The endocrine system in the progression of mild cognitive impairment to dementia

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In memoriam

Arto Nordlund, Ph.D.

(1962 – 2017)

To my parents and family

Denn das Selbst ist stark, genau in dem Maße, wie es aktiv tätig ist.

Erich Fromm, Die Furcht vor der Freiheit

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ABSTRACT

Background and aims: Endocrine alterations have been related to cognitive decline, but the role of hormones may vary along the progression to manifest dementia. The overall aim of this thesis was to assess whether thyroid hormones (THs) and insulin-like growth factor-I (IGF-I) are dysregulated around the onset of Alzheimer's disease (AD) and vascular dementia (VaD).

Methods: Patients were recruited from the Gothenburg MCI Study. At baseline, THs in serum (Study I – III) and cerebrospinal fluid (CSF, Study II) were evaluated as well as serum IGF-I (Study IV). Brain volumes were determined using magnetic resonance imaging (MRI; Study II and III).

Results: In Study I, low serum free triiodothyronine (FT3) was associated with increased risk of progression from subjective cognitive impairment (SCI) or mild cognitive impairment (MCI) to manifest AD. In Study II, serum free thyroxine (FT4) was elevated and FT3/FT4 ratio was decreased in mild AD dementia, whereas CSF TH levels were unchanged. Serum FT3 was associated with higher left amygdala volume in AD patients and total T3 with higher hippocampus volumes in the controls. In Study III, patients with AD and stable MCI displayed reduced serum FT3 and lower FT3/FT4 ratio. Only in AD patients, lower serum thyroid-stimulating hormone (TSH) and higher FT3 and FT3/FT4 ratio were associated with greater annual hippocampal volume loss. In Study IV, patients with low serum IGF-I had a twofold higher risk of conversion to VaD.

Conclusions: Overall, the results suggest that dysregulation of THs is associated with hippocampal volume loss and increased risk of progression to AD dementia, whereas altered IGF-I activity may contribute to VaD conversion.

Keywords: Thyroid hormones, IGF-I, Alzheimer's disease, vascular dementia

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SAMMANFATTNING PÅ SVENSKA

Åldersrelaterade endokrina förändringar har associerats med kognitiv dysfunktion, men hormonernas betydelse kan variera under utvecklingen mot manifest demens. Endast ett fåtal studier har tidigare undersökt betydelsen av hormonella nivåer i stadierna mellan normalt kognitivt åldrande och manifest demens. Det övergripande syftet med denna avhandling var att undersöka om tyreoideahormoner och insulin-like growth factor-I (IGF-I) är dysreglerade före och efter debuten av manifest Alzheimers sjukdom (AD) och vaskulär demens (VaD).

De ingående patienterna rekryterades från Gothenburg MCI Study vid minnesmottagningen på Sahlgrenska universitetssjukhuset, Mölndal, Sverige. Vid studiens start så mättes tyreoideahormoner i serum och cerebrospinalvätska (CSF) samt IGF-I i serum. Hjärnvolymer av betydelse för AD bestämdes med hjälp av magnetisk resonanstomografi (MRT).

I det första delarbetet så hade patienter med låg nivå i serum av tyreoideahormonet fritt trijodtyronin (FT3) en ökad risk för övergång från subjektiv kognitiv störning (SCI) eller mild kognitiv störning (MCI) till kliniskt manifest AD.

I andra delarbetet så var tyreoideahormonet fritt tyroxin (FT4) förhöjt, och kvoten mellan det biologiskt aktiva FT3 och prohormonet FT4 var sänkt, hos patienter med AD jämfört med de friska kontrollerna. I CSF så var alla tyreoideahormonerna oförändrade hos patienterna med AD. Vidare så var högre FT3 i serum korrelerat med högre volym av vänster amygdala hos AD-patienter och totalt T3 var associerat med högre hippocampusvolymer hos kontrollerna.

I tredje delarbetet så hade patienter med AD och stabil MCI minskad nivå i serum av FT3 och lägre kvot mellan FT3 och FT4. Endast hos AD-patienter var lägre nivå i serum av sköldkörtelstimulerande hormon (TSH), högre FT3, och högre kvot mellan FT3 och FT4 associerade med större årlig förlust av hippocampusvolym.

I fjärde studien så hade patienter med lågt serum IGF-I en dubbelt så hög risk för övergång från SCI eller MCI till manifest VaD.

Sammantaget så visar resultaten att dysreglerade nivåer av tyreoideahormon är relaterade till förlust av hippocampusvolym och ökad risk för övergång från SCI/MCI till AD, medan låg nivå av IGF-I kan bidra till utvecklingen av VaD.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Quinlan P, Horvath A, Wallin A, Svensson J. Low serum concentration of free triiodothyronine (FT3) is associated with increased risk of Alzheimer's disease. Psychoneuroendocrinology. 2019;99:112-119.

II. Quinlan P, Horvath A, Eckerström C, Wallin A, Svensson J. Altered thyroid hormone profile in patients with Alzheimer's disease. Psychoneuroendocrinology. 2020;121:104844.

III. Quinlan, P, Horvath, A, Eckerström, C, Wallin, A, Svensson, J. Higher thyroid function is associated with accelerated hippocampal volume loss in Alzheimer's disease. Psychoneuroendocrinology. 2022;139:105710.

IV. Quinlan P, Horvath A, Nordlund A, Wallin A, Svensson J. Low serum insulin-like growth factor-I (IGF-I) level is associated with increased risk of vascular dementia. Psychoneuroendocrinology. 2017;86:169-175.

CONTENT

ABBREVIATIONS

AD	Alzheimer's disease
APOE	Apolipoprotein *E*
APP	Amyloid precursor protein
Aβ	β-Amyloid
BBB	Blood-brain barrier
BMI	Body mass index
CNS	Central nervous system
CSF	Cerebrospinal fluid
CVD	Cerebrovascular disease
D 1-3	Deiodinase type 1 - 3
ELISA	Enzymelinked immunosorbent assay
FT3	Free triiodothyronine
FT4	Free tetraiodothyronine/thyroxine
GDS	Global deterioration scale
GH	Growth hormone
GHRH	Growth hormone-releasing hormone
GSK3β	Glycogen synthase kinase 3β
HDL	High-density lipoprotein
IGF-I	Insulin-like growth factor-I
IGFBP	Insulin-like growth factor-binding protein
LATE	Limbic-predominant age-related TDP-43 encephalopathy
LDL	Low-density lipoprotein
MAPK	Mitogen-activated protein kinases
MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
MTL	Medial temporal lobe
NFTs	Neurofibrillary tangles
NP	Neuritic plaques
OATP1	Organic anion-transporting polypeptide 1c1
PSD	Post stroke dementia
PSEN	Presenilin
P-tau	Phospho-tau

rT3	Reverse triiodothyronine
SCI	Subjective cognitive impairment
SSVD	Subcortical small vessel type of dementia
T3	Triiodothyronine
T4	Tetraiodothyronine/thyroxine
TH	Thyroid hormone
TSH	Thyroid-stimulating hormone/thyrotropin
TT3	Total triiodothyronine
TT4	Total tetraiodothyronine/thyroxine
T-tau	Total tau
VaD	Vascular dementia
WMC	White matter changes
WMH	White matter hyperintensities

DEFINITIONS IN SHORT

Alzheimer's disease	A neurodegenerative disease characterized by certain neuropathological changes and a typical pattern of progressive brain atrophy and cognitive decline leading to dementia.
Biomarkers	A biological parameter that is objectively, accurately and reproducibly measured as an indicator of normal or pathogenic biological processes.
Cognition	Mental processes by which knowledge is accumulated and manipulated such as perceiving, recognizing, conceiving, reasoning and decision making.
Dementia	A clinical syndrome due to brain injury or disease characterized by progressive loss of cognitive function severe enough to interfere with daily activities.
Hormones	Chemical substances produced and secreted by various endocrine glands and transported to distant target tissues to regulate the activity of specific cells or organs.
Mild cognitive impairment	A concept used to describe the stage between expected cognitive decline in normal aging and cognitive impairment in dementia.
Subjective cognitive impairment	Self-perceived cognitive deterioration that does not require objective neuropsychological confirmation.
Vascular dementia	A neurocognitive disorder caused by cerebrovascular lesions in the brain.

1 INTRODUCTION

The global population is aging considerably. Achievements in medical sciences and increasing social welfare have reduced young life mortality and favored longevity. Therefore, a dramatic shift in the population's age structure has occurred, with a dramatic increase not only in the number but also in the proportion of elderly individuals. By the year 2050, it is expected that 1.5 billion people will be above the age of 65 years and the number of persons over 80 years of age is expected to triple from 143 million people in 2019 to 426 million in 2050 [1].

A longer life span results in a prolonged opportunity for individuals to contribute to societies provided the individual's health and function is maintained at old age. However, aging is accompanied by a progressive deterioration of physical and mental health. Age is the largest risk factor for numerous diseases and beyond the age of 75 years, almost all elderly individuals will suffer from one or more chronic diseases [2]. Given the expected trends in population aging, age-related diseases will impose an increasing burden on patients, caregivers, and relatives and may challenge health and social care systems beyond sustainability.

Dementia is a chronic disorder and one of the major causes of disability and dependency among the elderly [3]. Dementia is a heterogeneous clinical syndrome characterized by progressive neurodegeneration and cognitive decline, which leads to the loss of ability for independent living and the need for assistance. The most common causes of dementia are Alzheimer's disease (AD) and vascular dementia (VaD), accounting for 70% of all dementia cases in the elderly population [4].

Dementia rarely affects the younger population, and less than 1% of individuals younger than 65 years suffer from dementia. Above the age of 65 years, the prevalence increases dramatically. In the western world, the prevalence of dementia is 2.5% at age 65 and doubles every five years to reach a prevalence of 33-35% at the age of 90 years [5]. Over 50 million people worldwide live with dementia, and this number is estimated to increase to 152 million by 2050 due to population aging [6]. The socioeconomic cost has been estimated at 818 billion US$ in 2015 and is predicted to grow 2 trillion to US$ by 2030 [7].

In most cases, dementia is a slowly progressing disease, which means a prolonged need for treatment and care for the patient. Medical costs account

for less than 20% of this amount, whereas the main cost consists of social care (care facilities) and informal care [8]. Patients with dementia require substantial levels of care, often provided by family caregivers. Family caregivers are often referred to as the invisible second patient as they may suffer from psychological distress, poorer physical health and social isolation. [9, 10]. Due to the extensive burden of dementia and the prognosis of a substantial increase in dementia patients, the World Health Organization (WHO) has declared dementia a global health priority [11].

No definite cause or effective treatment for dementia has yet been discovered, and disease mechanisms are insufficiently understood. Therefore, it is essential to identify modifiable risk factors early in the disease process. The concept of mild cognitive impairment (MCI) has been established to identify individuals with early signs of cognitive decline who are at high risk of developing dementia. MCI is often considered as a transitional state in which individuals experience greater cognitive decline than expected for normal aging, yet do not meet the criteria for dementia [12, 13]. MCI provides a window of opportunity to investigate risk factors, early pathological mechanisms, and potential treatments before complexity increases due to advanced, irreversible pathology and additional concomitant diseases. Although various genetic, biochemical, physiological, environmental, and lifestyle risk factors for dementia have been identified, the single most significant risk factor is age.

There are multiple alterations in the endocrine system during the aging process. As individuals age, the secretion of hormones decreases within most endocrine axes, as does the sensitivity to many hormones in the target tissues. The decline of hormone activity has been related to the aging phenotype, including altered metabolism and deterioration in immune function, but also to cardiovascular disease and cognitive decline. Moreover, the loss of metabolic, neurogenic, and neuroprotective actions of hormones may render the brain more susceptible to injury and insult and increase the risk of dementia.

Experimental, clinical and epidemiological studies have produced divergent results regarding the associations between endocrine factors and dementia. It is not well understood whether the complex alterations of the endocrine system are detrimental or beneficial for the aging process and related diseases. Furthermore, the role of hormone alterations could be different in the early versus late stages of disease progression. The studies presented in this thesis were carried out to investigate whether thyroid hormones (THs) and insulin-like growth factor-I (IGF-I) are associated with dementia and related neuropathology in the transitional stage between normal aging and manifest dementia.

1.1 DEMENTIA

1.1.1 DEFINITION AND CLASSIFICATION

Dementia is one of the most devastating diseases of old age for patients, their families and for societies worldwide. The term dementia does not specify a single disease, but is used to describe a syndrome caused by a variety of diseases and injuries that affect the brain.

Dementia is defined as a late-life disorder that, over the course of many years, is caused by the progressive and irreversible loss of cognitive function, to the extent that the person loses the ability for independent living and is in need of assistance. The Latin term dementia is well descriptive of the disorder as the "de" signifies "apart" and "mentia" translates into "mind", suggesting that the affected person drifts apart from their mind. The deterioration of cognitive domains varies depending on the underlying etiology, but usually comprises dysfunction of general learning and memory, language, attention and speed, perception and visuospatial abilities, and executive functions [14, 15].

In the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5)[16], dementia is subsumed under the newly established *major neurocognitive disorders*. However, the term dementia is retained as it customarily differentiates the primary degenerative disorders that affect older adults from the secondary neurocognitive disease that affect younger individuals, such as traumatic brain injury or HIV infection. The DSM-5 specifies eight different subtypes of dementia, whereupon AD and VaD are the most common forms of dementia, accounting for about 70% of all dementia cases above 65 years of age [4].

1.2 ALZHEIMER'S DISEASE

1.2.1 CLASSIFICATION OF ALZHEIMER'S DISEASE

Alzheimer's disease is the most prevalent cause of dementia, accounting for 54% of all dementia cases [17]. The disease was first described in 1907 by the German neurologist Alois Alzheimer after studying the case of Auguste D, who, at the age of 51 years, was admitted to the care of Dr. Alzheimer. The patient presented at the Frankfurt hospital with a set of symptoms, including memory loss, impaired comprehension, disorientation, and hallucinations that required intensive care [18]. After her death at 56 years of age, Dr. Alzheimer examined her brain using a novel silver staining histological technique. Alzheimer's observation of specific changes in the patient's brain, described

as “miliar foci” and “peculiar changes in the neurofibrils”, are known today as senile plaques and neurofibrillary tangles. These findings changed the history of dementia research and our understanding of the disease [18, 19].

Historically, the clinical diagnosis of AD was considered probable, as it was based on the set and course of prototypical symptoms after the systematic exclusion of other etiologies causing cognitive impairment. Established diagnostic criteria for AD were the DSM-IV [20], and The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria [21]. Both criteria defined AD by a gradual onset of cognitive decline and a progressive worsening of memory and a deterioration of at least one more cognitive domain with disturbances in social or occupational functions. With advances in basic research, it is now recognized that pathological changes occur several years prior to the clinical manifestations of AD, and that the spectrum of AD spans from clinically asymptotic individuals to severely impaired patients *(Fig. 1)*. In the current editions, the DSM-5 and the revised NINCDS-ADRDA criteria consider preclinical stages of the disease (minor neurocognitive disorder), the differentiation between sporadic and genetic causes, and, although not required for diagnosis, the diagnostic utility of biomarkers [16, 22-24].

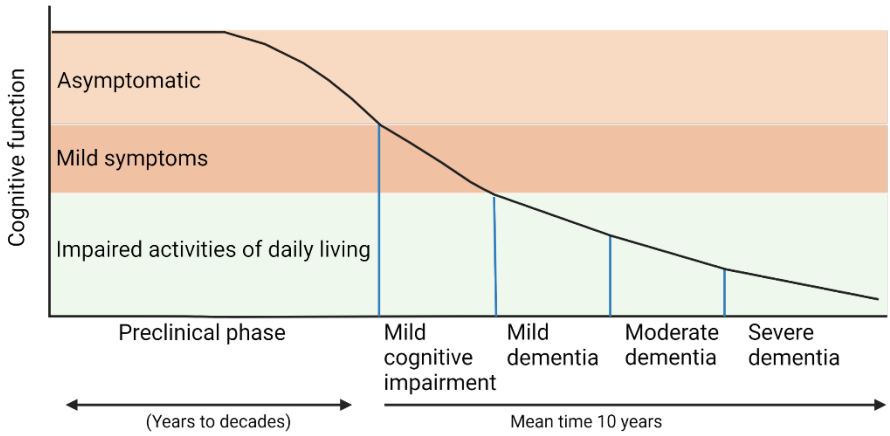

Figure 1. The continuum of Alzheimer’s disease (AD). AD progresses over many years, initially with a long, clinically asymptomatic interval of accumulating neuropathology burden, followed by the mild cognitive impairment (MCI) state with clinically detectable cognitive decline, and finally manifest dementia with severe functional impairment. Created with BioRender.com.

For reasons that are not well understood, AD pathology first occurs in brain structures and neuronal networks responsible for episodic memory before other networks that subserve executive function, language, visuospatial, and attention are affected. This pattern causes cognitive symptoms that are, although variable to some extent, pathognomonic for AD [25, 26]. Throughout the advancement of the disease, the affected patient may experience forgetfulness and getting lost in familiar places. Disturbances in the ability to form new memories may be the first indication of mild dementia. As the disease progresses, the ability to perform complex tasks deteriorates, followed by declining language skills and reasoning and the ability to make judgments. Depression, agitation and changes in personality often accompany cognitive decline. In advanced stages, a patient may become increasingly confused about time and space, fails to recognize relatives and friends, and needs extensive care [27].

1.2.2 PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

AD is divided into two major subtypes according to the age of onset. Early-onset AD usually manifests before the age of 65 years and accounts for 1 - 6% of all AD cases. This form is often a familial disease caused by mutations in genes encoding proteins such as amyloid precursor protein (APP), presenilin 1 (PSEN) 1, or PSEN 2 [28]. In contrast, the more common late-onset AD is considered as a sporadic disease as there is no known single necessary or sufficient cause for the disease [29]. Sporadic AD likely has a complex etiology involving environmental, genetic, and metabolic factors [30].

1.2.2.1 NEURODEGENERATION AND BRAIN ATROPHY

Macroscopically, the AD brain is characterized by widened sulci and narrowed gyri due to a stereotypical pattern of neurodegeneration and substantial loss of gray matter *(Fig. 2)*. In AD, the earliest pathological lesions occur in the medial temporal lobe (MTL) structures such as the entorhinal cortex, hippocampus, and amygdala [31]. During the progression of the disease, gray matter atrophy extends to other cortical areas along a temporal-parietal-frontal trajectory, sparing the primary motor, sensory and visual cortices until the late disease stages. Although the loss of brain volume is associated with the normal aging process, the pattern of atrophy is qualitatively and quantitatively different in AD. In normal aging, atrophy predominantly occurs in sensorimotor, visual cortices and frontal areas of the brain. Volume loss of the MTL due to normal aging is threefold milder compared to that in AD (5% and 18%, respectively), and the annual rate of MTL volume loss is significantly lower (1.45 vs 4.7%)

[32]. The total cortical volume loss was estimated to be 25% greater in AD patients compared with controls in a post-mortem study [33].

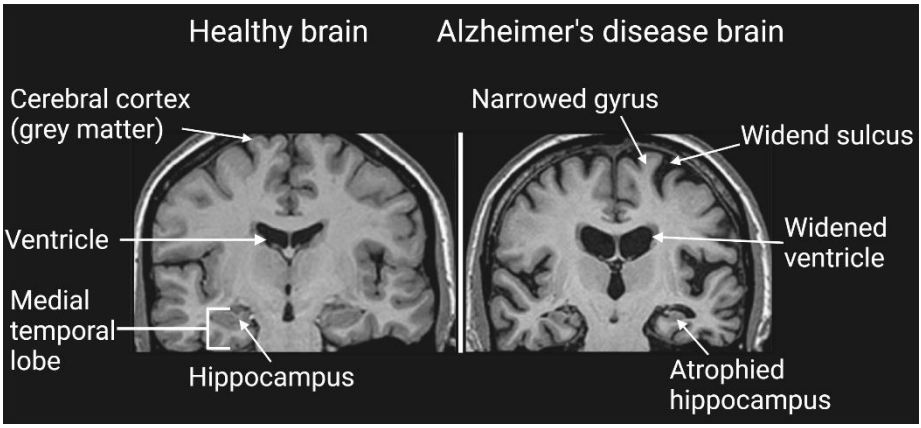

Figure 2. Magnetic resonance imaging (MRI)- derived coronal slices at the level of the medial temporal lobe in the healthy brain (left) and Alzheimer's disease brain (AD) (right). The image depicts characteristic changes of hippocampal volume loss, widened ventricles and sulci and narrowed gyri due to neurodegeneration and loss of gray matter in AD. Created with BioRender.com.

The hippocampus is, together with other structures of the MTL, essential for learning and explicit memory function [34]. Due to the consistent findings of hippocampal atrophy in AD, there is a general agreement on the clinical significance and diagnostic value of hippocampus volumetry using magnetic resonance imaging (MRI) [22, 35]. Furthermore, hippocampal volume loss is correlated with the severity of memory impairment and cognitive deficits in AD [36]. However, several longitudinal studies indicate that hippocampal atrophy may occur seven to ten years before manifest dementia and five years before the first cognitive symptoms [37, 38]. In addition, analyses of large-scale imaging databases suggest morphometric differences in the hippocampus in individuals who develop AD more than 40 years before dementia onset compared to non-demented individuals [39]. Hence, MRI volumetry of MTL structures may assist in the early diagnosis of AD, and can provide important information in terms of the cause and treatment of the disease.

Additional changes in AD include selective degeneration of neurons in nucleus basalis Meynert and cytopathological alterations in locus coeruleus (LC), leading to depletion of acetylcholine (ACh) and noradrenaline (NA), respectively [40, 41]. ACh is involved in hippocampus-dependent memory formation [42-44], and NA modulates vigilance, attention, working memory, planning [45, 46], and memory consolidation [47]. It has been hypothesized

that the depletion of these neurotransmitters may cause downstream synaptic dysfunction and loss of synaptic density [48, 49]. Indeed, in AD, the synaptic density was reduced in the hippocampus and the frontal and temporal cortex, which was associated with reduced cognitive performance [50-52]. Moreover, the neurodegeneration and loss of synaptic function found in AD have been the best predictor of cognitive dysfunction in the disease [53, 54].

1.2.2.2 NEUROFIBRILLARY TANGLES

The primary constituent of the neurofibrillary tangles (NFTs) is the cytoskeleton-associated protein tau. The tau protein is a multifunctional protein promoting the assembly and stability of microtubules in the cytoskeleton, which is essential for axonal functions such as the transport of vesicles and neuronal communication [55]. The biological activity of tau is regulated by the degree of phosphorylation. In the AD brain, tau is hyperphosphorylated, misfolded and aggregated into NFTs in the cytoplasma of nerve cells. Invariably accompanying NFTs are neuropil threads that result from the breakdown of dendrites and axons of neurons [56]. Brain levels of tau may be four- to eightfold increased in AD patients compared to controls due to the amount of hyperphosphorylated tau [57]. As a result of hyperphosphorylation, tau loses the ability to bind to tubulin in the microtubules to stimulate their assembly *(Fig. 3)*. This in turn leads to the disassembly of microtubules causing compromised axoplasmic flow and subsequent retrograde degeneration (loss of synapses) and finally, neuron death [58]. Glycogen synthase kinase 3β (GSK3β) and cyclin-dependent protein kinase-5 (Cdk5) have been identified as pivotal kinases in both the regular and pathological phosphorylation of tau [59]. Overall, neurofibrillary neurodegeneration is a major feature of AD dementia.

The development of NFTs appears to follow a predictable topographical pattern across the AD brain [60] . Braak and Braak distinguished six stages of the spatiotemporal pattern of NFTs. Initially, NFTs appear in the entorhinal cortex (stage I), followed by the CA1 region of the hippocampus (stage II) and the subiculum of the hippocampus formation (stage III). Next, NFTs appear across the amygdala, thalamus and claustrum (stage IV). Finally, all isocortical areas, including the associative areas (stage V) and primary sensory, motor and visual areas (stage VI) are affected [60, 61].

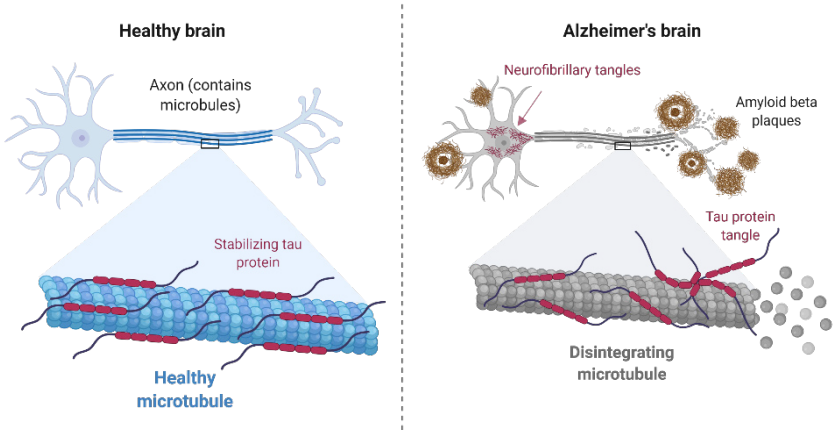

Figure 3. Schematic drawing of a healthy neuron (left) with axonal tree and functional cytoskeleton consisting of tau stabilized microtubules. The right panel displays hallmarks of Alzheimer's disease neuropathology. Abnormal formation of tau protein tangles disrupts microtubule stability causing the microtubule structures to collapse, leading to axonal degeneration. Neurofibrillary tangles composed of hyperphosphorylated tau are present in the cell body (red). Deposition of amyloid beta plaques (neuritic plaques) is displayed around the cell body and axon endings (brown). Created with BioRender.com.

The spatiotemporal distribution of NFTs is closely associated with the pattern of neurodegeneration and severity of cognitive symptoms [61-64]. Moreover, the neuropsychological profile of AD is highly dependent on the topographical distribution of NFTs *(Fig. 4)*. For example, the initial episodic memory impairment is correlated with the neurofibrillary degeneration of the MTL structures, whereas higher-order cognitive functions such as visuo-spatial deficits (occipitoparietal cortex), apraxia (parietal cortex), semantic memory (anterior temporal cortex) and visuo-perceptive deficits (occipitotemporal cortex) are related to the temporal-parietal-frontal trajectory of AD neuropathology [65].

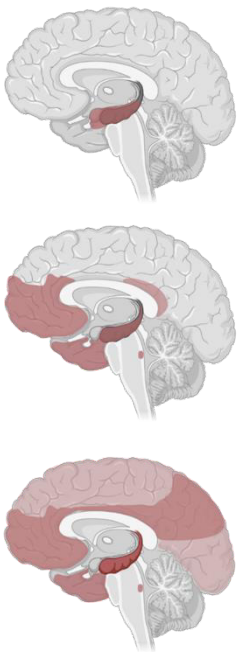

Figure 4. Schematic representation of the Braak staging system [60], illustrating the topographic progression of tau pathology and associated cognitive impairment [25, 26]. Adapted from Braak et al (1995) [60]. Created with BioRender.com.

1.2.2.3 AMYLOID PLAQUES AND THE AMYLOID CASCADE HYPOTHESIS

The central hallmark of AD neuropathology is the abnormal aggregation of the β-amyloid (Aβ) protein to the formation of extra cellular neuritic plaques (NP). Although Dr. Alois Alzheimer described neuritic plaques already in 1907, Aβ was not identified as the principal constituent of amyloid deposits until 1984 [66]. Aβ is derived after the sequential cleavage of the amyloid precursor protein (APP) by the enzymes β- and γ-secretase [67]. It has a peptide length of 39-43 amino acids, the most abundant being $A\beta_{40}$, but $A\beta_{42}$ is the most common form in neuritic plaques due to its fibrillization and insolubility [68, 69]. Aβ-containing plaques are morphologically distinguished between diffuse vs dense-core plaques. Diffuse plaques are commonly present in non-demented elderly people and show little neurotoxicity, whereas dense-core plaques are most often found in AD brains [70].

The discovery of Aβ resulted in the proposal of the “amyloid cascade hypothesis” [71]. This hypothesis incorporates histological and genetic

information, suggesting that the aggregation of Aβ to neuritic plaques is the primary cause of AD. Briefly, according to the "amyloid cascade hypothesis", the driving force of AD is the abnormal APP processing, leading to the deposition of Aβ into extra cellular neuritic plaques. This initiates a sequence of events, including the hyperphosphorylation of the tau protein by altering GSK3β activity and ultimately results in dementia [72]. Several possible mechanisms of Aβ toxicity have been identified, including neuroinflammatory reactions [73], oxidative stress caused by reactive oxygen species (ROS) [74], and N-methyl-d-aspartate receptor (NMDAR) mediated excitotoxicity [75]. However, the sequence and mechanisms of events in the "amyloid cascade hypothesis" are debated, as the factors that initiate this cascade are unclear. Aβ burden is less well correlated with the degree of cognitive impairment than NFTs, and some studies indicate that NFTs may precede the appearance of amyloid plaques [76, 77]. Moreover, several studies suggest that tau mediates, or my even be necessary, for Aβ toxicity [78-80].

The accumulation of amyloid plaques also follows a spatio-temporal pattern, although less predictable than NFT distribution. Braak and Braak distinguished three stages of amyloid deposition. In Stage A, amyloid aggregations are mainly found in the basal portions of the frontal, temporal and occipital lobes. In stage B, all isocortical association areas are affected. Notably, the hippocampus is only mildly involved. In Stage C, amyloid depositions appear in the primary isocortical areas as well as in subcortical structures such as the thalamus, hypothalamus and striatum [60].

1.3 VASCULAR DEMENTIA

Vascular dementia is a neurocognitive disorder that describes severe cognitive impairment that is directly related to cerebrovascular lesions to the brain. VaD is the second most common cause of dementia in the elderly, representing 15-20% of all dementia cases [81].

Cerebrovascular insufficiency has long been suspected of contributing to dementia in the elderly. Historically, it was hypothesized that vasoparalysis caused an impaired ability to adjust to the metabolic requirements causing a global hypoperfusion state of the brain and subsequent neuronal death and dementia [82]. This hypothesis was questioned when the measurement of cerebral blood flow became available, and it was demonstrated that cerebral blood vessels remained the ability to increase cerebral blood flow in cognitively impaired individuals [83]. Simultaneously, the concept of multi-infarct dementia was introduced, which proposes that cognitive decline is caused by a series of brain infarcts which was considered the primary

mechanism of VaD [84]. It was increasingly accepted that VaD is a heterogeneous condition and that various cerebrovascular diseases can cause cognitive impairment.

Several definitions and diagnostic criteria for VaD have been proposed. The most widely used are the International Workshop of the National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria [85]. The diagnosis of probable VaD required a decline in memory and impairment in two other cognitive domains which interfere with daily activities. Furthermore, there should be a history or clinical signs of stroke, and for the first time, neuroimaging evidence of vascular disease was required.

Although the VaD construct was comprehensive, including several phenotypes of vascular-related cognitive impairment, it did not account for the growing neuropathological evidence that neurodegenerative and vascular features act synergistically in most dementias. In addition, the VaD concept does not include cognitive impairment due to cerebrovascular disease (CVD), which may be significant but does not meet the criteria for a diagnosis of dementia [86].

The concept of vascular cognitive impairment (VCI) was introduced to capture the wide spectrum of cognitive disorders that are attributable to all forms of cerebrovascular brain injuries and acknowledges cognitive impairment without dementia [86]. Moreover, O'Brien and colleagues proposed that most VCI cases had a predominantly frontal lobe syndrome with preserved memory but impaired executive function. Recent guidelines from the Vascular Impairment of Cognition Classification Consensus Study (VICCCS) [87] distinguish mild VCI from major VCI, which replaces the VaD term and aligns with the revised classification of the minor and major neurocognitive disorders of the DSM-V.

1.3.1 PATHOPHYSIOLOGY OF VASCULAR DEMENTIA

VaD is a large heterogeneous group of disorders with a variety of complex pathogenic mechanisms. The clinical presentation of VCI and VaD depends on the location, extent, and type of cerebral damage, which determines whether the onset is sudden or insidious, followed by a gradual deterioration with a fluctuating course [88]. The most common causes of cognitive decline are ischemia, hypoperfusion and hemorrhagic brain lesions due to cerebrovascular

or cardiovascular diseases. Ischemic forms of VaD are broadly divided into large-vessel and small-vessel diseases [89].

1.3.1.1 LARGE VESSEL DISEASE

Archetypal for large vessel disease is post-stroke dementia (PSD), characterized by substantial cognitive impairment following stroke. The onset of cognitive impairment is usually within three months but can occur until 12 months after stroke. Cognitive decline post-stroke is common and may affect a third of patients who suffered a stroke [90]. PSD can occur after a single strategic infarct, e.g. thalamus, angular gyrus or hippocampus, each presenting with a characteristic cognitive syndrome. Dementia may also result from the cumulative effect of several infarcts as described in the construct of multi-infarct dementia, often referred to as cortical VaD (cVaD) [84].

The underlying mechanisms of PSD are not well understood since dementia is not always a direct consequence of the cerebrovascular lesion, and a progressive decline may indicate a neurodegenerative rather than a vascular origin. Moreover, large vessel disease is seldom found isolated, and covert small vessel disease is common in the elderly, accelerating the dementia progress [91]. Silent cerebral infarcts increase with advancing age and are considered to be a major contributor to cognitive decline [92]. Risk factors for PSD include age and low education, whereas the associations with vascular risk factors such as hypertension, diabetes, hyperlipidemia and smoking are less consistent [93, 94].

1.3.1.2 SMALL VESSEL TYPE DEMENTIA

Small vessel disease refers to a pathological process that causes damage to small end arteries, arterioles and brain capillaries, leading to reduced or interrupted perfusion of the brain parenchyma as well as multiple small infarcts, which eventually results in cerebral atrophy [95]. When the disease has progressed to manifest dementia [96], the term subcortical small vessel type of dementia (SSVD) can be used [97].

In SSVD, in contrast to cortical infarcts, small vessel infarcts or ischemia occurs in subcortical structures such as the cerebral white matter, basal ganglia and brain stem. On MRI examination, SSVD is characterized by ischemic white matter lesions denominated as white matter changes (WMC) or white matter hyperintensities (WMH). These changes are usually symmetrical and bilaterally situated. Also, lacunar infarcts, small cavitating lesions in the white brain matter and in the thalamus, basal ganglia and pons, can be attributed to small vessel disease [96, 98]. Cerebrovascular lesions in the subcortical areas

tend to cause a slow deterioration primarily in executive function, with a relative sparing of memory function, cognitive and motor slowing, as well as changes in personality, depression and motor disturbances [98]. The interruption of prefrontal-subcortical circuits by ischemic lesions has been suggested to underlie the executive dysfunction seen in SSVSD. In addition, interruption of the orbitofrontal-subcortical loop may cause personality changes [99].

Although white matter lesions contribute to the cognitive impairment in SSVD, white matter lesions can also be seen in normal aging. With increasing age, white matter lesions is a common neuroradiological finding in the elderly, affecting up to 65% of individuals over 65 years of age [100]. According to their location, they are classified as periventricular or subcortical white matter lesions [101]. It is debated whether the presence of white matter lesions can be asymptomatic [102], but in a longitudinal study of patients with mild cognitive complaints, the amount of white matter lesions was predictive of accelerated decline in global cognitive function [103].

1.3.2 MIXED DEMENTIA

Mixed dementia refers to the condition where more than one type of dementia coincides in the brain. The most common form is AD pathology combined with cerebral ischemic/hypoxic lesions related to concomitant VaD [104]. There is no consensus on the diagnosis of mixed dementia. In general, it is required that there is a clinically typical AD phenotype in combination with the presence of dementia-related cerebrovascular changes assessed by neuroimaging [104]. The synergistic contribution of both etiologies to cognitive decline in mixed dementia is not clear [105]. Population-based neuroimaging and neuropathological studies have shown that many AD patients have vascular involvement and vice versa [106-109]. On the basis of the considerable overlap of AD and VaD in the brain of dementia patients, the common risk factors and clinical symptoms, the dichotomy of AD and VaD has been questioned [110, 111].

1.4 MILD COGNITIVE IMPAIRMENT

Neurodegenerative cognitive disorders such as AD, pass through a transient phase of subtle cognitive dysfunction before the onset of manifest dementia *(Fig. 1)*. Mild cognitive impairment (MCI) is generally considered as an intermediate stage between normal cognitive aging and dementia and has been conceptualized as a diagnostic entity to identify individuals who depart from normal cognitive aging and may develop dementia. Individuals with MCI

experience loss of cognitive function to a greater extent than expected for their age, but this loss is not severe enough to meet the criteria for a dementia diagnosis [112].

The consensus conference of the International Working group on Mild Cognitive Impairment (IWGMCI) released diagnostical guidelines, which incorporated different clinical presentations of MCI [113]. The proposed MCI criteria include (i) the person is neither normal nor demented; (ii) there is objective and self and/or informant reported evidence of cognitive deterioration; and (iii) activities of daily living are preserved, and complex instrumental functions are either intact or minimally impaired. Four different clinical subtypes of MCI have been proposed, dividing MCI into amnestic (aMCI) and non-amnestic (naMCI) subtypes with single (MCI-SD) or multiple (MCI-MD) impaired cognitive domains. It has been suggested that the MCI subtypes have different underlying degenerative etiologies. For example, individuals with memory impairment are most likely to convert to AD. In contrast, non-amnestic subtypes have a higher likelihood of developing other forms of dementia, such as VaD or Lewy body dementia [13, 113].

In the general population over 60 years of age, the incidence of MCI increases exponentially with increasing age, and the prevalence of MCI has varied between 16% and 28% [114, 115]. Patients with MCI convert in general at an annual rate of 10% in clinical settings [116-118]. Reflecting the high percentage of AD within all dementia cases, the most common diagnosis of MCI cases converting to dementia is AD (53%), followed by MD (AD with concomitant cerebrovascular disease) (33%) and frontotemporal dementia (13%) [119].

The course of MCI is variable. While some cases convert to dementia, others remain stable or return to 'normal' cognitive function [120]. Patients with stable MCI (sMCI) are often naMCI and are less likely to show risk factors for AD compared to MCI cases that progress to dementia [121]. However, MCI criteria do not specify the etiology of the cognitive impairment, and MCI is therefore a heterogeneous condition. It is of major interest within the field of MCI research to identify factors that contribute to cognitive deterioration and subsequent conversion to dementia.

1.4.1 BRAIN ATROPHY IN MILD COGNITIVE IMPAIRMENT

Neuropathological studies of brains from patients with AD showed that the earliest neurodegenerative changes occur in the MTL. In accordance with these

findings, MRI studies have shown detectable hippocampal volume atrophy even before the first cognitive symptoms [37, 38]. Intermediate levels of entorhinal cortex and hippocampal volumes have been shown to discriminate patients with MCI from AD and healthy controls [122]. More importantly, low baseline hippocampal volume and a higher rate of annual hippocampal volume loss in MCI patients have been shown to identify individuals who progress from cognitively healthy to MCI or from MCI to manifest AD [123-125]. The prediction of AD conversion by MTL structure volume gained 90% sensitivity and 85% specificity [126]. Lateral differences in hippocampal volume loss may exist in MCI patients who convert to dementia, but reports are conflictive [127, 128]. Annually hippocampal volume loss in MCI has been reported up to 3,6% compared to 0,0-1,4 % in normal and stable MCI [129, 130]. Hippocampal atrophy was greater among individuals carrying the *APOE* ε4 allele [129, 131].

1.5 RISK FACTORS FOR DEMENTIA

Twin-studies conclusively suggest a role of genetic factors also in sporadic late-onset AD [132]. Today, only the ε4 allele of the apolipoprotein *E* (*APOE ε4*) on chromosome 19 has been coherently associated with sporadic AD [133, 134]. *APOE ε4* is a genetic susceptibility factor, increasing the risk of developing AD during the lifetime. Approximately, carriers of one *APOE ε4* allele have a threefold increased risk of AD, whereas for homozygous carriers the risk of AD is increased 15-fold in Caucasian individuals [135]. The effect of the APOE ε4 on AD risk diminishes with increasing age, being stronger in individuals between 55 and 65 [136].

Sporadic AD and VaD are considered to result from complex interactions between genetic, biological, environmental, and lifestyle factors across the life span. As for dementias in general, advancing age has been found to be the strongest determinant for AD [137] and VaD [138]. Cardiovascular risk factors such as hypertension, hyperlipidaemia, smoking, diabetes type 2 and metabolic syndrome, obesity and low physical activity have been shown to increase the risk of both AD and VaD [139-146]. Robust associations have been found between impaired cardiovascular risk factors in midlife and increased risk of AD later in life [147-154]. However, when measured in late life, the associations have been weaker [147, 149, 153, 155, 156]. Consequently, the abnormalities in cardiovascular risk factors like body weight, blood pressure, and serum lipids found in midlife are gradually reduced during the years preceding a diagnosis of AD [149, 152, 157, 158].

Psychosocial risk factors for dementia include stressful life events, depression and poor social networks [159-162]. Epidemiological studies have shown that education and lifetime occupational attainment may be a protective factors for dementia [163, 164]. Lifetime education and learning may establish a cognitive reserve, which can compensate for the loss of axon integrity and neurodegeneration during ongoing dementia pathological processes and maintain cognitive function and postpone dementia onset [165, 166]. The cognitive reserve has been shown to maintain cognitive function in AD in the presence of AD pathology [167, 168].

1.6 RELEVANCE OF THE ENDOCRINE SYSTEM IN AGING AND DEMENTIA

As a natural part of the aging process, hormone levels of most endocrine axes decline [169]. The occurrence of menopause, andropause, and somatopause, is marked by reduced circulating levels of estrogens, androgens and IGF-I. Also, complex alterations in the regulation of thyroid hormones and cortisol occur in aging individuals [170]. This endocrine senescence has been related to the aging phenotype, including altered metabolism, insulin resistance, cardiovascular disease, deterioration in immune function and decline in cognition, all conditions that have been shown to increase the risk of dementia [145, 169, 171-173].

Clinical studies suggest that the age-related decline in endocrine function may be more accentuated or accelerated in individuals who develop dementia [174-179]. The loss of the metabolic, neurogenic and neuroprotective actions of hormones may render the aging brain vulnerable to neurological diseases such as dementia. In contrast, age-associated dysregulation and especially raised levels of hormones such as cortisol have been associated with cognitive impairment [180] and may therefore increase the risk of developing dementia.

The importance of age-associated endocrine alterations has recently been debated. It has been argued that these changes may be beneficial adaptations to the aging process and therefore provide protection from adverse health outcomes [181]. Furthermore, clinical and epidemiological studies have shown divergent results as a deficiency as well as an excess of hormones have been associated with dementing disorders such as AD and VaD. Therefore, adverse versus beneficial effects of hormones may depend on the age of the individual or the stage of the disease. Understanding endocrine alterations and their associations with cognitive decline and dementia neuropathology may provide opportunities to discover underlying mechanisms and novel strategies for earlier interventions.

In this thesis, the importance of THs and IGF-I was evaluated in relation to the development of AD and VaD as well as the associations with biomarkers of neuropathology. The following sections introduce the physiology of THs and IGF-I and the potential mechanisms underlying the actions of THs and IGF-I.

1.7 HORMONES OF THE HYPOTHALAMUS-PITUITARY-THYROID AXIS

Thyroid hormone synthesis and secretion are regulated by the hypothalamus-pituitary-thyroid (HPT) axis. The HPT axis origins from the paraventricular nucleus of the hypothalamus, which secretes thyrotropin-releasing hormone (TRH) into the central eminence. At the anterior pituitary, TRH stimulates the synthesis and secretion of thyroid-stimulating hormone (TSH), which acts on the thyroid to stimulate the release of thyroxine (T4) and triiodothyronine (T3) into the bloodstream. The thyroid hormones T4 and T3 control the secretion of TRH and TSH by a negative feedback mechanism to maintain optimal TH levels *(Fig. 5)* [182].

In the circulation, 99% of T4 and T3 are bound to transport proteins, mainly thyroid-binding globulin, transthyretin, and albumin. These proteins then carry THs in the bloodstream to the target tissue. T4 and T3 are actively transported across cell membranes by monocarboxylate transporter (MCT)8, MCT10 and organic anion-transporting polypeptide (OATP)1C1.

Only a fraction of serum T3 is directly released from the thyroid [183]. Most T3 is formed by enzymes that locally convert T4, often considered as a prohormone, to the bioactive T3 in different tissues. The deiodinases type 1 (D1) and type 2 (D2) are the primary enzymes responsible for the intracellular conversion of T4 to T3. D2 is most prominent in the brain, whereas D1 is primarily expressed in the liver.

THs elicit substantial effects on physiological functions in virtually every organ including the central nervous system (CNS), heart, autonomic nervous system, liver, muscle and bone. THs act as key regulators of energy metabolism as they directly affect adenosine triphosphate (ATP) consumption and mitochondrial biogenesis and activation [184]. Moreover, THs regulate cholesterol synthesis, transport and metabolism [185] as well as carbohydrate metabolism, including enhancement of insulin-dependent glucose uptake into the cells, gluconeogenesis and glycolysis [184]. Consequently, overt

hypothyroidism is associated with dyslipidemia, atherosclerosis, decreased cardiac output, cardiovascular disease, hypertension and body weight gain.

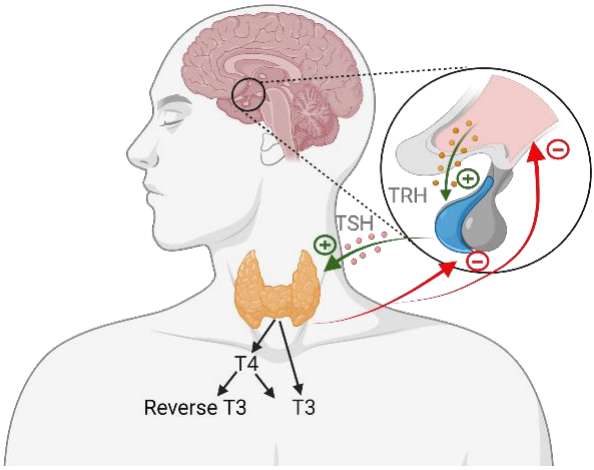

Figure 5. Schematic illustration of the hypothalamus-pituitary-thyroid axis. Thyrotropin-releasing hormone (TRH) is released from the hypothalamus to stimulate thyroid-stimulating hormone (TSH) secretion from the anterior pituitary. The thyroid hormones thyroxine (T4) and triiodothyronine (T3) are released from the thyroid in response to TSH and regulate further release of TSH and TRH b a negative feedback mechanism. T4 is converted into the inactive reverse T3 or the bioactive T3 in the local tissues. Created with BioRender.com.

1.7.1 THYROID HORMONES IN AGING

During aging, complex alterations occur in the regulation of the HPT axis. Several population-based studies have shown that normal aging is accompanied with a rise of TSH levels [186-188], although other studies have reported lower [189, 190] or unchanged [191] TSH levels. Free T4 (FT4) levels remain stable during aging [188], whereas serum free T3 (FT3) levels tend to decline [192]. Moreover, the prevalence of thyroid diseases increases with aging, and in particular subclinical disturbances are more frequent in elderly individuals [193].

Changes in thyroid function have commonly been related to the higher prevalence of cardiovascular disease, dyslipidemia and hypertension seen in the elderly. Suggestions have also been made that THs contribute to development of dementia. However, the clinical course of thyroid disease in elderly subjects differs from that in younger individuals. The results of recent

studies even suggest that low-normal TH levels in elderly individuals are associated with a beneficial effect for health status including reduced mortality and preserved physical function [194, 195]. In contrast, higher thyroid function was associated with risk factors for dementia and AD, such as atrial fibrillation and coronary heart disease [196, 197]. These results suggest not only a tolerance to reduced THs in the elderly population, but also that lower TH levels may be protective against several health risks. In summary, it is still unclear whether alterations in TH levels contribute to the development of age-associated diseases or whether they represent protective mechanisms to alleviate the consequences of aging.

1.7.2 THYROID HORMONES IN THE CENTRAL NERVOUS SYSTEM

1.7.2.1 THYROID HORMONE PATHWAYS IN THE BRAIN

Thyroid hormone receptors (TRs) are widely expressed in the brain, with a dense distribution in the hippocampus, suggesting that the CNS is particularly sensitive to the effects of THs [198, 199]. THs can enter the brain via the blood-brain barrier (BBB) or indirectly via the blood–cerebrospinal fluid (CSF)-barrier. The main entry route of T4 from the circulation is by crossing the endothelial cells of the BBB by the OATP1C1 transporter (high T4 affinity) into astrocytes. After entry, T4 can be converted into T3 via the D2 enzyme to supply the brain with sufficient T3. The transporter that mediates the efflux of T3 from the astrocytes has yet not been identified. Circulating T3 may also enter neurons and astrocytes directly via the MCT8 (high T3 affinity) transporter through gaps between the astrocyte feet. In the neuron, T3 levels are tightly regulated by the deiodinase type 3 (D3) enzyme that deactivates T3 and T4 by conversion to the inactive reverse T3 (rT3) and diiodothyronine T2 *(Fig. 6)* [200, 201].

The actions of THs are mediated through TRs, which are nuclear receptors and act as transcription factors, ultimately regulating the transcription of various genes. In the brain, THs regulate genes encoding proteins of myelin, mitochondria, neurotrophins and the cytoskeleton [202]. TRs also exert non-genomic effects coupled to second messenger activation that can affect brain function [203].

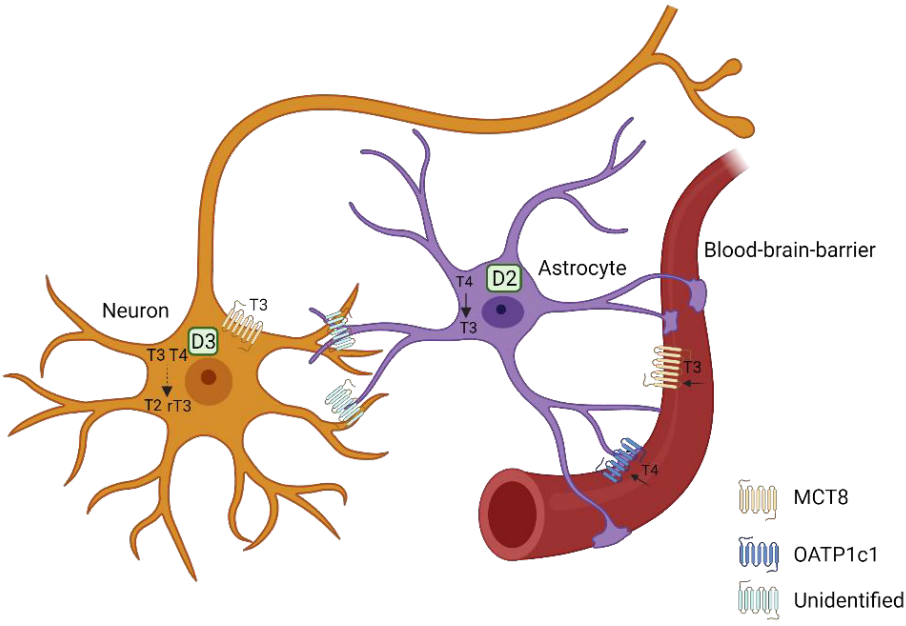

Figure 6. Schematic illustration of thyroid hormone transport in the brain, showing the main entry routes of thyroxine (T4) and triiodothyronine (T3) across the blood-brain-barrier into the astrocyte and the neuron via OATP1C1 and MCT8 transporters. Within astrocytes, T4 is converted to T3 by type 2 deiodinase (D2) and transferred via yet unidentified transporters to the neuron. Type 3 deiodinase (D3) converts T4 and T3 to the inactive metabolites diiodothyronine (T2) or reverse T3 (rT3). Created with BioRender.com.

1.7.2.2 THYROID HORMONE ACTIONS IN THE BRAIN

THs are essential for normal brain development. They promote neurogenesis, including cell proliferation, neuronal and glial cell differentiation, migration, as well as synaptogenesis and myelination [204, 205]. Even mild TH deficiency during critical transition periods in early brain development can lead to irreversible brain damage and severe impairment in specific cognitive domains [206, 207].

Although TH dysregulation has more detrimental consequences in early brain development, it has become evident that optimal TH levels are required even in adult neurogenesis [208]. In experimental animal models, TH deficiency resulted in reduced progenitor proliferation and decreased survival of new neurons in hippocampal structures [209-211]. Moreover, THs modulate long-term potentiation (LTP), the physiological correlate of memory consolidation and neuroplasticity [212, 213]. Hippocampal neurogenesis also occurs in healthy aged humans, whereas in AD, neurogenesis declines and could therefore be one of the factors contributing to the memory deficit in AD

patients [214]. However, whether altered TH levels contribute to the reductions in neurogenesis and brain plasticity in AD has yet not been studied.

The neuroprotective properties of THs may be increasingly important in the aging brain as the exposure to toxic and ischemic events is increased in old age. Cell death is mediated by excessive glutamate release from neurons and glia cells, resulting in a high influx of calcium ions (Ca^{2+}), which in turn causes prolonged depolarization of neurons and consequent excitotoxicity and brain damage [215]. Administration of THs *in vitro* limited excessive Ca^{2+} as well as excitotoxicity-induced neuronal death [216, 217]. However, also experimental hypothyroidism in rodents has been associated with attenuated glutamate release limiting excitotoxicity and neuronal death [218]. Furthermore, in humans, subclinical hypothyroidism may even be protective in acute ischemic stroke [219]. Thus, the results are not straightforward, and it is not well understood when the actions of THs are neuroprotective or even harmful. Although previous studies are lacking, it is conceivable that the effects of THs on cell survival may be influenced by the presence of neuropathology related to VaD or AD [218, 220].

1.7.2.3 THYROID HORMONES AND BRAIN MORPHOLOGY

Deficiency of THs in the developing brain may impair neurogenesis and brain growth to the extent that brain morphology is compromised. In congenital and maternal hypothyroidism, TH deficiency results in persistent reductions in hippocampal volumes, memory function and general intellectual abilities in the affected children [221, 222]. Moreover, in a large population-based study, grey matter volume and general intellectual abilities in children were associated with maternal FT4 levels in an inverted U-shape manner, indicating the importance of optimal TH levels during brain development [223]. However, whether mild deviations in maternal TH levels are important for cognitive functions in offspring is debated [224].

The associations between THs and adult human brain morphology have been scarcely studied. In a small cross-sectional study, right hippocampal volume was reduced by 12% in patients with untreated overt hypothyroidism compared with healthy controls [225]. In accordance, higher serum TSH levels were associated with lower total brain and hippocampal volumes in a population-based study, but this effect was only observed in individuals younger than 50 years of age [226]. In the Rotterdam Study, there was a positive association between serum FT4 and total brain volume in younger individuals, whereas, in older individuals, higher FT4 was related to lower brain volume [227]. Moreover, in the elderly population of the Rotterdam Scan Study, higher serum FT4 levels were associated with lower hippocampal and amygdalar volumes

[228]. Thus, serum TH levels have been associated with brain morphology in population-based human studies. This suggests that THs are not only essential for normal brain development but also for maintaining the adult brain. However, the associations between TH levels and brain morphology and function may be different in younger adults compared with those in older individuals.

1.7.3 THYROID HORMONES IN DEMENTIA DEVELOPMENT

Overt hypothyroidism in adults is associated with lethargy, hyporeflexia, poor motor coordination, affective mood disorders, and loss of cognitive function [229]. In elderly subjects, hypothyroidism is known to mimic dementia-like cognitive symptoms such as memory impairment, reduced mental speed, decreased word fluency, and language impairment as well as neuropsychiatric symptoms such as depression and anxiety [230-232]. This has given rise to the term pseudodementia. [231, 233].

In cross-sectional studies, even in the absence of overt thyroid disease, the decline of TH levels with advancing age may be accompanied by a deterioration of cognitive functions. In cross-sectional studies of elderly individuals, higher thyroid function within the normal reference range was associated with better memory performance [234] and higher global cognitive function [235]. Also, in a longitudinal study of older women, higher serum total T4 (TT4) levels provided protection from cognitive decline [236].

Conversely, there are epidemiological studies indicating that higher thyroid function, even within the normal reference range, is associated with cognitive decline. In elderly subjects aged 75–96 years included in the Kungsholmen Project, higher serum TSH levels were associated with better memory performance [237]. In a longitudinal follow-up of the previous study, declining serum TSH levels in the elderly subjects were accompanied by decreasing verbal fluency and reductions in visuo-spatial and memory functions [238]. Furthermore, in very old community-dwelling individuals, higher serum FT4 levels within the normal range were associated with lower global cognitive function at baseline and accelerated cognitive decline over time [239]. Altogether, the relation between the age-related decline in TH levels and cognitive decline is not fully clear as several cross-sectional studies have shown a positive association between TH levels and cognitive function, whereas the results of some longitudinal studies suggest that declining TH levels may be protective of cognitive decline.

Observational studies of the association between thyroid function and the risk of dementia have also reported conflicting results. Several cross-sectional studies linked TH deficiency to an increased risk of manifest dementia. For example, in a population-based, cross-sectional study, TSH above the upper limit of the reference range was associated with an increased risk of all-cause dementia [176]. Also, a history of hypothyroidism was more frequent in AD patients compared with controls in a re-analysis of eight case-control studies [240]. Moreover, a higher prevalence of hypothyroidism was observed in AD patients compared with patients having VaD or other dementia subtypes [241]. In contrast, some prospective population-based studies have found that excess rather than TH deficiency is associated with the risk of all-cause dementia and AD [242, 243]. Even within the normal reference range, low-normal TSH and high-normal total TT4 and FT4 were found to be risk factors for all-cause dementia and AD in elderly individuals [244-247]. Notably, only two epidemiological studies considered serum T3 levels, and in both studies, serum total T3 (TT3) was not associated with all-cause dementia or AD [196, 228].

Studies assessing TH levels in patients with already manifest dementia have shown variable results. Several cross-sectional studies have reported reduced serum levels of FT3 and TT3 but unchanged serum TSH or T4 levels in patients with AD compared to healthy controls [248-251]. One study showed higher serum TSH levels in serum and reduced TT4 levels in CSF of patients with AD compared with controls [252], whereas two other studies showed unchanged [253] or reduced CSF concentrations of TT3 [254]. A post-mortem study demonstrated reduced TT3 levels in the prefrontal cortex of AD brains [255]. Although not fully consistent, the results of most studies suggest that thyroid function is low-normal in manifest AD, and T3 levels may even be reduced in AD patients.

In summary, most cross-sectional studies have found that higher peripheral levels of THs are associated with better cognitive performance in non-demented elderly individuals. In contrast, several longitudinal population-based studies in elderly individuals suggest that higher TH levels predict cognitive decline over time and increased risk of AD. However, in manifest dementia, there are indications of reduced TH levels in serum and CNS. Therefore, although the underlying mechanisms are not fully known, one alternative is that the role of THs in the regulation of cognitive function may change with advancing age and with increasing AD neuropathology load.

1.7.4 THYROID HORMONES AND ALZHEIMER'S DISEASE NEUROPATHOLOGY

Experimental studies suggest that dysregulation of THs may promote the progression of AD neuropathology. *In vitro* studies have demonstrated that T3 suppresses the transcription of the APP gene [256, 257] and reduces the phosphorylation of the tau protein [258, 259]. Consistently, disruption of T3 signaling in rodents resulted in enhanced APP expression [260, 261]. These results may suggest that aberrant TH signaling may contribute to the production and accumulation of Aβ and increase the phosphorylation of tau. In rodent models of AD, induced hypothyroidism promoted increased levels of Aβ peptide, abnormal tau phosphorylation and cerebral atrophy [262, 263].

Few human studies have examined the relation between THs and AD neuropathology. In one study in which a comprehensive array of THs was determined by mass spectrometry in AD patients, none of the measured THs was associated CSF biomarkers of AD [253]. In a memory clinic population, serum and CSF levels of TSH and T3 were unrelated to CSF AD biomarkers, whereas CSF TT4 levels were inversely correlated with CSF total tau levels [252]. In contrast, in an autopsy substudy of the Honolulu-Asia Aging Study, serum TT4 levels were positively associated with the number of neocortical neuritic plaques and neurofibrillary tangles in the postmortem brain of elderly men [245].

In summary, experimental models of AD suggest that THs may directly affect the pathological processes in AD and promote the accumulation of NFTs and Aβ-containing neuritic plaques. In humans, there is scarce data on the associations between THs and AD neuropathology, and the results have been inconclusive.

1.8 THE SOMATOTROPIC AXIS

Insulin-like growth factor-I is the final substrate of the hypothalamic-pituitary-somatotropic axis. The somatotropic axis is mainly regulated by the stimulatory growth hormone-releasing hormone (GHRH) and the inhibitory somatostatin, which are both secreted by the hypothalamus. GHRH stimulates pulsatile growth hormone (GH) release from the pituitary, and in response to GH, IGF-I synthesis is then stimulated in various tissues *(Fig. 7)*. Liver-derived IGF-I can act as an endocrine hormone by being transported in the bloodstream to the target tissues. IGF-I synthesized in other tissues, including the brain, can exert local autocrine/paracrine effects. Both GH and IGF-I exert negative feedback on the somatotropic axis, either by inhibiting GH release

from the pituitary gland or indirectly via stimulation of somatostatin and inhibition of GHRH from the hypothalamus [264]. However, in elderly subjects, factors other than GH secretion, such as food intake and physical exercise, become increasingly important in the regulation of circulating IGF-I levels [265].

In plasma, about 1% of IGF-I circulates in the free form, while the remainder is bound to a family of binding proteins, which increases the half-life of IGF-I and regulates its availability to the target tissues. Thus, the six high-affinity IGF-binding proteins (IGFBPs) form an elaborate transport and regulatory system of IGF-I. The most abundant IGFBP in the circulation is IGFBP-3, binding almost 80% of circulating IGF-I [266].

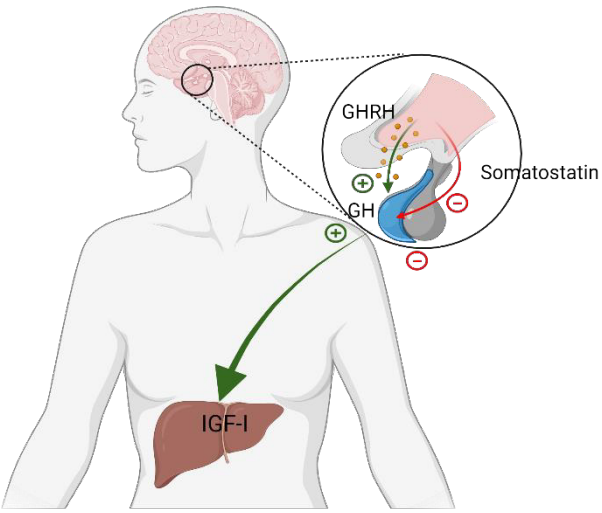

Figure 7. Schematic drawing of the somatotropic axis and the regulation of liver-derived insulin-like growth factor-I (IGF-I). Growth hormone (GH) secretion from the anterior pituitary is regulated by the stimulatory hypothalamic growth hormone-releasing hormone (GHRH) and the inhibitory somatostatin. GH then induces the production of IGF-I in the liver. Created with BioRender.com.

The biological effects of IGF-I are mainly mediated by the IGF-I receptor (IGF1R) and, to a lesser extent, by the insulin receptor. The binding of IGF-I to the IGF1R at the cell surface triggers a complex intracellular signaling cascade that activates the phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway, which promotes cell growth, maturation, and the mitogen-activated protein kinase (MAPK) signaling cascade. Among the downstream effects of the PI3K-Akt signaling pathway is the inhibition of GSK3β, which is involved

in the hyperphosphorylation of tau and the regulation of pathways influencing cellular aging, such as rapamycin (mTOR) and the Forkhead box O (FoxO) translocation [267].

The somatotropic axis undergoes significant changes throughout the life span. While prenatal serum levels are relatively low, there is a gradual increase in IGF-I levels in childhood, and after a peak at puberty, IGF-I levels gradually decline until only low levels can be detected in individuals over 60 years of age [268, 269]. This age-related decline in the somatotropic axis is often referred to as somatopause and may contribute to age-related catabolism, decreased physical performance, reduced immune function, increased risk of cardiovascular disease, and cognitive decline. [265]. However, the importance of the somatopause is debated [269, 270], and the extent to which the age-associated reductions in IGF-I levels affect the aging process and age-associated diseases is not fully clear.

1.8.1 THE IMPORTANCE OF IGF-I FOR THE BRAIN

In the brain, almost all cell types can produce IGF-I, although it is primarily expressed by neurons in the cortex, hippocampus, amygdala, and hypothalamus. The local expression of IGF-I in the brain peaks perinatally and declines after birth, highlighting the importance of liver-derived circulating IGF-I for adult brain function [271]. Circulating IGF-I can readily pass the BBB and subsequently bind to IGF1Rs in the various brain areas [272].

Early in life, the somatotropic axis is crucial for brain development as it promotes neurogenesis, cell survival, synaptogenesis and contributes to the formation of functional circuits in the hippocampus [272, 273]. Deficient IGF-I signaling results in microcephaly, loss of myelination, and behavioral deficits in mice [274, 275]. Conversely, overexpression of IGF-I in transgenic mice causes macrocephaly, increased number of neurons, and enhanced cortical volume [276, 277]. In humans, IGF-I gene mutations are associated with delayed psychomotor development and mental retardation [278-280].

Although the consequences of IGF-I deficiency may be less severe in the aging individual, experimental data suggest that IGF-I is important for the health and function of the adult brain. Peripheral IGF-I injection [281] and physical exercise, a condition that enhances IGF-I entry to the brain, increased the number of hippocampal neurons [282]. In addition, mice with inactivation of liver-derived circulating IGF-I displayed disrupted hippocampal LTP, which could be reversed by systemic IGF-I infusion [283]. Furthermore, IGF-I overexpression in mice resulted in increased synaptogenesis [284]. IGF-I

induced neuroprotection by inhibiting apoptosis [285], which has been shown to ameliorate neuronal loss following traumatic brain injury [286]. Finally, IGF-I administration improved recovery and cognitive outcomes after ischemic stroke [287].

In a human study of hypertensive adults, lower serum IGF-I was marginally associated with a widening of the radial width of the temporal horn, reflecting medial cerebral temporal lobe atrophy [288]. A positive correlation between serum IGF-I and hippocampal volume was observed in healthy individuals [289]. In the Framingham cohort, serum IGF-I concentration was positively associated with total brain volume [290]. However, serum IGF-I levels were not associated with later-life total brain or hippocampal volumes in a population-based study [291]. Therefore, circulating IGF-I levels are, at least to some extent, associated with total brain and hippocampal volumes in non-demented elderly individuals.

1.8.2 IGF-I AND COGNITIVE FUNCTION

The early cross-sectional studies showed a positive association between IGF-I and cognitive function in aging individuals [292-294]. Aleman et al. displayed that higher circulating IGF-I levels were associated with better performance in tests of mental processing speed, which is prone to decline during aging [295]. These results were supported by longitudinal analyses from the Rotterdam Study, which demonstrated that higher serum IGF-I levels were associated with slower cognitive decline over time in elderly individuals [296]. A large prospective study did not find any significant correlation between serum IGF-I and cognitive function instead, IGF-I levels below a certain threshold were associated with lower baseline levels and a greater decline in information processing speed [297]. Moreover, in a community-dwelling male population, higher circulating free IGF-I levels in midlife were predictive of lower global cognitive decline in late life [298]. In contrast, higher peripheral IGF-I was associated with greater cognitive decline in elderly men in one study [299], and in another study, there was no association between IGF-I and cognitive function [300].

1.8.3 IGF-I AND VASCULAR DEMENTIA

Considering that most studies have found that low circulating IGF-I is associated with cognitive decline in elderly subjects, it may be reasonable to assume that IGF-I dysregulation is involved in the development of dementia. Furthermore, the results of population-based studies have suggested that serum IGF-I levels are linked to vascular health in elderly individuals. In older men, high and low serum IGF-I levels were associated with increased risk of all-

cause mortality, whereas only low serum IGF-I levels were associated with increased risk of cardiovascular mortality [301]. In addition, studies of polymorphisms in the IGF-I gene identified a link between low serum IGF-I levels and measures of early atherosclerosis, such as increased carotid intima-media thickness [302, 303]. Thus, as low IGF-I is a risk factor for cardiovascular disease morbidity, there is a possibility that low IGF-I could promote the development of VaD, and also accelerate the cerebrovascular contribution in AD. In rat models of VaD, IGF-I and IGF-I mRNA levels were decreased in the hippocampus [304], and deficiency of circulating IGF-I exacerbated hypertension-induced microvascular rarefaction [305]. In humans, serum IGF-I was reduced in VaD patients and inversely associated with intima-media thickness [175], and a polymorphism in the IGF-I receptor gene was more common in female VaD patients than in female controls [306].

1.8.4 IGF-I AND EXPERIMENTAL ALZHEIMER'S DISEASE

Animal models have been used to study the relationship between IGF-I and AD neuropathology. Several *in vivo* animal studies suggest that IGF-I can increase the clearance of Aβ, thereby reducing Aβ burden in the brain. Interestingly, circulating IGF-I affected the clearance of Aβ by effects at the level of the BBB [307]. Moreover, systemic IGF-I treatment increased Aβ clearance by stimulating the neuronal release of Aβ, coupled with enhanced transport and elimination of Aβ in Tg2576 mice overexpressing a mutant form of human APP (APP695 KM670/671NL) [307]. In further experimental studies, systemic IGF-I infusion in transgenic mice expressing mutant forms of both APP and presenilin increased Aβ export to serum, reduced brain Aβ levels, and ameliorated spatial memory deficits [308]. Additionally, the anti-apoptotic properties of IGF-I have been shown to rescue hippocampal neurons that were exposed *in vitro* to Aβ-induced toxicity [309]. Also, in wild-type mice with centrally infused Aβ, systemic administration of IGF-I lowered Aβ toxicity, supporting that IGF-I treatment may be neuroprotective [310]. Finally, IGF-I deficiency led to the earlier formation of Aβ-containing plaques in an AD mouse model [311].

In addition to mediating Aβ clearance from the brain, IGF-I may also be involved in the phosphorylation of tau, thereby influencing the formation of NFTs in AD. In cultured human neuronal cells, IGF-I signaling regulated tau phosphorylation by inhibiting GSK3β activity, which is linked to reduced NFT formation in AD [312, 313]. In the IGF-I null mouse brain, the tau protein was hyperphosphorylated, supporting that IGF-I inhibits the phosphorylation of tau under normal conditions [314]. Disruption of IGF-I/insulin signaling

accelerated the accumulation of NFTs containing phosphorylated tau in the hippocampus of old mice [315]. Therefore, in experimental studies, there are several indications that IGF-I regulates the phosphorylation of the tau protein.

1.8.5 IGF-I AND HUMAN ALZHEIMER'S DISEASE

In one study, in line with the experimental results that IGF-I can affect Aβ and tau metabolism, serum IGF-I correlated with CSF $A\beta_{1-42}$, and CSF IGF-I correlated with CSF levels of total and phosphorylated tau in AD patients [316]. In the longitudinal, population-based Framingham Study, lower serum IGF-I levels were associated with a higher risk of AD [290]. In accordance, in a large study of 200 AD patients, lower baseline serum IGF-I levels were associated with faster cognitive decline within two years of follow-up [317].

In some contrast, in the Rotterdam cohort, higher IGF1R stimulation activity, as determined using an IGF-I kinase receptor activation assay, was related to an increased risk of all-cause dementia and AD [318]. The latter finding may be consistent with the postmortem findings of resistance to IGF1R signaling in the AD brain. In one study of postmortem AD brains, advancing Braak Stage of AD neuropathology was associated with progressively reduced IGF-I mRNA levels along with resistance to IGF-I signaling [319]. Other postmortem studies also found evidence of IGF-I resistance in the AD brain, which may occur prior to manifest dementia and could deprive the brain of trophic signals with consequent neurodegeneration [320, 321].

Cross-sectional studies in patients with manifest AD have produced variable results as circulating or CSF levels of IGF-I have been low [175, 322, 323], unchanged [324-326] or increased [325-327] compared with healthy controls. It has been hypothesized that these discrepant results are due to IGF-I resistance in early AD dementia, which could induce a compensatory increase in IGF-I levels [328]. Then, as AD progresses, there could be a gradual development of IGF-I deficiency resulting in low IGF-I levels [328].

As the majority of association studies implicated a beneficial role of IGF-I in maintaining cognitive function in old age, intervention studies to increase the activity of the somatotropic axis have been performed. However, the approach of administering GH to increase IGF-I levels has produced moderate results in terms of cognitive function. In adult patients with hypopituitarism and severe GH deficiency, a meta-analysis showed that GH replacement therapy improved cognitive performance, particularly attention and memory [329]. Two treatment studies in elderly individuals did not find any improvement in cognitive function following daily administration of GH or recombinant IGF-

I for six and twelve months, respectively [330, 331]. In contrast, in a randomized, placebo-controlled study, daily treatment of healthy elderly individuals with GHRH for six months improved cognitive function by approximately six percent compared with the placebo group [332]. Furthermore, in a larger randomized controlled trial, five months of GHRH treatment induced a marked increase in circulating IGF-I and improved cognitive function in MCI patients as well as healthy subjects [333]. However, treatment of patients with mild to moderate AD with a GH secretagogue over twelve months did affect the rate of AD progression [334].

In summary, although there are discrepant results, IGF-I has been positively associated with cognitive function in elderly subjects. Low IGF-I may result in dysregulation of the brain vasculature and could therefore be a risk factor for the development of VaD. In terms of AD, the observed resistance to IGF1R signaling in the AD brain could obscure possible associations between serum IGF-I and the risk of AD dementia.

2 AIM

Dysregulation of IGF-I and THs has been associated with the aging phenotype, including cognitive decline in elderly individuals. However, previous studies have produced conflicting results on whether levels of THs and IGF-I are associated with dementia development. This may at least partly be due to heterogeneity within and between study populations, differences in the applied diagnostic criteria, and that the disease stages have not been clearly defined. This thesis aimed to determine the importance of THs and IGF-I in disease stages around the onset of manifest dementia at a single memory clinic.

The specific aims of this thesis are:

- To determine whether serum TH levels are associated with the risk of progression from subjective (SCI) or objective mild cognitive impairment (MCI) to all-cause dementia, AD, or VaD (Study I).

- To determine serum and CSF levels of THs in AD patients and healthy controls and to investigate whether THs are related to CSF biomarkers of AD (Study II).

- To assess whether serum THs are associated with baseline levels and longitudinal changes in MRI-estimated hippocampal volumes in AD patients and healthy controls (Study III).

- To determine whether serum IGF-I levels are associated with the risk of progression from SCI or MCI to all-cause dementia, AD, or VaD (Study IV).

3 MATERIAL AND METHODS

3.1 THE GOTHENBURG MCI STUDY: SETTING AND PARTICIPANT ENROLLMENT

The Gothenburg mild cognitive impairment (MCI) study is an on-going, longitudinal study that was initiated in 1999. This single-center study is conducted at the memory clinic at Sahlgrenska University Hospital, Mölndal, Sweden. The overall objective is to investigate the early and manifest phases of AD, VaD and mixed dementia and to characterize the mechanisms underlying the progression from MCI to manifest dementia. In the Gothenburg MCI study, an extensive characterization of the participants was performed using clinical, neuropsychological, neurochemical, and neuroimaging methods [335].

All patients in Study I-IV were recruited from the Gothenburg MCI study. They had been referred to the memory clinic by other caregivers, e.g. primary health care centers, or by self-referral for assessment of the experienced cognitive deficits. All patients underwent a thorough baseline investigation, including medical history and physical, radiological, neurological and psychiatric examinations. Guidelines for inclusion of patients comprised age > 40 and < 79 years, Mini Mental State Examination (MMSE) score > 19, and self- or informant-reported cognitive decline with a duration $\geqslant$ 6 months. The exclusion criteria were designed to prevent the enrollment of patients with somatic and psychiatric disorders that could cause cognitive impairment. Hence, patients with subdural hemorrhage, and malignant diseases including brain tumor, encephalitis, and unstable heart disease were excluded, as well as patients with major affective disorder, schizophrenia, substance abuse, and confusion. The patients were then followed biannually according to a similar type of examination protocol as that at baseline.

In the Gothenburg MCI study, the healthy controls were recruited through senior citizen organizations or among spouses of the patients. To be considered as a healthy control, the individuals should not experience or show signs of cognitive deterioration at the time of inclusion in the study. In the controls, the exclusion criteria and the study procedures were similar to those in the patients.

3.2 ETHICAL CONSIDERATIONS

The Gothenburg MCI study was approved by the local ethics committee (diary number: L091-99 15 March 1999/T479-11 8 June 2011). Oral and written informed consent was obtained from all participants. The studies were performed in accordance with the Declaration of Helsinki.

3.3 DIAGNOSTIC PROCEDURES

3.3.1 ASSESSMENT OF COGNITIVE DECLINE

The degree of cognitive impairment was classified using the global deterioration scale (GDS), in which GDS stage 1 corresponds to no cognitive deficit, and stage 4 indicates probable mild dementia. Stage 2 equals SCI, and stage 3 equals MCI [336]. The classification is based on the medical history (self-reported and medical record review) and the assessment of cognitive symptoms, including the cognitive variables 13-20 of the Stepwise Comparative Status Analysis (STEP), covering memory disturbance, disorientation, impaired abstract thinking, impaired spatial functioning, poverty of language, agnosia and apraxia [337]; I-FLEX, a short form of the Executive Interview (EXIT) [338]; MMSE [339]; and the Clinical Dementia Rating Scale (CDR) [340]. The CDR rating is based on information provided by the participant as well as an informant.

The algorithm for being considered as cognitively healthy (GDS 1) was: STEP = 0, I-FLEX = 0, CDR $\leqslant$ 0.5, MMSE $\geqslant$ 29. The algorithm for GDS 2: STEP = 0; I-FLEX < 3; CDR $\leq$0.5; MMSE $\geq$ 28 plus additional subjective complaints reported in the clinical interview. GDS stage 3 was defined using the algorithm: STEP $\leq$ 1; IFLEX $\leq$ 3; CDR > 0.5; MMSE $\geq$ 26. GDS stage 4 was determined according to the algorithm: STEP > 1; IFLEX > 3; CDR > 1.0; MMSE $\leqslant$ 26.

3.3.2 DEMENTIA SUBTYPE DIAGNOSIS

For patients classified as probable dementia (GDS = 4), a diagnostic process was applied to diagnose the specific dementia subtypes. The clinicians who determined the specific dementia diagnoses had access to clinical symptomatology and MRI data but were blinded to the results of imaging volumetry/rating scales, CSF biomarker levels and neuropsychological test data. For the diagnosis of AD, the 1984 criteria of The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's

Disease and Related Disorders Association (NINCDS-ADRDA) were used [21].

VaD was diagnosed either as cortical vascular dementia (cVaD) according to the National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l'Enseignement en Neurosciences criteria [85] or as SSVD according to the Erkinjuntti criteria [341]. A diagnosis of mixed dementia was given when AD patients had concomitant MRI findings of cerebral WMHs (moderate or severe according to Fazekas classification) [342] with no predominant frontal lobe syndrome or if AD patients exhibited a mild degree of WMHs combined with a marked frontal lobe syndrome.

3.4 PARTICIPANTS IN STUDY I – IV

All participants included in Study I-IV were recruited from the Gothenburg MCI study. Consequently, all participants matched the inclusion and exclusion criteria of the Gothenburg MCI study. However, for the purpose of the study objectives, additional inclusion and exclusion criteria were applied for the individual studies, as described below.

3.4.1 STUDY I

Study I was a longitudinal study which investigated whether serum TH levels are associated with the risk of conversion to AD or VaD in a memory clinic population. Of the available 751 patients, exclusions were made due to lack of adequate blood sample (n = 121), manifest dementia (n = 206), lack of follow-up visit (n = 95), and levothyroxine treatment (n = 27). The final study population, therefore, consisted of 302 patients with SCI or MCI. All participants were euthyroid as determined by clinical and laboratory parameters, and none received drug treatment altering TH levels such as amiodarone, lithium, and thyreostatics. Patients who were diagnosed with AD during follow-up visits but also showed concomitant MRI findings of cerebral white matter changes (n = 21) were classified as AD since a vascular contribution is common in AD [343]. Thus, in total, 55 patients converted to AD and 17 to VaD. Ten patients converted to dementias other than AD or VaD. Lewy body dementia (n = 2) and primary progressive dementia (n = 1) were diagnosed as described previously [335, 344]. Seven patients converted to unspecified dementia. The follow-up time was calculated from the inclusion to the date of conversion to dementia (generally at one of the follow-up visits) or, for those who remained stable, to the last follow-up examination. The mean follow-up was 2.8 (SD 1.3) years.

3.4.2 STUDY II

Study II was a cross-sectional study with the primary objective of investigating serum and CSF levels of THs in AD patients (n = 36) and healthy controls (n = 34). The secondary aim was to investigate whether THs in serum and CSF were associated with CSF AD biomarkers and MRI-estimated hippocampal and amygdalar volumes. Patients with mixed forms of AD and VaD were excluded as vascular burden may confound the associations between THs and AD neuropathology. Thus, in Study II, it was required that AD patients had predominantly parietotemporal lobe symptoms and no or only a small amount of cerebral WMHs as estimated using MRI. Additional exclusion criteria comprised diabetes mellitus, thyroid disease established by clinical or laboratory parameters, levothyroxine therapy, and other medications known to affect TH levels, such as amiodarone, methimazole, propylthiouracil, lithium, and 5-fluorouracil.

3.4.3 STUDY III

The objective of Study III was to investigate the associations between serum THs and MRI-estimated hippocampal volumes at baseline, as well as the associations with changes in hippocampal volumes over time. We included patients with AD (n = 55), patients with cognitive impairment that did not progress to dementia [stable MCI (sMCI), n = 84], and healthy controls (n = 29). Of the 55 AD patients, 17 had MCI at baseline but converted to AD during the study period. Patients (n = 22) who fulfilled the AD criteria but also had concomitant MRI findings of cerebral WHMs were included in the AD group. Participants with thyroid disease, diabetes mellitus or treatment with medications altering TH levels such as levothyroxine, amiodarone, lithium, and thyreostatics were excluded from the study.

3.4.4 STUDY IV

Study IV sought to investigate whether serum IGF-I levels are associated with the conversion to AD or VaD in a memory clinic population. Of the 751 available patients in the Gothenburg MCI study, 499 were classified as SCI or MCI at baseline. Of these, 109 were excluded as they had no follow-up visit, and 48 patients were excluded due to a lack of adequate blood samples. Thus, 342 patients with SCI or MCI at baseline were included in Study IV. The follow-up time was calculated from the inclusion to the date of conversion to dementia or the last follow-up visit for those who remained stable. The mean follow-up was 3.6 (1.8) years, and the maximum follow-up time was six years. During the study period, 95 patients converted to dementia (VaD, n = 42; AD, n = 37). Of the 42 patients with VaD, 35 had SSVD, 6 had mixed forms of

SSVD and cVaD, and one patient had cVaD. Patients with a considerable vascular contribution to the cognitive impairment, i.e. patients developing mixed dementia, were included in the VaD group. Sixteen patients progressed to dementia other than AD or VaD (Lewy body dementia, n = 2; frontotemporal dementia, n = 2; and primary progressive aphasia, n = 1).

3.5 ASSESSMENT OF COVARIATES

Clinical characteristics and lifestyle factors were recorded at baseline and at each follow-up visit by a specialist physician. Body weight was determined to the nearest 0.5 kg, and body height was measured to the nearest 0.5 centimetres. Body mass index (BMI) was calculated as kilograms per meter squared (kg/m^2). Hypertension was defined as a systolic blood pressure ≥ 140 and a diastolic blood pressure ≥ 90 and/or receiving hypertension treatment. Medications such as beta-blockers, smoking habits, and the presence of diabetes mellitus were evaluated at each visit.

3.6 BIOCHEMICAL METHODS

3.6.1 BLOOD SAMPLES

At each visit, blood samples were collected in the fasted state between 8 AM and 10 AM and prepared into serum and plasma. All blood samples were aliquoted and then stored at -80˚C pending biochemical analyses. Low-density lipoprotein (LDL)-cholesterol was calculated according to Friedewald's formula [345] based on routine clinical measurements of total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides. *APOE* (gene map locus 19q13.2) genotyping was performed by minisequencing as described previously [346].

In Study I, III and IV, all analyses of baseline serum samples were carried out at one occasion in 2015 at the central laboratory of Sahlgrenska University Hospital. Serum levels of TSH, FT4, and FT3 were determined using Elecsys electrochemiluminescent immunoassays on a Cobas 8000 instrument (Roche Diagnostics Scandinavia AB, Stockholm, Sweden). The reference ranges were: TSH: 0.30 – 4.2 mlU/l, FT4: 12 - 22 pmol/L, and FT3: 3.1 – 6.8 pmol/L. The serum concentration of IGF-I was determined using a chemiluminescent immunometric assay (IDS-iSYS; Immunodiagnostic Systems Limited, Boldon, United Kingdom) on an IDS-iSYS automated system (IS31040; Immunodiagnostic Systems Limited). The IDS-iSYS IGF-I assay has been calibrated against the WHO International Standard 02/254.

In Study II, serum levels of TSH, FT4, TT4, FT3 and TT3 were analyzed using electrochemiluminescence immunoassays (Roche Diagnostics, Mannheim, Germany). All samples were analyzed at the central laboratory of Sahlgrenska University Hospital. The reference ranges in serum were: TSH: 0.30–4.2 mlU/l, TT4: 76.1–170 nmol/L, FT4: 12–22 pmol/L, TT3: 1.3 – 3.1 nmol/L and FT3: 3.1–6.8 pmol/L.

3.6.2 CEREBROSPINAL FLUID SAMPLES

In Study II, CSF samples were collected by lumbar puncture through the L3/L4 or L4/L5 interspace between 8 AM and 10 AM to avoid fluctuations in biomarker levels due to circadian fluctuations. The first portion of the CSF sample was discarded to avoid blood contamination. Thereafter, 20 mL CSF was collected in polypropylene tubes and gently mixed by inverting the tube. Afterwards, the CSF was centrifuged at room temperature at 2,000 *x g* for 10 minutes. CSF samples were stored at -80˚C pending biochemical analyses.

CSF levels of total (T)-tau, phosphorylated (P)-tau181 and Aβ amino acids 1 to 42 ($A\beta_{1\text{-}42}$) were determined using sandwich ELISAs (INNOTEST, Fujirebio, Gent, Belgium). In addition, CSF levels of TSH, total T4 and total T3 were determined at one occasion in 2017 using electrochemiluminescence immunoassays (Roche Diagnostics, Mannheim, Germany).

3.6.3 MRI PROCEDURES AND BRAIN VOLUMETRY

Magnetic resonance data acquisition was performed using a 1.5 tesla MRI scanner (Siemens Symphony, Erlangen, Germany). The imaging protocol and sequence as well as volumetric procedures have previously been described in detail [347]. To obtain estimated intracranial, hippocampal, and amygdalar volumes, T1-weighted images were analyzed using FreeSurfer automated segmentation software (version 5.3.0; https://surfer.nmr.mgh.harvard.edu/) [348]. To reduce head size variability, all regional brain volumes were corrected for intracranial volume (ICV) using the residual normalization method [349]. Briefly, a regression analysis was performed in the control group between the region of interest raw volume and the ICV to obtain the regression coefficient β. Then, the regression coefficient β was applied to the entire study sample, and adjusted regional brain volumes were calculated according to the formula: $Volume_{adjusted\ i} = Volume_{raw\ i} - \beta(ICV_{raw\ i} - ICV_{mean})$. Brain volumes in Study II and III are presented in cm^3.

3.6.4 STATISTICAL ANALYSES

All statistical methods in Study I-IV were performed using SPSS for Windows SPSS, Chicago, IL, USA). Versions 21, 24, and 25 of SPPS were used. With the exception of Study II, between-group differences were examined using chi-square tests for categorical data and analyses of variance (ANOVA) for continuous variables, followed by Tukey's honestly significant difference for post-hoc analyses. A p-value < 0.05 was regarded as significant in Study I-IV.

In Study II, non-parametric tests were utilized due to the non-normal distribution of the TH variables. Median and interquartile ranges (25th – 75th percentile) were used for the presentation of descriptive data. For continuous variables, differences between two groups were carried out using Mann-Whitney U-tests. The Kruskal-Wallis test was applied to assess differences in serum and CSF TH levels between *APOE ε4* allele distribution groups. We used the Spearman rank-order correlation test to estimate whether THs were associated with CSF AD biomarkers and MRI-estimated hippocampal and amygdalar volumes.

In Study I and IV, we used Cox proportional hazards regression analyses to examine whether serum levels of THs (Study I) and IGF-I (Study IV) were associated with the risk of conversion to manifest dementia. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated to evaluate the association between each individual hormone (Study I: TSH, FT4, FT3; Study IV: IGF-I) and all-cause dementia, AD and VaD. In Study I, all endocrine parameters were entered as continuous variables as well as cubic expressions to test for linear and non-linear relationships with the risk of dementia. In the second step, serum TH levels were stratified into quartiles and entered into the models. In Study IV, serum IGF-I was stratified into quartiles, and HRs were calculated for low IGF-I (quartile 1) and high IGF-I (quartile 4) compared with intermediate IGF-I (quartiles 2-3) for the risk of converting to dementia. In further Cox proportional hazards regression analyses, the risk of VaD in the lowest IGF-I quartile (quartile 1) was compared with that in the three higher quartiles (quartile 2–4). Finally, we evaluated the independent effects of THs (Study I) and IGF-I (Study IV) on the risk of conversion to dementia by including multiple covariates in the Cox proportional hazards regression models.

In Study III, hierarchical linear regression analyses adjusted for age, sex and education were applied to evaluate whether serum TH levels at baseline were associated with baseline hippocampal volumes as well as the changes in hippocampal volumes over time. In these analyses, the longitudinal changes in

hippocampal volumes were expressed as annualized percent changes. We calculated the annualized percent changes as: the volume at the last available MRI scan minus the volume at the baseline MRI scan divided by the volume at the baseline MRI scan divided by the duration (years) between the measurements (x100). Furthermore, the hierarchical linear regression analyses consisted of three sequentially built models. In the total study population, the first model included a variable for study group membership and covariates. In the next step, a TH variable was added to the model, and the independent effect on hippocampal volume was evaluated. Finally, a two-way interaction term (TH x study group) was included in the models to evaluate if the associations between THs and hippocampal volumes differed between the study groups. We then performed post-hoc analyses using univariate linear regression to quantify the associations between THs and hippocampal volumes in each study group.

4 RESULTS

4.1 STUDY I

This longitudinal study examined, in 302 patients with SCI or MCI, whether serum TH levels were associated with the risk of progression to manifest AD or VaD. Eighty-two (28%) patients progressed to manifest dementia during a mean follow-up time of 2.8 (SD 1.3) years. Of these, 55 (18%) were diagnosed with AD and 17 (6%) with VaD (Table 1).

At baseline, serum FT3 quartiles differed in terms of age, sex, education, and smoking status. Furthermore, none of the measured THs differed between patients with no, one or two *APOE ε4* alleles. However, adjustment for the measured descriptive variables at baseline and *APOE ε4* allele status did not alter the results of the Cox proportional hazard regression analyses.

Table 1. Brief summary of clinical characteristics and endocrine measurements at baseline in Study I and IV.

	Study I	**Study IV**
	(n = 302)	(n = 342)
Study design	Longitudinal	Longitudinal
Baseline diagnosis	SCI/MCI	SCI/MCI
Dementia conversion, n (%)	82 (28)	95 (28)
Demographic variables		
Age (years)	65 (7.8)	64.6 (7.8)
Men/women, n (%)	138/164 (46/54)	148/194 (43/57)
Education (years)	12.5 (3.4)	12.5 (3.5)
MMSE score	28.5 (1.4)	28.5 (1.4)
Endocrine variables		
Thyroid variables		
TSH (mlU/L)	2.1 (1.2)	-
FT4 (pmol/L)	15.6 (2.2)	-
FT3 (pmol/L)	4.8 (0.5)	-
IGF-I (ng/ml)	-	116 (33)

If not stated otherwise, values are given as means (SD).
Abbreviations: AD = Alzheimer's disease, FT3 = Free triiodothyronine, FT4 = Free thyroxine, IGF-I = Insuline-like growth factor-I, MCI = Mild cognitive impairment, MMSE = Mini Mental State Examination, SCI = Subjective cognitive imapairment, TSH = Thyroid-stimulating hormone, VaD = Vascular dementia.

In the Cox proportional hazard regression analyses, higher serum FT3 levels were associated with a lower risk of conversion to AD (per 1 pmol/L increment

in FT3, HR = 0.53; 95% CI = 0.32 – 0.92). Additional Cox proportional hazard regression analyses showed that patients in the lowest FT3 quartile had a more than twofold increased risk of progressing from SCI/MCI to manifest AD compared with those in the highest FT3 quartile (quartile 1 vs. quartile 4: HR = 2.63; 95% CI = 1.06 – 6.47) Moreover, cumulative survival curves further illustrated that low FT3 was associated with increased risk of AD (log-rank p-value=0.026 between all quartiles; *Fig. 8*).

Finally, serum FT3 was not associated with the risk of VaD in the Cox proportional hazard regression analyses, and there were no associations between serum TSH or FT4 levels and the risk of conversion to dementia.

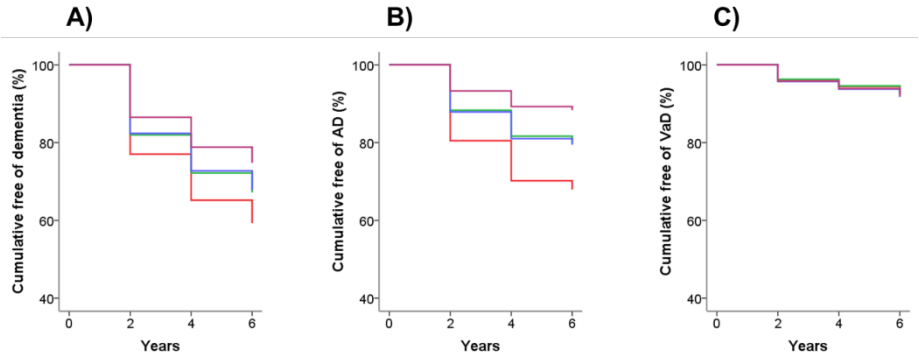

Figure 8. Low serum FT3 is associated with increased risk of AD. Kaplan-Meier survival curves of (A) all-cause dementia (log-rank test: P = 0.245), (B) AD (log-rank test: All quartiles, P = 0.026; quartile 1 vs. quartile 4, P = 0.005) and (C) VaD (log-rank test: P = 0.996) by serum FT3 concentration. Red, low FT3 (quartile 1); green, intermediate-low FT3 (quartile 2); blue, intermediate-high FT3 (quartile 3); purple, high FT3 (quartile 4). Reproduced with permission from Elsevier, Quinlan et al. Psychoneuroendocrinology. 2019;99:112-119.

4.2 STUDY II

In this cross-sectional study, we examined serum and CSF levels of THs in 36 patients with mild AD dementia and 34 cognitively healthy controls. Furthermore, it was investigated whether THs in serum or CSF were associated with CSF AD biomarkers and MRI-estimated brain region volumes. All participants were euthyroid and free of levothyroxine treatment.

Analyses of the clinical characteristics showed, expectedly, that the MMSE score was lower and the frequency of the *APOE ε4 allele* was higher in the AD patients. The AD patients also displayed impaired CSF AD biomarker levels ($A\beta_{1\text{-}42}$, T-tau and P-tau) and reduced hippocampal and amygdalar volumes compared with the controls.

The AD patients showed moderate alterations in serum TH levels compared with the healthy controls. Serum levels of FT4 were elevated ($p < 0.05$) and also TT4 levels tended to be higher ($p = 0.05$) in the AD patients compared with the controls. Moreover, serum FT3/FT4 and TT3/TT4 ratios were reduced in the AD patients (both $p < 0.05$ vs. the controls). Serum levels of TSH, FT3, and TT3 were unchanged (Table 2).

CSF levels of all THs (TSH, TT4, TT3, and TT3/TT4 ratio) were similar in both study groups. Also, the CSF/serum ratios of all the measured THs did not differ between the groups.

In the correlation analyses, serum and CSF levels of THs did not correlate with the CSF levels of $A\beta_{1\text{-}42}$, T-tau, or P-tau in the AD group or in the control group. Next, we investigated whether THs were correlated with MRI-estimated amygdalar and hippocampal volumes. In the AD patients, serum FT3 correlated positively with left amygdalar volume ($r_s = 0.42$, $p = 0.03$), but was not correlated with hippocampal volumes. In the controls, serum TT3 was correlated with left ($r_s = 0.51$, $p < 0.01$) and right ($r_s = 0.48$, $p = 0.01$) hippocampal volumes. Serum levels of other THs were not associated with amygdalar or hippocampal volumes in AD patients or healthy controls. Finally, CSF TH levels did not correlate with the measured brain region volumes.

4.3 STUDY III

This prospective study investigated whether serum THs at baseline were associated with baseline levels and longitudinal changes in MRI-estimated hippocampal volumes. The study included 139 patients (AD, n=55 and sMCI, n=84) and 29 cognitively healthy controls in whom baseline serum TH measurements and baseline MRI had been performed. Of the 139 patients and 29 healthy controls, 73 patients (sMCI, n = 47; AD, n = 26) and 13 controls had at least one follow-up MRI.

None of the participants received levothyroxine treatment or other treatments that could affect serum TH levels. None of the participants had serum TSH levels outside the reference range or showed clinical signs of thyroid disease; hence all participants were regarded as euthyroid.

At baseline, serum levels of FT3 and the FT3/FT4 ratio were reduced in patients with AD ($p < 0.05$) and sMCI ($p < 0.05$) compared with the controls (Table 2). Serum TSH and FT4 levels were unaltered. In subanalyses, carriers of the *APOE ε4 allele* did not differ in serum levels of any TH compared with non-carriers.

Table 2. Brief summary of clinical characteristics and endocrine measurements at baseline in Study II and III.

	Study II		**Study III**		
	Controls	AD	Controls	Stable MCI	AD
Demographic variables					
N	34	36	29	84	55
Age (years)	64 (6.8)	68 (6.2)	61 (6.9)	64 (8.5)	68 (7.2)[a,b]
Men/women, n (%)	12/22 (35/65)	15/21 (42/58)	13/16 (45/55)	32/52 (38/62)	26/29 (47/53)
Education (years)	12.4 (2.5)	11.7 (3.6)	13.1 (3.0)	13.4 (3.3)	13.0 (3.9)
MMSE score	29.4 (0.9)	23.1 (4.5)[a]	29.2 (1.0)	28.5 (1.5)[d]	25.8 (2.9)[a,c]
Thyroid variables					
TSH (mlU/L)	2.1 (0.9)	2.0 (1.1)	2.2 (0.7)	1.9 (0.8)	2.0 (0.8)
FT4 (pmol/L)	14.1 (2.1)	15.1 (1.9)[d]	15.9 (1.7)	15.8 (2.3)	16.0 (2.1)
FT3 (pmol/L)	4.7 (0.5)	4.6 (0.5)	5.3 (0.6)	4.9 (0.6)[d]	4.9 (0.5)[d]
FT3/FT4 ratio, %	34.2 (6.0)	31.1 (31.1)[d]	33.7 (4.1)	31.5 (4.1)[d]	31.1 (4.9)[d]

If not stated otherwise, values are given as means (SD). Abbreviations: AD = Alzheimer's disease, FT3 = Free triiodothyronine, FT4 = Free thyroxine, MCI = Mild cognitive impairment, MMSE = Mini Mental State Examination, TSH = Thyroid-stimulating hormone.

[a] $p < 0.001$ vs controls; [b] $p < 0.05$ vs stable MCI; [c] $p < 0.001$ vs stable MCI; [d] $p < 0.05$ vs controls

In the cross-sectional analyses at baseline, there was no overall association between serum FT4 and hippocampal volumes. However, we found a significant interaction (FT4 x study group), and in further analyses, higher serum FT4 was associated with lower left baseline hippocampal volumes only in the AD group (β = -0.311, 95% CI = -0.17 to -0.02, p = 0.02). In addition, serum FT3/FT4 ratio were associated with greater left (β = 0.17, 95% CI = 0.01 – 0.04, p = 0.004) and right (β = 0.13, 95% CI = 0.00 – 0.04, p = 0.03) baseline hippocampal volumes in the entire study population. There was no association between serum TSH and FT3 levels and baseline hippocampal volumes.

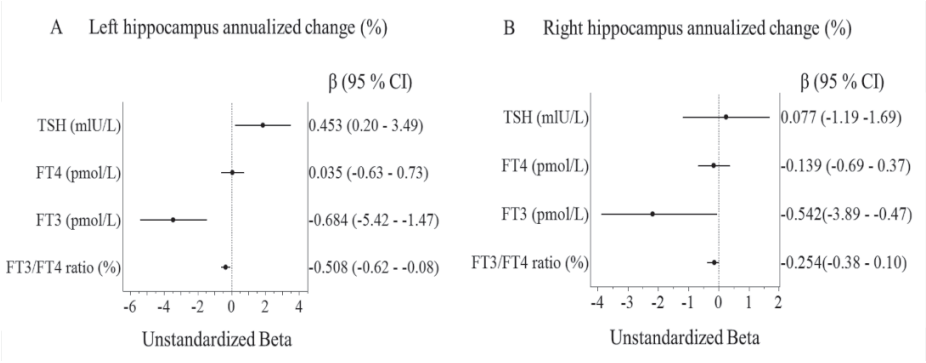

Figure 9. Effect sizes of the associations between thyroid hormones and the annualized percent changes in a) left and b) right hippocampal volumes in AD patients. The forest plot displays unstandardized Beta values and 95% confidence intervals derived by linear regression analyses.

In the longitudinal analyses, there were no overall associations between serum levels of TSH, FT4 and FT3/FT4 ratio and the annual hippocampal volume change. In contrast, higher serum FT3 levels were associated with greater annual volume loss in the entire study population. However, in the post-hoc analyses, lower serum TSH was associated with larger annual volume loss of the left hippocampus (β = 0.45, 95% CI = 0.20 – 3.49, p = 0.03) only in the AD patients. Also only in the AD patients, higher serum FT3 levels were related to grater annual decline of the left (β = -0.68, 95% CI = -5.68 to -1.47, p < 0.01) and right (β = -0.54, 95% CI = -3.89 to -0.47, p = 0.02) hippocampus. Furthermore, higher serum FT3/FT4 ratio was related to greater annual volume loss of the left hippocampus (β = -0.51, 95% CI = -0.62 to -0.08, p = 0.01;) (*Fig. 9 and 10*).

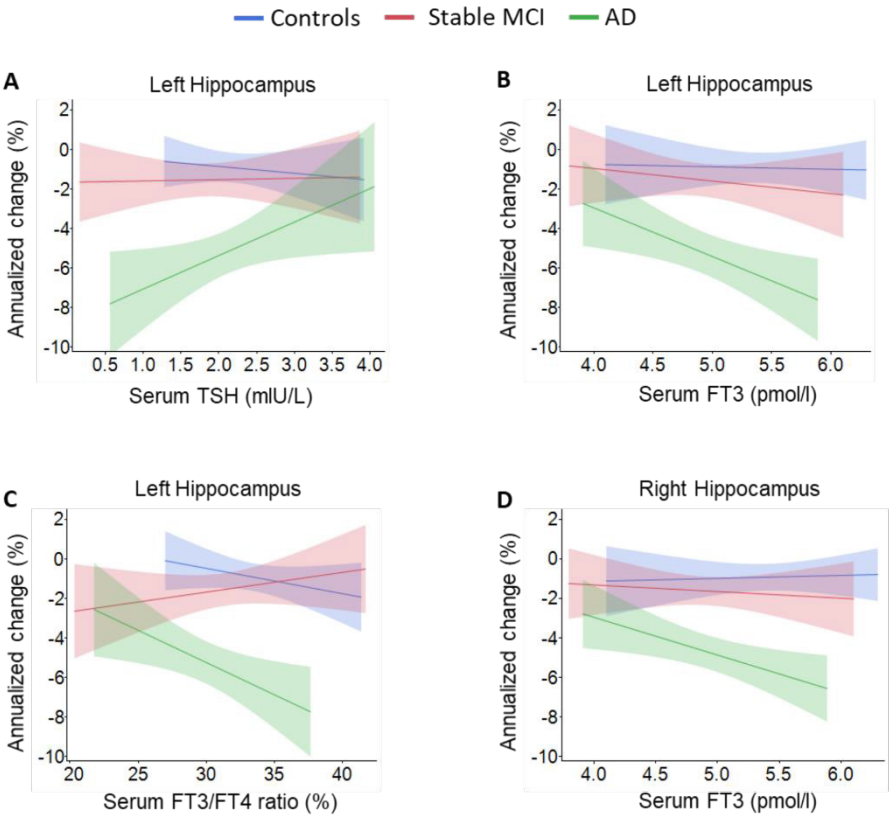

Figure 10. Associations between serum TSH, FT3 and FT3/FT4 ratio and the annualized percent changes of hippocampal volumes in healthy controls (n = 13), sMCI patients (n = 47) and AD (n = 26) patients. Lower serum TSH (A), higher serum FT3 (B), and higher serum FT3/FT4 ratio (C) were associated with larger annual loss of left hippocampal volume in AD patients but not in sMCI and healthy controls. Similarly, serum FT3 (D) was associated with larger annual loss of right hippocampal volume only in AD patients. Regression lines and 95% confidence intervals (shaded areas) are shown. Reproduced with permission from Elsevier, Quinlan et al. Psychoneuroendocrinology. 2022;139:105710.

4.4 STUDY IV

The objective of this study was to investigate the association between serum IGF-I and the risk of conversion from SCI and MCI to AD or VaD. At baseline, 342 patients suffered from SCI or MCI. Of these, during the follow-up (mean 3.6 years), 95 (28%) converted to all-cause dementia [AD, n = 37 (11%) and VaD, n = 42 (12%)].

Baseline analyses of quartile groups of serum IGF-I showed that the patients in the lowest IGF-I quartile (quartile 1) tended to be older, had lower BMI and were more likely to be female. Other variables were similar at baseline in the IGF-I quartile groups.

In Cox proportional hazard regression analyses, patients with low serum IGF-I had a more than twofold increased risk of converting to VaD compared with the patients in the two intermediate IGF-I quartiles (quartile 1 vs quartile 2-3: HR = 2.22, 95% CI: 1.13-4.36). This association remained after full adjustment for covariates (HR = 2.21, 95% CI: 1.05–4.63). Moreover, cumulative survival curves further demonstrated that low IGF-I increased the risk of VaD (log-rank test: p = 0.01 quartile 1 vs. quartiles 2–3; *Fig. 11*).

High serum IGF-I (quartile 4) was not associated with the risk of conversion to VaD in the Cox proportional hazard regression analyses. Furthermore, low IGF-I (quartile I) or high IGF-I (quartile 4) was not associated with the risk of all-cause dementia or AD compared with intermediate IGF-I levels (quartiles 2-3).

In additional Cox proportional hazard regression analyses, we compared the risk of VaD in the lowest IGF-I quartile compared with that in the three upper quartiles (quartiles 2-4). In these analyses, the risk of conversion to VaD was increased the lowest IGF-I quartile (quartile 1 vs. quartiles 2-4: HR = 2.19. 95% CI: 1.19-4.04; fully adjusted: HR = 2.20, 95% CI: 1.10–4.38). Thus, in summary, low serum IGF-I levels were associated with a higher risk of converting to VaD, but not with the risk of converting to AD without major vascular contribution.

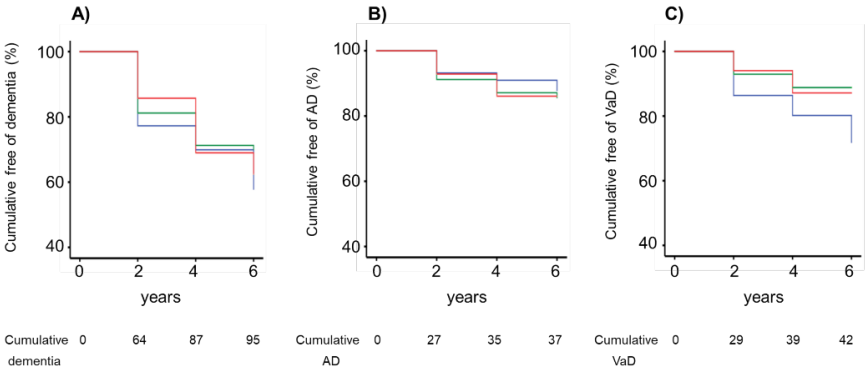

Figure 11. Low IGF-I is associated with increased risk of VaD. Kaplan-Meier survival curves for (A) all-cause dementia (log-rank test: P =0.22 quartile 1 vs. quartiles 2–3, P= 0.84 quartile 4 vs. quartiles 2–3), (B) AD (P =0.55 quartile 1 vs. quartiles 2–3, P= 0.87 quartile 4 vs. quartiles 2–3), and (C) VaD (P =0.01 quartile 1 vs. quartiles 2–3, P= 0.93 quartile 4 vs. quartiles 2–3) by serum IGF-I concentration. Blue, low IGF-I (quartile 1); green, intermediate IGF-I (quartile 2–3), red, high (quartile 4) serum IGF-I concentration. Please note that patients with mixed forms of AD and VaD were included in the VaD group. Reproduced with permission from Elsevier, Quinlan, P. et al. Psychoneuroendocrinology. 2017;86:169-175.

5 DISCUSSION

5.1 RESEARCH QUESTION AND MAIN FINDINGS

Altered TH and IGF-I regulation during aging has been associated with the decline of several physiological functions, including brain functions and cognition. However, whether altered levels of THs and IGF-I are associated with the risk of dementia remains unclear since previous studies have produced conflicting results. Conceivably, as most dementing diseases progress over many years, hormone levels and their associations with dementia risk may vary along the course of the disease. Therefore, this thesis examined the importance of THs and IGF-I in MCI and mild dementia to assess their relationships with dementia progression and associated markers of neuropathology in well-defined study populations recruited at a single memory clinic.

Study I was a longitudinal study of patients with SCI and MCI at baseline. The results showed that low baseline serum FT3 was associated with increased risk of progression to AD dementia, whereas the measured THs were unrelated to the risks of all-cause dementia and VaD. Patients with serum FT3 levels in the lowest FT3 quartile had a more than twofold increased risk of conversion to AD (Study I).

The cross-sectional analyses (Study II and III) demonstrated that serum THs are altered in AD patients. In mild AD dementia, serum FT4 levels were moderately elevated and serum T3/T4 ratios were decreased, whereas CSF TH levels were unchanged (Study II). In a larger population of AD patients, serum levels of FT3 and FT3/FT4 ratio were reduced compared with healthy controls (Study III).

In Study II and III, it was investigated whether THs were associated with CSF AD biomarkers and brain region volumes (Study II+III). There were no associations between THs and CSF AD biomarkers ($A\beta_{1-42}$, T-tau and P-tau; Study II). However, higher serum TH levels were associated with larger annual hippocampal volume loss in AD patients (Study III).

In Study IV, low baseline serum IGF-I level was associated with increased risk of conversion from SCI/MCI to VaD. Patients in the lowest IGF-I quartile had a more than two-fold increased risk of VaD compared with patients having higher serum IGF-I levels.

5.2 THYROID HORMONES

5.2.1 TRIIODOTHYRONINE AND THE PROGRESSION OF ALZHEIMER'S DISEASE

Several population-based studies have suggested that even within the normal reference range, excess rather than deficiency of circulating THs is associated with increased risk of dementia [196, 228, 244-247, 350]. However, the results of studies in manifest dementia are less clear, and there are few studies of the role of THs in memory clinic patients. Furthermore, the usefulness of serum T3 to predict the risk of subsequent dementia has previously not been evaluated at a memory clinic.

In Study I, which was performed in help-seeking patients with SCI and MCI at a single memory clinic, we found that serum FT3 levels were inversely associated with the risk of progression to AD dementia. The risk of progressing to manifest AD was more than twofold increased in patients in the lowest FT3 quartile compared with those in the highest FT3 quartile. Serum levels of TSH or FT4 were not related to the risk of conversion to dementia. The results remained significant after adjustment for several covariates, including hypertension, LDL/HDL ratio, BMI and *APOE ε4* genotype, suggesting that serum FT3 is independently associated with the risk of conversion to AD.

In addition to our study, only one previous study has investigated circulating THs in relation to the risk of dementia in a memory clinic population [247]. In that study (n = 93), TSH levels obtained from medical records were inversely related to the risk of subsequent AD (OR=3.5 per 1 mU/L decrease in TSH) [247]. Furthermore, in the population-based Korean Longitudinal Study on Health and Aging, eight of 76 individuals who were defined as MCI at baseline converted to all-cause dementia; the risk of converting to dementia was 6.8 times greater per 1 mlU/L decrease in serum TSH [350]. Thus, in contrast to the results of our study, the previous studies demonstrated an inverse association between TSH and the risk of dementia. However, the earlier studies did not evaluate serum FT3 and relatively few MCI patients were included [247, 350]. In addition, one of the previous studies did not exclude patients with thyroid disease or levothyroxine treatment [247]. Finally, in two population-based studies in older adults, no associations were detected between serum TT3 and all-cause dementia or AD [196, 228].

The results of several, mostly epidemiological, studies suggest that high-normal thyroid function (higher FT4 and lower TSH) is associated with increased risk of all-cause dementia and AD [196, 228, 244-247, 350]. We

have explored the time window in which the patients suffer from SCI or MCI without meeting the criteria for manifest dementia. The findings of Study I extend the previous literature by demonstrating that in SCI and MCI patients, serum TSH or FT4 are unrelated to the risk of conversion to dementia, whereas low serum FT3 is predictive of increased risk of conversion to AD.

5.2.2 ALTERED THYROID HORMONE CONCENTRATIONS IN ALZHEIMER'S DISEASE

In manifest AD dementia, THs have been unchanged or reduced in most studies [248-252]. This may be in some contrast to the observation in epidemiological studies that higher levels of THs are associated with increased risk of all-cause dementia and AD [196, 228, 244-247, 350]. Therefore, we investigated TH concentrations in serum (Study I - III) and CSF (Study II) in patients with AD and sMCI and also compared these values with those in cognitively healthy controls from the Gothenburg MCI study.

In patients with mild AD dementia (mean MMSE score = 25) (Study II), serum FT4 levels were elevated and serum TT4 tended to be increased compared with healthy controls, whereas serum FT3/FT4 and TT3/TT4 ratios were decreased. In Study III, AD patients as well as sMCI patients had lower serum FT3 levels and FT3/FT4 ratios compared with the controls. In addition, in Study I, serum FT3 levels were lower in patients who later developed AD dementia. Overall, these results suggest that peripheral THs are dysregulated in the relatively early phases of AD with low-normal serum FT3 as well as reduced FT3 in relation to FT4. Moreover, the reduced serum T3/T4 ratios in both Study II and Study III suggest that the peripheral conversion of T4 to the bioactive T3 is decreased in AD patients.

Serum FT4 was elevated in the AD patients included in Study II, whereas serum FT4 levels were not significantly altered in the AD group in Study III. Taken together, the possibility cannot be excluded that there is a minor elevation of serum FT4 also around the onset of manifest AD, which would be in line with previous epidemiological data [244-247, 350] and the two earlier studies in MCI [247, 350]. Furthermore, population-based data suggest that serum FT4 and TT4 levels are elevated already eight years before the onset of manifest AD dementia [246]. In contrast, although this has been scarcely studied, serum T3 levels have not been elevated in the asymptomatic stages of AD [196, 228]. Later on, when the disease has progressed to clinically manifest AD, serum TSH levels have been variable, serum FT4 levels have mostly been unchanged, whereas serum FT3 and TT3 levels have been unchanged or even reduced [248-250, 252-254]. Altogether, the results of Study I - III combined

with the results of other studies suggest that that TH levels gradually decline along the course of AD with higher TH levels in the very early stages followed by relatively lower values in AD dementia.

Few studies have examined the peripheral T4 deiodination in relation to cognitive impairment and dementia. In very old individuals, lower serum FT3 levels and lower FT3/FT4 ratios were associated with accelerated cognitive decline and increased mortality, respectively [195]. Furthermore, at a geriatric ward, reduced serum FT3/FT4 ratio was an independent predictor of cognitive decline even in old patients with unchanged FT3 levels [351]. The importance of the peripheral conversion of T4 to T3 in AD may be further supported by the finding that carriers of a common polymorphism (D2-Thr92Ala) in the DIO2 gene, which encodes the T4 to T3 converting type 2 deiodinase (D2) enzyme, showed transcriptional alterations associated with neurodegeneration [352]. Thus, there are several indications that lower peripheral conversion of T4 to T3 is associated with increased risk of cognitive decline. Although the underlying mechanisms are not fully clear, one possibility is that reduced peripheral conversion of T4 to T3 is the primary dysfunction in AD, which in turn results in a compensatory increase in T4 levels. However, it is also possible that reduced T3/T4 ratio in AD is a protective action in order to alleviate the harmful effects of high T4 on the brain.

In contrast to TH levels in serum, we did not observe any alterations in CSF TH levels in mild AD dementia compared with the control group. In previous cross-sectional studies of patients with mild to moderate AD dementia, CSF TH levels were unchanged in one study [253], whereas CSF TT4 levels were reduced in another study [252]. Furthermore, in advanced AD (mean MMSE score = 8), Sampaolo et al. found marked reductions in CSF levels of TT3 and TT3/TT4 ratio compared with controls [254]. These results suggest that T3 levels decline in CSF when AD progresses to the more severe stages of the disease. This assumption is supported by the findings of a study by Davis et al., which demonstrated low T3 but unchanged T4 in the postmortem prefrontal cortex in advanced AD (Braak stage V-VI) but not in AD with mild neuropathological burden (Braak stage I-II) [255]. Therefore, major changes in the central nervous levels of THs probably do not occur until the later stages of AD.

5.2.3 THYROID HORMONES AND CSF-BIOMARKERS OF ALZHEIMER'S DISEASE

Experimental studies have shown that aberrant TH signaling promotes AD neuropathology [256, 257, 263]. However, the association between THs and CSF AD biomarkers has been insufficiently studied in humans. We therefore investigated TH levels in serum and CSF in relation to CSF AD biomarkers.

In Study II, serum or CSF levels of THs did not correlate with the CSF levels of $A\beta_{1-42}$, T-tau and P-tau in AD patients or in healthy controls. One previous study of AD patients at a memory clinic showed that higher serum TSH levels were associated with lower CSF $A\beta_{1-42}$ levels in AD patients, and CSF TT4 was inversely correlated with CSF T-tau levels in the entire study population [252]. In the Honolulu-Asia Aging Study, higher serum TT4 levels were associated with a higher burden of neuritic plaques and neurofibrillary tangles in the post-mortem neocortex of participants with and without dementia [245]. Another study failed to show any associations between THs and the core AD biomarkers [253]. Thus, the associations between TH levels and markers of Aβ and tau metabolism have been variable in humans.

Experimental studies support that THs could influence AD pathogenesis as T3 suppressed the transcription of the APP gene *in vitro* [256, 257] and reduced the phosphorylation of the tau protein [258, 259]. Accordingly, induced hypothyroidism in rodent models of AD promoted Aβ accumulation and abnormal tau phosphorylation [262, 263]. Although experimental studies suggest that inadequate TH levels favor the expression of AD neuropathology, the results of human studies are inconsistent. The extent to which THs can directly affect Aβ and tau metabolism in humans is therefore not fully clear, but there is also a possibility that THs can interact with AD pathogenesis through indirect actions such as the production of reactive oxygen species, modulation of inflammation or glutamate release leading to excitotoxicity and neurodegeneration [218, 220, 353-355].

5.2.4 THYROID HORMONES AND BRAIN MORPHOLOGY

Atrophy of the mediotemporal lobe, which includes the hippocampus and amygdala, is one of the first signs of AD neuropathology and is strongly associated with cognitive decline [38, 39]. THs promote a number of processes in the adult hippocampus such as neurogenesis, myelination, plasticity and neuroprotection [208, 217]. However, no previous study has investigated whether THs are associated with brain region volumes in human AD.

In cross-sectional analyses in Study II, we evaluated whether serum and CSF levels of THs were related to MRI-estimated hippocampal and amygdalar volumes. In the AD group, serum FT3 levels correlated positively with left amygdalar volumes but not with hippocampal volumes. Furthermore, in the healthy controls, higher serum TT3 levels were associated with larger hippocampal volumes. In Study III, in which the number of participants was larger than in Study II, higher FT3/FT4 ratio was associated with greater baseline left and right hippocampal volumes in the total cohort. In the sMCI patients and the healthy controls, this was the only association that could be detected between thyroid function and hippocampal volumes. Therefore, the combined results of Study II and III suggest that in non-demented individuals, higher T3 in relation to T4 is associated with larger baseline hippocampal volumes. Finally, the CSF levels of THs were not associated with brain volumes in AD patients or healthy controls (Study II).

In study III, only in the AD group, there was also a baseline association between higher serum FT4 and lower left hippocampal volume. In previous population-based studies, higher serum FT4 has been related to smaller total brain volumes [227] as well as lower hippocampal and amygdalar volumes in elderly individuals [228]. Thus, high-normal FT4 may have an adverse effect on hippocampal size, and our results suggest that higher serum FT4 could be more detrimental in AD patients.

The associations between serum THs and longitudinal changes in MRI-estimated hippocampal volumes were determined in Study III. The results showed that only in the AD group, lower serum TSH and higher serum levels of FT3 and FT3/FT4 ratio were associated with greater annual hippocampal volume loss. In the AD patients, the annual loss of left hippocampal volume was 3.5% greater with each serum FT3 pmol/L increment. Similar effect sizes were found for the associations between serum TSH and FT3/FT4 ratio and the annual left hippocampal volume change. Thus, exclusively in the AD patients, higher thyroid function was associated with accelerated hippocampal volume loss.

Limbic-predominant age-related Tar DNA binding protein 43 (TDP-43) encephalopathy (LATE) is associated with altered TH levels, reduced cognitive function, and reduced hippocampal volume due to hippocampal sclerosis [356, 357]. Especially in AD patients over 80 years of age, co-morbid LATE neuropathology may be present, which in turn could worsen the hippocampal atrophy [358]. In Study II and III, it was not possible to evaluate the co-existence of LATE neuropathology due to the lack of autopsy confirmation. However, as all the included AD patients were below 80 years

of age at baseline, it is less likely that co-existence of LATE was of major importance for the results of Study II and III.

Overall, the results of Study II and III suggest that higher serum T3 in relation to T4 is associated with increased hippocampal volume in non-demented individuals. In contrast, in AD patients, higher thyroid function was associated with greater annual loss of hippocampal volume. Therefore, the associations between serum THs and hippocampal volumes in healthy controls and a group with cognitive impairment (sMCI) were partly different from those in AD patients. Speculatively, the associations found only in AD patients could be due to interactions between THs and AD neuropathology, whereas it is less likely that the AD-specific associations are due to cognitive impairment in general.

5.3 GENERAL CONCLUSION STUDY I - III

The results of Study I - III clearly show that TH levels are dysregulated in AD and related to the progression of the disease. Low serum FT3 was associated with increased risk of conversion to AD dementia. In manifest AD, the serum ratio between FT3 and FT4 was reduced as a consequence of low-normal serum FT3 and high-normal serum FT4. Furthermore, in manifest AD, higher thyroid function was associated with accelerated hippocampal volume loss. Thus, the levels as well as the role of THs may be different during the various phases of AD progression. However, it is not clear whether the dysregulation of THs represents a causal mechanism underlying AD or whether the changes in TH levels mainly stand for adaptive mechanisms in response to the increasing AD neuropathological load during the progression of the disease.

5.4 INSULIN-LIKE GROWTH FACTOR-I AND VASCULAR DEMENTIA

Studies in experimental animal models as well as in humans have suggested that deficiency of IGF-I, or resistance to IGF-I receptor signaling, may deprive the brain of neuroprotective and AD pathology regulating properties [307, 309, 318]. Low IGF-I levels have also been linked to increased risk of atherosclerosis and cardiovascular disease [301, 359]. However, in humans, the importance of IGF-I for VaD development has previously not been investigated in detail. Study IV was therefore the first prospective study to investigate the association between serum IGF-I and the risk of progression to all-cause dementia, AD or VaD in a memory clinic population.

In Study IV, low serum IGF-I levels were associated with increased risk of conversion to VaD. SCI and MCI patients who had serum IGF-I levels in the lowest IGF-I quartile at baseline had a more than twofold increased risk of subsequent VaD compared with patients having higher IGF-I levels. Some of the clinical baseline characteristics differed across the IGF-I quartile groups as the patients in the lowest IGF-I quartile were older, had lower BMI, and were more likely to be female. However, in the Cox proportional hazard regression analyses, all results remained statistically significant after adjustment for age, gender, BMI, and several other covariates. In contrast, high serum IGF-I at baseline was not associated with the risk of VaD. Furthermore, there was no association between low or high serum IGF-I and the risk of all-cause dementia or AD.

Previous studies have given some indications that IGF-I may be of importance for the development of VaD. Serum IGF-I was reduced in VaD patients in one study [175], and in another, a polymorphism in the IGF-I receptor gene was more frequently observed in female VaD patients compared with non-demented controls [306]. Furthermore, the results of experimental animal studies suggest that IGF-I can affect several processes related to VaD development such as alterations in endothelial cells and astrocytes, development of neurovascular uncoupling, and regulation of the number of oligodendrocytes and myelin production [360-363].

There have been discrepant results in terms of the relation between IGF-I and AD. A prospective analysis based on the Framingham Study cohort demonstrated that low serum IGF-I was associated with increased risk of AD and reduced total brain volume [290]. In contrast, in the Rotterdam Study, higher IGF-I receptor stimulating activity was associated with increased risk of dementia, suggesting IGF-I resistance in prodromal AD [318]. This would be in line with the experimental [364] and postmortem human [320, 365] findings of brain resistance to IGF-I receptor signaling in manifest AD dementia. Therefore, it could be speculated that high serum IGF-I would be a sign of IGF-I resistance in early AD stages. However, our results showed no association between low or high serum IGF-I and the risk of AD without concomitant brain vascular pathology. Therefore, the results of Study IV could suggest that the brain resistance to IGF-I receptor signaling is not present in AD until the disease is clinically manifest. Alternatively, in AD, the serum levels of IGF-I may not be representative of the brain resistance to IGF-I action.

5.5 STRENGTHS AND LIMITATIONS

To further understand the role of IGF-I and THs in relation to dementia, we examined help-seeking patients included in the longitudinal Gothenburg MCI study. This single-center study is conducted at the memory clinic at Sahlgrenska University Hospital, Mölndal, Sweden, and there are both strengths and limitations that need to be considered

5.5.1 STRENGTHS

The Gothenburg MCI Study was specifically designed to investigate the early and manifest phases of AD, VaD and mixed dementia and to characterize the mechanisms underlying the progression of MCI to manifest dementia. The strengths of the single-center Gothenburg MCI study included that all participants underwent a strict study protocol administered by experienced clinicians at a specialized memory clinic, which likely reduced the variability in the diagnostic assessments. In addition, the participants were well characterized as there were comprehensive assessments including medical history as well as physical, radiological, neurological and psychiatric examinations.

In addition, the exclusion criteria were designed to prevent the enrollment of patients with somatic and psychiatric disorders that could cause cognitive impairment. In the present studies, we applied additional exclusion criteria that could interfere with IGF-I or TH levels, including diabetes mellitus, treated or untreated thyroid disease or medication that could alter hormone levels. Thus, compared with most other studies, the included study populations were rather homogenous and free of comorbid conditions, which reduced the number of factors that could have obscured or biased the associations between hormone levels and dementia-related outcomes. In addition, for confounders that could not be controlled by exclusion, there was sufficient data to adjust the statistical models to estimate the independent effects of IGF-I or TH levels on the risk of dementia.

The longitudinal design of the Gothenburg MCI Study with close follow-up intervals allowed the investigation of THs and IGF-I levels in close proximity to the onset of dementia. In addition, our studies examined the critical time window in which patients have SCI, MCI, or only mild dementia. Thus, we were able to examine the associations between THs and IGF-I and dementia-related outcomes both before and after the onset of clinically manifest AD and severe dementia. In addition, the longitudinal design of Study III allowed us to examine whether THs were associated with annual hippocampal volume loss. In contrast, in previous population-based studies, hormone levels were

measured at baseline, and follow-up intervals were usually long until the onset of manifest dementia. Previous clinical trials were generally cross-sectional studies that included AD patients with advanced dementia. Therefore, most previous studies omitted the stage of MCI.

Another strength of Study I, III, and IV is that all blood samples were analyzed on one occasion in 2015. In Study II, the blood and CSF samples were analyzed in 2017. All analyses were performed using established assays in certified laboratories. These procedures likely reduced assay variability as well as the measurement errors in terms of IGF-I and THs concentrations.

5.5.2 LIMITATIONS

5.5.2.1 GENERALIZABILITY

Although studying a well-characterized study population of a single memory clinic has several strengths as confounding factors were minimized, it limits the generalizability of our results. The exclusion criteria of the Gothenburg MCI Study were rather restrictive. As a result, the study populations in Study I-IV were relatively homogenous and may therefore differ from the general population of individuals with cognitive disorders. Our participants were relatively free of psychiatric and somatic diseases; thus, our findings may not be applicable to individuals with comorbid conditions that could interact with the activity of THs and IGF-I. In Study I-III, we additionally excluded patients with thyroid disease including subclinical thyroid dysfunction, and our results may therefore not be valid in individuals with TH levels outside the normal reference range. Finally, the patients in Study II and III were in the early stages of AD dementia; the findings of these studies may not be applicable in more advanced stages of the disease.

5.5.2.2 METHODOLOGICAL AND STATISTICAL CONSIDERATIONS

A limitation of the studies presented in this thesis is that blood samples were only available at baseline, and no conclusions can therefore be made in terms of the trajectories of hormone levels during the progression of the cognitive disorders. Moreover, the lack of hormone measurements across the follow-up period could have underestimated the true association between hormone levels and dementia-related outcomes in the longitudinal studies (Study I, III and IV). Also, the relationship between IGF-I and THs in the circulation and the central nervous system is not well defined, limiting the conclusion about causality and underlying mechanisms of the association between serum hormone levels and the risk of conversion to dementia (Study I and IV), and hippocampal volume

loss (Study III). Preferably, future studies should include repeated hormone measurements in serum and CSF.

As in most single-center studies, the populations in the present studies were relatively small. Although the conversion rates of SCI/MCI to dementia were similar to those seen in other studies [118], the relatively small number of patients that progressed to dementia in Study I and IV may have limited the statistical power to detect the true associations between hormone levels and the risk of dementia. Furthermore, the study populations were of small to moderate size in Study II and III, and it cannot be excluded that some of the negative findings were due to limited statistical power. Moreover, it should be noted that in studies with small sample sizes, the risk of inflated effect sizes increases. In this regard, the relatively small number of participants with follow-up data in Study III may have resulted in an overestimation of the effect sizes in the regression models.

5.5.3 ETHICAL CONSIDERATIONS

The Gothenburg MCI study was approved by the local ethics committee (diary number: L091-99 15 March 1999/T479-11 8 June 2011). The studies were performed in accordance with the Declaration of Helsinki. Oral and written informed consent was obtained from all participants. However, an important ethical consideration in clinical dementia research concerns the informed consent process. In the Gothenburg MCI study, all patients had voluntarily sought help at the memory clinic due to cognitive complaints. Moreover, to be included in the Gothenburg MCI study, patients were not allowed to suffer from more severe cognitive impairment than that corresponding to mild dementia. All participants that could not provide free and informed consent were not included. Participants were assured that they could withdraw from the study at any time without repercussions for their continued treatment.

All participants were informed about the study procedures and examinations and received an additional briefing on invasive methods such as lumbar puncture. Both lumbar puncture and MRI are routine procedures in the clinical setting when assessing cognitive decline. Furthermore, additional MRI is often performed during follow-up examinations in the clinical setting. Before the clinical examinations, participants were informed of potential risks (especially headache after lumbar puncture and claustrophobia during MRI). After being informed of the potential risks, participants could decline to participate. Finally, an advantage of participation was that participants were examined by specialized physicians and experienced personnel according to a highly standardized protocol.

6 CONCLUSION

6.1 CONCLUSIONS OF INDIVIDUAL STUDIES

6.1.1 STUDY I

In patients with SCI or MCI at baseline of the Gothenburg MCI study, low serum FT3 was specifically associated with increased risk of conversion to AD dementia, whereas there was no association with the risk of conversion to all-cause dementia or VaD. None of the other measured THs were associated with the risk of conversion to dementia of any type. Overall, these findings suggest that it could be of value to monitor serum FT3 in patients with cognitive complaints.

6.1.2 STUDY II

In a cross-sectional study, serum FT4 levels were increased, and serum FT3/FT4 and TT3/TT4 ratios were decreased in AD patients compared with controls. CSF concentrations of THs were unchanged, and there were no correlations between THs and CSF AD biomarkers. However, serum FT3 was positively associated with amygdalar volume in AD, and in the healthy controls, there were positive correlations between serum TT3 and left and right hippocampal volumes. These results suggest that the peripheral conversion of T4 to T3 could be of importance in AD and that adequate T3 levels are required to maintain hippocampal volumes in healthy elderly individuals.

6.1.3 STUDY III

At baseline of the Gothenburg MCI study, serum levels of FT3 and FT3/FT4 ratio were reduced in patients with AD and sMCI compared with healthy controls. Furthermore, serum FT3/FT4 ratio was positively associated with baseline hippocampal volumes in all study groups, whereas higher serum FT4 levels were associated with lower baseline hippocampal volume only in the AD group. Also, exclusively in AD patients, lower serum TSH and higher serum levels of FT3 and FT3/FT4 ratio were associated with greater annual loss of hippocampal volumes. Thus, the associations between serum THs and hippocampal volumes were different in AD patients compared with sMCI patients and healthy controls. These observations suggest that THs could exert detrimental effects on the hippocampus in the presence of established AD neuropathology.

6.1.4 STUDY IV

During follow-up in the Gothenburg MCI study, low serum IGF-I at baseline was associated with increased risk of subsequent VaD, but not with the risk of all-cause dementia or AD without major concomitant brain vascular pathology. High serum IGF-I was not associated with the risk of subsequent dementia. This suggests that low serum IGF-I is associated with dysfunction of the brain vasculature, which in turn increases the risk of VaD.

6.2 GENERAL CONCLUSION

The results of the included studies indicate that THs are involved in the progression of AD, and that IGF-I is involved in VaD development. Low serum IGF-I is a predictor of the risk of conversion from SCI or MCI to clinically manifest VaD, which likely reflects that IGF-I interacts with the brain vasculature. Furthermore, low serum FT3 was associated with increased risk of AD. However, in contrast, higher thyroid function was associated with accelerated loss of hippocampal volume in manifest AD. Therefore, the role of THs may be different in manifest AD compared with that in the preclinical stages of AD or that in healthy individuals.

7 FUTURE PERSPECTIVES

In the Gothenburg MCI study, hormone measurements were available only at baseline. In consequence, individual set points of IGF-I or THs as well as potential variations of hormone levels across the study period could not be considered. Further studies are therefore needed to investigate the trajectories of hormone levels during cognitive decline and the development of AD or VaD. Optimally, levels of THs and IGF-I could be determined already in the very early, preclinical disease phases and then followed throughout the disease progression.

In Study I and Study IV, the associations between THs and IGF-I, respectively, and the risk of conversion to dementia were determined. However, THs and IGF-I were determined in serum. In future studies, it would be of interest to assess whether CSF levels of THs and IGF-I also are related to the risk of subsequent dementia.

In the included studies, it was not possible to determine the causality of the associations between hormone levels and dementia progression. Therefore, there is a need to assess whether alterations in hormone levels are causally related to the risk of dementia or if they reflect adaptations to the increased pathological load during dementia development. This information is, in turn, required to determine whether the observed changes in THs and IGF-I exert long-term beneficial or detrimental effects on dementia progression.

In Study II, TH levels did not correlate with the CSF AD biomarkers. Therefore, further studies are required to determine the mechanisms underlying the associations between TH levels and AD progression. Potentially, alterations in TH levels could affect inflammation and other pathways of neurodegeneration or interact with metabolism, and such effects could be dependent on whether there is ongoing neuropathology. Also, in terms of IGF-I, it is important to determine the mechanisms of how IGF-I affect the regulation of the brain vasculature and in what way this could affect the development of VaD.

A better understanding of the regulation and the actions of hormones during dementia development may not only provide opportunities to predict the conversion to dementia but could also influence the future treatment of dementia. Currently, there are no truly effective treatments for dementing disorders available. Therefore, any beneficial effects of hormonal modulation would be of value. For instance, levothyroxine treatment of hypothyroid

patients could be optimized if the effects of THs on cognition are delineated in more detail. Furthermore, IGF-I is closely related to insulin, and it would be of interest to determine how diabetic medications affect IGF-I activity as well as the risk of cognitive decline.

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