P*<0.05). Furthermore, after this exclusion, the level of serum transthyretin remained elevated only in AD patients compared to healthy controls (*P*<0.01) (*Paper III*).

CSF TSH level was similar in all study groups (*Paper III*). Total T_4 level in CSF was lower in patients with AD and in patients with other dementias vs. SMCI patients (both *P*=0.01) and vs. controls (both *P*=0.001). Furthermore, the ratio between total T_3 and total T_4 level in CSF was higher in patients with AD and other dementias compared to healthy controls (Fig. 3) (*P*<0.01 and *P*<0.05, respectively). We were unable to measure transthyretin level in CSF.

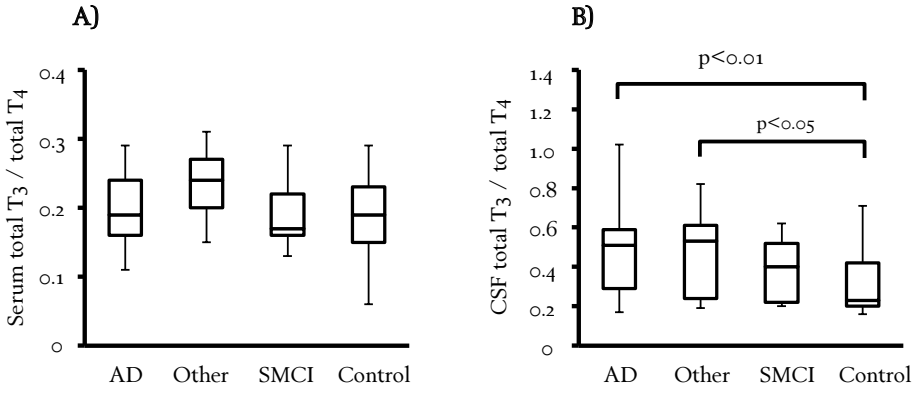

Fig. 3

Ratios between total T_3 and total T_4 in A) serum and B) CSF in the study population of patients with AD (n=31), other dementias (n=15), SMCI (n=13), and healthy controls (n=19). Values in the box plots are given as medians (horizontal lines), 25th-75th percentiles (boxes), and ranges (whiskers). Between-group differences were assessed using the Kruskal-Wallis test for multiple variables, followed by the Mann-Whitney U test for pair-wise comparisons.

The observed between-group differences remained after exclusion of patients with elevated TPO-Ab levels (CSF T_4; AD and other dementias vs. control (both P=0.001; CSF T_3/T_4 ratio; and AD and other dementias vs. control, P<0.01 and P<0.05, respectively) (*Paper III*).

In *Paper III,* in the total study population (n=78), serum TSH levels correlated positively with CSF levels of TSH (r=0.62, P<0.0001), T-tau (r=0.26, P<0.05; Fig. 1A), and P-tau (r=0.24, P<0.05), and negatively with CSF total T_4 level (r=-0.37, P<0.01). Moreover, total T_4 levels in CSF correlated positively with serum free T_4 level and MMSE score (both r=0.26, P<0.05), and negatively with CSF T-tau level (r=-0.23, P<0.05). Serum transthyretin levels correlated negatively with the CSF level of sAPP-α (r=-0.27, P<0.05) (*Paper III*). Otherwise, there were no correlations between thyroid hormone levels and CSF biomarkers, MMSE score or anthropometric data (data not shown).

In the AD group (n=31), serum TSH level or CSF total T_4 level did not correlate with CSF T-tau level or MMSE score (data not shown). However, serum TSH correlated inversely with CSF $A\beta_{1-42}$ level in the AD group (r=-0.41, $P<0.05$) and serum transthyretin correlated negatively with sAPP-α (r=-0.51, $P < 0.01$) and sAPP-β (r=-0.50, $P<0.01$) (*Paper III*).

Sex steroids and cortisol

In *Paper IV,* individuals medicating with estradiol, selective estrogen receptor modulators (SERMs), estriol, or plant extracts with sex steroid activity were excluded. Aiming to compare gender-related differences, we merged all patient groups (AD, other dementias, and SMCI) into one large group (D group). Next, we performed analyses between D group and controls (C group) in the total study population and performed separate analyses to determine any between-differences in men or women.

D group patients showed higher levels of cortisol in 24-h urine and increased adrenal-derived androgen precursors DHEAS and DHEA in serum. We also observed higher serum concentrations of ADT, E1, and E1S in D group compared to C group (Fig. 4). Serum levels of other sex steroids were similar in both groups in the total study population. When men and women were analyzed separately, serum levels of E1 and E1S were higher in both males and females in D group compared to C group (Fig. 4). In addition, the 24-hour urine level of cortisol and serum concentrations of DHEA, 4-dione, and ADT were higher in D group females compared to C Croup females (*Paper IV*).

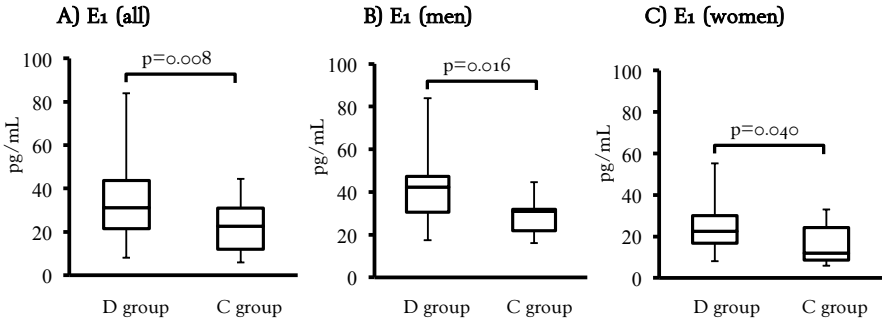

Fig. 4

Serum E1 concentration in A) the total study population, B) men, and C) women in 50 patients with cognitive impairment (26 men; D group) and 18 healthy controls (9 men; C group). Values in the box plots are given as medians (horizontal lines), 25th-75th percentiles (boxes), and ranges (whiskers). Between-group differences were assessed using the Mann–Whitney U-test.

Serum SHBG concentration was similar in D group and C group in the total study population and also in men and women analyzed separately (*Paper IV*). We found measurable levels of SHBG in the CSF of all patients and controls, but such levels did not differ between groups in the total study population or in men. However, the CSF concentration of SHBG in D group women was lower than that in healthy female controls (*Paper IV*).

Despite advanced technology, we were unable to determine the levels of most sex hormones in the CSF (*Paper IV*). Using GC-MS, the level of 4-dione was ≥ the detection limit in 25 of the D group patients and 8 controls, and levels did not differ between groups ($p=0.83$). We observed measurable T levels in the CSF of 15 men and detected no difference between groups (data not shown). Other sex steroids were not measurable in CSF using GC-MS or LS-MS (*Paper IV*).

Compared to the remaining patients in D group (n=25), subanalyses showed that AD patients (n=25) had similar values for sex steroids and cortisol (*Paper IV*). Differences between AD patients and healthy controls were similar to the differences observed in the total study population, with AD patients having higher serum levels of DHEAS, E1, E1S, and ADT (all $p<0.05$ vs. controls; data not shown). The CSF level of SHBG was lower, and the 24-hour urine and CSF cortisol levels were higher in AD patients compared to controls (all $p<0.05$, data not shown) (*Paper IV*).

Serum and CSF concentrations of cortisol correlated positively in the total study population (n=64; $r=0.61$, $p<0.001$), in men (n=32; $r=0.54$, $p=0.001$), and in women (n=32; $r=0.69$, $p<0.001$) (*Paper IV*). CSF and 24-hour urine cortisol also correlated positively in the total study population as well as in men and women (data not shown). CSF cortisol correlated negatively with MMSE score in the total study population ($r=-0.33$, $p=0.01$) as well as in women ($r=-0.52$, $p<0.01$). Furthermore, in the total study population with measurable 4-dione in CSF, 4-dione correlated positively with that in serum (n=33; $r=0.68$; $p<0.001$). Serum and CSF concentrations of SHBG correlated positively in the total study population (n=68; $r=0.59$, $p<0.001$), in men (n=35; $r=0.54$, $p=0.001$), and in women (n=33; $r=0.63$, $p<0.001$). We observed no correlation between CSF levels of 4-dione or SHBG and MMSE score (data not shown).

Correlation analyses were performed between MMSE score and serum levels of cortisol and sex hormones (*Paper IV*). In the total study population

(n=68), MMSE score correlated negatively with serum ADT (-0.40, p<0.01) and serum DHEA (r=-0.29, p<0.05). In men (n=35), MMSE score correlated negatively with serum levels of ADT (r=-0.54, p=0.001), E1 (r=-0.46, p<0.01), DHEAS (r=-0.40, p<0.01), DHEA (r=-0.38, p<0.05), E1S (r=-0.36, p<0.05), and 4-dione (r=-0.35, p<0.05). In women (n=33), MMSE score correlated negatively with serum levels of cortisol (-0.42, p<0.05), ADT (-0.42, p<0.05), E1 (r=-0.39, p<0.05), and E1S (r=-0.36, p<0.05) (*Paper IV*).

Leukocyte telomere length

In *Paper V*, LTL in patients with AD (n=32) and in patients with other dementias (n=15) was similar to that in healthy controls (n=20). However, patients with SMCI (n=13) had shorter LTL compared to AD patients (p=0.02) and controls (p=0.008; Fig. 1A in *Paper V*). Subanalyses within the AD group showed that LTL in patients with MCI that later converted to AD (n=7) was similar to that in patients who received a clinical diagnosis of AD dementia during primary evaluation (n=25) and healthy controls, but higher compared to the SMCI group (p=0.02) (Fig 1B in *Paper V*). LTL in AD patients (n=24) who were highly positive for CSF biomarker levels (arbitrarily defined as $A\beta_{1\text{-}42}$ 530 ng/l, T-tau>350 ng/l, and P-tau>60 ng/l) was similar to LTL in the remaining AD patients (n=8; Fig. 1C in *Paper V*). LTL did not associate with APOE genotype (*Paper V*).

We observed no correlations between LTL and age, MMSE score, or any of the anthropometric variables in the total study population or in the AD group (*Paper V*, data not shown). Additionally. LTL did not correlate with CSF levels of $A\beta_{1\text{-}42}$ (r=0.10), T-tau (r=0.07), and P-tau (r=0.21) in the total study population or in the AD group (Fig. 5) (r=0.22, r=0.13, and r=0.18, respectively) (*Paper V*).

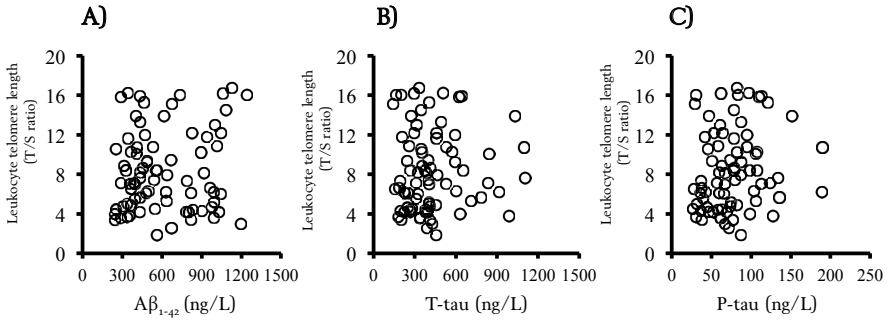

Fig. 5

In the total study population (n=80), leukocyte telomere length was not correlated with cerebrospinal fluid (CSF) levels of A) $A\beta_{1\text{-}42}$ (r=0.10) B) T-tau (r=0.07), or C) P-tau (r=0.21). Correlations were sought using the Spearman rank order correlation test.

DISCUSSION

Diagnostic accuracy of Alzheimer's disease biomarkers in cerebrospinal fluid

In the multivariate analysis presented in *Paper I*, the AUROC level was 0.97 [237]. Therefore, the sensitivity and specificity of the combination of the biomarkers $A\beta_{1-42}$, T-tau and P-tau for AD vs. controls is among the highest reported in the literature. Furthermore, patients with clinically detectable AD at evaluation had similar CSF levels of $A\beta_{1-42}$, T-tau, and P-tau compared to patients with AD first detected clinically during follow-up visits. This finding concurs with previous results showing that core biomarkers are stable over time during the progression AD [53,56,57]. The high accuracy of $A\beta_{1-42}$, T-tau and P-tau in *Paper I* could be explained by the homogeneity of our mono-center study population. Also, we characterized study participants in detail regarding medical history including cardiovascular risk factors and vascular disease components. We excluded patients receiving acetylcholine esterase inhibitor therapy. Furthermore, we collected samples at a standardized time-point, and all samples were analyzed simultaneously, eliminating between-assay and batch-to-batch variations for the analytical kits.

Any biomarker study using clinical diagnosis as the reference standard is limited by the uncertainty of clinical diagnosis toward pathological confirmation. Autopsy studies have revealed errors in clinical diagnosis even in patients followed for years at expert research centers, with sensitivities and specificities around 80% and 70%, respectively [9]. However, some argue that the accuracy of clinical diagnosis has improved over the years [36,240]. Whatever the specific precision, the remaining error is ultimately reflected as in the inaccuracy of diagnostic tests constructed to use clinical diagnosis as the reference standard. To overcome this, CSF biomarkers can be used for diagnostic purposes. When evaluated directly toward autopsy-confirmed AD, $A\beta_{1-42}$ and T-tau in CSF are highly sensitive (96% for CSF $A\beta_{1-42}$) [241]. CSF P-tau has been investigated mainly for specificity toward non-AD dementia, with specificities in the 60-100% range for different dementias [242]. In *Paper I*, the triad of $A\beta_{1-42}$, T-tau, and P-tau in CSF were excellent tools to distinguish

AD from other forms of dementia in patients under primary evaluation for cognitive decline as well as healthy controls.

Also in *Paper I*, amyloid-related CSF biomarkers (i.e. $A\beta_{x-38}$, $A\beta_{x-40}$, $A\beta_{x-42}$, sAPP-α, and sAPP-β) yielded only marginal improvement in diagnostic accuracy, increasing the AUROC level in multivariate analysis from 0.97 to 0.98. This increase resulted mostly from the addition of $A\beta_{x-42}$. Compared to healthy and MCI controls, univariate analysis showed significantly lower $A\beta_{x-42}$ in CSF in AD patients, and in multivariate analysis $A\beta_{x-42}$ discriminated AD from the combined groups of healthy controls and stable MCI. Although $A\beta_{x-38}$, $A\beta_{x-40}$, sAPP-α, and sAPP-β were lower in patients with dementias other than AD compared to most of the other study groups, these biomarkers did not significantly distinguish AD patients from the combined groups of healthy and MCI controls in univariate or multivariate analysis. In summary, in a highly defined cohort of untreated patients the additional amyloid-related biomarkers provided little extra information about AD-diagnosed vs. healthy controls compared to core biomarkers $A\beta_{1-42}$, T-tau, and P-tau.

Hormonal levels in serum and cerebrospinal fluid in Alzheimer's disease

IGF-I and insulin in Alzheimer's disease

In *Paper II*, serum IGF-I levels were higher in AD patients and patients with other dementias and serum IGFBP-3 levels were higher in AD and SMCI patients compared to healthy controls. In contrast, CSF levels of IGF-I and IGFBP-3 were similar in all groups. Some previous studies have shown elevated levels of circulating IGF-I in AD patients [143-145]. We observed increased levels of serum IGF-I in AD patients with only moderate reductions in MMSE score. Others have suggested that the discrepant results in clinical studies could be explained by resistance to IGF-I action in early AD (reflected as increased serum IGF-I) followed by deficiency along disease progression (reflected as low serum IGF-I) [243]. Our cross-sectional study did not allow us investigate changes over time. However, patients may gradually become more isolated and immobilized as AD progresses, possibly resulting in malnutrition and reduced muscle mass associated with low circulating IGF-I level [131]. In turn, lowered circulating IGF-I could accelerate the disease progression [243]. Furthermore, reduced levels of circulating IGF-I in AD patients carrying the Swedish APP 670/671 mutation [140] and in patients with

marked AD, as determined by low MMSE score [141] could support the notion that the circulating IGF-I decreases with advancing disease.

In Paper II, and also in studies by Tham et al. [143], Watanabe et al. [141], and Duron et al. [142], AD patients did not receive medical treatment with acetylcholine esterase inhibitors; it is unclear whether AD patients received such treatment in studies by Mustafa et al. [140], Vardy et al. [144], and Salehi et al. [145]. Duron et al. [142], reported low serum levels of IGF-I and IGFBP-3 in AD men but not in AD women. The reason that serum IGF-I and IGFBP-3 in our AD patients differed from those in the Duron et al. study is proven elusive [142]. Their multi-center study [142] (n=694) was considerably larger than ours and their MMSE score was only marginally lower compared to ours[142]. However, strictly defined lumbar puncture and laboratory assay procedures were used to study a well-characterized mono-center population of non-diabetic patients in *Paper II*. Furthermore, our patients and controls were matched for age, gender, BMI, and waist:hip ratio, thus yielding highly controlled parameters regarding the IGF-I system. In the study by Duron et al. [142] AD patients were older than the controls, which could have been important because serum IGF-I levels decrease gradually during normal aging [131].

In *Paper II*, CSF IGF-I in the control group was approximately 50-fold lower in CSF compared to that in serum, concurring with the results of previous studies [143,145]. Additionally, CSF IGF-I levels did not differ between groups in *Paper II*. The few studies that have evaluated IGF-I levels in CSF reported unchanged [143] and increased [145] levels of IGF-I in the CSF of AD patients. In other studies, the brain expression of IGF-I as measured by RT-PCR was decreased, as did IGF-I signaling in advanced human AD [135,244]. A rigorous study by Talbot et al. [148] confirmed resistance to IGF-I signaling in the AD brain.

In addition to being affected by nutritional status and body composition, serum IGF-I levels are largely regulated by GH secretion [131]. Experimental data show that GH receptors and IGF-I receptors are expressed in neural progenitor cells [110]. A recent study demonstrated that GH mediates exercise-dependent activation of neural precursor cells in the subventricular zone of aged mice [245]. Furthermore, age and exposure to environmental stimuli can endogenously regulate the production of GH in the hippocampus [246,247]. In adult hypopituitary patients, systemic GH treatment increased IGF-I and IGFBP-3 in the circulation and the CSF [248]. Other studies reported no major changes in AD patients regarding spontaneous GH secretion or GH-releasing hormone (GHRH)-stimulated GH response [140,249]. In *Paper II*, high serum levels of IGF-I and IGFBP-3 might imply increased secretion of GH.

However, we did not perform provocative testing or repetitive blood sampling to assess GH secretion and thus could not evaluate whether GH secretion or the expression of GH or its receptor influenced our results. IGFBP-3 is the major IGF-I binding protein in the circulation [132]. In *Paper II*, the unchanged IGF-I/IGBP-3 ratio in both serum and CSF suggests that free bioactive IGF-I was unaffected. However, increased total IGF-I levels and an unchanged IGF-I/IGFBP-3 ratio in the circulation likely suggest an increased pool of circulating IGF-I that could be delivered to appropriate tissues. Because other IGFBPs, such as IGFBP-2 [111], may participate importantly in the CNS, it is difficult to evaluate whether free IGF-I levels in the CNS were unchanged. Furthermore, the CSF/serum IGFBP-3 ratio decreased. However, the exact mechanisms underlying the reduced CSF/serum IGFBP-3 ratio in *Paper II* are not known.

In *Paper II,* the changes observed in the IGF-I system correlated with disease status, as evaluated by CSF levels of AD biomarkers. In the total study population (n=80), serum levels of IGF-I and IGFBP-3 correlated negatively with $A\beta_{1-42}$ levels in CSF, and in AD patients (n=32) CSF/serum IGF-I ratio correlated positively with CSF P-tau levels. However, further longitudinal studies must determine whether increased serum IGF-I in early AD is a compensatory mechanism that protects the brain by increasing Aβ clearance, as suggested by Carro and coworkers [136], or whether increased IGF-I activity causes AD by increasing the level of toxic soluble Aβ oligomers [139].

Other studies have reported an association between type 2 diabetes mellitus and increased risk of AD [150,151]. Although circulating insulin does not seem to be an independent risk factor for the development of dementia [152], deficient insulin signaling, as observed in autopsied frontal cortices from AD patients [250], may contribute to this association. Similarly, a recent study reported reduced insulin signaling in the IR→IRS-1→PI3K pathway and diminished responses to IGF-1 in the IGF-1R→IRS-2→PI3K pathway in the brains of AD patients without diabetes mellitus [148]. Although CSF insulin may not fully reflect brain insulin levels (or signaling), the results of previous studies support both low [251] and unchanged [252] CSF insulin levels in human AD. Because *Paper II* observed no between-group differences comparing either serum or CSF values of insulin, we confirmed a previous observation of unchanged CSF insulin levels [252]. Differences in the size of study populations and the stage of dementia might explain the conflicting data with previously reported low CSF insulin in AD [251]. We closely matched *Paper II* participants for variables that influence IGF-I and insulin levels, and we excluded subjects with diabetes mellitus. Therefore, our results do not support a role of insulin

levels in serum or CSF in non-diabetic patients with early AD after correction for age and body composition.

Passage through the blood-brain barrier

Before initializing the studies included in this thesis, we hypothesized that serum levels of IGF-I would be low in AD. *Paper II* demonstrates that this is not true, at least not in early AD. Therefore, the age-related reduction in serum IGF-I [70] does not appear to participate in the development of AD. However, this does not exclude low IGF-I activity in the pathophysiology of AD. Talbot et al. [148] confirmed that resistance to IGF-I signaling is present in the AD brain. Furthermore, the results of *Paper II* could suggest reduced passage of IGF-I through the BBB.

CSF/serum ratios might indicate the efficacy of the BBB in transporting IGF-I into the CNS. In *Paper II*, the observed reduction in CSF/serum IGF-I ratio in AD patients might suggest compromised BBB function in those patients. The lack of correlation with albumin ratio, the best established biomarker for general BBB function [223], argues against generally reduced transport of proteins into the CNS in demented patients, and such effect may therefore be specific to IGF-I.

In accord with the results of *Paper II*, Carro et al. [243] proposed a specific BBB disruption of serum IGF-I entrance into the brain in AD. A rodent model of AD [137] showed that the choroid plexus receptor megalin induces IGF-I transport across the BBB. Moreover, megalin manipulation offered protection against AD, IGF-I increased megalin levels, and megalin abundance declined in AD mice [137]. In AD, reduced IGF-I transport through the BBB potentially could result in low levels of brain IGF-I. Speculatively, the higher levels of serum IGF-I observed in *Paper II* could be a compensatory action to maintain sufficient transport of IGF-I across the BBB. However, mechanisms other than reduced BBB efficacy could underlie a dissociation between CSF and serum levels of IGF-I in AD. An increase of serum IGF-I levels could be a compensatory mechanism to counteract low local production of IGF-I in the CNS. Furthermore, serum IGF-I might increase in AD independently of CSF levels because circulating IGF-I may increase Aβ clearance by independent actions at the level of the BBB [136].

In *Paper II*, we not only found a discrepancy between serum and CSF levels regarding IGF-I, but also showed a reduced CSF/serum ratio of IGFBP-3 in AD patients. Furthermore, as described in *Paper III*, we observed low levels of total T_4 in serum but not in CSF. However, regulation of T_4 transport over

the BBB differs from that of IGF-I. Thyroid hormones are synthesized in the thyroid gland and then transported into the CNS [253]. Although interstitial levels of thyroid hormones in the brain are normally at near constant levels [254], some factors (e.g. mutations of the gene encoding MCT8) can influence the transport of thyroid hormones into the CNS, possibly causing an imbalance between systemic and brain levels of thyroid hormones [254,255].

Paper IV showed no discrepancy between serum and CSF levels of cortisol, and serum and CSF concentrations correlated positively in the total study population as well in men as in women. The levels of sex hormones in the CSF levels were too low to allow any conclusions in this respect.

Thyroid hormones in Alzheimer's disease

In serum, TSH levels were higher in AD patients compared to patients with other dementias or SMCI, and AD patients also tended to have higher levels of serum TSH compared to healthy controls (*Paper III*). These results (as well as other differences between patients with AD or other dementias vs. controls) remained after we excluded participants with TPO-Ab levels exceeding the upper normal range. Serum levels of total and free T_4 and T_3 did not differ between study groups. Our results suggest marginally low thyroid function in AD patients, as determined in serum samples. This finding concurs somewhat with the results of epidemiological studies that demonstrated an inverse association between AD and peripheral thyroid hormone levels [117,162]. However, several epidemiological studies have also shown that subclinical hyperthyroidism could be a risk factor for cognitive decline [157-161].

Serum transthyretin concentration was higher in AD patients compared to healthy controls. In the circulation, thyroid hormones, particularly T_4, are transported through the choroid plexus bound to transthyretin and subsequently released in the CSF [256]. In line with a possibly relative hypothyroidism in the AD patients included here, a previous study of patients with hypothyroidism revealed increased levels of circulating transthyretin levels [257]. In CSF, transthyretin may affect the deposition of Aβ protein fibrils and, therefore, may affect AD status [256]. In a previous study, CSF transthyretin was similar in AD patients and controls, but CSF transthyretin levels were decreased in AD patients receiving cholinesterase inhibitors compared to those who did not [258]. However, transthyretin levels in CSF were not measureable in *Paper III*.

The ratio between CSF levels of total T_3 and total T_4 increased in AD patients compared to controls. The activity of two deiodinases, type 2 (D_2) and type 3 (D_3), [254] regulates the thyroid hormone metabolism in the CNS. The supply of T_3 in the brain depends mainly on the intracellular deiodination of T_4 by D_2 [254]. Therefore, the findings of *Paper III* (i.e. unchanged CSF level of total T_3 and increased total T_3 / total T_4 ratio in CSF) may represent a compensatory increase in D_2 activity to counteract the effects of reduced total T_4 levels in the CSF of AD patients. A previous study of AD patients with markedly low mean MMSE score observed a reduced total T_3 / total T_4 ratio and lower total T_3 levels in CSF [259]. The patients in *Paper III* were in the early phases of cognitive impairment/dementia and had a relatively moderate reduction of mean MMSE score. Therefore, it could be hypothesized that D_2 activity decreases gradually during the increasing duration of AD, resulting in low CSF total T_3 level over time.

The CSF level of total T_4 correlated positively with MMSE score in the total study population whereas serum levels of thyroid hormones did not correlate with cognitive function. The hippocampus contains a high density of thyroid hormone receptors [164], and an earlier study showed a reduction in thyroid hormone receptor mRNA in the hippocampus area of AD patients [260]. This suggests that reduced CSF levels of total T_4 in CSF (*Paper III*) or reduced responsiveness to thyroid hormones in the hippocampus could be functionally important for cognitive function in AD patients.

In the total study population in *Paper III*, total T_4 in the CSF correlated negatively with CSF T-tau levels, and serum TSH levels correlated positively with CSF levels of T-tau and P-tau. In the AD group (n=31), TSH levels in serum or total T_4 in CSF did not correlate with CSF T-tau levels or MMSE score, whereas serum TSH levels correlated negatively with CSF $A\beta_{1\text{-}42}$ levels. Thus, thyroid hormone levels associate with the activity of neuronal degenerative processes in AD. However, it remains unclear whether these relations reflect mere associations or cause and effect relationships. Furthermore, experimental studies suggest that thyroid hormones may affect APP gene splicing and processing as well as the secretion of APP peptides [261]. Serum transthyretin levels correlated negatively with CSF level of sAPP-α in the total study population and with both sAPP-α and sAPP-β in the AD group. Otherwise, we observed no correlations between thyroid hormones in serum or CSF and CSF levels of sAPP-α or sAPP-β.

Sex hormones and Alzheimer´s disease

In the total study population in *Paper IV*, patients with cognitive impairment (D group) had higher serum levels of DHEA, DHEAS, ADT, E1, and E1S compared to healthy controls (C group), whereas serum levels of glucuronidated androgen metabolites were unchanged, suggesting a similar total peripheral pool of androgens [262]. When men and women were analyzed separately, only serum levels of E1 and E1S were higher in both male and female D patients compared to C group. E1 and E1s exert weak estrogenic effects and some [263-265] but not all [266] previous studies displayed that high circulating E1 level is a predictor of cognitive decline. High E1 concentrations in midlife were associated with smaller right and left brain occipital volumes in late life in men [264]. In community-dwelling postmenopausal women, high circulating E1 levels were related to declining verbal fluency over 4 years [265]. However, one study reported that high circulating E1 level was associated with reduced risk of MCI [266]. Other changes in sex steroid beside E1 and E1S were only seen in female D patients and are for greater detail discussed below under the heading *Gender-related differences*.

In subanalyses in *Paper IV*, we observed no differences in sex steroid levels between different groups of dementia, possibly due to the relatively few subjects in each subgroup. However, the subanalyses showed that AD patients had higher serum levels of DHEAS, E1, E1S, and ADT compared to healthy controls. Because our cross-sectional study design did not allow longitudinal follow-up regarding changes in sex steroid concentrations, it is unclear whether the observed changes reported in *Paper IV* mainly reflect a more marked dysregulation of sex steroid levels with increasing dementia or whether a cause and effect relationship exists between sex steroid levels and cognitive function. Therefore, additional prospective studies are needed to investigate whether sex steroid levels in healthy elderly subjects associate with later onset of cognitive impairment.

Cortisol and Alzheimer's disease

In *Paper IV*, analyses showed that 24-hour urine cortisol as well as CSF cortisol levels were increased in D group compared to the controls. Our findings regarding higher cortisol levels concur with the notion that the central drive of the HPA-axis increases in AD patients [267]. In experimental studies, hypercortisolism influenced degenerative brain aging, notably hippocampal hypotrophy [268]. Furthermore, hypercortisolism affects hippocampus-associated cognitive dysfunction [269-271] and could be important for the pathophysiology of AD [268]. In some studies, patients with AD showed

slightly increased circulating levels of cortisol [272-274], insensitivity to GC (dexamethasone) feedback, and weakened ACTH responses to CRH [275]. It may be that characteristics of hypercortisolism in peripheral tissues are relatively sparse in AD patients, which could be due to altered metabolism of cortisol, as has been indicated in women with mild to moderate AD [276]. It is therefore possible that an activation of the HPA axis is secondary to changes in cortisol clearance in AD. Local brain amplification of GCs by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) might be important for age-related memory deficits. 11β-HSD1 deficient mice are protected from age-related spatial memory impairments, but the underlying mechanisms have not been fully explored [269]. It has recently been indicated that increased systemic 11β-HSD1 activity predicts brain atrophy and cognitive decline in older men [270], implying that use of 11β-HSD1 inhibitors might ameliorate age-related memory impairments [271]. Because we did not measure the activity of 11β -HSD, its significance in *Paper IV* is unknown.

Gender-related differences

In *Paper IV*, in line with the results of some other studies [276,277] there were gender-related differences in terms of sex hormones. Both men and women in D group had increased serum E1 and E1S levels whereas other changes were gender-specific and only seen in D group women. The gender-related differences observed in sex steroid concentrations in D group patients may occur because elderly postmenopausal women have much lower circulating sex steroid concentrations than men. Women undergo rapid declines in serum estrogen levels after menopause [70] whereas ongoing production of androgens by male gonads decreases slowly over time [70,182]. In elderly men, gonad-produced T can be processed in the peripheral tissues to sex steroid metabolites, including estrogens [182], and approximately 80% of circulating E2 in men derives from androgens [182]. Circulating levels of E2 in elderly men therefore exceed such levels in postmenopausal women [182]. Accordingly, men in our study had higher serum levels of androgens and estrogens compared to women. In female D patients, in addition to increased serum E1 and E1S levels, there were also increases in serum concentrations of DHEA, 4-dione and ADT compared to the female controls. Since the adrenal glands are the major source of circulating sex steroids in postmenopausal women [174,233], these results demonstrate that cognitive impairment in women is associated with increased adrenal secretion of sex steroids such as DHEA. DHEA can then be processed to a number of sex steroid metabolites (Fig. 1 *Paper IV*).

In *Paper IV* in the total study population, as previously observed for DHEA and DHEAS [184], the much higher serum levels correlated positively with the

much lower CSF levels in terms of SHBG and when measurable, 4-dione. The lower CSF levels of SHBG might suggest that not only serum levels, but also CSF levels of sex steroids could be altered in D group females. However, the metabolism of sex steroids in CNS is complex. Increased brain production of DHEA has been demonstrated in AD that correlate with increased levels of CSF levels of DHEA [183], but the increased CSF levels of DHEA in patients with AD or VAD were accompanied by a reduced level of DHEAS and unchanged levels of the metabolites 7α-hydroxy-DHEA, 7β-hydroxy-DHEA, and 16α-hydroxy-DHEA in another study [184]. The relative importance for brain function of sex steroids produced within the CNS vs. that of passage of circulating sex steroids through the BBB into the brain is not fully clear [174,177,182].

An increase in the total peripheral pool of androgens did not accompany increased serum levels of androgens in D group women, as the glucuronidated androgen metabolites (ADTG + 3G + 17G) were unchanged. Percutaneous administration of DHEAS increases serum levels of androgens to a much higher extent than serum levels of glucuronidated androgen metabolites in postmenopausal women [278]. Therefore, it is possible that there is some resistance to the peripheral actions of DHEA in elderly postmenopausal women. The results of *Paper IV* may suggest that such peripheral resistance was greater in D group women compared to healthy elderly postmenopausal women. It was not possible to evaluate whether the total peripheral pool of estrogens was affected in D group women because there is at present no marker for the total peripheral activity of estrogens.

In D group women (*Paper IV)*, it is possible that the increased cortisol levels might reduce peripheral androgen processing, which could provide an additional explanation for the unchanged levels of glucuronidated androgen metabolites (ADTG + 3G + 17G). Increased cortisol levels in D group women could also impair both TSH secretion [279] and the somatotropic axis [280]. On the other hand, increased levels of androgens in D group women might increase the activity of the somatotropic axis [281] The extent to which there were gender-related differences in terms of the IGF-I system (*Paper II*) and thyroid hormones (*Paper III*) was not investigated, mainly because the statistical power for such analyses was considered too low.

Hormonal levels in other dementias

In *Paper II* elevated serum IGF-I and a decreased CSF/serum IGF-I ratio were observed not only in AD patients, but also in the group of other dementias, which had different clinical presentation and pathogenesis compared to AD. Earlier studies reported that serum IGF-I level correlated positively with functional improvement after ischemic stroke [282,283]. Furthermore, in *Paper III*, CSF total T_4 level was reduced and the ratio between total T_3 and total T_4 in CSF was increased in the other dementia group compared to the control group. It was not evaluated in more detail whether there were alterations regarding cortisol and sex hormones in the other dementia group in *Paper IV*. In *Paper V*, LTL was unchanged in the other dementia group. In the studies included in this thesis, it was not possible to determine whether hormonal levels were affected in each specific diagnosis in the other dementia group (such as VAD and DLB). However, at least in terms of the IGF-I/insulin system, there are some indications that the pattern of dysregulation could be disease-specific. In a previous study, patients with PDD/DLB displayed molecular abnormalities regarding the insulin/IGF-I system that overlapped with, but were distinguishable from AD [284]. The results of another study further suggested that the pattern of IGF-I/insulin dysregulation could be specific for the neurodegenerative disease studied [285].

Leukocyte Telomere Length

The results in *Paper V* demonstrate shorter LTL in patients with SMCI compared to healthy controls, patients with MCI that later converted to AD, or patients with established AD. This far, the association between LTL and cognitive performance in healthy non-demented people is not conclusive [236]. Some studies have observed an association between LTL and age-related cognitive decline in elderly subjects [200,202]. However, other studies have shown no or only a modest relation between LTL and cognitive aging [203-206]. Our data (i.e. reduced LTL in SMCI that did not convert to dementia) might indicate that these patients form the part of the normal population with the most pronounced biological aging regarding cognitive function.

Patients with an AD diagnosis as well as patients with MCI that later converted to AD had similar LTL as healthy controls in *Paper V*. Furthermore, we observed no difference in LTL between AD patients arbitrary defined as highly positive for the CSF biomarkers $A\beta_{1-42}$, T-tau and P-tau levels compared to the remaining AD patients or healthy controls.

There were no correlation between the core CSF biomarker levels and LTL and there was no association between LTL and APOE ε4-carrier status. The results of *Paper V* therefore support the previous findings from very old demented subjects [215,216] showing that LTL could not be used as a biomarker for AD. Moreover, the data in *Paper V* confirm the lack of correlation between the telomere length and the APOE polymorphism [215], which is a well-known genetic risk factor associated with old age dementia [286]. Thus, although age is a risk factor for AD, the specific processes in AD do not appear to be primarily driven by increased biological aging.

In *Paper V*, LTL was measured using an established quantitative PCR assay used in several previous studies [197,287-289]. This assay is reproducible [197] and can track the attrition of mean telomere length with increasing passage number of cells in culture [197]. Furthermore, the results of the quantitative PCR assay highly correlate with those obtained by Southern blot technique [197,234]. However, there are also some limitations in *Paper V*. Clinical rather than autopsy examination served as the diagnostic standard. Furthermore, the included subjects were not longitudinally followed the possibility cannot be excluded that the absence of an association between LTL and dementia status was due to the cross-sectional design, the relatively small study population, or that most AD patients were evaluated in the early phases of the disease with relatively moderate reductions of MMSE scores. However, the lack of correlation between LTL and age is in some accordance with the observations of a weaker association between LTL and age in elderly subjects, especially elderly men [235,290]. Furthermore, it is not fully clear whether telomere lengths in leukocytes are representative of the processes that occur in other somatic cells. Telomere length may differ by cell type and cell culture [192], but there are correlations between telomere length in different tissues of an individual [291,292]. In patients with AD, telomere lengths in peripheral leukocytes correlated positively with those in cerebellum [293]. This suggests that telomere length in leukocytes could serve as a surrogate marker for relative telomere length in other tissues including the brain. Therefore, although it is not fully clear whether a cause and effect relationship exists between telomere length and age-related phenotypes, telomere length in leukocytes appears to be representative of telomere length in other somatic cells and a marker of aging.

Stable mild cognitive impairment

MCI is a heterogeneous condition characterized by cognitive changes between normal aging and dementia. Some forms of MCI are regarded as potential preclinical forms of dementia. The etiology of MCI, which is varied and

includes cerebrovascular risk factors, also associates with metabolic and endocrine factors [294].

In this thesis, it has been studied whether there are changes in LTL and hormonal levels in MCI patients that did not convert to a dementing disorder. In *Paper V*, the SMCI patients displayed reduced LTL as a possible sign of increased cognitive aging in this group. Furthermore, in *Paper II*, serum IGFBP-3 level was increased and the CSF/serum ratio of IGFBP-3 was decreased in SMCI patients compared to controls. In *Paper III*, serum transthyretin level was increased in SMCI patients, mainly due to that 2 SMCI patients had serum TPO-Ab levels above the upper reference range. It was not evaluated in more detail in *Paper IV* whether sex hormones and cortisol differed between SMCI patients and controls. In summary, LTL may be decreased in SMCI and although less marked than in AD patients, there are also some hormonal aberrations in SMCI patients. However, whether the hormonal changes could in any way cause SMCI or whether these changes merely are consequences of SMCI remains to be determined in further studies.

CONCLUSIONS

Concentrations of hormones decrease and the risk of AD accelerates with increasing age. The studies included in this thesis used a well-characterized, mono-center study population to evaluate hormone concentrations, CSF biomarkers for AD, and LTL, a marker of aging. The main conclusions can be summarized as follows:

The CSF biomarkers $A\beta_{1-42}$, T-tau and P-tau were highly accurate in diagnosing AD vs. controls and SMCI in a well-defined study cohort of untreated patients. The addition of other biomarkers only marginally increased diagnostic accuracy (*Paper I*).

Serum but not CSF levels of IGF-I and IGFBP-3 were higher in AD patients compared to controls. The increased ratios between CSF and serum levels of IGF-I show that the IGF-I transport of through the BBB may be lower in AD (*Paper II*). Serum and CSF insulin levels were similar in all study groups *(Paper II).*

The CSF level of total T_4 was lower in AD patients, possibly suggesting a relative brain hypothyroidism in these patients. This was not detectable in serum values except for marginally increased serum TSH levels in the AD group (*Paper III*).

Patients with cognitive impairment displayed gender-specific changes in cortisol and precursor sex steroids. Both men and women displayed higher levels of serum estrone and estrone sulfate. Other changes were only noted in cognitively impaired women (*Paper IV*).

Reduced LTL in patients with SMCI not progressing to AD suggests an association between cognitive symptoms and biological aging in this group. In contrast, the absence of an association of LTL and AD suggests that biological aging is not a primary driver of AD-specific pathology(*Paper V*).

Patients with other dementias displayed changes similar to those in AD patients regarding the IGF-I system and thyroid hormones (*Papers II and III*). However, the other dementia group was relatively small and included few

cases of each specific diagnosis. Therefore, further studies are needed to explore whether the pattern of hormonal dysregulation is disease-specific in dementing disorders.

FUTURE PERSPECTIVES

The extent to which the observed changes in hormonal levels are specific for AD or whether they are relevant also for other dementias remains undetermined. Further studies are in addition needed to elucidate whether altered hormonal levels play a pathogenic role or are secondary to the decline in cognitive function.

AD affects the hippocampal formation, a key area for learning and memory processes, early during the course of the disease. Several hormones may affect the development and progression of AD, including those that readily enter the brain through the BBB and those produced locally within the brain. Future prevention and treatment of AD may involve manipulating the effect of hormones on brain cells. The possibility that hormonal intervention could reduce risk or postpone the clinical onset of dementia, including AD, remains undetermined. Scientific research could yield cost-effective approaches to early diagnosis and care that might help societies anticipate and manage future costs [295]. Preventive interventions that lower incidence, improve treatment and care, and slow disease progression might substantially modify future projections of the number of people with dementia.

Moreover, further elucidation of the nature and contribution of genetic factors in AD and related disorders will accelerate the use of genotype-phenotype correlations in dementia classification. Achieving similarly high diagnostic results for core biomarkers in clinical practice (*Paper I)* will require a high level of stringency in all steps of the procedure. Detailed characterization of patients' medical history will improve differential diagnostics, particularly toward VAD, and cognitive testing and lumbar puncture must adhere to highly standardized procedures. Nonetheless, batch-to-batch variations in analytical kits may still result in reduced diagnostic accuracy in the clinical setting.

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SAMMANFATTNING (SVENSK TEXT)

Allt fler i befolkningen uppnår en allt högre ålder. En stor utmaning för samhället blir att hantera en tilltagande sjukdomsbörda, då det med högre ålder följer en ökad risk för ett flertal sjukdomar. Hit hör inte minst hjärnans sjukdomar. Som en effekt av den naturliga åldringsprocessen ses en nedgång i halterna av många hormoner såsom insulinliknande tillväxtfaktor-I (IGF-I), sköldkörtel- respektive könshormoner. Det är inte klarlagt om dessa sänkta nivåer är av betydelse för åldersrelaterade kognitiva besvär, med ytterst demenssjukdomar såsom Alzheimers sjukdom (AD).

I diagnostiken av AD spelar biomarkörer en viktig roll. Genom bestämning av dessa ämnen i ryggvätskan (*cerebrospinal fluid*, CSF) erhålls central information för prognos och val av behandling. Föga är känt om hormonella nivåer är relaterade till dessa AD biomarkörer eller på markörer för åldrande.

Ett syfte med denna avhandling är att validera CSF biomarkörer för de typiska förändringarna för AD och relaterade tillstånd (såsom amyloid- respektive axonal patologi).

En central målsättning är att öka förståelsen av i vilken utsträckning hormonella avvikelser bidrar till kognitiva symtom. Tillvägagångssättet har varit att i en homogen kohort samla in data för såväl patienter som en grupp av friska jämförelsepersoner (kontroller).

Konsekutiva patienter (n = 60) som remitterats för utredning av kognitiv svikt samt frivilliga kontrollpersoner (n=20) ingick. För att kunna påvisa eventuella gruppskillnader i hormonellt eller endokrint perspektiv exkluderades personer med känd benägenhet för avvikelser i de hormonsystem vi avsåg mäta (t ex diabetes mellitus).

Kliniska diagnoser för samtliga patienter ställdes av en oberoende demensexpert. Patienterna indelades sedan i grupper med AD demens eller lindrig kognitiv störning (*mild cognitive impairment*, MCI) med diagnosen AD vid uppföljning (n=32), stabil MCI (SMCI, n=13), eller andra demenssjukdomar (n=15). Alla försökspersoner undersöktes av mig och alla förfaranden, inklusive kognitiva tester och lumbalpunktion utfördes under standardiserade förhållanden. Blod, CSF samt urin samlades för klinisk kemisk analys. Åldersmarkören telomerlängd i vita blodkroppar analyserades med kvantitativ PCR-teknik.

Vi fann (*delarbete I*) att de etablerade AD biomarkörerna (amyloid beta [$A\beta_{1-42}$], totalt tau [T-tau] och fosforylerat tau-protein [P-tau]) visade en hög förmåga att diagnosticera AD gentemot de kombinerade grupperna av kontroller och SMCI, med mycket god träffsäkerhet AUROC 0,97 (95 % konfidensintervall 0,93–1,00, p<0,0001) och att kompletterande analyser endast marginellt ökade den diagnostiska noggrannheten. I delarbete II sågs att serum-IGF-I-nivån ökade hos patienter med AD eller andra demenssjukdomar jämfört med friska kontroller (p=0,01 respektive p<0,05), medan nivån av IGF-I i CSF var oförändrad. I *delarbete III* sågs en marginell ökning av serumnivån av tyreoideastimulerande hormon (TSH) hos AD-patienter. Än mer uttalade var förändringarna i CSF med total tyroxin (T_4) lägre hos patienter med AD eller andra demenssjukdomar jämfört med friska kontroller (båda p=0,001). I *delarbete IV* hade både manliga och kvinnliga patienter ökade serumkoncentrationer av östron (E1) och östronsulfat (E1S) jämfört med kontroller av samma kön, medan förhöjda serumnivåer av andra könshormon och kortisol endast sågs hos de kvinnliga patienterna. I *delarbete V* framgår att AD-patienter och kontroller hade liknande LTL medan SMCI patienterna hade minskat LTL jämfört med AD patienter (p=0,02) och kontroller (p=0,008).

Sammantaget hade biomarkörerna $A\beta_{1-42}$, T-tau och P-tau mycket hög noggrannhet för att diagnosticera AD i en väl definierad studiepopulation. Det fanns flera förändringar i hormonella värden hos patienter med AD. Med avseende på IGF-I och tyroxin tyder resultaten på minskad transport av dessa hormoner genom blod-hjärnbarriären. Avvikelser i könssteroider och kortisol var mer markerade hos kvinnliga patienter. Låg LTL indikerar möjligen ett tydligare biologiska åldrande som en orsak till kognitiva symtom hos SMCI patienter. Låg LTL verkar däremot inte vara en riskfaktor för övergång från MCI till AD.

POPULÄR SAMMANFATTNING (SVENSK TEXT)

Bakgrund

Redan i förhistorisk tid fanns det människor som uppnådde vad vi idag menar med hög ålder. Dessa individer utgjorde emellertid en mycket liten andel av befolkningen. Genom framsteg inom flera områden uppnår glädjande nog allt fler en allt högre ålder. Emellertid medför hög ålder försämring i flera kroppsliga funktioner och leder till ökad risk att drabbas av flera sjukdomar. Det är ingen djärv gissning att inte minst förekomsten av hjärnans sjukdomar kommer att öka mycket kraftigt i en åldrande befolkning. En stor utmaning för samhället blir att hantera de sociala och medicinska problem som följer av detta.
Ett perspektiv på det naturliga åldrandet är att halten av flera hormoner ändras. Ofta handlar det om en nedgång, såsom i fallet med insulinliknande tillväxtfaktor-I (IGF-I), sköldkörtel- respektive könshormoner. Man vet inte säkert om vilken roll dessa lägre nivåer spelar för åldersrelaterade minnesbesvär med ytterst demenssjukdomar, där den vanligaste är Alzheimers sjukdom (AD).
Diagnos möjlig AD kan ställas på kliniska grunder, men det först när man kan koppla relevant klinisk information till speciella vävnadsförändringar i hjärnan som den definitiva Alzheimerdiagnosen erhålls. Av lätt insedda skäl kan detta i regel göras först efter döden. I syfte att bringa ökad klarhet i vilket slags sjukdom som ligger bakom den enskilde patientens besvär kan man göra flera kompletterande undersökningar. I Sverige finns en stark tradition av att mäta halterna av äggviteämnen i ryggvätskan. Dessa så kallade biomarkörer kan vara vägledande för prognos och val av behandling.

Ett delmål med min avhandling är att undersöka värdet av biomarkörer för AD. En annan central målsättning är att öka förståelsen av i vilken utsträckning ändrade hormonnivåer för kvinnor respektive män bidrar till glömska eller andra kognitiva symptom.

Vi har undersökt patienter som remitterats för utredning av minnesbesvär och jämfört dem med en grupp friska jämförelsepersoner (kontroller). Eftersom vi

var angelägna om att undersöka hormonnivåer var vi mycket restriktiva i urvalet av vilka som fick delta i studien. Till exempel var diabetes eller övervikt skäl för att inte kunna ingå, eftersom dessa tillstånd har känd benägenhet för att ge avvikelser i de hormonsystem som vi avsåg mäta.

Resultat

I den första artikeln beskriver vi att de etablerade AD biomarkörerna hade mycket god träffsäkerhet för att bidra till den kliniska diagnosen.

I det andra delarbetet sågs serum-IGF-I-nivån hos patienter med demens vara ökad jämfört med friska kontroller, medan nivån av IGF-I i ryggvätskan var oförändrad.

I det tredje delarbetet sågs hos AD-patienter en marginell ökning i serum av det hypofyshormon som stimulerar sköldkörteln. Än mer uttalade var förändringarna i ryggvätskan avseende det dominerande sköldkörtelhormonet.

I det fjärde delarbetet sågs såväl manliga som kvinnliga patienter ha ökade serumkoncentrationer av könshormonet östron jämfört med kontroller av samma kön, medan förhöjda serumnivåer av andra könshormon och kortisol endast sågs hos de kvinnliga patienterna.

I det femte delarbetet framgår att AD-patienter och kontroller hade liknande nivåer av åldersmarkören telomerlängd (här mätt i vita blodkroppar). De patienter som hade en mindre allvarlig kognitiv svikt och inte utvecklade demens, hade däremot minskad telomerlängd. Detta skulle kunna stödja att AD inte är en naturlig följd av avancerat åldrande.

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APPENDICES

Appendix I

International Classification of Diseases, ICD-10

F00 Dementia in Alzheimer's disease

Alzheimer's disease (AD) is a primary degenerative cerebral disease of unknown etiology, with characteristic neuropathological and neurochemical features. It is usually insidious in onset and develops slowly but steadily over a period of years. This period can be as short as 2 or 3 years, but can occasionally be considerably longer. The onset can be in middle adult life or even earlier (AD with early onset), but the incidence is higher in later life (AD with late onset). In cases with onset before the age of 65–70, there is the likelihood of a family history of a similar dementia, a more rapid course, and prominence of features of temporal and parietal lobe damage, including dysphasia or dyspraxia. In cases with a later onset, the course tends to be slower and to be characterized by more general impairment of higher cortical functions. Patients with Down's syndrome are at high risk of developing AD. There are characteristic changes in the brain: a pronounced reduction in the population of neurons, particularly in the hippocampus, substantia innominata, locus ceruleus, and temporoparietal and frontal cortex; appearance of neurofibrillary tangles made of paired helical filaments: neuritic (argentophil) plaques, which consist largely of amyloid and show a definite progression in their development (although plaques without amyloid are also known to exist); and granulovacuolar bodies. Neurochemical changes have also been found, including a pronounced reduction in the enzyme choline acetyltransferase, in acetylcholine itself, and in other neurotransmitters and neuromodulators. As originally described, the clinical features are accompanied by the above brain changes. However, it now appears that the two do not always progress in parallel: one may be indisputably present with only minimal evidence of the other. Nevertheless, the clinical features of AD are such that it is often possible to make a presumptive diagnosis on clinical grounds alone. Dementia in AD is at present irreversible.

Diagnostic guidelines

The following features are essential for a definite diagnosis:
a) Presence of a dementia as described above.
b)Insidious onset with slow deterioration. While the onset usually seems difficult to pinpoint in time, realization by others that the defects exist may come suddenly. An apparent plateau may occur in the progression.
c) Absence of clinical evidence, or findings from special investigations, to suggest that the mental state may be due to other systemic or brain disease which can induce a dementia (e.g. hypothyroidism, hypercalcemia, vitamin B12 deficiency, niacin deficiency, neurosyphilis, normal pressure hydrocephalus, or subdural hematoma
d) Absence of a sudden, apoplectic onset, or of neurological signs of focal damage such as hemiparesis, sensory loss, visual field defects, and incoordination occurring early in the illness (although these phenomena may be superimposed later).
In a certain proportion of cases, the features of AD and vascular dementia may both be present. In such cases, double diagnosis (and coding) should be made. When the vascular dementia precedes the AD, it may be impossible to diagnose the latter on clinical grounds.
Includes: primary degenerative dementia of Alzheimer's type.

Differential diagnosis.

Consider: a depressive disorder (F30–F39); delirium (F05.–); organic amnesic syndrome (F04); other primary dementias, such as in Pick's, Creutzfeldt-Jakob or Huntington's disease (F02.–); secondary

dementias associated with a variety of physical disease, toxic states, et c. (F02.8); mild, moderate or severe mental retardation (F70–F72).
Dementia in AD may coexist with VaD (to be coded F00.2), as when cerebrovascular episodes (multi-infarct phenomena) are superimposed on a clinical picture and history suggesting AD.
Such episodes may result in sudden exacerbations of the manifestations of dementia. According to post-mortem findings, both types may coexist in as many as 10–15% of all dementia cases.

F00.0 Dementia in AD with early onset

Dementia in AD beginning before the age of 65. There is relatively rapid deterioration, with pronounced multiple disorders of the higher cortical functions. Aphasia, agraphia, alexia, and apraxia occur relatively early in the course of the dementia in most cases.

Diagnostic guidelines

As for dementia, described above, with onset before the age of 65 years, and usually with rapid progression of symptoms. Family history of AD is a contributory but not necessary factor for the diagnosis, as is a family history of Down's syndrome or of lymphoma.

F00.1 Dementia in AD with late onset

Dementia in AD where the clinically observable onset as after the age of 65 years and usually in the late 70s or thereafter, with a slow progression, and usually with memory impairment as the principal feature.

Diagnostic guidelines

As for dementia, described above, with attention to the presence or absence of features differentiating the disorder from the early onset subtype (F00.0).

Appendix II

NINCDS-ADRDA

Clinical diagnosis of AD: Report of the Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease, 1984

I. The criteria for the clinical diagnosis of "probable Alzheimer disease" include:

- Dementia established by clinical examination and documented by the Mini-Mental Test, Blessed Dementia Scale (to be exact, the Dementia Test Score), or some similar examination, and confirmed by neuropsychological tests.
- Deficits in two or more areas of cognition.
- Progressive worsening of memory and other cognitive functions.
- No disturbance of consciousness.
- Onset between ages 40 and 90, most often after age 65.

Absence of systemic disorders or other brain diseases that in and of themselves could account for the progressive deficits in memory and cognition.

II. The diagnosis of "probable Alzheimer disease" is supported by:

- Progressive deterioration of specific cognitive functions such as language (aphasia), motor skills (apraxia), and perception (agnosia).
- Impaired activities of daily living and altered patterns of behavior.
- Family history of similar disorders, particularly if confirmed neuropathologically.
- Laboratory results of:
  - Normal lumbar puncture as evaluated by standard techniques.
  - Normal pattern or non-specific changes in EEG, such as increased slow-wave activity.
- Evidence of cerebral atrophy on CT with progression documented by serial observation.

III. Other clinical features consistent with the diagnosis of "probable Alzheimer disease", after exclusion of causes of dementia other than Alzheimer disease, include:

- Plateaus in the course of progression of the illness.
- Associated symptoms of depression, insomnia, incontinence, delusions, illusions, hallucinations, catastrophic verbal, emotional, or physical outbursts, sexual disorders, and weight loss.
- Other neurological abnormalities in some patients, especially with more advanced disease and including motor signs such as increased muscle tone, myoclonus, or gait disorder.
- Seizures in advanced disease.
- CT normal for age.

IV. Features that make the diagnosis of "probable Alzheimer disease" uncertain or unlikely include:

- Sudden apoplectic onset.
- Focal neurological findings such as hemiparesis, sensory loss, visual field deficits, and incoordination early in the course of the illness.
- Seizures or gait disturbances at the onset or very early in the course of the illness.

V. Clinical diagnosis of "possible Alzheimer disease":

- May be made on the basis of the dementia syndrome, in the absence of other neurological, psychiatric, or systemic disorders sufficient to cause dementia, and in the presence of variations in the onset, in the presentation, or in the course.

- May be made in the presence of a second systemic or brain disorder sufficient to produce dementia, which is not considered to be the cause of the dementia.
- Should be used in research studies when a single, gradually progressive severe cognitive deficit is identified in the absence of other identifiable cause.

VI. Criteria for diagnosis of "definite" Alzheimer's disease are:

- The clinical criteria for probable Alzheimer's disease and histopathological evidence obtained from a biopsy or autopsy.

VII. Classification of Alzheimer's disease for research purposes should specify features that may differentiate subtypes of the disorder such as:

- Familial occurrence;
- Onset before age of 65;
- Presence of trisomy-21; and
- Coexistence of other relevant conditions such as Parkinson's disease.

Appendix III

SOME MILESTONES IN THE PROCESS OF EXPLORING DEMENTIA

1822	First description of dementia paralytica [296]
1881	Apoplectic dementia [297]
1880	Depressive pseudodementia [298]
1892	Pick's disease, lobar atrophy [299,300]
1893	Dementia praecox [301]
1894	Presenile dementia, Binswanger's disease [302]
1906	First report on Auguste D by A Alzheimer [303]
1907	First publication of Alzheimer's disease [304]
1910	Alzheimer's disease (AD) [305], Pathology of AD [306]
1911	Pathology of Pick's disease [307]
1912	Lewy body [308]
1913	Treponema pallidum in GPI [309]
1917	Treatment of GPI (Wagner-Jauregg) [310]
1923	Dementia in Parkinson's disease [311]
1927	Staging of Pick's disease [312]
1929	Down syndrome dementia [313]
1933	Normal senile involution (N Gellerstedt) [314]
1938	Congophilic angiopathy [315]
1952	The three-stage model of AD [316]
1961	Pseudodementia [317]
1962	Benign senescent forgetfulness [17]
1963	Paired helical filaments [318,319]
1965	Normal pressure hydrocephalus [14]
1968	Brain imaging [320]
1969	CIBA-foundation: AD [321]
1974	Multiinfarct dementia [322]
1975	Ischemic Score [323]
	MMSE [23]
1976	Cholinergic deficiency in AD [324,325]
	Aluminum and other metals in AD [326]
1981	White Matter Disease (WMD) in AD [327]
	NbM in AD [328-330]
1982	Reversible dementias [13]
1983	Treatable dementias [331]
1984	Amyloid in AD and DSD [332]
1985	Tau protein [333,334]
	Vascular degenerative overlap [327,335,336]
1986	Age associated memory impairment [16]
	Cholinergic treatment
1987	Ubiquitin in NFT and plaque [337]
	Leukoaraiosis [338]
	APP characterization, mutations, markers [339,340]
1988	Strategic infarct dementia [341]
1991	Dementia with Lewy bodies
1992	Semantic dementia [342]

	Amyloid cascade hypothesis [40]
	Swedish mutation [343]
1993	Apolipoprotein E [222]
1994	Frontotemporal dementia [68]
1996	MCI [20]
1996	CADASIL [344]
1998	Frontotemporal lobar degeneration [69]
2007	Dubois criteria [27]
2008	SBU-project: Dementia [4]
2011	NIA-criteria [29,30]

Modified from Dementia Report by The Swedish Council on Health Technology Assessment [4].