

The role of insulin-like growth factor-I in Alzheimer's disease and vascular dementia

Alexandra Horvath

Department of Internal Medicine and Clinical Nutrition

Institute of Medicine

Sahlgrenska Academy, University of Gothenburg



UNIVERSITY OF GOTHENBURG

Gothenburg 2024

The role of insulin-like growth factor-I in Alzheimer's disease and
vascular dementia

© Alexandra Horvath 2024
alexandra.horvath@gu.se

ISBN 978-91-8069-559-6 (PRINT)
ISBN 978-91-8069-560-2 (PDF)

Printed in Borås, Sweden 2024
Printed by Stema Specialtryck AB



“It is better to create something that others criticize than to create nothing and criticize others.”

Ricky Gervais

ABSTRACT

ABSTRACT

Background and purpose: Insulin-like growth factor-I (IGF-I) is involved in normal brain function, but little is known whether IGF-I activity affects the cognitive continuum of dementing disorders. The overall purpose of this thesis was to examine whether changes in IGF-I concentrations are linked to the development and progression of Alzheimer's disease (AD) and vascular dementia (VaD).

Methods: Study participants derived from the prospective Gothenburg Mild Cognitive Impairment study, which is performed at a single memory clinic. IGF-I was analyzed in serum (Study I-IV) and cerebrospinal fluid (Study II). Magnetic resonance imaging-estimated brain volumes were investigated in Study III and IV. In Study IV, neuropsychological test performance was also assessed.

Results: Patients with subjective or objective cognitive impairment (SCI/MCI) having low circulating IGF-I levels had a doubled risk of developing VaD (Study I). In AD, serum but not cerebrospinal fluid concentrations of IGF-I were higher than in the cognitively intact controls (Study II). In stable MCI, but not in AD, higher serum IGF-I was related to larger baseline volumes of the hippocampus and amygdala, and several brain lobes. Furthermore, in stable MCI, lower serum IGF-I was associated with accelerated loss of hippocampal volume over time (Study III). In SCI/MCI, the positive relationships between baseline IGF-I and white matter volumes at baseline and after 2 years were no longer present following correction for multiple variables. However, in the adjusted analyses, lower serum IGF-I was associated with decreased processing speed and executive function in both SCI/MCI and AD patients (Study IV).

Conclusion: Low serum IGF-I levels in SCI or MCI patients were associated with reduced neurocognitive performance and volumes of the gray but not the white brain matter. Low IGF-I was related to an increased risk of developing VaD in SCI and MCI patients. Conversely, in AD, IGF-I serum concentrations were elevated, which supports the hypothesis of IGF-I receptor resistance in the AD brain.

Keywords: IGF-I, mild cognitive impairment, Alzheimer's disease, vascular dementia

ISBN 978-91-8069-559-6 (PRINT)

ISBN 978-91-8069-560-2 (PDF)

SAMMANFATTNING PÅ SVENSKA

SAMMANFATTNING PÅ SVENSKA

Den globala befolkningen blir allt större vilket medför en ökad förekomst av kognitiv dysfunktion och olika demenssjukdomar. Trots stora forskningsframsteg så är fortfarande mycket okänt, men det finns ett visst belägg för att åldersrelaterade hormonella förändringar påverkar den kognitiva förmågan. Insulin-like growth factor-I (IGF-I), ett hormon som är viktigt för hjärnans utveckling men också för normal hjärnfunktion i vuxen ålder, har i ett fåtal tidigare studier kunnat påverka de olika stadierna vid kognitiv svikt. Den centrala målsättningen med denna avhandling var att utforska huruvida förändringar i nivåer av IGF-I påverkar förloppet, både innan och efter klinisk manifestation, av olika demenssjukdomar innefattande Alzheimers sjukdom (AD) och vaskulär demens (VaD).

Samtliga patienter ingick i Gothenburg Mild Cognitive Impairment study som bedrivs på Minnesmottagningen, Sahlgrenska Universitetssjukhuset, Mölndal, Sverige. IGF-I mättes i både blod (serum) och ryggvätska (CSF). Hjärnavbildningar för deltagarna bedömdes med hjälp av magnetisk resonanstomografi. Kognitiv funktion, både global och specifika kognitiva domäner, mättes med olika kognitiva tester.

I det första delarbetet så studerades patienter med subjektiv eller objektiv mild kognitiv störning (SCI eller MCI). Patienterna med SCI/MCI som hade låga nivåer av serum IGF-I hade en dubblerad risk att utveckla klinisk manifest VaD.

I det andra delarbetet så hade patienter med AD, jämfört med friska kontroller, högre nivåer av IGF-I i serum men inte i CSF.

I det tredje delarbetet, hos patienter med stabil MCI (sMCI), så observerades signifikanta korrelationer mellan högre serum IGF-I och större volymer av flertalet av de mätta hjärnområdena (hippocampus, amygdala samt frontal-, temporal- och parietalloberna). Dessa samband förelåg inte hos AD-patienterna. Vidare så var högre IGF-I nivåer i serum korrelerade till minskad årlig förlust av hippocampusvolym i sMCI-gruppen.

I det fjärde delarbetet så var högre serum-nivåer av IGF-I hos SCI/MCI korrelerade till större volymer av den totala vita substansen i hjärnan och corpus callosum inklusive dess ingående delstrukturer. Emellertid, efter justering för potentiella förväxlingsfaktorer, så kvarstod inga korrelationer mellan IGF-I och hjärnvolymer i SCI/MCI-gruppen eller AD-gruppen. Slutligen, efter justering för förväxlingsfaktorer, så var högre nivåer av serum

IGF-I nivåer korrelerat med bättre exekutiv funktion samt uppmärksamhet hos både SCI/MCI-patienter och AD-patienter.

Sammanfattningsvis så var låga nivåer av serum IGF-I hos patienter med SCI eller MCI associerade med ökad risk för att utveckla VaD, mindre hjärnvolymer i den grå men inte den vita hjärnsubstansen, och sämre kognitiv förmåga. Nivåerna av serum IGF-I var högre hos patienter med manifest AD jämfört med friska kontroller och lägre serum IGF-I var korrelerat med sämre kognitiv förmåga i AD-gruppen. Sammantaget så verkar effekterna av IGF-I att skilja sig åt beroende på underliggande hjärnsjukdom och sjukdomsfas, och den skyddande effekten av IGF-I verkar vara sänkt i hjärnans grå substans hos patienter med AD.

LIST OF PAPERS

LIST OF PAPERS

The current thesis is based on the following Studies, referred to in the text by their Roman numerals. All reprints in the thesis were made with copyright permission from the publishers.

- I. Quinlan, P., Horvath, A., Nordlund, A., Wallin, A., Svensson, J. 2017. Low serum insulin-like growth factor-I (IGF-I) level is associated with increased risk of vascular dementia. *Psychoneuroendocrinology*. 86: 169-175
- II. Horvath, A., Salman, Z., Quinlan, P., Wallin, A., Svensson, J. 2020. Patients with Alzheimer's disease have increased levels of insulin-like growth factor-I in serum but not in cerebrospinal fluid. *Journal of Alzheimer's Disease*. 75: 289-298
- III. Horvath, A., Quinlan, P., Eckerström, C., Åberg, ND., Wallin, A., Svensson, J. 2022. Low serum insulin-like growth factor-I is associated with decline in hippocampal volume in stable mild cognitive impairment but not in Alzheimer's Disease. *Journal of Alzheimer's Disease*. 88: 1007-1016.
- IV. Horvath, A., Quinlan, P., Eckerström, C., Åberg, ND., Wallin, A., Svensson, J. 2024. The associations between serum insulin-like growth factor-I, brain white matter volumes, and cognition in mild cognitive impairment and Alzheimer's disease. Accepted. *Journal of Alzheimer's Disease*.

CONTENTS

CONTENTS

ABBREVIATIONS	VII
DEFINITIONS IN SHORT	IX
INTRODUCTION	3
MILD COGNITIVE IMPAIRMENT	4
<i>BRAIN ATROPHY PATTERN IN MILD COGNITIVE IMPAIRMENT</i>	6
DEMENTIA	7
<i>DEFINITION</i>	7
ALZHEIMER'S DISEASE	8
<i>CLASSIFICATION AND DIAGNOSIS</i>	8
<i>GENETIC RISK FACTORS</i>	10
<i>ALZHEIMER'S DISEASE NEUROPATHOLOGY</i>	11
THE AMYLOID CASCADE HYPOTHESIS	11
TAU HYPERPHOSPHORYLATION AND NEUROFIBRILLARY TANGLES	13
<i>BRAIN ATROPHY PATTERN IN ALZHEIMER'S DISEASE</i>	15
VASCULAR DEMENTIA	18
<i>THE HISTORIC CONCEPT OF VASCULAR DEMENTIA</i>	18
<i>PATHOPHYSIOLOGIC FINDINGS OF VASCULAR DEMENTIA</i>	19
SUBCORTICAL SMALL VESSEL DISEASE	19
CEREBRAL LARGE VESSEL DISEASE	20
MIXED DEMENTIA	21
RISK FACTORS FOR DEMENTIA	21
INSULIN-LIKE GROWTH FACTOR-I	23
<i>THE SOMATOTROPIC AXIS</i>	26
<i>INSULIN-LIKE GROWTH FACTOR-I IN THE CENTRAL NERVOUS SYSTEM</i>	30
<i>INSULIN-LIKE GROWTH FACTOR-I AND THE COGNITIVE SPECTRUM</i> 31	
INSULIN-LIKE GROWTH FACTOR-I AND ITS RELATION TO COGNITIVE DECLINE	31
INSULIN-LIKE GROWTH FACTOR-I AND BRAIN MORPHOLOGY	32
INSULIN-LIKE GROWTH FACTOR-I AND DEMENTIA AND ITS RELATED RISK FACTORS	33
INSULIN-LIKE GROWTH FACTOR-I AND EXPERIMENTAL ALZHEIMER'S DISEASE	34
INSULIN-LIKE GROWTH FACTOR-I AND ALZHEIMER'S DISEASE IN HUMANS	35
INSULIN-LIKE GROWTH FACTOR-I IN CEREBROVASCULAR AGING AND VASCULAR DEMENTIA	37
AIM OF THESIS	41
MATERIALS AND METHODS	43

THE GOTHENBURG MCI STUDY – DESIGN AND ELIGIBILITY	43
<i>ELIGIBILITY CRITERIA</i>	43
ELIGIBILITY CRITERIA FOR THE GOTHENBURG MCI STUDY	43
SPECIFIC INCLUSION AND EXCLUSION CRITERIA FOR STUDY I-IV	44
DIAGNOSTIC PROCEDURES	45
<i>GRADING OF COGNITIVE FUNCTION</i>	45
<i>DIAGNOSTIC CATEGORIES</i>	47
PARTICIPANTS	47
<i>STUDY I</i>	47
<i>STUDY II</i>	48
<i>STUDY III</i>	48
<i>STUDY IV</i>	49
COVARIATE ASSESSMENT	49
CEREBROSPINAL FLUID AND BLOOD SAMPLES.....	49
BIOCHEMICAL AND NEUROCHEMICAL ASSESSMENTS	50
NEUROPSYCHOLOGICAL ASSESSMENTS.....	51
MAGNETIC RESONANCE IMAGING ASSESSMENTS	51
STATISTICAL ANALYSES	53
ETHICAL PERMITS.....	54
RESULTS.....	57
STUDY I.....	57
STUDY II.....	60
STUDY III	62
STUDY IV	65
DISCUSSION	69
IGF-I AND RISK OF DEMENTIA	69
INSULIN-LIKE GROWTH FACTOR-I AND INSULIN LEVELS IN HEALTHY PERSONS VS IN ALZHEIMER’S DISEASE	70
<i>CEREBROSPINAL FLUID INSULIN-LIKE GROWTH FACTOR-I LEVELS</i> ..	72
INSULIN-LIKE GROWTH FACTOR-I AND GRAY MATTER BRAIN MORPHOLOGY	73
IGF-I AND WHITE MATTER BRAIN MORPHOLOGY AND COGNITIVE FUNCTION	75
STRENGTHS AND LIMITATIONS	78
<i>STRENGTHS</i>	78
<i>LIMITATIONS</i>	79
ETHICAL DISCUSSION.....	80
CONCLUSION.....	83
FUTURE PERSPECTIVES	87
ACKNOWLEDGEMENT	89

REFERENCES..... 93

ABBREVIATIONS

ABBREVIATIONS

AD	Alzheimer's disease
ADL	Activities of daily living
APOE	Apolipoprotein <i>E</i>
APP	Amyloid precursor protein
A β	β -Amyloid
BBB	Blood-brain barrier
BMI	Body mass index
cVaD	Cortical vascular dementia
CC	Corpus callosum
CNS	Central nervous system
CSF	Cerebrospinal fluid
ELISA	Enzyme-linked immunosorbent assay
GDS	Global deterioration scale
GH	Growth hormone
GHRH	Growth hormone-releasing hormone
HDL	High-density lipoprotein
ICV	Intracranial volume
IGFBP	Insulin-like growth factor-binding protein
IGF-I	Insulin-like growth factor-I
IGF-IR	Insulin-like growth factor-I receptor
LDL	Low-density lipoprotein
MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
PET	Positron emission tomography
P-TAU	Phosphorylated tau
SCI	Subjective cognitive impairment
SMCI	Stable mild cognitive impairment
SSVD	Subcortical small vessel type of dementia
T-TAU	Total tau
TMT	Trail Making Test
VaD	Vascular dementia
WMH	White matter hyperintensity

DEFINITIONS IN SHORT

DEFINITIONS IN SHORT

Alzheimer's disease	A progressive neurodegenerative dementia associated with pathological protein deposits leading to brain damage and subsequently cognitive dysfunction.
Biomarker	A quantifiable parameter that is pathognomonic for a certain condition or biological state.
Cognition	The mental ability to process, understand, and act based on acquired knowledge, previous senses, and experiences.
Dementia	An umbrella term encompassing various diseases and illnesses of the brain, clinically observed as cognitive impairment interfering with activities of daily living.
Hormones	Signaling molecules secreted by endocrine glands and transported in the bloodstream, exerting their effects in various distant target tissues.
IGF-I	A hormone primarily acting as a mediator of growth of which 70% in serum is produced by the liver. It is also produced locally by various cells

	including neurons and astrocytes.
Mild cognitive impairment	A condition characterized by greater cognitive dysfunction than age matched counterparts and is a preceding stage of manifest dementia.
Mixed dementia	A state wherein an individual meets the criteria for more than one dementing disorder. A common combination includes Alzheimer's disease and vascular dementia.
Stable mild cognitive impairment	A diagnosis of mild cognitive impairment that remains stable throughout life without the progression to dementia.
Subjective cognitive impairment	Subjective complaints of cognitive deficits without any objective verifiable findings.
Vascular dementia	A dementia mainly caused by various small and large vessel-related events.

INTRODUCTION

INTRODUCTION

In 2023, the World Health Organization (WHO) reported that dementia is the 7th leading cause of death worldwide with an estimated incidence of 55 million individuals. Over 10 million persons are diagnosed with dementia annually, resulting in 150 million dementia cases by the year 2050¹. A recent publication proposed that the current global cost for persons with dementia is approximately 1313 billion US dollars². Since the prevalence of dementing disorders is rapidly increasing, it is not surprising that the economic burden will accumulate³. However, dementing disorders are not only costly but also have a large impact on demented patients and their families. Sadly, family caregivers of dementia patients tend to develop psychiatric symptoms including grief, anxiety, and symptoms of depression^{4,5}.

As dementing diseases continue to surge globally without any curative treatment available, there is a pressing need to identify factors which might influence the onset as well as the progression of cognitive impairment. Following decades of research, it is now widely accepted that aging is associated with hormonal changes, which could have clinical consequences^{6, 7}. Notably, levels of several circulating hormones decline with age including insulin-like growth factor-I (IGF-I), estrogens and androgens leading to onset of somatopause, menopause and andropause, respectively⁸. These age-related phenomena are also associated with obesity, osteoporosis, cardiovascular events, insulin resistance as well as increased risk of mortality in population-based studies⁹⁻¹⁵. Moreover, the endocrine senescence seems to accelerate cognitive decline and increase the risk of dementia development^{16, 17}. While recognition of their pathophysiological intersection is growing, it is not yet clear whether endocrine alterations are indeed detrimental during the aging process¹⁸. For instance, both reduced and excessive hormone concentrations have been related to an increased risk of progression to several subtypes of dementing disorders such as Alzheimer's disease (AD) and vascular dementia (VaD).

While the current evidence is evasive, one hormone has received specific attention due to its regulation of normal brain health. IGF-I has an important role for the central nervous system (CNS) as it promotes proliferation and differentiation of neuronal cells, and influences growth

and mental development¹⁹⁻²¹. Although rare, mutations in the IGF-I gene are linked to mental retardation²². In addition to having pleiotropic effects, the IGF-I receptor (IGF-IR) is abundantly expressed in the brain throughout life demonstrating its importance in aging individuals^{23, 24}. In support of this, higher serum IGF-I has been related to better selective attention, executive function, and working memory in cognitively intact individuals^{25, 26}. Furthermore, a few longitudinal investigations have shown that decreased IGF-I is related to dementia-related risk factors such as insulin resistance, hypertension, atherosclerosis, and ischemic stroke²⁷⁻²⁹.

The findings of previous research indicate that IGF-I may be involved in the pathological processes of AD and VaD. However, most previous clinical data are based on epidemiological studies, while studies investigating IGF-I and cognition in memory clinic settings are lacking. The rationale of this thesis relies on previous research efforts demonstrating not only a relationship between IGF-I and risk factors for dementia, but also that altered IGF-I levels in serum and cerebrospinal fluid (CSF) seem to influence dementia onset and disease progression. This warrants longitudinal clinical studies in the memory clinic setting to elucidate how IGF-I interacts with dementia development.

MILD COGNITIVE IMPAIRMENT

Cognitive decline is part of the normal aging process leading to both structural as well as functional alterations in the brain. Increasing age is closely related to deterioration in certain cognitive abilities while other functions are more resilient to brain aging^{30, 31}. In some instances, the cognitive decline is more severe than the normal age-related cognitive deficits.

In the context of medicine, cognitive impairment is described as a clinical syndrome that includes the spectrum of individuals from subjective or mild cognitive impairment (SCI/MCI) to dementing disorders (Figure 1). SCI is characterized by subjective cognitive complaints and is often considered as a putative stage before MCI and dementia onset³². Individuals with SCI are twice as likely to develop MCI than their cognitively intact counterparts, and 7% of SCI patients are given a dementia diagnosis later on in life³³. Persons with MCI in

addition have objective findings of a more marked cognitive decline than that in normal elderly persons³⁴.

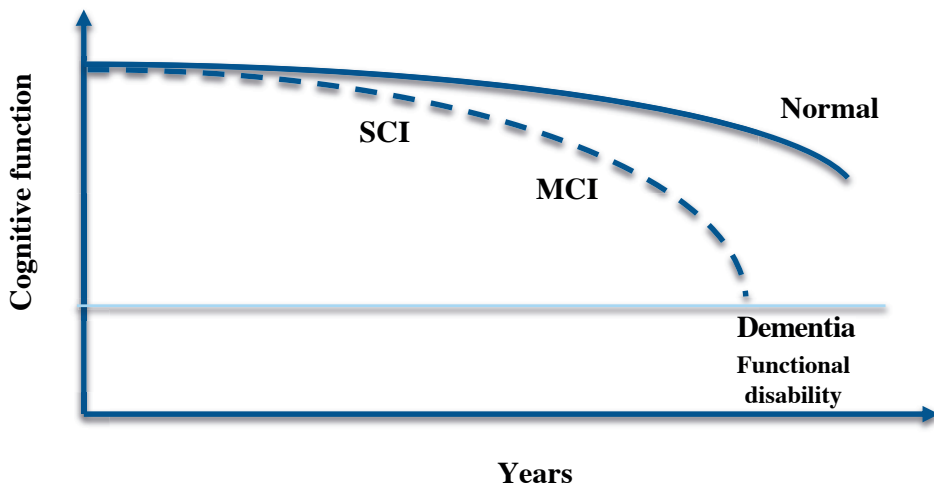


Figure 1. The cognitive continuum throughout the lifespan. MCI; mild cognitive impairment, SCI; subjective cognitive impairment. Created with Microsoft PowerPoint.

In the 5th version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), individuals with MCI display a *modest* cognitive decline in at least one cognitive area (social cognition, language, perceptual motor skills, learning and memory, executive function, or attention). Compared to a dementia diagnosis, patients with MCI still have the ability to conduct activities of daily living (ADL)³⁵. MCI can be challenging to diagnose due to its heterogeneity and there are no cognitive tests or biomarkers that correlate with, or predict the risk of MCI³⁶. Although MCI can be a transient phase between preclinical and clinically established dementia, some patients remain in stable MCI (sMCI) without dementia development³⁷. Traditionally, MCI has been regarded as two distinct subtypes depending on the underlying etiology. Amnesic MCI is mainly considered as a putative stage prior to the onset of AD, whereas non-amnesic MCI demonstrate deficits in other cognitive domains and may progress to AD or other dementia diseases³⁸.

Previous meta-analyses of MCI patients have concluded that the annual conversion rate to dementia is approximately 5-10%. Out of these patients, 9.6% progress to AD and 8.1% to VaD³⁹. In the short-term, a multimodal intervention approach involving physical and cognitive exercises as well as vitamin supplements, the latter in individuals with malnutrition, may enhance global cognition as well as other cognitive functions in MCI⁴⁰. However, the relatively high number of dementia converters in this subset of patients³⁹ may argue against long-term protection of such interventions. In summary, individuals with cognitive impairment develop a broad range of symptoms, and in its severest form, the cognitive decline results in a dementia diagnosis.

BRAIN ATROPHY PATTERN IN MILD COGNITIVE IMPAIRMENT

Earlier, it was believed that the topological progression of brain atrophy was a typical phenomenon of aging⁴¹. However, later investigations have confirmed that brain regional volume loss varies in healthy individuals, MCI, and dementia patients, and this difference becomes more marked with increasing cognitive disability⁴²⁻⁴⁴. MCI typically show signs of cortical thinning and degeneration of the medial temporal lobe (MTL) consisting of the hippocampus, parahippocampal regions, amygdala, and entorhinal cortex. These characteristics are central features of AD, although not all MCI patients with these imaging results develop AD dementia⁴⁵⁻⁴⁷. Moreover, as MCI progresses, the MTL atrophy is accompanied by hypoactivation of MTL circuits, especially during memory tasks⁴⁸. Additionally, brain autopsies extend previous brain imaging findings as more than half of MCI patients demonstrate AD-like neuropathology⁴⁹.

Like in AD, the hippocampus seems especially vulnerable to degeneration in MCI as its annual atrophy rate was 3.0% compared to 1.1% observed in normal aging⁵⁰. Within the hippocampus, volume loss of the CA1-CA2 transition zone appears to be superior in distinguishing cognitively intact persons from those with MCI⁵¹. Additionally, MCI patients who later developed AD dementia displayed smaller cortical brain volumes pathognomonic of AD (entorhinal, temporoparietal, posterior cingulate, and orbitofrontal cortex) than those remaining as stable MCI⁵².

Furthermore, ischemic lesions in the brain white matter visualized on magnetic resonance imaging (MRI) fluid attenuated inversion recovery (FLAIR) sequences; white matter hyperintensities (WMHs), are commonly observed in elderly individuals with a prevalence increasing with the aging population⁵³. It is generally believed that the amount of WMHs can be used as a biomarker of cerebrovascular dysfunction in the smaller arteries. In MCI, these WMHs seem to involve the temporal, prefrontal and parietal lobes⁵⁴. A recent meta-analysis of MCI patients demonstrated that WMH load has a medium-sized relationship with several cognitive domains such as executive function, attention, and processing speed⁵⁵.

Altogether, these findings confirm that MCI is a heterogenous disease and may show signs of pathological changes that precede the onset of dementia, including AD and VaD.

DEMENTIA

DEFINITION

Historically, the phrase dementia stems from *demens*, a Latin word meaning "without mind", indicating that an individual becomes disconnected from their own mind. In 1797, Philippe Pinel was the first to describe the medical term dementia, but it has been used as a phrase as early as the 13th century⁵⁶. The characteristics of dementia were later on expanded by Pinel's student, Etienne Esquirol, who described dementia as an acquired, rather than a developmental syndrome affecting the brain⁵⁶.

For many years, the term dementia has been considered a negative label and even stigmatic given the meaning of *demens*⁵⁷. As a result, in comparison to DSM-4, dementia has been rephrased as *major neurocognitive disorders* in DSM-5 and is distinguished from mental illnesses. Herein, "cognitive" reflects a person's abilities to think and reflect while "neurocognitive" is an umbrella term for brain diseases and impairments of brain function causing neurocognitive disorders³⁵. However, in both the social and research milieu, dementia is still used universally and for this reason, this term will be used in the thesis.

Moreover, similar to the classification of MCI, dementia is diagnosed in individuals with persistent and progressive decline in at least one cognitive domain³⁵. In addition, it is required that the person has lost the ability to perform everyday tasks that can be quantified using various scales^{35, 58}. Thirteen dementia subtypes have been included in the DSM-5; in individuals over the age of 65 years, 70% of dementia is related to AD³⁵.

ALZHEIMER'S DISEASE

CLASSIFICATION AND DIAGNOSIS

Between 1901-1906, the psychiatrist Alois Alzheimer documented the symptoms of the female patient Auguste D at the Frankfurt Psychiatric Hospital. She presented with and developed disturbances in sleep and memory, and demonstrated behavioral changes including aggressiveness, and confusion. Following her death, Alzheimer performed an autopsy of her brain and found two prominent histological processes, which is today recognized as amyloid- β ($A\beta$) plaques and neurofibrillary tangles (NFTs). Originally, the condition of Auguste D was described as *presenile dementia* although it was later changed to carry his name; *Alzheimer's disease*⁵⁹.

AD is the most abundant dementia etiology, representing 70% of all cases. In the 7th and 8th decade of life, the incidence increases exponentially⁶⁰. To have standardized criteria for the diagnosis of clinical AD, the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) created a work group and published their initial recommendations in 1984. They provided three possible classifications of AD depending on the degree of certainty; *possible AD*, *probable AD*, and *definite AD*, the latter based on histopathologic findings from autopsies or biopsies⁶¹. However, the NINCDS-ADRDA criteria have been updated to harmonize with later research findings providing higher specificity and sensitivity for an AD diagnosis.

Previously, it was hypothesized that symptoms of AD gradually and slowly deteriorate, especially memory function in combination with deterioration in at least one other cognitive function. In contrast,

accumulating evidence now suggests that the neuropathological processes leading to cognitive dysfunction start years or even decades before the clinical manifestations are evident. These alterations include CSF biomarkers of brain amyloid accumulation and neuronal injury as well as changes on brain imaging^{38, 62, 63}. Moreover, the Rotterdam study displayed that memory deficits in AD patients were present up to 16 years before manifest dementia⁶⁴. Based on previous findings, the revised NINCDS-ADRDA criteria relies on the notion that AD is a continuum from the preclinical stages of disease to severe AD dementia (Figure 2)^{38, 62, 63}. In parallel with this revision, the criteria for clinical AD were similarly revised in both the DSM-5 as well as the International Classification of Diseases 10th Revision (ICD-10)^{35, 65}.

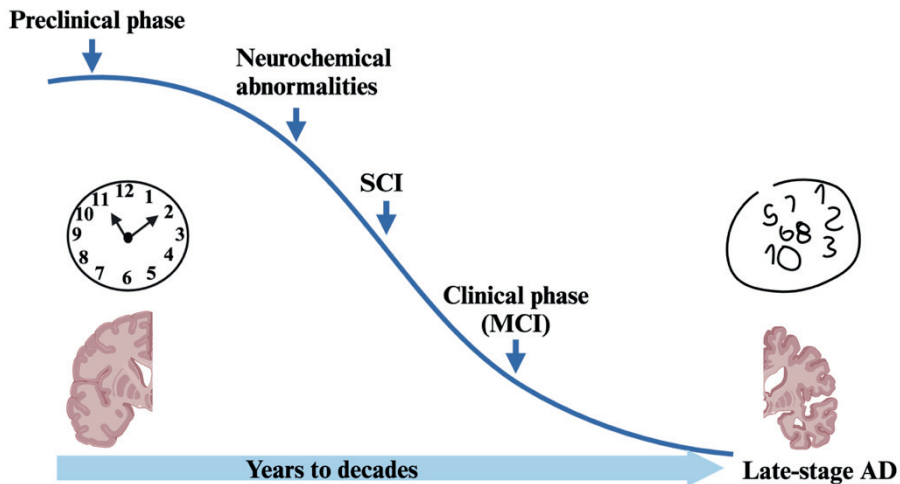


Figure 2. Continuum of AD. Neurochemical brain alterations including accumulation of P-tau and $A\beta$ possibly debut years or even decades prior to the onset of manifest AD whereas brain volumes and cognition remains intact during the initial stages of AD. However, late-stage AD is associated with pronounced brain atrophy and cognitive impairment as demonstrated by the Clock-test. $A\beta$; Amyloid beta protein, AD; Alzheimer's Disease, MCI; mild cognitive impairment, P-tau; phosphorylated tau protein, SCI; subjective cognitive impairment. Created with BioRender.com

GENETIC RISK FACTORS

AD is categorized as either early-onset debuting <65 years of age, or as late-onset that occur in individuals >65 years. Early-onset AD (EOAD) is rare and found in 1-5% of all AD cases and usually progresses more rapidly than late-onset AD (LOAD), the latter representing the majority of AD patients ($\approx 95\%$). In general, AD in individuals with onset <65 years of age is inherited in a Mendelian pattern and include mutations in three distinguished genes that regulate the metabolism of A β ; amyloid precursor protein (*APP*) and presenilin-1/2 (*PSEN1/PSEN2*). Since these genes have a high penetrance (85%) and autosomal dominance, they can be used as diagnostic biomarkers for EOAD^{66, 67}.

Moreover, the apolipoprotein *E* (*APOE*) gene has been established as an important mediator of LOAD susceptibility⁶⁸. Human genetic studies have shown that the *APOE* gene is present in three alleles; $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ with corresponding frequencies of 8.4%, 77.9% and 13.7%, respectively⁶⁹. The ApoE protein is an important regulator of lipid metabolism in peripheral organs, while in the brain, this protein is involved in the transportation of cholesterol to neuronal cells and is a mediator of A β metabolism. Astrocytes are the main producers of the ApoE protein in the brain, and while the exact mechanisms are unclear, A β are able to bind to the ApoE protein, indicating a direct association between these proteins⁷⁰. The $\epsilon 2$ allele confers protection against AD development, whereas $\epsilon 3$ is neutral in terms of AD risk. Conversely, there is a dose-dependent relationship between the number of *APOE* $\epsilon 4$ alleles and the risk of AD dementia⁷¹. In postmortem studies, the $\epsilon 4$ allele was related to a more pronounced turnover of A β to senile plaques and ApoE was more commonly deposited in neuritic plaques than in noncarriers of the $\epsilon 4$ ^{72, 73}. However, the etiology behind LOAD is probably multifactorial and likely involves additional environmental and metabolic processes⁷⁴.

ALZHEIMER'S DISEASE NEUROPATHOLOGY

Initially, AD was thought to develop because of reduced synthesis of acetylcholine in the presynaptic regions. In fact, most of the available drug therapies for AD relies on the cholinergic hypothesis⁷⁵. Nevertheless, the pathophysiologic processes involved in AD was later expanded as the amyloid cascade is now considered an established theory of AD causation, subsequently leading to the tau cascade. Although the latter two processes are associated with AD, it cannot be excluded that AD is the result of various genes that are beyond amyloid and tau depositions.

THE AMYLOID CASCADE HYPOTHESIS

Senile or neuritic plaques containing aggregates of the β -amyloid protein have been firmly implicated in the neuropathology of AD and are considered as one of the hallmarks of the disease. Of particular pertinence, these plaques are found in the extracellular space of the brain and are mainly composed of the $A\beta_{42}$ isoform due to its hydrophobic character (Figure 3). However, there is also evidence of folded $A\beta_{40}$ within the neuritic plaques⁷⁶.

The amyloid- β protein is a residual product from the APP metabolism. APP is a protein found in intracellular vesicles or on the cell surface of various neuronal cells. However, APP is expressed in other peripheral tissues as well⁷⁷. APP is degraded sequentially by the cleaving enzymes β -secretase and γ -secretase, which results in various amino acid lengths of amyloid- β ⁷⁸.

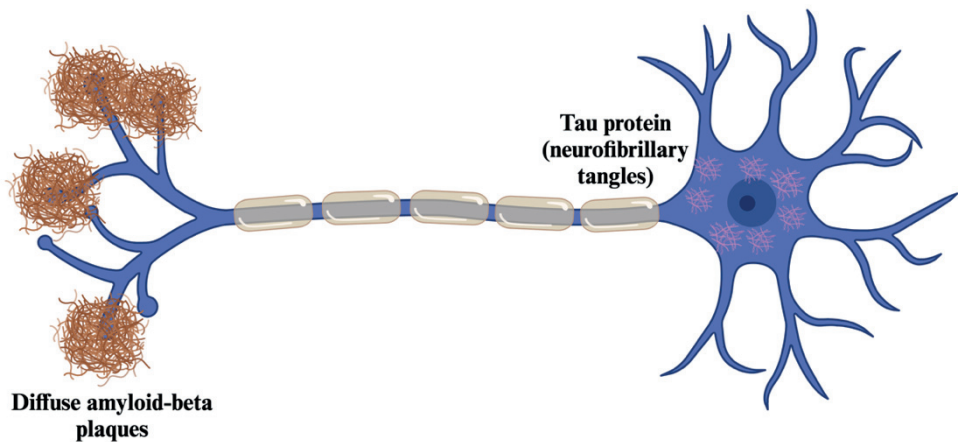


Figure 3. Neuronal cell with AD pathology. β -amyloid plaques are formed in the extracellular space while neurofibrillary tangles containing P-tau are found within the cell. AD; Alzheimer' disease, β -amyloid; beta-amyloid, P-tau; phosphorylated tau. Created with BioRender.com

The A β protein reaches its final form through degradation by neprilysin and insulin-degrading enzyme. Normally, large quantities of the A β remain undegraded and several mechanisms at the level of the blood-brain barrier (BBB) can result in release of A β into the circulation⁷⁹. An experimental study of transgenic mice concluded that plasma A β correlates with the burden of A β deposition in the hippocampal and cortical brain regions⁸⁰. Moreover, the A β_{42} /A β_{40} ratio seems to be linked to age at disease manifestation in familial AD⁸¹.

Although not completely understood, preclinical experiments have illustrated that AD is delineated by an imbalance in the production and elimination of A β . This discrepancy results in aggregation of the amyloid- β protein with subsequent deposition, and finally the formation of neuritic plaques⁸². Accumulation of A β exerts neurotoxic effects by stimulating glial cells (astrocytes and microglia), damaging dendrites and axons, and causing dysfunctional synapses⁸³. Mechanisms contributing to A β toxicity include N-methyl-d-aspartate receptor (NMDAR) activity⁸⁴, neuroinflammatory responses⁸⁵, and oxidative stress through reactive oxygen species (ROS)⁸⁶.

Based on its importance in AD neuropathology, A β is used as a fluid biomarker in CSF⁸⁷ as well as on A β positron emission tomography (A β -PET) imaging, where brain amyloidosis can be visualized^{88, 89}. Deranged A β levels confirmed in CSF and on PET scans is regarded as the earliest sign of AD neuropathology^{90, 91}. It has been postulated that in AD, roughly 50% of the decrease in CSF A β_{42} levels are the result of A β aggregation in neuritic plaques. The concentrations of A β_{42} in CSF has been extensively used as diagnostic tool for AD in both clinical and research settings, demonstrating high validity and sensitivity of approximately 80%^{92, 93}. Similar to the analyses of CSF A β for the prediction of AD, recent studies have shown that A β -PET is reliable for predicting AD conversion and highly correlates with post-mortem amyloid load^{89, 94, 95}. However, A β -PET has been somewhat questioned as elders with normal cognition display relatively high levels of A β deposition⁹⁶. In the recent guidelines by the National Institute on Aging and Alzheimer's Association (NIA-AA), it is purported that cognitive decline is not driven by β -amyloidosis alone, but β -amyloidosis may affect other downstream pathways (tau hyperphosphorylation and neurodegeneration), eventually causing cognitive impairment⁹⁷.

TAU HYPERPHOSPHORYLATION AND NEUROFIBRILLARY TANGLES

A widely held view is that increased brain amyloid levels cause abnormal phosphorylation of tau although some studies propose that neuritic plaques are preceded by NFT development possibly enhancing existing A β toxicity^{98, 99}. Tau is a microtubule-related protein that is a component of the neuronal cytoskeleton enabling axonal transport by stabilizing the microtubules¹⁰⁰. The activity of tau is inversely correlated with the amount of phosphorylation, thereby modulating its binding capacity to the microtubules¹⁰¹. *In vitro*, tau phosphorylation stimulates its self-aggregation into paired helical filaments (PHFs) with consequential dissemble of microtubules¹⁰². In turn, retrograde synapse loss occurs followed by neuronal death and subsequent leakage of tau¹⁰³. In the 1980s, it was demonstrated that PHFs observed in NFTs of AD brains consist of tau¹⁰⁴.

The results of multiple studies suggest that the distribution of intraneuronal neurofibrils and NFTs in AD follows a distinct arrangement¹⁰⁵⁻¹⁰⁸. Followingly, Braak and Braak proposed a spatiotemporal pattern involving six stages¹⁰⁹. In the first stage (Stage I), NFTs can be found in the entorhinal cortex while the second stage additionally involves the hippocampal CA1 area. NFTs then spread to include the subicular complex of the hippocampus (Stage III), followed by the thalamus, amygdala, and claustrum (Stage IV). In Stages V and VI, NFTs are also visible in the isocortex of the associate areas and the motor, sensory, and visual areas, respectively¹⁰⁹. The level of AD-like atrophy pattern on brain MRI (structural abnormality index scores (STAND)) in living individuals strongly correlates with postmortem Braak staging¹¹⁰. However, comparably to the depositions of A β , NFTs can be identified in individuals free from dementing disorders¹¹¹.

Given that tau proteins eventually spread into the extracellular matrix of AD brains, tau is used for diagnostic purposes; total tau (T-tau) and phosphorylated tau (P-tau). T-tau reflects the total amount of neuronal or axonal injury and demonstrate a high sensitivity in AD¹¹². However, increments in CSF T-tau levels are also present in other neurological disorders such as Creutzfeldt-Jakob disease and stroke^{113,114}. In contrast, P-tau is AD-specific and can differentiate AD from other brain-related conditions¹¹⁵. Almost all AD patients show increments in CSF concentrations of T-tau and P-tau₁₈₁ with sensitivities of approximately 80% and 85%, respectively¹¹². In recent years, immunoassays for plasma have been developed that are able to differentiate AD from cognitively healthy individuals using blood P-tau_(s202)¹¹⁶. In all, the NIA-AA recommends that an AD diagnosis should be given in individuals when there is evidence of abnormal biomarker levels of both A β and tau^{97,117}.

Levels of NFTs have been linked to the distribution of brain atrophy¹¹⁸, cognitive function¹¹⁹, and dementia severity¹²⁰. In individuals spanning from normal aging to AD, higher tau PET signaling was associated with lower performance in memory, language, executive function, and global cognition¹²¹⁻¹²³ as well as Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog) scores¹²⁴. In similar patient

populations undergoing PET scans, increased uptake of tau in the neocortex and MTL correlates well with positive amyloid PET imaging^{125, 126}.

BRAIN ATROPHY PATTERN IN ALZHEIMER'S DISEASE

Following the same topographic pattern as tau formation and Braak staging, degeneration of the MTL is the earliest brain imaging sign of AD disease¹²⁷⁻¹²⁹. This brain area is mainly involved in learning and memory¹³⁰. Atrophy of the MTL has been shown to predict future cognitive deterioration, progression from MCI to AD, and distinguishing probable AD from normal aging¹³¹⁻¹³³. Based on its sensitivity to detect AD, scoring systems of MTL atrophy (MTA score/Shelten's score)¹¹⁰ and entorhinal cortical atrophy (ERICA score)¹³⁴ have been developed showing high diagnostic accuracy for AD.

Morphologic changes to the hippocampus found early in the disease course makes it a valuable marker of AD¹³⁵. Compared to cognitively intact individuals, patients with AD demonstrate 15-30% reduced volumes of the hippocampus in the early disease stages with an annual decline that was 3.3% higher than their healthy counterparts¹³⁶. Prospective studies further illustrate that hippocampal degeneration precedes the onset of symptoms and correlates with deterioration in visual and verbal memory¹³⁷. Hippocampal atrophy seems to be comparable between EOAD and LOAD¹³⁸ although it lacks specificity for AD as morphological changes in the hippocampus can be observed in other dementias such as VaD¹³⁹ and frontotemporal lobe dementia¹⁴⁰.

Similar to the small vessel type of dementia (SSVD), widespread atrophy of the brain white matter and the presence of WMHs also exists in the AD brain, predominantly the posterior parts of the brain¹⁴¹⁻¹⁴³. The burden of WMHs is positively associated with MTA and AD severity, demonstrating a stronger relationship than in MCI patients¹⁴⁴. Moreover, longitudinal studies converge to propose that the corpus

callosum (CC), fornix, and the cingulum are the best brain white matter markers of AD progression (Figure 4)^{145, 146}.

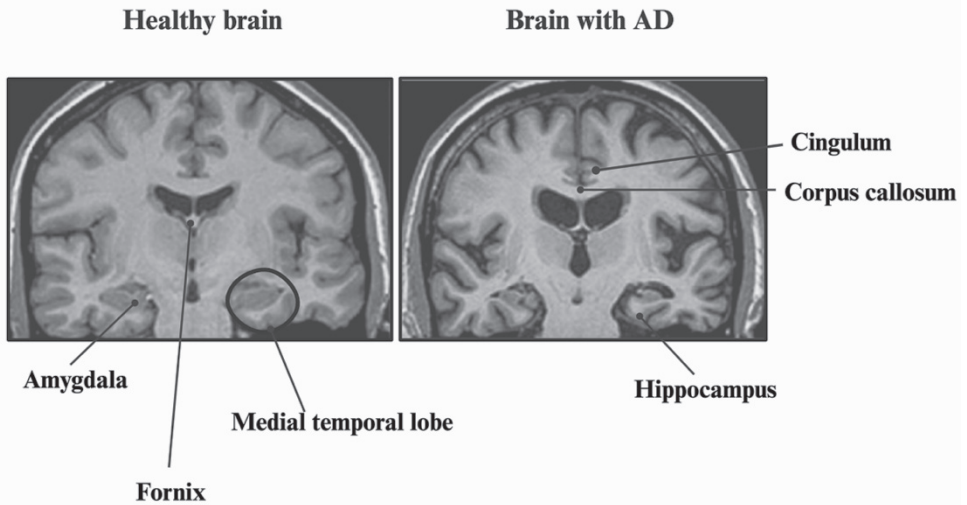


Figure 4. Coronal slices of a healthy brain (left) and a brain with AD (right), visualized on T1-weighted MRI. AD; Alzheimer's disease, MRI; magnetic resonance imaging. Created with BioRender.com

Parallel with AD severity, brain atrophy spreads in a stereotypical pattern to involve other neocortical areas extending in a temporal-parietal-frontal trajectory. Finally, other brain regions responsible for visual, motor, and sensory functions usually become involved in late-stage AD¹⁴⁷. In addition to this pathognomonic brain degeneration, gross findings in late disease stages typically encompass diffuse and gyral brain atrophy and enlargement of the lateral ventricles and Sylvian fissure (Figure 5)¹⁴⁸.

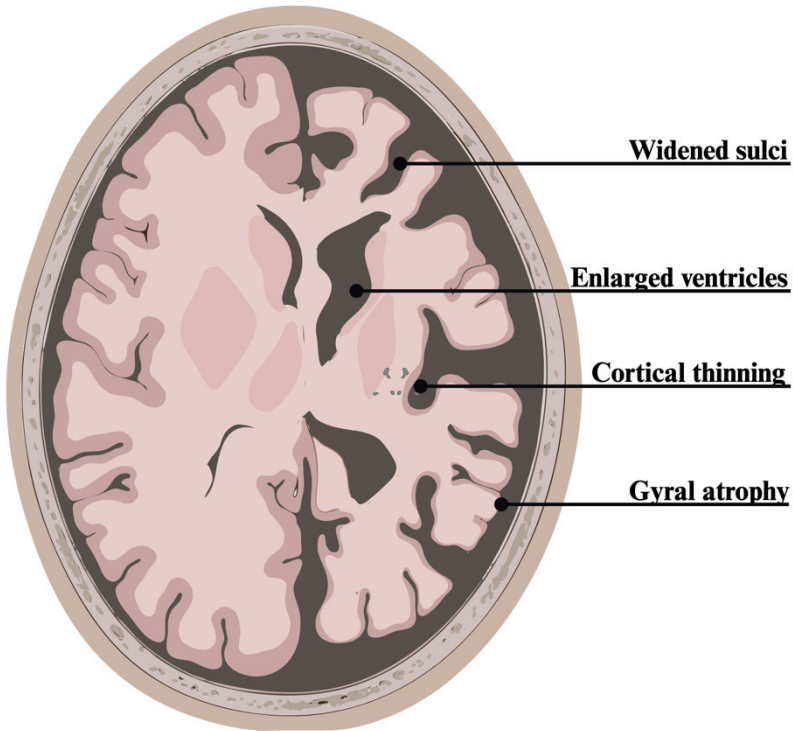


Figure 5. Axial slice of the brain. Brain atrophy pattern in late-stage AD is characterized by widening of the sulci, enlargement of ventricles (the lateral ventricles shown on image), thinning of cerebral cortex as well as general and gyral atrophy. AD; Alzheimer' disease. Created with BioRender.com

VASCULAR DEMENTIA

THE HISTORIC CONCEPT OF VASCULAR DEMENTIA

Since the late 19th century, the clinical contribution and definition of VaD have fluctuated remarkably. It received special attention in 1894 by Otto Binswanger and Alois Alzheimer who posited the existence of four VaD subtypes: arteriosclerotic brain degeneration, vascular cortical atrophy, subcortical encephalopathy (Binswanger's disease) and dementia post-apoplexy (today recognized as post-stroke dementia)¹⁴⁹. However, during the decades to follow, dementia based on cerebrovascular disease (arteriosclerosis) was abandoned as a differential diagnosis. Mainly, it was challenged by other post-mortem findings of Dr. Alzheimer, including those typical of AD (A β plaques and NFTs) which was considered being the main cause of dementia¹⁵⁰.

VaD as a possibly cause of dementia started to come back in the 1970s and the previously denominated *cerebral arteriosclerosis* was renamed as *multi-infarct dementia* (MID). MID was defined as involving multiple small or large ischemic infarcts, resulting in cognitive decline¹⁴⁹. Following several years of additional research, VaD was again believed to be a heterogenous brain disease, which led to the consensus on diagnostic VaD criteria in 1993 by the Neuroepidemiology Branch of the National Institute of Neurological Disorders and Stroke (NINDS) together with the Association Internationale pour la Recherche et l'Enseignement en Neurosciences (AIREN)¹⁵¹. According to the NINDS-AIREN criteria, patients with probable VaD must demonstrate memory loss and reduced function of two additional cognitive domains leading to impaired ADL function. In addition, patients with VaD need to present with a history of stroke, with or without neurological sequelae. This in turn led to the requirement of using brain imaging for clinical diagnosis¹⁵¹.

More recently, the Vascular Impairment of Cognition Classification Consensus Study (VICCCS) redefined VaD as *major vascular cognitive impairment* (VCI) given the complex and heterogeneous pathological processes leading to VaD¹⁵². According to the VICCCS study group, VaD is divided into several subtypes depending on the underlying

pathology including post-stroke dementia, multi-infarct dementia, SSVD and mixed dementia (e.g., AD+VaD). Moreover, several familial variants have been identified as causation of VaD. Thus, the diagnosis of VaD is based on the magnitude, location, quantity, and lesion type causing the development of VaD-associated symptoms although disease-specific biomarkers are still missing¹⁵³. Patients with VaD either present with a sudden or insidious symptom debut with a gradual worsening of symptoms. Compared to AD, the symptoms often fluctuate¹⁵³. These criteria are similar to that of DSM-5 where major and minor neurocognitive disorders are considered as separate diagnostic entities.

PATHOPHYSIOLOGIC FINDINGS OF VASCULAR DEMENTIA

Following AD, VaD is considered the second most common dementia form in an elderly population with an associated prevalence of 15-20%¹⁵⁴. Although VaD is differentiated into several familial and sporadic subvariants, VaD mainly is the cause of changes in the smaller or larger arterial vessels together with other underlying mechanisms.

SUBCORTICAL SMALL VESSEL DISEASE

Typically, patients with SSVD demonstrate pronounced WMHs and possibly lacunar infarcts¹⁵⁵. In tandem with WMHs, they are believed to mediate the progression of cognitive decline. A recent longitudinal study demonstrated that both brain atrophy (including subcortical and cortical brain atrophy, and MTA) as well as WMHs independently contribute to cognitive impairment in individuals free from dementia. Moreover, the same study showed that a reduction in brain volume potentiates the effects of WMHs and lacunar infarcts on cognitive deterioration¹⁵⁶.

SSVD is primarily a microvascular disease with several concomitant cascades causing alterations of the brain parenchyma, myelin morphology, and arterioles. It has been proposed that SSVD is associated with an impaired BBB function, consequently leading to chronic leakage of larger molecules and fluid in the white matter tracts. Possibly, these changes later lead to edema and the formation of gliosis in the brain white matter^{157, 158}. In turn, the periventricular spaces,

especially the lateral ventricles of the deep white matter expand and are often accompanied by perivascular hemorrhage (hemosiderin)¹⁵⁹. Microvascular disease is further related to structural changes in the arterioles including wall thickening (lipohyalinosis), arteriosclerosis and fibroid necrosis. The morphological alterations of the arterioles may contribute to impaired tissue perfusion and cerebral blood flow causing lacunar infarcts as well as microinfarcts^{160, 161}. Other pathophysiologic processes causing SSVD include oxidative stress, endothelial dysfunction, neuroinflammation, chronic intermittent hypoxia, and hypertension^{162, 163}.

CEREBRAL LARGE VESSEL DISEASE

Large vessel disease usually affects the medium or larger arteries of the cerebral circulation comprising intima proliferation and the formation of atherosclerotic plaques¹⁶⁴. Rupture of atherosclerotic plaques can lead to thrombotic occlusions causing infarcts of various size. This form of VaD is referred to as post-stroke dementia and is mainly the result of larger infarcts although the disease can also be caused by a strategic infarct of smaller size (e.g. infarcts in the basal ganglia, hypothalamus or hippocampus)¹⁶⁵. Alternatively, large vessel occlusion or dysfunction of distal arterial blood flow can arise due to carotid atherosclerotic stenosis leading to watershed infarcts¹⁶⁶. Individuals with post-stroke dementia generally develop cognitive dysfunction within a couple of months although symptom onset can occur as late as 12 months after the cerebral insult¹⁶⁷. Repeated cortical infarcts (MID) are also regarded as large vessel disease and are commonly known as cortical VaD (cVaD)¹⁶⁸.

The clinical contribution of large vessel disease has long been debated since it may be difficult to establish whether the functional disability is induced by cognitive or physical impairment¹⁶⁹. Studies converge to suggest that large vessel disease seldom give rise to VaD alone, but rather is a mix of various cerebrovascular pathologies. For instance, lacunar infarcts are often observed with other cerebrovascular lesions including large ischemic infarcts¹⁷⁰. Parallel with aging, the presence of silent infarcts increases although it is uncertain whether these infarcts predict the conversion to dementia^{171, 172}. Moreover, as found in postmortem studies, microinfarction is considered as a major risk factor

for VaD although the majority of microinfarcts are not visible on 1.5 and 3.0 T MRI used in the clinical setting^{173, 174}.

MIXED DEMENTIA

Mixed dementia is a nosological term indicating the presence of symptoms that are associated with at least two dementia subtypes. It does not explicitly state the underlying conditions as it may be a combination of any dementia etiologies¹⁷⁵. Typically, a mixed dementia diagnosis is given to individuals with symptoms of AD in tandem with significant vascular contributions on brain imaging making AD+VaD the most common form of mixed dementia¹⁷⁶. In a previous publication, patients with mixed dementia of the AD+VaD type presented with biomarkers typical of AD and SSVD-like neuropsychological profile¹⁷⁷. While AD and VaD have long been considered as separate diagnostic entities, an appreciable number of studies of the post-mortem brain have demonstrated that AD often co-exist with cerebrovascular disease including microangiopathy and large infarcts¹⁷⁸⁻¹⁸¹. In contrast, the brains of VaD patients often display signs of hippocampal atrophy and NFTs containing hyperphosphorylated tau^{182, 183}. Given their converging pathologies, researchers have been faced with the conundrum of their combined contributions to cognitive decline although it is most likely that cerebrovascular disease accelerate AD-like neuropathology.

RISK FACTORS FOR DEMENTIA

In addition to genetic predisposition for dementia development, a growing body of literature convey that several acquired factors influence dementia onset. Many of these factors seem to increase the risk of dementia when they present in midlife, and represent a complex interaction between lifestyle, vascular, metabolic, and environmental components.

Observational data consistently show that high age is the most salient risk factor for developing a dementing disorder including AD and VaD, with a rapid rise in dementia incidence between the ages 75 to 85 years¹⁸⁴⁻¹⁸⁶. Furthermore, hypertension has primarily been related to VaD, but also to AD, most likely because of the long-term effects of

elevated systolic blood pressure leading to impaired cerebral blood flow. As a result, this may accelerate cerebrovascular disease and cognitive impairment¹⁸⁷⁻¹⁸⁹. Physical activity also seems to modulate the risk of dementia to some degree. A meta-analysis of 16 longitudinal studies demonstrated that higher physical activity level was related to a lower relative risk of dementia by any cause (28%), and AD (45%)¹⁹⁰. Lastly, history of hyperinsulinemia, diabetes mellitus and obesity have been suggested as risk factors for both AD and VaD¹⁹¹⁻¹⁹⁴. It has been estimated that diabetes mellitus, hypertension and physical inactivity are associated with 30% of all AD cases¹⁹⁵. Therefore, an aggressive approach to treat these modifiable factors in midlife might subsequently minimize the possibility of dementia onset, progression, and severity.

As found for the somatic risk factors, multiple studies have found associations between social behavior, cognitive status, and dementia risk. For instance, pooled data analyses have shown that individuals experiencing loneliness as well as those who are less engaged in social activities and contact have increased risk of incident dementia¹⁹⁶. Moreover, lower education level is a risk factor for dementia, being more evident in developed regions than in developing regions¹⁹⁷. Finally, it has been proposed that in the event of brain damage, some individuals seem to be more resilient to cognitive decline and dementia than others. This concept, the *cognitive reserve*, is believed to rely on two strategies; *neural reserve* and *neural compensation*. The former involves the ability of using existing functional neuronal sequences while the latter includes the capability to utilize complementary cognitive mechanisms or neuronal sequences to overcome the cognitive decline^{198, 199}. Higher cognitive reserve, in cognitively intact individuals as well as in subjects with prodromal AD, provides protection from the risk of conversion to AD (Figure 6)²⁰⁰.

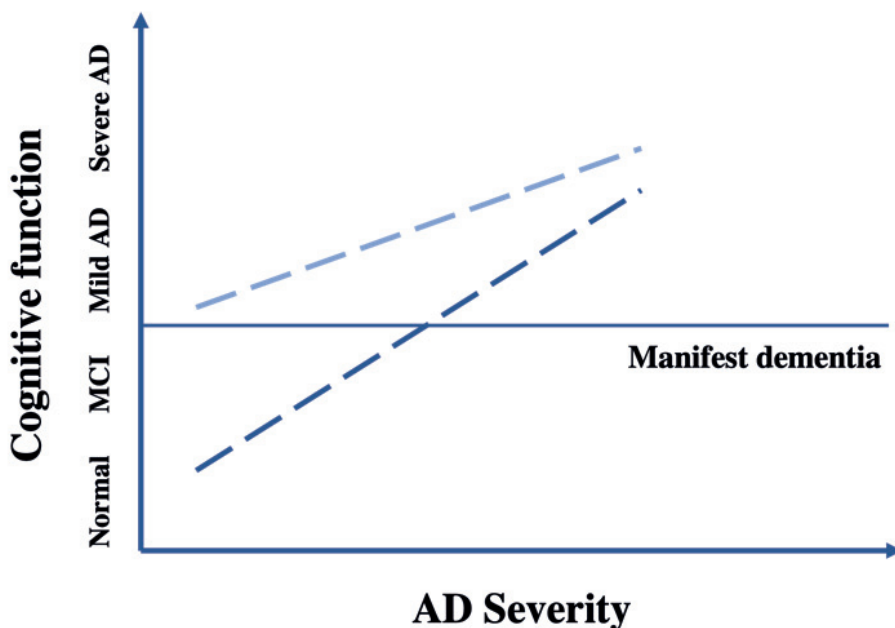


Figure 6. Theoretical concept of AD neuropathology and the cognitive reserve. Persons with mild AD neuropathology (AD severity) and low cognitive reserve might already show symptoms of AD compared to those having higher cognitive reserve. The light blue dotted line denotes low cognitive reserve while the dark blue dotted line denotes high cognitive reserve. AD; Alzheimer's disease, MCI; mild cognitive impairment. Created with Microsoft PowerPoint.

INSULIN-LIKE GROWTH FACTOR-I

The role of the insulin-like growth factor (IGF)/insulin system is complex, and members of this system regulate development, growth, and cellular metabolism throughout life^{201, 202}. This family of hormones display some identical amino acid sequences and includes IGF-I, IGF-II, proinsulin, and insulin. IGF-I and IGF-II are transcribed from two separate genes and together with insulin, they act as ligands to the membrane-bound tyrosine kinase receptors; IGF-I receptor (IGF-IR), IGF-II receptor (IGF-IIR), insulin receptor A (IR-A), and insulin receptor B (IR-B)²⁰³. The IGF-IR/IIR demonstrates 60% homology with the IR-A/B, but the effects exerted by these receptors differ²⁰⁴.

Moreover, the structural similarities of these hormones and their corresponding receptors make it possible for them to bind to each other's receptors but with various degree of affinity²⁰⁵. During normal physiologic conditions, the IGF-IR and the IR can form a hybrid heterodimeric receptor that binds with higher affinity to IGF-I than insulin²⁰⁶. In comparison to other tyrosine kinase receptors, the insulin and IGF receptors are covalent dimeric structures although all receptors of this family dimerize or oligomerize following activation²⁰⁷. Interestingly, in mammals, insulin and IGF-I have their own receptors whereas in invertebrates such as *Drosophila* and *C.elegans*²⁰⁵, insulin and IGFs share the same tyrosine kinase receptor²⁰⁸.

Binding of IGF-I on the cell surface to the IGF-IR triggers intracellular autophosphorylation on the tyrosine residues creating docking stations for other substrates. This leads to signal transduction involving of a network of lipids and kinases such as MAP kinases (MAPK) and the phosphoinositide 3-kinase (PI3K)-Akt pathway. These signaling pathways elicit effects on the metabolism, cell survival, and gene transcription (Figure 7)^{209, 210}. In the brain, IGF-I-induced activation of phospho-Akt interacts with the production and translocation of the glucose transporter protein type-4 (GLUT4) of neurons²¹¹. This pathway, under the influence of IGF-I activity also inhibits glycogen synthetase kinase 3 β (GSK3 β) activity. For instance, mice with IGF-I null brains exhibited increased levels of phosphorylated tau, a protein that belongs to the GSK3 β family and is involved in one of the key pathologic processes of AD²¹². Thus, in particular, the Akt-pathway seems to have a significant role in the regulation of glucose homeostasis as well as neurofilament stabilization of neuronal cells.

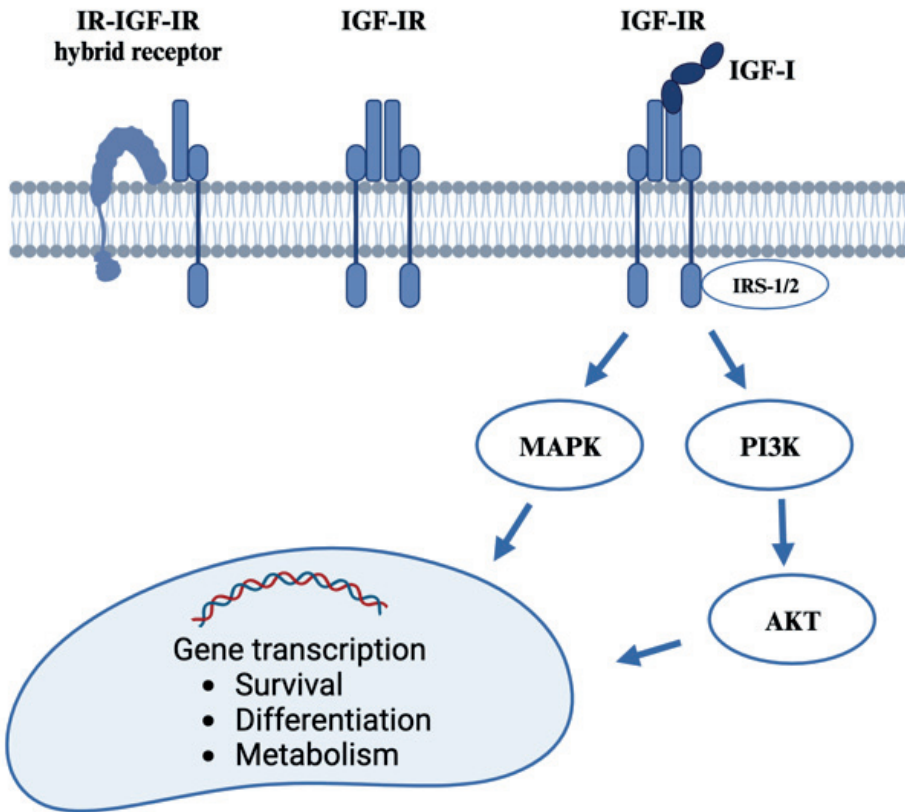


Figure 7. The IR and IGF-IR receptor can form a hybrid receptor (IR-IGF-IR) with relatively high affinity to IGF-I. Docking of IGF-I to the IGF-IR initiates an intracellular cascade, with activation of the MAPK and PI3K-Akt pathways being two crucial signaling cascades. Ultimately, these signaling pathways lead to gene transcription within the nucleus promoting several cell-related functions including survival, differentiation, and metabolism. IGF-I; insulin-like growth factor-I, IGF-IR; insulin-like growth factor-I receptor, IR; insulin receptor, IRS; insulin receptor substrate, MAPK; map-kinase, PI3K; phosphoinositide 3-kinase. Created with BioRender.com

Several genetic studies confirm that the IGF-I gene has been relatively stable during the evolution in species such as flies, reptiles, birds, and mammals^{208, 213, 214}. In biologic fluids, most of IGF-I is transported by insulin-like growth factor-binding proteins (IGFBPs) in binary complexes²¹⁵. Seven IGFBPs have been identified so far, with IGFBP 1 to 6 demonstrating the highest affinity to IGFs²¹⁶. In adults, >90% of serum IGF-I is bound to IGFBP-3²¹⁷, whereas IGFBP-2 is the main transporter of IGF-I in the CSF²¹⁸. These binding proteins are degraded by various proteases resulting in free forms of IGF-I which enables the activation of the IGF-IR²¹⁹. In addition to prolonging the half-life of IGF-I²²⁰, the IGFBPs can to some degree modulate the actions of IGF-I by interacting with the IGF-IR although these findings have been somewhat of a controversy²²¹. Likewise, experimental models have shown that some IGFBPs can influence mitosis, growth and cell migration in an IGF-I-independent manner²²²⁻²²⁴.

Moreover, in mammals, IGF-I can also form ternary complexes encompassing IGF-I, IGFBP-3 or IGFBP-5, and the acid-labile subunit (ALS) found in the circulation. It is postulated that this ternary configuration acts as an IGF-I reservoir²²⁵. The ALS has little or no affinity to unbound IGFBP3 and free IGF-I, respectively²²⁶. Compared to binary complexes of IGF-I and IGFBPs, ternary complexes are not able to cross the endothelial vessels resulting in high concentrations of IGF-I in serum²²⁷. The half-life of free circulating IGF-I is rather short (approximately 10 minutes)²²⁰ which in ternary complexes is extended to more than 12 hours²²⁸.

THE SOMATOTROPIC AXIS

The somatotropic axis, which consists of growth hormone (GH) and IGF-I, is an important mediator of growth and development²²⁹. Persons with Laron syndrome (IGF-I deficiency) or childhood onset GH deficiency present with dwarfism and short stature, while excessive GH and IGF-I in gigantism are associated with tall stature, and acromegaly with soft tissue and bone overgrowth^{230, 231}. Physiologic GH is released predominantly nocturnally in a pulsatile manner from the anterior pituitary gland. GH levels therefore fluctuate extensively during a 24-hour period. GH release is under the positive influence of GH-releasing hormone (GHRH), whereas it is negatively affected by somatostatin. Both GHRH as well as somatostatin derive from the hypothalamus and

reach the anterior pituitary gland through the portal venous system^{232, 233}.

One key action of GH is to stimulate the liver to secrete IGF-I into the bloodstream. While GH release is not consistent throughout the day, IGF-I release is rather constant, resulting in stable levels of IGF-I in serum. Therefore, at least to some extent, IGF-I can be used in the diagnosis and monitoring of GH-related disorders²³³. The hepatocytes produce ~70% of circulating IGF-I, which elicits endocrinologic actions in the target tissues. Inactivation of rodent liver-derived IGF-I did not influence postnatal growth to any major extent, which suggests that GH induces major effects on growth by direct effects or by stimulating autocrine/paracrine IGF-I production²³⁴. In a negative feedback loop, both GH and IGF-I can influence somatotropic activity by regulating the release of somatostatin and GHRH from the hypothalamus or by inhibiting GH secretion from the anterior pituitary gland (Figure 8)²³⁵.

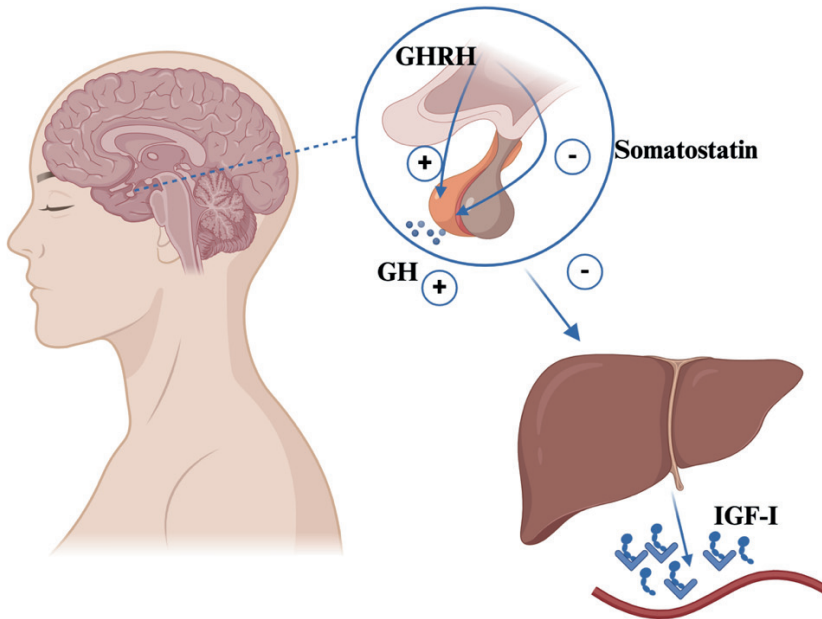


Figure 8. In humans, pulsatile secretion of GH from the anterior hypopituitary gland is under the dual control by GHRH (+) and somatostatin (-). GH can then activate the liver cells (hepatocytes) leading to the release of IGF-I into the bloodstream. The majority of IGF-I in the circulation is bound to transporter proteins; IGF-BPs. Please note that the blue shaped dots right beneath the liver represent free and bound IGF-I and is a didactic illustration. IGF-I; insulin-like growth factor-I, IGF-BP; insulin-like growth factor-binding protein, GH; growth hormone, GHRH; growth hormone-releasing hormone. Created with BioRender.com

Furthermore, several shifts can be observed in the somatotrophic axis from the prenatal period to aging. During pregnancy, the production rate of serum IGF-I is rather low, while in childhood when GH pulses are markedly elevated, serum IGF-I levels steadily increase and reach a peak in early adolescence^{236, 237}. However, in the third decade, concentrations of IGF-I gradually start to decrease with relatively low levels found in individuals over 60 years of age^{238, 239}.

GH levels also decrease during aging, and this phenomenon is often referred to as the somatopause. It is estimated that the rates of GH production decrease by 14% per decade, which is the result of both central and peripheral processes. Firstly, decline in cholinergic activity in the hypothalamus results in elevated somatostatin secretion and therefore blocks GHRH-related GH secretion²⁴⁰. Secondly, peripheral IGF-I release following GH activation gradually decreased leading to reduced secretion of both GH and IGF-I in the long-term²⁴¹. While IGF-I release is highly dependent of GH activity in younger individuals, not only GH but also physical exercise and nutritional status is important regulators of IGF-I levels in the aging individual²⁴².

The somatopause has been associated with adverse effects during aging such as reductions in lean body mass, muscle strength, and aerobic capacity²⁴³. Additionally, some studies have found associations between the somatopause and increased risk of for instance renal disease, cardiovascular disease, and cognitive impairment^{22, 244}. Nonetheless, the functional benefits of GH treatment as a modifier of the somatopause are controversial as the effects of GH supplemental have been varying¹⁸. Moreover, whether the drop in GH and IGF-I levels during aging are protective or detrimental in terms of age-related diseases are not fully understood. In experimental studies, increased longevity has been observed in animal models with reduced GH/IGF-I signaling, possibly due to better resistance to oxidative stress²⁴⁵. Moreover, high IGF-I may result in uncontrolled cell division with subsequent cancer development²⁴⁶. In some agreement, in several population-based studies, higher circulating IGF-I was associated with increased cancer risk²⁴⁷⁻²⁴⁹.

INSULIN-LIKE GROWTH FACTOR-I IN THE CENTRAL NERVOUS SYSTEM

IGF-I is imperative for brain development and is involved in neurogenesis, synaptogenesis, axonal growth, and cortical maturation²⁵⁰. Experimental studies have also confirmed that IGF-I regulates the myelin by interactions with the oligodendrocytes and IGF-I may therefore influence the integrity of the white matter of the brain²⁵¹⁻²⁵³.

Moreover, disruption of the IGF-I gene results in loss of neurons, reduced brain volume and impaired myelination of axons in the CNS as well as behavioral changes in mice²⁵⁴. Consistent with this concept, astrocyte-specific overexpression of IGF-I led to greater myelination and brain size in an experimental mouse model²⁵⁵. In humans, preterm children with defects in the IGF-I gene display mental retardation and delayed psychomotor function^{256, 257}, and low serum IGF-I levels were associated with smaller brain size²⁵⁸.

Almost all cell types in the brain are capable of IGF-I production although it is mainly expressed by neuronal cells in areas with great remodeling and renewal²⁵⁹. Paracrine production of IGF-I is relatively high in the prenatal brain but gradually decreases during the following years. Conversely, the IGF-IR show a stable expression pattern and is widely distributed in the brain tissue, but like IGF-I production, it is condensed in regions with rich brain activity^{23, 24}.

In terms of peripheral IGF-I, it enters the BBB at the choroid plexus via megalin/low-density lipoprotein receptor-related protein-2 (LRP2), a cargo transporter also involved in AD pathology²⁶⁰. Similarly, following neuronal activation, binding of IGFBP-3 to the LRP1 receptor increased the transcytosis of IGF-I across the choroid plexus²⁶¹. Moreover, in a previous study of adult rodents, circulating IGF-I levels as well as exercise led to increased choroid plexus-related megalin density, supporting the hypothesis that megalin is involved in adult neuroprotection through exercise-induced elevations of IGF-I²⁶².

Therefore, the persistent expression of the IGF-IR in addition to reduced paracrine IGF-I production indicate that peripheral IGF-I has an important role for the adult brain. However, along with aging in the rabbit pup model, transportation of IGF-I across the BBB gradually

declined, indicating the importance of IGF-I for development of the CNS²⁶³.

While the exact mechanisms for IGF-I induced neuroprotection have yet to be established, adult IGF-I seems to stimulate neuronal plasticity, survival of neurons, cell reparation, and reducing cell death²⁶⁴⁻²⁶⁶. However, in mice, local brain IGF-I production did not compensate for the age-related decline in peripheral IGF-I levels demonstrating a causal relationship with decline in cognitive function²³⁹.

Despite these findings, IGF-I seem to have salutary effects in the context of acute cerebral insults. For instance, increased IGF-I levels by intraventricular IGF-I infusions inhibited apoptosis of oligodendrocytic progenitor cells²⁶⁷ which could partly underly the greater brain white matter recovery observed following traumatic brain injury in patients with high circulating IGF-I²⁶⁸. Similarly, after an ischemic stroke, higher serum IGF-I was associated improved cognitive and functional recovery up to 24 months after the cerebral insult^{269, 270}.

Overall, experimental studies consistently demonstrate that IGF-I is important for normal development of the CNS while in the adult brain, the protective role of both local and systemic IGF-I is less apparent in human studies.

INSULIN-LIKE GROWTH FACTOR-I AND THE COGNITIVE SPECTRUM

INSULIN-LIKE GROWTH FACTOR-I AND ITS RELATION TO COGNITIVE DECLINE

The thalamus, hippocampus, amygdala, and choroid plexus demonstrate higher adult IGF-IR expression than other brain areas²³. It has been postulated that the age-related decrease in IGF-I levels parallel with dysfunction of these regions, which in turn could be important for the cognitive impairment seen in elderly individuals^{25, 271}. In some cross-sectional analyses, higher serum IGF-I appeared to be associated with better selective attention, executive function and working memory^{26, 272}. In another cross-sectional study, lower serum IGF-I was related to some protection against cognitive decline late into the tenth decade of life²⁷³.

Notably, these findings have not always been consistent between genders, with results limited to only women or men in some of the previous studies²⁷²⁻²⁷⁴. Moreover, most cognitive studies on IGF-I have focused on healthy individuals, whereas there are few studies in patients with MCI. However, in a study of MCI patients by Doi and colleagues²⁷⁵ reduced serum IGF-I levels were associated with impaired attention, executive function, processing speed, and visuospatial function²⁷⁵. Altogether, lower peripheral IGF-I concentrations seem to be linked to impaired function of several cognitive domains in individuals free from dementia.

Similar to interventional research aiming to reverse the somatopause, the somatotrophic axis has previously been manipulated (through administration of GHRH injections, GH/IGF-I analogues or with the use of physical exercise) in an attempt to interfere with age-associated cognitive decline. In some of these experiments, elevation of circulating IGF-I levels resulted in increased hippocampal volume through increased neurogenesis with subsequent improvement in learning and memory²⁷⁶⁻²⁷⁸. However, the reversal of memory deficits was not observed in a 1-year trial of women (mean age 70.6 years) receiving IGF-I injections²⁷⁹.

INSULIN-LIKE GROWTH FACTOR-I AND BRAIN MORPHOLOGY

Since degeneration of the hippocampus is one of the first brain areas to be involved in older persons²⁸⁰, it is reasonable that the main focus of research has been on IGF-I and its relationship to the hippocampal regions. In a small interventional study of elder individuals, exercise-induced increments in circulating IGF-I concentrations were related to greater volume of the hippocampus but not with total gray matter volume²⁷⁸. This positive association was also seen in a cohort study of considerable size ($n = 370,000$)²⁸¹. In contrast, the Framingham study showed that higher IGF-I concentrations were positively related to the total brain volume, while IGF-I did not show any relationship with hippocampal volume²⁸². In some accordance with the results in the Framingham cohort, circulating IGF-I in hypertensive adults was marginally related to the radial width of the temporal horn, a measure that reflects MTA²⁸³. Thus, although not consistently reported, the

results of some previous reports indicate that higher serum IGF-I is linked to greater volumes of the hippocampus in cognitively intact individuals.

Another brain structure to degenerate with age, but not as evidently as the MTL, is the brain white matter¹⁴⁴. Clinical studies on the associations between IGF-I and brain atrophy or lesions in the white matter are relatively scarce. An observational study found that IGF-I correlated positively with WMH volume but not with the total brain white matter²⁸⁴.

Contrary, results based on the UK Biobank showed that individuals with higher serum IGF-I had larger total brain white matter volume, while IGF-I was inversely related to the amount of WMHs²⁸¹. However, the results from the British 1946 birth cohort did not confirm an association between serum IGF-I and the burden of WMHs²⁸⁵. In conclusion, the relationship between circulating IGF-I and brain white matter volume and disease seem to be inconsistent between epidemiological studies warranting additional research on the topic.

INSULIN-LIKE GROWTH FACTOR-I AND DEMENTIA AND ITS RELATED RISK FACTORS

Some longitudinal investigations have shown that lower circulating IGF-I is related to dementia-related risk factors including insulin resistance, hypertension, atherosclerosis, and ischemic stroke²⁷⁻²⁹. Therefore, as altered IGF-I levels to some extents are associated with cognitive decline and other risk factors for dementia, several studies have determined whether circulating IGF-I concentrations can predict the risk of dementia development. In epidemiological studies, both higher IGF-IR activity measured using an IGF-I kinase receptor activation assay and low circulating IGF-I levels have been related to increased risk of dementia by any type^{282, 286}. Interestingly, results from UK Biobank studies showed that the relationship between IGF-I concentrations and dementia disease risk was U-shaped after both 5 and 12 years of follow-up.^{281, 287} In another population-based study, IGF-I levels did not influence the risk of dementia conversion²⁸⁸. Finally, no relationship was found between circulating IGF-I and dementia risk in a study of male individuals²⁸⁹. In summary, although low serum IGF-I

levels appear to be related to dementia-related risk factors, observational studies demonstrate divergent results in terms of serum IGF-I levels and the risk of dementia.

INSULIN-LIKE GROWTH FACTOR-I AND EXPERIMENTAL ALZHEIMER'S DISEASE

Several lines of research propose that the IGF-I signaling pathway is involved in AD pathology. Experimental data suggest that IGF-I confer protection against the accumulation of A β . In mice, circulating IGF-I mediated transportation of the A β -protein across the choroid plexus via interactions at the BBB level with LRP2, thereby promoting the clearance of A β from the brain²⁶². The notion that circulating IGF-I regulates A β was further supported by the finding that deficiency of liver-derived IGF-I in mice with mutations in the *APP* and *PS1* genes demonstrated early formation of A β plaques²⁹⁰. Furthermore, in a mouse model (*APP/PS2*) mimicking the AD phenotype, systemic IGF-I treatment resulted in improved spatial learning and memory, and decreased brain A β load²⁹¹. Moreover, administration of IGF-I to older rats ameliorated the formation of hippocampal and cortical A β plaques with subsequent increase in CSF A β levels in these brain regions²⁶⁰. Finally, IGF-I was able to rescue hippocampal neurons exposed to amyloidogenic peptides *in vitro*²⁹².

IGF-I is not only involved in the clearance of A β , but also influences the process of tau phosphorylation and its aggregation to form NFTs. In fact, mice with IGF-I null brains exhibit large proportions of hyperphosphorylated neurons²¹². IGF-I activity lowered the burden of tauopathy through the inhibition of the GSK3 β pathway in cultured human neurons²⁹³. Finally, accumulation of NFTs encompassing hyperphosphorylated tau was observed in the aging hippocampus in mice with perturbation of the insulin receptor substrate 2 (IRS-2), a downstream signaling molecule of the insulin/IGF-I axis²⁹⁴. In conclusion, these previous findings illustrate that disturbed IGF-I activity is possibly linked to the configuration of A β plaques and NFTs in experimental models of AD.

INSULIN-LIKE GROWTH FACTOR-I AND ALZHEIMER'S DISEASE IN HUMANS

As described above, there is increasing evidence from experimental animal studies linking the IGF-I signaling pathway to the progression of AD neuropathology. However, in humans, controversy continues to surround such findings with most available human studies being cross-sectional in their nature. Nevertheless, in a two-generation observational study, individuals in the lowest quartile of serum IGF-I had a 51% increased risk of progression to AD when compared with the three higher IGF-I quartiles²⁸². In line with these findings, the Rotterdam Study demonstrated that 1 standard deviation (SD) increment in IGF-IR activity was related to a 17% increase in incident AD and 41% increase in prevalent AD²⁸⁶. In an extended follow-up of the same cohort, the risk of AD dementia was higher in persons with one or two alleles of the *APOE ε4* gene in the middle and highest tertiles of IGF-IR activity²⁹⁵. The risk of developing AD has also been investigated in case-controls in which the presence of the IGF-I polymorphism rs972936 was associated with a higher risk of AD development in both Southern European and Han Chinese populations^{296, 297}. However, serum IGF-I did not influence the AD risk in a mendelian randomization study²⁹⁸.

In smaller cross-sectional investigations, circulating IGF-I concentrations were lower²⁹⁹⁻³⁰², unchanged³⁰³ or higher^{304, 305} in AD patients compared with cognitively intact individuals. Similarly, two previous meta-analyses showed no difference (n = 9 studies)³⁰⁶ or lower circulating IGF-I levels (n = 9 studies)³⁰⁷ in AD patients. A large population-based study (n = 395,769) demonstrated that an increasing number of *APOE ε4* alleles was positively associated with serum IGF-I levels³⁰⁸. Similar discrepancies have been found in terms of CSF levels of IGF-I as AD patients exhibited comparable³⁰³, higher³⁰⁵ or lower³⁰⁹ IGF-I levels in CSF than healthy controls.

The ambiguous evidence regarding IGF-I and its relationship with clinical AD dementia is not fully understood but it has been postulated that early AD is characterized by resistance to the IGF-IR³¹⁰⁻³¹². In the postmortem AD brain, the IGF-IR resistance became more evident with the advancement of the disease using Braak staging and paralleled with reduced IGF-I and IGF-IR messenger ribonucleic acid (mRNA) concentrations³¹³. In other postmortem studies, it has been hypothesized that development of IGF-IR resistance is initiated prior the clinical phases of AD^{311, 312}.

Possibly, increments of IGF-I in the early phases of dementia with AD neuropathology might be a protective mechanism to overcome IGF-IR resistance in order to elicit neurotropic effects and counteract neurodegeneration^{311, 312}. In support of this hypothesis, AD neurons show less response to IGF-I in the IGF-IR→IRS-2→PI3K signaling pathway (Figure 7) and thus, increasing IGF-I levels may serve as a protector against impaired brain function^{239, 311}. In addition, both IGF-I in CSF and the ratio between CSF and serum IGF-I have been positively related with amount of CSF P-tau in AD, the latter reflecting increased levels of tau phosphorylation as well as neuronal death^{303, 304, 314}. However, along with the progression of AD, IGF-I levels might be diminished as a result of IGF-I deficiency³¹⁵. This, in conjunction with worsened ADL function, could promote isolation, immobilization, malnutrition and reduced lean body mass in AD patients, all of which are related to lower peripheral IGF-I²². In turn, decrements in serum IGF-I could potentially exacerbate the AD course^{260, 315}. This notion was supported by Vidal et al.³¹⁶ as older individuals with AD dementia and decreased baseline circulating IGF-I concentrations had a more rapid deterioration in global cognition over a 2-year period³¹⁶. Moreover, non-responders to donepezil treatment had lower peripheral IGF-I concentrations and worse Mini-Mental State Examination (MMSE) scores prior to AD treatment than responders³¹⁷. However, in a clinical trial, 12 months of treatment with a GH secretagogue with increased IGF-I levels did not alleviate symptoms in patients with mild to moderate AD³¹⁸.

In summary, the results of several studies implicate that altered IGF-I signaling in brains with AD is followed by IGF-I deficiency in later stages of clinical AD. However, as longitudinal studies are currently lacking, it is unclear how alterations in IGF-I activity influence the progression of AD dementia.

INSULIN-LIKE GROWTH FACTOR-I IN CEREBROVASCULAR AGING AND VASCULAR DEMENTIA

A substantial body of evidence indicate that IGF-I activity may regulate vascular and neurotropic functions that promote brain health. In observational studies of older individuals, decline in circulating IGF-I was related to early-onset atherosclerosis and increased the risk of both cardiovascular and cerebrovascular disease²⁷⁻²⁹. Experimental models mimicking the aging phenotype have shown comparable findings as knockout of liver-specific IGF-I mice demonstrate decreased microvascular density (microvascular rarefaction), a mechanism believed to adversely affect cerebral perfusion³¹⁹. In addition to this age-related process, neurovascular uncoupling and microvascular endothelial dysfunction have been confirmed in brains of IGF-I knockout mice³²⁰ and absence of IGF-I led to reduced numbers and function of astrocytes and oligodendrocytes²³⁵. These observations support the notion of cerebrovascular aging being a multifactorial process that is partly regulated by IGF-I signaling.

In VaD, the role of IGF-I has been less studied. However, since altered IGF-I activity is related to cerebrovascular aging and dementia-related risk factors, it is plausible that IGF-I is involved in VaD progression. Indeed, in a rodent model of VaD, the expression of IGF-I mRNA markedly decreased in the hippocampal region³²¹. A human study found an increased risk of VaD in female carriers of a polymorphism in the *IGF-IR* gene compared with a female control population³²². Lastly, IGF-I levels have been lower in VaD patients^{323, 324}, and were inversely associated with carotid intima media thickness³⁰².

In conclusion, the IGF-I system has mainly been evaluated cross-sectionally in dementia populations, or prospectively in cognitively intact individuals with relatively extensive follow-up time. Previous

studies have not been designed to observe discrete pathological changes in proximity to the onset of manifest dementia. Consequently, this could have led to inadequate knowledge of how hormonal status is related to the onset and progression of dementia. Longitudinal trials are therefore warranted in the memory clinic setting. Especially in SCI or MCI, which are often considered as pre-stages of dementia, the timing and nature of such events in relation to hormonal levels can be investigated. Overall, in a memory clinic population, the complexity of the IGF-I system can be studied in both the preclinical and clinically manifest stages of dementing disorders.

AIM OF THESIS

AIM OF THESIS

The results of previous research suggest that altered IGF-I activity participates in the pathological processes seen in normal aging as well as in cognitively impaired persons. However, the findings have not been consistent between studies. The purpose of the current thesis was to explore whether IGF-I concentrations are related to the progression of cognitive dysfunction in a memory clinic population. The design of the thesis enabled a time window wherein potential effects could be observed in various stages of cognitive impairment.

This thesis has namely four central specific aims including:

1. To investigate whether serum IGF-I levels at baseline can predict the risk of progression to dementia by any type, and the AD or VaD subtypes in patients with SCI or MCI (Study I).
2. To determine whether IGF-I concentrations in serum and CSF differ between AD patients without major cerebrovascular disease and healthy subjects, and if IGF-I is related to CSF AD biomarker concentrations (Study II).
3. To explore if baseline circulating IGF-I is related to brain MRI measurements at baseline and longitudinally in patients with sMCI or AD dementia (Study III).
4. To examine whether baseline circulating IGF-I is associated with brain white matter MRI measures and cognitive performance in patients with SCI/MCI or AD (Study IV).

MATERIALS AND METHODS

MATERIALS AND METHODS

THE GOTHENBURG MCI STUDY – DESIGN AND ELIGIBILITY

All patients and controls in Study I-IV were recruited from the Gothenburg MCI study. This is a longitudinal mono-center study carried out at the memory clinic, Sahlgrenska University Hospital, Mölndal, Sweden. The Gothenburg MCI study commenced in 1999 with the main goal of characterizing various traits and phases of dementia development and progression, especially AD, SSVD, and their overlaps (mixed dementia). The study comprises patients across the cognitive continuum, including those with SCI to those with manifest dementia. All study subjects in the Gothenburg MCI study were initially individuals seeking medical attention at the memory clinic, Sahlgrenska University Hospital. These patients were either self-referred, or referred by other departments, mainly from primary care units. Healthy controls have also been recruited to the study, mainly via senior citizen organizations while several of the controls were relatives of the patients at the memory clinic³²⁵.

Participants in the Gothenburg MCI study underwent extensive baseline evaluation including clinical, neuropsychological, biochemical, neurochemical, and neuroimaging examinations. Most of these examinations were repeated at biannual follow-up visits³²⁵.

ELIGIBILITY CRITERIA

To fulfill the purpose of each Study, study-specific eligibility criteria were added to the general criteria used in the Gothenburg MCI study.

ELIGIBILITY CRITERIA FOR THE GOTHENBURG MCI STUDY

Patients were included if they were ≥ 50 and ≤ 79 years of age with a MMSE score > 18 , and self- or informant-reported cognitive impairment during the last 6 months. However, patients were excluded if they suffered from systemic or psychiatric conditions potentially causing

cognitive impairment including subdural hemorrhage, hydrocephalus, brain tumor, encephalitis, hypothyroid disease except for treated hypothyroidism, unstable heart disease, schizophrenia, major depression, bipolar disease, abuse of alcohol or other substances, and delirium.

As for the healthy controls, they were eligible if they did not demonstrate symptoms of cognitive decline, were between 50-79 years of age, and scored >26 on the MMSE test. The same exclusion criteria were applied for the healthy controls as those employed in the patients with cognitive decline³²⁵.

SPECIFIC INCLUSION AND EXCLUSION CRITERIA FOR STUDY I-IV

In Studies I-IV, we used the general eligibility criteria for the Gothenburg MCI study (given above) in addition to the study-specific inclusion and exclusion criteria (given below).

Study I: Patients were enrolled if they were diagnosed at baseline with SCI or MCI and had at least one available follow-up visit. Only patients with baseline serum IGF-I samples were eligible for inclusion.

Study II: Inclusion criteria were available serum and CSF for analysis of IGF-I and that the participant was classified as a healthy control or suffering from AD. Those with mixed dementia (AD+VaD), and participants with concomitant diabetes mellitus were excluded since these conditions may influence IGF-I levels.

Study III: In this Study, patients were eligible if they were classified as sMCI or AD. The patients were included if they had baseline data on serum IGF-I as well as brain MRI scans.

Study IV: Patients were enrolled if they had a diagnosis of SCI/MCI or AD and accessible samples at baseline for determination of circulating IGF-I. Additional inclusion criteria were available baseline MRI measurements of brain white matter variables and neuropsychological tests evaluating global cognition, attention, and executive function.

Diabetes mellitus was an exclusion criterion since it can accelerate the amount of WMHs.

DIAGNOSTIC PROCEDURES

GRADING OF COGNITIVE FUNCTION

The staging of cognitive function was determined using the Global Deterioration Scale (GDS)³²⁶. Participants assessed as GDS 1 were considered as having no cognitive deficits, GDS 2 corresponds to SCI, GDS 3 equals MCI, and GDS 4 reflects possible mild dementia. The GDS grading relies on information concerning the cognitive and functional performance of the individual, including a review of medical history (self-reported and medical chart review), checklists, and various instruments: MMSE³²⁷; variables 13 to 20 on the Stepwise Comparative Status Analysis (STEP) including disorientation, memory or visuospatial disturbances, reduced abstract thinking, visual agnosia, apraxia, poverty of language, and sensory aphasia³²⁸; I-FLEX, a short version of the Executive Interview comprising number-letter task, Luria hand sequences, counting and interference tasks, anomalous sentence repetition, and word fluency,³²⁹; and Clinical Dementia Rating (CDR)³³⁰. To determine the appropriate CDR score, information was retrieved from both the participant and an informant³²⁵.

The algorithm for the GDS assessment was:

- GDS 1: MMSE ≥ 29 , STEP = 0, I-FLEX = 0, and CDR ≤ 0.5
- GDS 2: MMSE ≥ 28 , STEP = 0, I-FLEX < 3 , and CDR ≤ 0.5
- GDS 3: MMSE ≥ 26 , STEP ≤ 1 , I-FLEX ≤ 3 , and CDR > 0.5
- GDS 4: MMSE ≤ 25 , STEP > 1 , I-FLEX > 3 , and CDR > 1.0 .

If the guidelines were not applicable, the specialist psychiatrists/neurologists together determined the appropriate GDS score³²⁵.

In Study I, the subjects were considered as SCI or MCI if they were categorized as GDS 2 or 3 at the baseline examinations. In Study III, sMCI was defined as a baseline diagnosis of MCI without conversion to dementia during the follow-up. In Study IV, only SCI or MCI patients who remained stable throughout the study period were categorized as SCI or MCI in the analyses.

For participants staged as GDS 4 (possible dementia), the definite dementia etiology was determined by a specialist physician (Figure 9). During the diagnostic process, only clinical information and MRI images were available for examination, whereas the clinicians were blinded to neuropsychological test scores (others than those included in the GDS rating; MMSE, STEP variables 13-20, I-FLEX, and CDR), and MRI volumetric results, and CSF biomarkers.

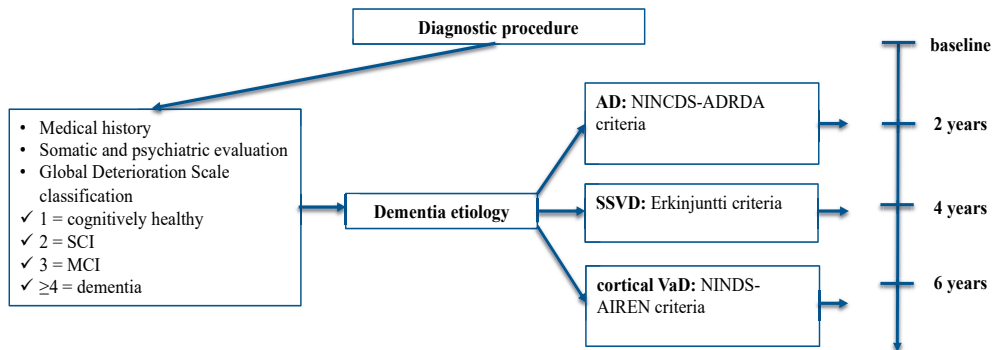


Figure 9. Diagnostic process for participants in the Gothenburg MCI study. The timeline to the right denotes the scheduled follow-up examinations in the Gothenburg MCI study. AD; Alzheimer’s disease, MCI; mild cognitive impairment SCI; subjective cognitive impairment, SSVD; subcortical small vessel type of dementia, VaD; vascular dementia. Created with Microsoft PowerPoint.

DIAGNOSTIC CATEGORIES

The diagnosis of AD was set using published criteria from 1984 by NINCDS-ADRDA⁶¹. Participants with VaD were either diagnosed as suffering from SSVD as defined by Erkinjuntti et al.³³¹, or as having cVaD according to the NINDS-AIREN criteria¹⁵¹. A mixed dementia diagnosis was set when participants had a combination of AD+SSVD or AD+cVaD. Patients with AD+SSVD exhibited a parietotemporal lobe syndrome (reflecting clinical AD symptomatology) and WMHs seen on MRI. Specifically, the WMHs were either mild in junction to symptoms reflecting a marked frontal and parietal lobe syndrome or moderate to severe with the absence of a predominant frontal lobe syndrome. Patients were classified as cVaD if the dementia was related to a single- or multi-infarct stroke³²⁵. Additionally, Lewy body dementia was determined using the guidelines by McKeith et al.³³², frontotemporal lobe dementia according to Neary et al.³³³, and primary progressive aphasia was diagnosed in line with the criteria by Gorno-Tempini et al.³³⁴. Lastly, a diagnosis of non-ultra descriptum (NUD) was given to patients who fulfilled the criteria for manifest dementia but did not demonstrate traits for a specific dementia etiology³²⁵.

PARTICIPANTS

STUDY I

Study I was a prospective observational study aimed to investigate whether serum IGF-I was related to the risk of developing dementia by any cause, AD or VaD in individuals diagnosed with SCI or MCI. In total, 499 patients with SCI or MCI were potentially eligible of which 342 patients met the inclusion criteria. Reasons for exclusion included inadequate blood samples of IGF-I ($n = 48$) and no follow-up visit ($n = 109$). Patients were followed for a maximum of 6 years with a mean follow-up of 3.6 (SD 1.8) years. This was determined as the time point of inclusion to when the patients had developed dementia (usually observed at a follow-up) while for stable SCI or MCI, the last available follow-up visit.

Ninety-five patients progressed to dementia of whom 42 patients developed VaD and 37 AD. To assemble all patients with major cerebrovascular load as a causative factor for cognitive impairment, patients with a mixed dementia diagnosis were merged with those progressing to VaD. Furthermore, within the VaD group, 35 patients developed SSVD, six patients mixed forms of cVaD and SSVD, and one patient cVaD. Totally, 16 patients were diagnosed with other forms of dementia [frontotemporal dementia (n = 2), dementia with Lewy bodies (n = 2), primary progressive aphasia (n = 1), and dementia NUD (n = 11)].

STUDY II

Study II had the main objective to cross-sectionally compare IGF-I levels in serum and CSF between cognitively intact controls and patients with AD dementia. Secondary aims were 1) to evaluate the importance of symptomatic AD medications on IGF-I levels and, 2) whether IGF-I in serum or CSF was associated with the core CSF biomarkers of AD ($A\beta_{1-42}$, T-tau, P-tau₁₈₁) and CSF/serum albumin ratio. As a secondary aim, we also compared serum insulin levels between the two study groups. In total, 36 healthy controls and 40 AD patients met the inclusion and exclusion criteria and were included. In the AD group, 18 were symptomatically treated with acetylcholinesterase (AChE) inhibitors and/or NMDAR antagonists.

STUDY III

Study III was a longitudinal study exploring the relationship between baseline circulating IGF-I and MRI measured brain regional volumes at the baseline evaluation and over time. This study included 110 patients with sMCI and 60 patients with AD, all of whom had baseline data on serum IGF-I and brain MRI scans. In the AD group, 37 patients had AD dementia without major vascular contributions whereas 23 patients suffered from mixed AD and SSVD. Moreover, 17 of the AD patients had a baseline diagnosis of SCI or MCI but who developed AD during the follow-up period and were therefore included in the AD group. Fifty-eight patients with sMCI and 29 patients with AD dementia had an available longitudinal brain MRI scan (sMCI: 2 years; n = 47, 4 years; n = 2, 6 years; n = 27, and AD: 2 years; n = 27, 4 years; n = 1, 6 years; n = 7). The mean follow-up time was 3.3 (SD 1.9) years.

STUDY IV

Study IV had the objective to examine the association between circulating IGF-I, MRI measurements of brain white matter structures and neuropsychological test performance reflecting global cognition, attention, and executive function. Study IV encompassed 106 patients with SCI (n = 56) or MCI (n = 50, defined as the SCI/MCI group) and 59 patients with AD. Of the patients with AD, 16 of them were diagnosed with SCI or MCI at the baseline evaluation but who developed AD dementia at the final follow-up (the 2-year visit) and were therefore included in the AD group. Among patients with baseline MRI data (n = 165), 75 patients underwent a brain MRI scan at the 2-year follow-up (SCI/MCI: n = 48, AD: n = 27).

COVARIATE ASSESSMENT

Body mass index (BMI) was determined using the formula: weight in kilograms (kg) per height in meters (m) squared; (kg/m^2). Blood pressure was measured in the sitting position and calculated as the mean of two measurements acquired at different visits. The mean arterial pressure (MAP) was computed according to the formula: diastolic blood pressure + $1/3$ *(systolic blood pressure – diastolic blood pressure). The value of low-density lipoprotein-cholesterol/high-density lipoprotein cholesterol (LDL/HDL) ratio was calculated as the LDL concentration divided by the HDL concentration. Furthermore, at each study visit, the status of current medications, smoking history as well as the presence of hypertension and diabetes mellitus were documented by a specialist physician.

CEREBROSPINAL FLUID AND BLOOD SAMPLES

Samples of CSF were collected at the lumbar L3/L4 or L4/L5 level using standard equipment. In brief, the procedure was performed during aseptic conditions and usually, the participants received local anesthetic (lidocaine) at the level of puncture. Followingly, a Quincke cutting-edge needle (0.7 mm/22 gauge) was inserted, with the bevel parallel to the fibers of the dura mater. During the initial step of the procedure, the first collection of CSF was disposed to minimize the risk of blood contamination. Overall, 20 ml of CSF was collected from each study participant in polypropylene tubes³²⁵.

The samples with CSF were then mixed by inverting the tubes, followed by 10 minutes of centrifugation at room temperature at 2,000 x g. Blood and CSF samples were collected in the fasted state between 8.00 AM-10.00 AM, and 8.00 AM-12.00 AM, respectively, and stored at -80° C, pending analyses.

BIOCHEMICAL AND NEUROCHEMICAL ASSESSMENTS

The biochemical serum assessments were conducted at the Clinical Chemistry Laboratory, Sahlgrenska University Hospital, while the CSF samples were analyzed at the Clinical Neurochemistry Laboratory. The core AD biomarker ($A\beta_{1-42}$, T-tau, P-tau₁₈₁) concentrations were assessed using sandwich enzyme-linked immunosorbent assays (ELISA, Innogenetics, Ghent, Belgium). To reduce the inter-assay variability for the AD core biomarkers, the laboratory examined at least two more internal CSF control samples (aliquots of pooled CSF) in each analysis.

In Study I, III, and IV, serum IGF-I was analyzed on a single occasion in June 2015 with a chemiluminescent immunometric assay (IDS-iSYS; Immunodiagnostic Systems Limited, Boldon, UK) on an IDS-iSYS automated system (IS31040; Immunodiagnostic Systems Limited, Boldon, UK). The inter- and intra-assay variability for IGF-I measurements were $\leq 3.7\%$ and $\leq 8.7\%$, respectively³³⁵. The IDS-iSYS assay for serum IGF-I was calibrated according to the WHO International Standard (02/254).

In Study II, concentrations of serum and CSF IGF-I were measured in May 2017 on one occasion with the use of a sandwich ELISA (Mediagnost, Reutlingen, Germany). Serum samples were diluted to 1:21 with an inter- and intra-assay variation lower than 6.8% and 6.7%, respectively³³⁶. The maximum number of dilutions corresponding to detectable IGF-I levels in CSF was 1:2 according to the manufacturer. The assay for IGF-I analyses system was calibrated in agreement with the WHO/NIBSC International Standard (02/254).

The Friedewald's formula³³⁷ was used to calculate the LDL levels based on the values of total cholesterol, triglycerides and HDL. Total cholesterol, triglycerides, LDL/HDL, serum insulin, and the ratio of CSF/serum albumin were analyzed using routine clinical assessment methods. Genotyping of *APOE* (gene map locus 19q13.2) was conducted using minisequencing technique³³⁸.

NEUROPSYCHOLOGICAL ASSESSMENTS

In the Gothenburg MCI study, a battery of neurocognitive examinations evaluating both global and specific cognitive functions were administered at each visit. The neuropsychological tests were led by licensed psychologists or by psychologist trainees supervised by a licensed psychologist³²⁵. The tests were administered using standardized protocols and performed during two sessions. In Study I-IV, global cognition was evaluated using the MMSE test which examines visuospatial skills, orientation, verbal memory, naming, concentration, and attention³²⁷. Episodic memory was graded with the Rey Auditory Verbal Learning Test (RAVLT; Study II and III)³³⁹. In Study IV, the domains of complex attention, processing speed, and visual scanning were assessed using the Trail Making Test (TMT) which consists of two parts; part A (TMT-A), followed by part B (TMT-B)³⁴⁰. Interference control was evaluated with the Stroop Test (I-III), Victoria version which is a shorter version of the original test developed by Stroop in 1935³⁴¹.

MAGNETIC RESONANCE IMAGING ASSESSMENTS

In Studies III and IV, brain imaging was conducted on a 1.5 T Siemens Symphony MRI (Erlanger, Germany). The protocol for the scanning and sequence generation for the MRI scans as well as the volumetric calculations were conducted according to standardized procedures^{138, 177}. T1-weighted images were analyzed with the free software package, FreeSurfer, version 5.3.0. (<https://surfer.nmr.mgh.harvard.edu/>, Figure 10). In brief, all volumetric assessments for Study III and IV were completed using T1 3D inversion recovery images (repetition time 1610 ms, echo time 2.38 ms, flip angle 15°, coronal axis, field of view 250 x 203 mm, 1 mm thickness of slice, pixel spacing and matrix size 0.49 x 0.49 mm and 512 x 416, respectively). Following volumetric assessment, a two-

step blinded quality examination was performed with the FreeSurfer graphical user interface Freeview (<https://surfer.nmr.mgh.harvard.edu/fswiki/FreeviewGuide/FreeviewIntroduction>)^{138, 177}. The FreeSurfer outputs were not normalized for total intracranial volume (ICV) as there were no statistical differences between study groups (Study III; sMCI vs. AD and Study IV; SCI/MCI vs. AD). All brain-related MRI measurements in Study III and IV are given in cm³.

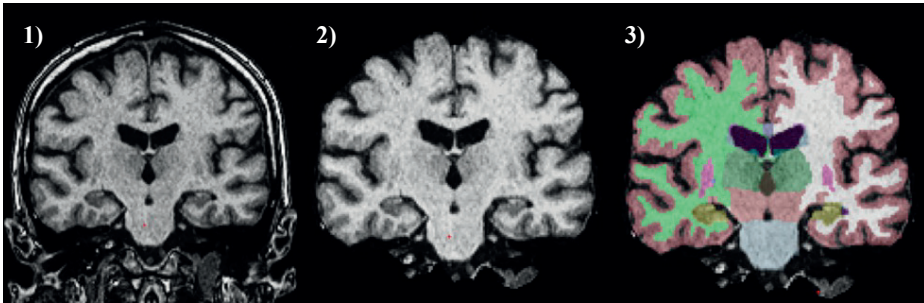


Figure 10. Brain images were assessed with the FreeSurfer software (v. 5.3.0). Coronal T1-weighted brain images; 1) Original image, 2) Removal of the cranium and, 3) Complete segmentation.

In Study III, the brain regions of interest included hippocampus, amygdala and the frontal, parietal, occipital and temporal lobes. The labels for the cortical segment/parcellation and brain lobes were created in accordance with the Desikan-Killiany cortical atlas³⁴². Endpoint brain volumes were defined as the brain volumes at the last follow-up visit. The annualized change for the brain regions of interest was calculated as: $(\text{endpoint MRI volume} - \text{baseline MRI value}) / (\text{by the number of years between the two MRI scans})$.

In Study IV, the investigated brain regions comprised the total brain white matter, total WMH amount, and CC (total and subsections; anterior, posterior, and central parts). The changes for the brain white matter volumes were calculated by subtracting the baseline MRI value from the 2-year MRI value.

STATISTICAL ANALYSES

Continuous data are expressed as means and SD (Study I and II) or as the median and the 25th-75th percentiles (Study III and IV). In Study II, the standard error of the mean was additionally provided in some of the analyses. Between-group differences were calculated with the Chi-square test for categorical data (Study I-IV) and analysis of variance (ANOVA; Study I and II) or the Mann-Whitney U test (Study III and IV) for continuous variables. In Study I, when ANOVA was used, *post hoc* testing was performed using Tukey's adjustment for multiple comparisons (Study I). In Study II, analysis of covariance (ANCOVA) was also performed using age, duration of education years, gender, and BMI as covariates as they seem to be related to dementia with AD neuropathology. In all the presented ANCOVA analyses, the ANCOVA assumptions had been tested and were met.

In Study I, Cox proportional hazards regressions were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for the relationship between circulating IGF-I levels and the risk of developing dementia by any cause, AD or VaD during the follow-up period. Circulating IGF-I was divided into quartiles and comparisons were made for the lowest (quartile 1) and highest (quartile 4) quartiles against quartiles 2-3. Moreover, quartile I was also compared with the other quartiles (2-4) to determine the risk of VaD using Cox proportional hazards regressions. Lastly, in Study I, we also determined the independency of circulating IGF-I on the risk of conversion to dementia and was evaluated by sequential inclusion of various covariates in the Cox proportional hazards regression analyses. First, in Model A, the analysis was adjusted for age and gender, while Model B was further adjusted for education (years), BMI (log-transformed), LDL-cholesterol, and current smoking (yes/no). Finally, Model C, included the variables in Model B and in addition hypertension, diabetes mellitus, and *APOE ε4* status.

In Studies I and II, correlation analyses were conducted with the Pearson's linear correlation coefficient. Furthermore, in Study II, binary logistic regression analyses were conducted to calculate the odds ratios (OR) and 95% CIs for the risk of prevalent AD per SD increase in serum IGF-I.

In Studies III and IV, the Spearman rank order correlation test was conducted to analyze whether serum IGF-I levels were related to baseline volumes and alterations over time in MRI measurements of brain region variables (Study III and IV) as well as cognitive test performance (Study IV). Moreover, in Study IV, partial correlation analyses were conducted to investigate the relation between baseline circulating IGF-I and brain white matter structures as well as between baseline serum IGF-I and neuropsychological test results [controlled for age, gender, BMI, smoking status, LDL/HDL ratio, and MAP]. In the partial correlation analyses between baseline circulating IGF-I and brain white matter values, ICV was additionally included as a covariate. Finally, in the partial correlation analyses, the logarithmic value of serum IGF-I was applied as it was skewedly distributed.

ETHICAL PERMITS

Ethical approval was granted by the Regional Ethical Committee at the University of Gothenburg (diary numbers L091-99 15; March 1999 and T479-11; 8 June 2011) and the Swedish Ethical Review Authority (diary number 2020-06733; 15 March 2021). All participants provided oral and written informed consent for participation. The included Studies followed the ethical principles of the Helsinki Declaration.

RESULTS

RESULTS

STUDY I

This prospective cohort study determined if baseline serum IGF-I predicted the risk of conversion to dementia by any cause and dementia subtypes in individuals with SCI or MCI. A total of 95 patients (28%) developed dementia [VaD: n = 42 (12%), AD: n = 37 (11%), other dementia forms: n = 16 (5%)] during a mean follow-up of 3.6 (SD 1.8) years (Table 1).

Baseline values of age, gender distribution, and BMI differed between serum IGF-I quartile groups. However, the duration of education (years), MMSE score, and LDL-cholesterol concentration were statistically similar in all the IGF-I quartile groups. The frequencies of the *APOE* $\epsilon 4$ alleles, current smoking (yes/no), hypertension and diabetes mellitus were similar in patients with low (quartile 1), intermediate (quartiles 2-3), and high (quartile 4) serum IGF-I levels.

Table 1. Baseline demographic and biochemical attributes of participants in Study I.

(n = 342)

Dementia conversion, n, (%)	95 (28)
Demographic characteristics	
Age (years)	64.6 (7.8)
Male/female, n, (%)	148/194 (43/57)
Education (years)	12.5 (3.5)
MMSE score	28.5 (1.4)
BMI (kg/m ²)	25.1 (3.6)
<i>APOE</i> $\epsilon 4$ allele (0/1/2; n, (%))	170/125/34 (52/38/10)
Biochemical characteristics	
Serum IGF-I (ng/mL)	116 (33)
LDL-cholesterol (mmol/L)	3.45 (0.89)

All variables are presented as mean (SD) if not stated otherwise. Abbreviations = *APOE*; apolipoprotein E, BMI; body mass index, IGF-I; insulin-like growth factor-I, LDL; low-density lipoprotein, MMSE; Mini-Mental State Examination, SD; standard deviation. Adapted from Table 1 in Psychoneuroendocrinology, Vol. 86, Quinlan

et al., Low serum insulin-like growth factor-I (IGF-I) level is associated with increased risk of vascular dementia, p. 169-175, 2017, with permission from Elsevier.

The Cox proportional hazard regression analyses showed that neither low nor high serum IGF-I concentrations [quartile 1 or 4 vs. quartiles 2-3] were related to the risk of conversion to dementia by any cause, or dementia with AD in persons with SCI or MCI.

Conversely, the Cox proportional hazard regression demonstrated that low levels of serum IGF-I in SCI/MCI patients were related to a two-fold increased risk of developing VaD [quartile 1 vs. quartiles 2-3, crude HR = 2.22, 95% CI: 1.13-4.36]. Following full adjustment for covariates, this association remained statistically significant [quartile 1 vs. quartiles 2-3, HR = 2.21, 95% CI: 1.05-4.63]. These findings were further illustrated by Kaplan-Meier survival curves displaying a higher risk of developing VaD in patients with either SCI or MCI having low circulating IGF-I (log-rank test: $p = 0.01$, quartile 1 vs. quartiles 2-3, Figure 11). In contrast, the highest IGF-I quartile in SCI/MCI patients did not influence the risk of conversion to VaD (quartile 4 vs quartiles 2-3). Moreover, in subanalyses, the lowest IGF-I quartile was associated with an increased risk of VaD when compared with the 3 highest quartiles of IGF-I [crude HR = 2.19, 95% CI: 1.19-4.04].

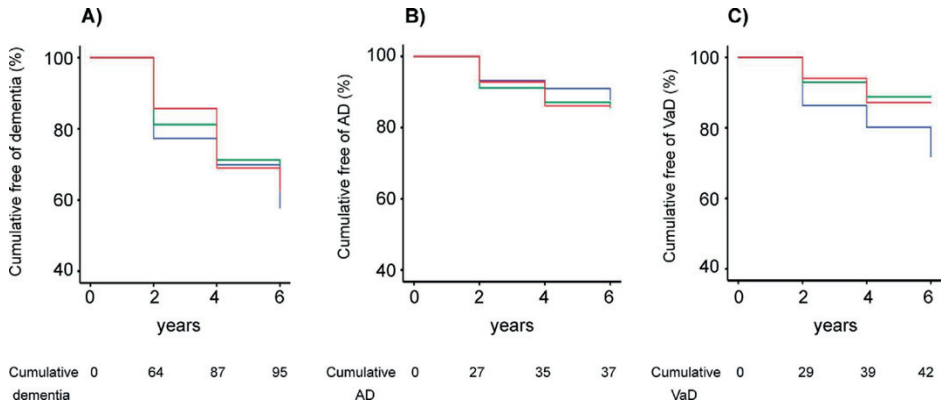


Figure 11. Low IGF-I is associated with increased risk of VaD. Kaplan-Meier survival curves for (A) all-cause dementia (log-rank test: $P = 0.22$ quartile 1 vs. quartiles 2–3, $P = 0.84$ quartile 4 vs. quartiles 2–3), (B) AD ($P = 0.55$ quartile 1 vs. quartiles 2–3, $P = 0.87$ quartile 4 vs. quartiles 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. Reprinted from *Psychoneuroendocrinology*, Vol. 86, Quinlan et al., Low serum insulin-like growth factor-I (IGF-I) level is associated with increased risk of vascular dementia, p. 169-175, 2017, with permission from Elsevier.

STUDY II

Study II was a cross-sectional study aiming to determine serum and CSF concentrations of IGF-I as well as serum insulin in non-diabetic AD patients (n = 40) and cognitively normal controls (n = 36).

Age, gender distribution, duration of education years, and BMI were statistically similar between patients with AD and healthy controls. When compared with the healthy controls, the AD patients scored lower on the MMSE and RAVLT tests and had deranged values of CSF A β ₁₋₄₂, T-tau, and P-tau₁₈₁. As expected, the *APOE* ϵ 4 allele distribution was statistically different between AD patients and controls.

In ANOVA analyses, serum and CSF concentrations of IGF-I were similar between the study groups. However, in ANCOVA analyses [controlled for age, gender, duration of education years, and BMI], IGF-I levels in serum ($p = 0.04$), but not in CSF, were significantly higher in the AD patients compared with the healthy controls. In line with these findings, the binary logistic regression model adjusted for the same covariates demonstrated an increased risk of prevalent AD per SD increment in circulating IGF-I [OR = 1.83, 95% CI: 1.01-3.32]. After removal of AD patients receiving AChE inhibitors and/or NMDAR antagonists (n = 18), the relationship between circulating IGF-I and higher risk of prevalent dementia with AD was more pronounced [OR = 2.23, 95% CI: 1.10-4.48].

Additionally, no differences were found between AD patients and healthy controls in terms of serum insulin and CSF/serum IGF-I ratio in the unadjusted analyses. Furthermore, ANCOVA analyses for serum insulin and CSF/serum IGF-I ratio were not conducted as these variables did not meet the assumptions for ANCOVA.

In both the total study population ($n = 76$) and AD patients ($n = 40$), circulating IGF-I was inversely associated with CSF $A\beta_{1-42}$ ($r = -0.25$, $p = 0.03$, and $r = -0.41$, $p < 0.01$, respectively, Figure 12). In the entire study group, higher CSF IGF-I was related to higher CSF/serum albumin ratio ($r = 0.54$, $p < 0.001$) as well as the CSF levels of T-tau ($r = 0.41$, $p < 0.001$) and P-tau₁₈₁ ($r = 0.37$, $p = 0.001$, Figure 12). Similar findings were observed in the AD group as IGF-I in CSF was positively related to the CSF/serum albumin ratio ($r = 0.70$, $p < 0.001$), CSF T-tau ($r = 0.35$, $p = 0.03$) and CSF P-tau₁₈₁ ($r = 0.33$, $p = 0.04$, Figure 12).

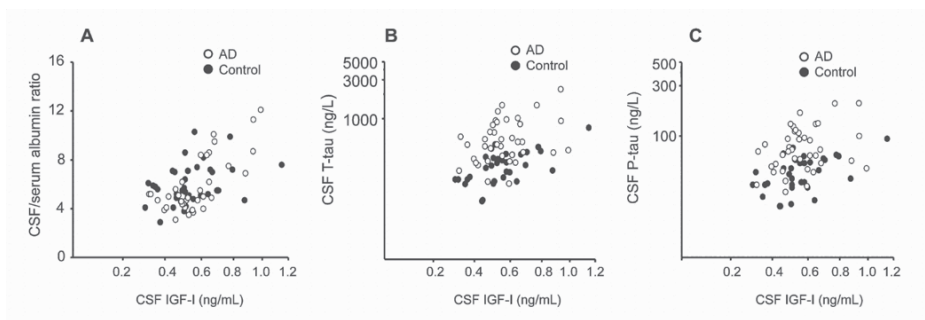


Figure 12. A) CSF IGF-I correlated positively with CSF/serum albumin ratio in the total study population ($n = 76$; $r = 0.54$, $p < 0.001$) and in the AD group ($n = 40$; $r = 0.70$, $p < 0.001$). B) The positive correlation between CSF IGF-I and the CSF AD biomarker T-tau in the total study population ($n = 76$; $r = 0.41$, $p < 0.001$) and in the AD group ($n = 40$; $r = 0.35$, $p = 0.03$). C) CSF IGF-I correlated positively with CSF P-tau in the total study population ($n = 76$; $r = 0.37$, $p = 0.001$) and in the AD group ($n = 40$; $r = 0.33$, $p = 0.04$). In all panels, values in AD patients are marked with white circles and values in controls are marked with black circles. Note the logarithmic scale on the x-axis in all panels and on the y-axis in (B) and (C). Correlations were sought using Pearson's linear correlation coefficient. Reprinted from *Journal of Alzheimer's Disease*, Vol. 75, Horvath et al., Patients with Alzheimer's Disease Have Increased Levels of Insulin-like Growth Factor-I in Serum but not in Cerebrospinal Fluid, p. 289-298, 2020, with permission from IOS PRESS.

STUDY III

The purpose of Study III was to explore whether serum IGF-I was related to brain MRI measurements at baseline and changes over time, defined as annualized changes, with a maximum follow-up of 6 years. Altogether, 110 sMCI patients and 60 AD dementia patients were enrolled, of which 87 patients had at least one available MRI follow-up (sMCI: n = 58, AD: n = 29).

Serum IGF-I at baseline was comparable in the sMCI and AD patients although there was a tendency towards lower IGF-I levels in sMCI (Table 2). Additionally, sMCI patients exhibited larger baseline and endpoint brain volumes of the hippocampus and amygdala, and frontal, parietal, and temporal lobes. In contrast, occipital lobe volume did not differ between the two groups.

Table 2. Brief overview of baseline clinical and neuropsychological data of participants in Study III and Study IV.

	Study III		Study IV	
	sMCI (n = 110)	AD (n = 60)	SCI/MCI (n = 106)	AD (n = 59)
MRI follow-up, n	58	29	48	27
Clinical characteristics				
Age (years)	64.0 (58.0–71.0)	69.0 (62.0–75.0)	64.0 (59.5–71.0)	69.0 (62.0–75.0)
Male/female, n, (%)	40/70 (36/64)	28/32 (47/53)	34/72 (32/68)	28/31 (47/53)
Education (years)	13.5 (11.0–16.0)	12.5 (9.0–15.0)	14.0 (11.0–16.0)	13.0 (9.0–15.0)
Serum IGF-I (ng/mL)	112 (94–139)	123 (96–146)	113 (95–139)	118 (95–146)
Cognitive test performance				
MMSE scores	29 (28–30)	26.5 (24–28)	29 (28–30)	27 (25–28)
RAVLT delayed recall scores	8.0 (5.0–11.0)	1.0 (0.0–3.0)	-	-

All variables are presented as the median (25th-75th percentiles) if not stated otherwise.

Abbreviations = AD; Alzheimer's disease, IGF-I; insulin-like growth factor-I, MCI; mild cognitive impairment, MMSE; Mini-Mental State Examination, MRI; magnetic resonance imaging, RAVLT; Rey Auditory Verbal Learning Test, SCI; subjective cognitive impairment, sMCI; stable mild cognitive impairment

In sMCI patients, higher baseline circulating IGF-I was related to larger volumes at baseline in terms of the hippocampus ($r_s = 0.32, p < 0.01$) and amygdala ($r_s = 0.27, p < 0.01$). At baseline, serum IGF-I in the sMCI group was also associated with the frontal ($r_s = 0.26, p < 0.01$), parietal ($r_s = 0.22, p = 0.02$) and temporal ($r_s = 0.25, p = 0.02$) lobes, but not with the occipital lobe. Additionally, lower baseline concentrations of IGF-I correlated with greater annual volume decline of the hippocampus ($r_s = 0.32, p = 0.02$), Figure 13), whereas IGF-I did not associate with the annualized changes of the other brain measures in sMCI. In subgroup analyses, sMCI patients having IGF-I below the median exhibited a more marked annual volume loss of the hippocampal regions compared to sMCI patients displaying IGF-I concentrations above the median ($p = 0.02$).

Conversely, in patients with AD, IGF-I was not related to any of the measured brain structures at the baseline evaluation or with the longitudinal changes. Finally, MRI characteristics were comparable between AD patients with circulating IGF-I levels above or below the median.

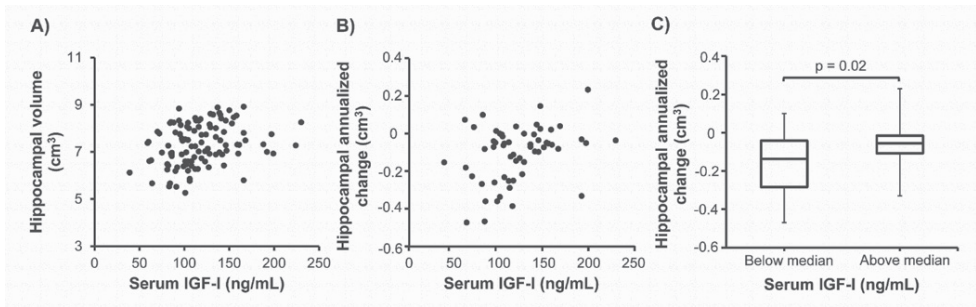


Figure 13. Higher serum insulin-like growth factor-I (IGF-I) concentration is associated with larger hippocampal volume in stable mild cognitive impairment (sMCI). In the sMCI patients, serum IGF-I correlated positively with (A) baseline hippocampal volume ($n = 110$; $r_s = 0.32$, $p < 0.01$) and (B) the annualized change in hippocampal volume ($n = 58$; $r_s = 0.32$, $p = 0.02$). (C) In the sMCI group ($n = 58$), patients having serum IGF-I concentrations above the median had a less prominent decrease in hippocampal volume during the follow-up than those having serum IGF-I below the median ($p = 0.02$). Data in the box plots are presented as medians (horizontal lines), 25th–75th percentiles (boxes), and ranges (whiskers). Correlations were sought using the Spearman rank order correlation test and between-group differences were investigated using the Mann-Whitney U test. Reprinted from *Journal of Alzheimer's Disease*, Vol. 88, Horvath et al., Low Serum Insulin-like Growth Factor-I Is Associated with Decline in Hippocampal Volume in Stable Mild Cognitive Impairment but not in Alzheimer's Disease, p. 1007-1016, 2022, with permission from IOS PRESS.

STUDY IV

Study IV was a longitudinal study of SCI/MCI and AD patients examining the relationship between serum IGF-I and MRI-estimated white matter brain measures as well as cognitive status reflecting global cognition and the cognitive domains attention and executive function. Patients were evaluated at baseline (SCI/MCI: $n = 106$, AD: $n = 59$) and after 2 years (SCI/MCI: $n = 48$, AD: $n = 27$).

SCI/MCI and AD patients had similar serum levels of IGF-I at baseline (Table 2). In the SCI/MCI group, higher baseline serum IGF-I was related to greater baseline volumes of the total white matter ($r_s = 0.26$, $p < 0.01$), but not with the amount of WMHs in the Spearman rank order correlation analyses. Additionally, baseline IGF-I was positively associated with the baseline volumes of total CC ($r_s = 0.29$, $p < 0.01$) and CC sub-portions (anterior; $r_s = 0.28$, $p < 0.01$, central; $r_s = 0.29$, $p < 0.01$, and posterior; $r_s = 0.26$, $p < 0.01$; Figure 14). Similar to the analyses between circulating IGF-I levels and white matter brain variables, higher serum IGF-I in SCI/MCI patients was also related to better baseline performance in some of the tests evaluating attention and executive function (TMT-A; $r_s = -0.22$, $p = 0.04$, Stroop Test II; $r_s = -0.28$, $p < 0.01$ and Stroop Test III; $r_s = -0.21$, $p < 0.05$). In the AD patients, no correlations were observed in the Spearman rank order correlation analyses between baseline IGF-I and baseline brain white matter measures or cognitive test scores.

Secondly, we also performed longitudinal analyses investigating the relationship between baseline serum IGF-I levels and 2-year values of MRI-estimated brain white structures and cognitive test performances as well as changes in the variables. In SCI/MCI, there were positive associations between baseline IGF-I and the volumes after 2 years of the total white matter ($r_s = 0.41$, $p < 0.01$) and total CC ($r_s = 0.34$, $p = 0.03$). Contrary, in AD, baseline IGF-I was not related to the 2-year brain white matter volumes. Moreover, in both groups, no relationships were found between baseline serum IGF-I and any of the neuropsychological test scores after 2 years. Furthermore, in SCI/MCI patients as well as in AD patients, baseline IGF-I was not related to the changes from baseline to the 2-year follow-up in any of the brain white matter structures or the neuropsychological test performances.

In partial correlation analyses following adjustment for multiple covariates [age, gender, BMI, smoking status (never/previous/current), LDL/HDL ratio, MAP, and ICV], baseline serum IGF-I was no longer significantly correlated with any of the investigated brain white matter measures at baseline or the 2-year follow-up in SCI/MCI patients. Comparable results were observed in the AD group as the partial correlation analyses (adjusted for the same variables) did not show any relationship between serum IGF-I and brain white matter volumes at any time point.

However, at baseline, serum IGF-I was related to better baseline performance in terms of MMSE ($r = 0.24, p = 0.04$) and Stroop Test II ($r = -0.29, p = 0.01$) in SCI/MCI whereas in AD, serum IGF-I was inversely associated with TMT-B ($r = -0.40, p = 0.04$) and Stroop Test I ($r = -0.39, p = 0.049$) scores in the partial correlation analyses (adjusted for the same confounders except for ICV). Lastly, the adjusted partial correlation analyses did not show any significant association in either SCI/MCI or AD patients between baseline serum IGF-I and 2-year cognitive test scores.

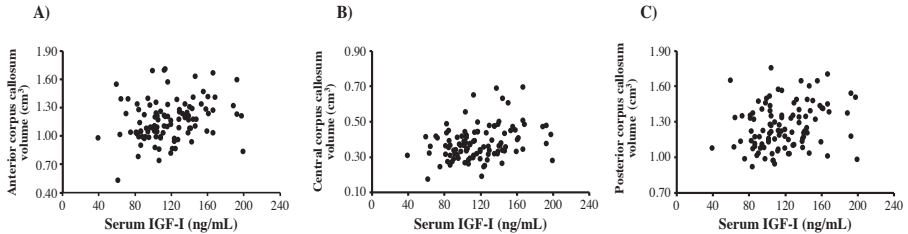


Figure 14. Higher serum IGF-I is associated with larger baseline volumes of subsections of corpus callosum in SCI/MCI patients ($n = 106$) in unadjusted analyses. Serum IGF-I was positively correlated with the (A) anterior ($r_s = 0.28, p < 0.01$), (B) central ($r_s = 0.29, p < 0.01$), and (C) posterior ($r_s = 0.26, p < 0.01$) parts of the corpus callosum at baseline in the SCI/MCI group. Taken from Study IV, unpublished. Created with Microsoft PowerPoint and Excel.

DISCUSSION

DISCUSSION

IGF-I AND RISK OF DEMENTIA

Experimental studies suggest that lack of circulating IGF-I accelerates cerebrovascular aging as deficiency of endocrine liver-derived IGF-I led to decreased microvascular density and endothelial dysfunction in the mouse brain^{319, 320}. However, it has been less clear whether alterations in circulating IGF-I levels influence the risk of VaD. Furthermore, IGF-I has also been linked to AD neuropathology. In mouse AD models, IGF-I and A β interacts at the level of the BBB, where lower circulating IGF-I results in reduced clearance of A β from the brain, and higher A β load in the brain causes decreased transport of IGF-I across the BBB into the brain³⁰⁹. In addition, severe IGF-I resistance has been demonstrated in experimental studies as well as in the postmortem human AD brain^{239, 311}, which could possibly preclude IGF-I from exerting neuroprotection in AD. In a memory clinic setting, the aim of Study I was therefore to examine whether altered IGF-I levels influence the risk of dementia by any etiology, AD, or VaD in patients with SCI/MCI at baseline.

Patients with SCI/MCI having low circulating IGF-I concentrations had a doubled risk of conversion to VaD (Study I). This relationship was present in both the univariate analysis and after the correction of variables known to influence IGF-I levels such as age, gender, and BMI. The majority of the VaD patients were diagnosed with SSVD which is characterized by events such as arteriolosclerosis, demyelination, neurovascular uncoupling, and impaired endothelial function of the brain vasculature, all of which are influenced by IGF-I activity^{254, 319, 320}. It could therefore be hypothesized that IGF-I is related to the progression of VaD. This notion is in agreement with the results of previous studies showing reduced IGF-I levels in VaD patients^{302, 323, 324} and increased risk of VaD in carriers of a polymorphism in the *IGF-IR* gene³²².

In Study I, neither low nor high circulating IGF-I concentrations predicted the risk of developing dementia by any cause, or dementia with AD. In line with the absence of relationship between IGF-I and all-cause dementia in Study I, no relationship was found between IGF-I and dementia risk in the Caerphilly Prospective Study²⁸⁸. In some contrast,

the results of a study based on the UK Biobank showed a U-shaped association between IGF-I and dementia risk²⁸¹. Discrepant results have also been found in terms of the association between IGF-I and the risk of AD. The prospective Framingham cohort study displayed that individuals with low circulating IGF-I had an increased risk of developing AD dementia²⁸². In the same year, the Rotterdam cohort study showed an increased AD risk in individuals with higher IGF-I kinase receptor activity²⁸⁶. In the latter study, it was hypothesized that higher IGF-IR activity is closely related to the brain resistance to IGF-I signaling previously found in the early phases of AD. It is conceivable that alterations in IGF-I activity starts in the prodromal stages of AD but is not displayed in serum IGF-I early in the AD course.

INSULIN-LIKE GROWTH FACTOR-I AND INSULIN LEVELS IN HEALTHY PERSONS VS IN ALZHEIMER'S DISEASE

In addition to the discrepant results between serum IGF-I levels and AD risk, several cross-sectional analyses have shown higher^{303, 304}, unchanged³⁴³, or lower³⁰⁵ levels of serum and CSF IGF-I in AD patients. The main objective of Study II was therefore to examine whether concentrations of serum IGF-I and insulin as well as IGF-I in CSF was different between cognitively intact controls and patients with AD.

In the ANCOVA analyses controlled for BMI, duration of education years, age, and gender, the adjusted mean circulating IGF-I level was approximately 16% higher in patients with AD than that in the controls (Study II). The multivariate logistic regression analyses adjusted for the same covariates further supported these findings as the risk of prevalent AD was almost doubled per SD increase in serum IGF-levels.

In addition to the increasing resistance to IGF-IR signaling with the advancement of AD^{239, 311-313}, the higher serum IGF-I concentrations observed in our AD patients could be explained by several other potential mechanisms. In experimental models, IGF-I reduced the burden of A β plaques and influenced phosphorylation of the tau-protein^{212, 260}. Increments of serum IGF-I might therefore be a compensatory mechanism to counteract the increasing load of A β in AD. Additionally, decreased passage of IGF-I across the BBB²⁶² could

further underlie the increased serum IGF-I levels observed in the AD patients (Study II). Speculatively, the increase in circulating IGF-I might be a protective effect to uphold the entrance of IGF-I into the CNS and counteract the perturbed IGF-I activity found in the AD brain^{312, 313}.

In several cross-sectional analyses of IGF-I concentrations in AD, the patients suffered from AD combined with major cerebrovascular disease^{304, 344} whereas this was not clarified by others^{303, 345, 346}. However, consideration of cerebrovascular pathology may be necessary since decline in IGF-I levels results in the formation of carotid atherosclerosis³²⁴, impaired microvascular density in the brain³¹⁹, and is linked to VaD³²³. Taking this into account, in Study II, patients with AD who demonstrated white matter disease (Fazekas scale grade II (moderate) and III (severe)) were not eligible³⁴⁷. Speculatively, the role of IGF-I may be different in vascular and non-vascular AD as IGF-I deficiency could aggravate cerebrovascular disease, which in turn could accelerate AD progression.

After the exclusion of AD patients with symptomatic AD medication, we found that the risk of prevalent AD per SD increment in IGF-I levels was more marked than that in the total AD group (Study II). In some of the earlier studies on IGF-I, the AD patients did not receive AChE inhibitors^{301, 304}, while this was not specified in other studies^{346, 348}. As IGF-I in tandem with acetylcholine decline in the AD brain³¹³, it could be argued that symptomatic AD medications act as modulators of serum IGF-I levels. Indeed, in a randomized controlled trial of male patients with AD, 8 weeks of treatment with donepezil counteracted the age-related decline in the concentrations of members of the GH/IGF-I system³⁴⁹.

In conclusion, our AD patients demonstrated higher IGF-I levels in serum compared with healthy controls (Study II), which is in agreement with some of the previous IGF-I studies in AD^{303, 304, 345}. It is important to acknowledge that factors such as concomitant cerebrovascular pathology and AD medications might influence serum IGF-I levels in AD and perhaps provide some explanation to the diversity of previous IGF-I studies. Additionally, it is plausible that factors such as age, severity of AD symptomology³⁰⁶ and presence of IGF-I

polymorphisms²⁹⁶ may affect IGF-I levels and should therefore be considered in future IGF-I studies in AD.

Insulin and IGF-I belongs to the same family of hormones. Therefore, a secondary objective of Study II was to investigate insulin levels in AD and healthy controls. CSF insulin levels were not available, but serum insulin levels were comparable in the AD patients and healthy controls. In recent years, the insulin pathway has emerged as one contributor in the pathogenesis of AD dementia and AD has been referred to as ‘‘type 3 diabetes’’³⁵⁰. Moreover, type 2 diabetes mellitus increased the risk of AD^{351, 352} and resistance to insulin activity was confirmed by postmortem brain examinations of humans with AD³⁵³. Consistent with the findings of Study II, a previous study did not find statistical differences in serum insulin levels between healthy individuals and AD patients³⁰⁴. In contrast, Craft et al.³⁵⁴ showed lower CSF insulin and higher plasma insulin in AD than in healthy controls. These differences were more pronounced with increasing AD dementia severity³⁵⁴. Similar to the discrepancy of IGF-I studies, the lack of adjustment for factors associated with CSF and serum insulin levels might explain why the results of insulin studies in AD including Study II have been divergent.

CEREBROSPINAL FLUID INSULIN-LIKE GROWTH FACTOR-I LEVELS

In the elderly brain, the local production of IGF-I gradually declines, which contrasts with the relatively stable expression pattern of the IGF-IR throughout life^{23, 24}. In adulthood, the majority of CSF IGF-I derives from the circulation and enters the brain at the level of the choroid plexus via the megalin/LRP2 receptor, which also acts as an exporter of A β ²⁶². While this pathway seems to be disrupted in the experimental AD brain³⁵⁵, clinical studies have shown divergent results as CSF IGF-I has been increased³⁰⁵ or unchanged^{303, 304, 343} in AD.

In Study II, we found that IGF-I concentrations in CSF were comparable between the AD group and the cognitively healthy control group, whereas serum IGF-I concentrations were higher in the AD patients after adjustment for covariates. The unchanged CSF IGF-I levels together with elevated serum IGF-I in our AD patients supports the hypotheses of IGF-IR resistance in combination with a reduced passage of circulating IGF-I across the BBB^{262, 355}. The notion of BBB-dependent

passage of serum IGF-I into the CNS is possibly supported by the positive association between IGF-I in CSF and the ratio between CSF and serum albumin that was observed in our AD patients (Study II), the latter ratio being an established measure of BBB integrity³⁵⁶.

The unchanged CSF IGF-I concentrations in patients with AD (Study II) is in accordance with two previous AD studies^{303, 304, 343}, while another study found increased CSF levels of IGF-I³⁰⁵. The exact mechanisms have not been elucidated in detail, but it has been suggested that increments in circulating IGF-I concentrations reflect altered sensitivity to IGF-IR signaling in brains with AD neuropathology. As the dementia progresses, the IGF-I concentrations decrease (reflecting deficiency), which could be secondary to malnutrition, impaired body composition, isolation and immobilization. These factors are commonly associated with low serum IGF-I levels²². Speculatively, during AD progression, this could result in gradual decline of IGF-I passage across the BBB and could provide some insight to the discrepant CSF IGF-I studies.

In addition, CSF levels of A β ₁₋₄₂ in AD are reduced, most likely because of deposition in amyloid plaques within the brain followed by elevations of CSF P-tau and T-tau^{356, 357}. In the AD patients (Study II), serum IGF-I was inversely related to CSF A β ₁₋₄₂ concentrations, which supports previous findings of peripheral IGF-I interacting with A β and its transportation from the brain^{260, 262}. Conversely, high CSF IGF-I in the AD patients was correlated with increased CSF T-tau and P-tau₁₈₁ (Study II). The IGF-I null brain of mice is characterized by hyperphosphorylation of tau, and as tau is a subunit of the microtubules, it is most likely that central but not circulating IGF-I regulates tau²¹².

INSULIN-LIKE GROWTH FACTOR-I AND GRAY MATTER BRAIN MORPHOLOGY

The MTL is the first brain structure to degenerate in AD^{358, 359} and atrophy of its constituent parts parallel each other at several stages of the disease progression³⁶⁰. With the severity of AD dementia, the neuronal atrophy spreads from the MTL and becomes evident in a temporal-parietal-frontal trajectory^{361, 362}. Previous research indicate that IGF-I is essential for neuronal survival and functionality of the rodent hippocampus^{363, 364} as well as other brain areas²⁵⁴. However, whether

IGF-I confers neuroprotection in the human brain has been less clear. Higher serum IGF-I has been associated with larger total brain volume and hippocampal volume in healthy individuals^{278, 282}, whereas these findings have not been replicated in other studies^{283, 285}. Additionally, no previous study has investigated the relationship between IGF-I and brain regional volumes in patients with cognitive impairment.

In Study III, we determined whether baseline serum IGF-I was related to MRI-determined structures with a high density of gray matter in sMCI and AD. At the baseline evaluation, sMCI patients but not AD patients with high serum IGF-I levels had larger volumes of all investigated brain regions apart from the occipital lobe. Additionally, higher serum IGF-I in sMCI was associated with decreased annual atrophy of the hippocampus but was not associated with the annualized changes of other brain regions. In AD, baseline IGF-I was not related to the longitudinal brain MRI measures.

Among the baseline and longitudinal brain volumes examined in Study III, the strongest relationship was observed between IGF-I and the hippocampal region in sMCI patients. Interestingly, in a previous study of MCI, higher circulating IGF-I was related to superior scores in tests evaluating memory and learning, which are mainly regulated by the hippocampus³⁶⁵. In the postmortem human brain, a greater density of IGF-IRs has been observed in the hippocampus than in other brain regions³⁶⁶. Furthermore, experimental studies illustrate that expression of the IGF-IR increases in the aging hippocampus of rodents, perhaps acting as a compensatory mechanism for the age-related decrease in IGF-I activity^{367, 368}. Altogether, the results of Study III extend the previous knowledge by showing a close relation in sMCI between circulating IGF-I and the hippocampal regions.

In Study III, we did not find any relationship between circulating IGF-I and the investigated brain MRI variables in patients with AD. This supports earlier reports showing reduced mRNA expressions of IGF-I and IGF-IR in areas with a high density of gray matter in the human postmortem AD brain^{312, 313}. Perhaps, these findings together with brain IGF-IR resistance and reduced passage of IGF-I across the BBB^{239, 262, 311} provide some explanation to the lack of association between IGF-I and the gray matter in our AD patients.

In conclusion, the findings of Study III indicate that IGF-I is linked to the maintenance of the adult hippocampus in patients with mild cognitive dysfunction. However, the neuroprotective effects normally induced by IGF-I may be reduced in established AD due to altered IGF-IR activity or by reduced passage of IGF-I across the BBB.

IGF-I AND WHITE MATTER BRAIN MORPHOLOGY AND COGNITIVE FUNCTION

Although not being one of the central pathophysiological processes, atrophy of the brain white matter is often observed in MCI and more markedly in AD³⁶⁹. As a result, in Study IV, we examined the association between circulating IGF-I and white matter brain structures.

In both the baseline and the 2-year visit data using the Spearman rank order correlation test, SCI/MCI patients showed positive correlations between serum IGF-I and volumes of the total brain white matter and CC, but not with lesions in the white matter (WMHs, Study IV). In subanalyses, at baseline, higher IGF-I was associated with larger volumes of all CC substructures in the SCI/MCI group including the anterior, central and posterior parts. Contrary, in SCI/MCI, baseline IGF-I did not correlate with the changes from baseline to the 2-year follow-up in any of the brain white matter structures. Furthermore, in the partial correlation analyses controlled for ICV and other covariates in SCI/MCI, serum IGF-I was no longer significantly associated with any of the brain white matter volumes (Study IV).

Patients with AD did not exhibit any significant relationship between circulating IGF-I and the examined white matter brain variables in neither the Spearman rank order correlation test, nor the partial correlation analyses (Study IV). This is coherent with Study III as in the AD group, circulating IGF-I did not relate to the evaluated baseline or annual decrease of various volumes reflecting the gray matter of the brain. Speculatively, the absence of associations between serum IGF-I and gray and white matter in AD could at least to some extent be the result of IGF-IR resistance^{239, 311-313}.

Degeneration of the brain white matter is complex, and involves impaired function of oligodendrocytes, myelin sheaths and axons³⁷⁰. *In*

vivo animal as well as *in vitro* experimental studies demonstrate that brain white matter degeneration is more evident in the setting of IGF-I deficiency²⁵¹⁻²⁵⁴. Especially, IGF-I may play a key role for the maintenance of CC. IGF-I null mice displayed a more prominent decrease in CC thickness compared with the decrease of the whole brain²⁵⁴, while lack of the IGF-IR in oligodendrocyte precursor cells resulted in reduced number of oligodendrocytes in the CC³⁷¹. It is unclear whether IGF-I has a similar role in terms of the human CC. Nevertheless, the relationship between IGF-I and CC volume in humans may perhaps be of special interest to investigate since the CC is one of the largest white matter structures of the brain and is susceptible to degeneration in elders¹⁴⁶.

While experimental data propose a close relationship between IGF-I and brain white matter integrity, population-based association studies provide contrasting findings with respect to the relationship between IGF-I and WMHs. In one study, higher serum IGF-I was related to decreased volume of WMHs²⁸⁴. Contrary, no relationship was found between IGF-I and WMH volume in another epidemiological study²⁸⁵, which is in agreement with the findings of Study IV in SCI/MCI patients. Lastly, a prospective cohort of individuals free from brain-related disease showed that higher serum IGF-I related to larger total brain white matter and decreased burden of WMHs at baseline²⁸¹. In conclusion, although some experimental and population-based studies suggest that IGF-I is related to the brain white matter^{281, 284}, it is unclear whether IGF-I provide neuroprotective signals in the brain white matter in individuals with cognitive dysfunction. Therefore, additional studies are warranted to clarify this issue in patients with cognitive impairment.

The presence of WMHs is associated with decline in various cognitive domains including executive function, speed, and visuopractical skills³⁷². Interestingly, previous research of older individuals has shown that both low and high serum IGF-I levels were implicated in executive function, processing capacity, and attention^{272, 275, 373}. Additionally, MCI patients with diabetes mellitus type 2 having low serum IGF-I/IGFBP-3 ratio performed worse on tests evaluating executive function, processing speed, and attention³⁷⁴. Therefore, another objective of Study IV was to determine if circulating IGF-I levels are related to cognitive tests that

are partly influenced by the brain white matter (TMT-A/B and Stroop test I-III).

In Study IV, we found that at baseline, higher serum IGF-I was related to better scores of TMT-A and Stroop tests II and III in SCI/MCI. Furthermore, in SCI/MCI patients, baseline serum IGF-I still correlated with better baseline neuropsychological test scores (MMSE and Stroop test II) in the partial correlation analyses with adjustment for multiple covariates (Study IV). Contrary, Study IV did not find significant relationships between circulating IGF-I levels and the 2-year scores of the neuropsychological tests in the Spearman or partial correlation analyses in the SCI/MCI group. Overall, our results support some of the previous observational studies suggesting that IGF-I may provide some protection against cognitive dysfunction in individuals with SCI or MCI. This assumption is further supported by previous interventional studies where raised IGF-I levels showed some²⁷⁷ or no²⁷⁹ improvement in cognitive functioning in cognitively intact individuals and MCI patients.

In AD, there was no relationship between serum IGF-I and neuropsychological test performance in the Spearman rank order correlation analyses, but after covariate adjustment in the partial correlation analyses, higher baseline serum IGF-I was related to better baseline scores of TMT-B and Stroop Test I. While the relation between IGF-I in serum and cognitive function in AD has not been studied in detail, a longitudinal study spanning over 2 years demonstrated a more marked decline in MMSE scores in AD patients with lower baseline serum IGF-I concentrations³¹⁶. It cannot be excluded that altered sensitivity to IGF-IR signaling is not only present in the gray matter of the AD brain^{239, 311}, but also in the white matter. However, as serum IGF-I was significantly associated with some of the baseline neuropsychological test scores in the AD patients in Study IV, resistance to IGF-IR signaling may not be as evident in the brain white matter.

In conclusion, based on the results of Study IV, it is possible that IGF-I can induce neuroprotection only to a moderate extent in SCI/MCI and AD in cognitive functions that are partly regulated by the brain white matter.

STRENGTHS AND LIMITATIONS

STRENGTHS

The Gothenburg MCI study was designed to examine phenotypes of SCI and MCI patients as well as the clinically manifest stages of AD, VaD and mixed dementia. All participants in the Studies of this thesis derive from this single center cohort study taking place at a specialized outpatient clinic. The memory clinic setting offered the advantage of utilizing the experience of clinical professionals who treat patients with cognitive deficits on a regular basis. All participants underwent comprehensive examinations using a multimodal approach to characterize their physical, psychoneurological, radiological and biochemical features at baseline and biannual follow-up visits. As the included participants were more extensively characterized than in most previous studies, the findings of this thesis will likely advance the current knowledge.

The exclusion criteria in the Gothenburg MCI study prevented the inclusion of patients having severe psychiatric or somatic conditions that are known to affect cognitive function. In addition to these general exclusion criteria in the Gothenburg MCI study, we used specific criteria in Studies II-IV to exclude patients with co-morbidities that could have biased the results. These additional exclusion criteria included diabetes mellitus (Study II-IV) and concomitant major cerebrovascular disease in AD (Study II). Thus, in comparison with previous cross-sectional and longitudinal investigations, the strict eligibility criteria of the included Studies likely produced homogenous study populations as well as the removal of factors potentially obscuring the relationships between IGF-I and the progression of cognitive impairment.

An additional strength of Studies I-IV is that all measurements of CSF and serum IGF-I were analyzed by qualified laboratory personnel who did not receive any clinical information about the study participants. In Study I, III and IV, serum IGF-I was measured at one timepoint in 2015. As for Study II, CSF and serum IGF-I concentrations were assessed in 2017 at a single occasion. All blood and CSF specimens were analyzed using well established techniques, which reduced the risk of measurement bias in the included Studies. Lastly, in terms of the evaluation of the Stroop effect (Study IV), in the Gothenburg MCI

study, a shorter version of the Stroop test was used (Victoria version). This version is commonly employed in geriatric and brain-injured patients who are more susceptible to fatigue during cognitive assessment and therefore minimizes the risk of discontinuation of the test³⁷⁵.

LIMITATIONS

This thesis has some limitations that need to be addressed. For instance, the patients included in Studies I-IV were on average younger and had milder cognitive impairment compared with most earlier reports. This could in turn compromise the external validity of the results and reduce the transferability to patients with more advanced dementias.

Overall, the number of participants in each Study was rather small, which could have reduced the statistical power. Specifically, in Study I, only 95 patients (28%) converted to VaD, AD, and other dementia subtypes. In Study II, we were only able to recruit 40 individuals with AD dementia and 36 controls (free from cognitive dysfunction). In addition, the cross-sectional nature of Study II had the consequence that alterations over time in IGF-I levels in serum and CSF could not be evaluated in AD patients. In Study III and IV, not all included patients had available longitudinal values of MRI measures and neuropsychological test scores.

Other limitations include that IGF-I-related factors such as IGFBPs and IGF-IR activity were not examined, and analysis of CSF IGF-I was only performed in Study II. Finally, as concentrations of serum and CSF IGF-I were analyzed at baseline, we were unable to uncover whether alterations in IGF-I levels influence brain imaging characteristics and neurocognitive performance in patients with various levels of cognitive dysfunction (SCI to dementia).

ETHICAL DISCUSSION

The Gothenburg MCI study, which the current thesis is based on, has received ethical approval from the Regional Ethical Committee at the University of Gothenburg (diary numbers L091-99 15; March 1999 and T479-11; 8 June 2011) and the Swedish Ethical Review Authority (diary number 2020-06733; 15 March 2021). All included Studies followed the Helsinki Declaration for medical research involving humans. In addition, all participants in the Gothenburg MCI study provided oral and written informed consent before enrollment.

In all the included Studies, the participants underwent extensive diagnostic evaluation including clinical, neuropsychological, biochemical, neurochemical, and MRI examinations³²⁵. Most of these procedures were repeated at biannual follow-up visits. At each evaluation, the participants were invited to these examinations including lumbar puncture and brain imaging using MRI. Furthermore, prior to the examinations, the participants were informed about the potential risks of the examinations and the possibility of declining to participate. Potential risks included, but were not limited to, post-lumbar puncture headache as well as claustrophobia during the MRI scan. Concerning the lumbar puncture test, the participants were provided information on how to handle the phenomenon of post-lumbar puncture headache if it should occur³²⁵. However, it is important to acknowledge that these tests are the gold standard and are routinely used in the clinical setting when evaluating patients with cognitive impairment. Therefore, the participants were not subjected to additional risks compared with the risks of a normal clinical evaluation. Rather, the participants included in Study I-IV were given the opportunity to be evaluated by staff personnel specialized in cognitive impairment. They also underwent a more in-depth evaluation than regular patients seeking help for cognitive deficits, who are normally evaluated at primary care clinics where resources are limited.

Many of the participants included in Study I-IV had manifest dementia, which is a vulnerable population, especially in research situations. Consequently, it is of uttermost importance that the advantages of the research outweigh the risks. However, all the recruited participants had voluntarily sought medical care at the memory clinic at the Sahlgrenska University Hospital, Mölndal, Sweden. Moreover, individuals with

major cognitive impairment, who could not provide independent and informed consent, were not considered for enrollment in the Gothenburg MCI study. As a result, we excluded participants who required an informant or a relative to provide informed consent for the study.

The central objective of this thesis was to gain further insight into the pathological cascades of dementing disorders. Previously, four principles of bioethics were proposed to minimize the risk of subjecting participants to unnecessary harm including autonomy/integrity, justice, beneficence and non-maleficence³⁷⁶. These ethical aspects relate to all the included Studies. For instance, the autonomy/integrity of the study participants were respected as those who could not express their own free will were not included. In addition, the global dementia prevalence is forecasted to increase three-fold by the year 2050³⁷⁷, and there are no established curative treatments. Because of this, it is important to deepen the knowledge of the endocrine system as a potential modifiable risk factor for the onset and progression of dementia. Such findings could pave the way for future prevention strategies or superior evidence-based medications for patients with cognitive dysfunction or dementia resulting in benefits for future patients. This might in turn enhance the quality of life for future demented patients without compromising their ethical rights. Lastly, participants in the Gothenburg MCI study were not exposed to any additional harm or unnecessary risks compared with those applied in the routine diagnostic procedures in patients with cognitive dysfunction (justice and non-maleficence).

CONCLUSION

CONCLUSION

In Study I, low baseline serum IGF-I in patients with SCI or MCI was associated with increased risk of developing clinically manifest VaD. In contrast, low and high levels of IGF-I did not influence the risk of developing all-cause dementia or dementia with AD. These findings suggest that serum IGF-I may be a risk marker for the progression from SCI/MCI to VaD.

In the cross-sectional Study II, higher serum IGF-I concentrations were observed in patients with AD than that of cognitively intact controls. CSF IGF-I, serum insulin, and CSF/serum IGF-I ratio were comparable between the two groups. These findings give additional support for the hypothesis that IGF-I activity is altered in brains with AD neuropathology, speculatively due to resistance to IGF-IR signaling or via reduced passage of IGF-I across the BBB.

In Study III, serum IGF-I at baseline did not differ significantly between patients with sMCI and AD. However, in sMCI patients, IGF-I was positively related to the baseline volumes of hippocampus and amygdala as well as all brain lobe volumes except for the occipital lobe. Moreover, in sMCI, lower IGF-I was related to a more marked annual decline in hippocampal volume. Conversely, in AD, there were no relationships between circulating IGF-I and the baseline volumes or annualized alterations in the investigated brain regions. These results propose protective effects of IGF-I in the sMCI brain, whereas the gray matter of the AD brain appears to be resistant to the effects of IGF-I.

In Study IV, baseline serum IGF-I was statistically similar in patients with SCI/MCI and AD. In the Spearman rank order correlation analyses in SCI/MCI patients, higher serum IGF-I correlated with greater baseline brain white matter volumes while these associations were lost in the partial correlation analyses following adjustment for multiple covariates. Moreover, after correction for covariates at baseline, SCI/MCI and AD patients with higher serum IGF-I showed better performance in executive function. Furthermore, in both study groups, IGF-I did not associate with lesions in the white matter (WMH volumes) or with the longitudinal alterations of the investigated variables. These observations indicate that in individuals spanning from SCI to AD

dementia, IGF-I may have a small protective role in the brain white matter. However, as the associations between serum IGF-I and the measured variables were somewhat more evident in SCI/MCI, it is plausible that resistance to IGF-IR signaling exist even in the brain white matter in the presence of AD neuropathology but not as prominently as in the gray matter.

Overall, the findings of this thesis suggest that altered IGF-I activity may be involved in the progression of AD and in the development of VaD. Low serum IGF-I concentrations in patients with SCI or MCI were associated with impaired executive cognitive functions as well as reduced volumes of the grey but not white matter brain structures. In SCI/MCI patients, low serum IGF-I was also identified as a risk factor for conversion to VaD, which suggests a link between IGF-I and cerebrovascular functions. In AD, most of the relationships between serum IGF-I and investigated brain variables and functions seen in SCI/MCI were absent, which may be a result of reduced passage of IGF-I through the BBB or due to resistance to IGF-IR signaling in brains with AD. Therefore, the increased serum IGF-I levels observed in AD could be a compensatory mechanism to maintain IGF-I levels in the brain affected by AD neuropathology. In summary, IGF-I levels are altered in cognitive disorders, but the degree of IGF-I dysregulation and its consequences may be dependent on the underlying brain neuropathology and the stage of disease progression.

FUTURE PERSPECTIVES

FUTURE PERSPECTIVES

The link between IGF-I and cognitive decline has been researched for decades, but the extent to which alterations in IGF-I activity affect the disease spectrum is still unsettled. It is yet unclear whether changes in IGF-I levels in dementing disorders are the result, or the cause of the progression of AD and other cognitive diseases.

Most of the previous research have solely analyzed the relationship between IGF-I levels in various biological fluids, especially in serum, and its impact on cognitive function. Conversely, studies on IGFBPs are less common and the results are disparate. Therefore, additional studies investigating IGFBPs are welcomed with the possibility of providing a deeper understanding of the actions of IGF-I in the context of cognitive decline.

Both experimental and clinical studies repeatedly infer that there is altered sensitivity to IGF-IR signaling in the gray matter of AD brains. While higher IGF-IR activity has been related to a higher prevalence and incidence of AD, the contribution of IGF-IR resistance to AD pathology is still uncertain. Therefore, it would be valuable to unveil the underlying mechanisms and the consequences of resistance to IGF-IR signaling in AD. Hypothetically, such findings could eventually lead to the development of new drugs modulating IGF-IR sensitivity in AD dementia and reduce the deprivation of neurotropic IGF-I actions.

Moreover, it is plausible that IGF-I resistance is present also in other dementia forms although such findings have not been previously reported. It would be of great interest to confirm this notion and, like in AD, potentially increase the understanding of the processes involved in dementia.

Lastly, the results of all studies (Study I-IV) included in this thesis indicate that altered IGF-I levels may have a role in the development and progress of cognitive decline, but they are based on relatively small sample sizes. This warrants further large-scale studies determining the importance of IGF-I activity in individuals with cognitive disease.

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

Lastly, I would like to express my appreciation to all of you who helped and gave me support during my PhD studies. I am utterly grateful for all the advice, discussions, and knowledge you have forwarded and the patience you have shown during this period of my life.

I would especially thank and mention the following persons and organizations:

Firstly, the **Department of Internal Medicine and Clinical Nutrition at the Institute of Medicine, Sahlgrenska Academy, University of Gothenburg** for accepting me as a doctoral student early on and supporting young researchers such as myself in gaining a doctorate degree.

My main supervisor **Johan Svensson**, thank you for taking me under your wings and giving me your time. Little by little I learnt “by doing” which I am most certain of will help me in the future as well. You have always been very meticulous with my work and that has taught me that I can always push boundaries and strive to become better at whatever I do.

My co-supervisors **Anders Wallin** and **David Åberg**, thank you for giving great input and advice throughout this journey. I appreciate that you always challenged me to expand my knowledge base and reasoning. Your expertise on dementia and endocrinology is quite unique and was valuable for the projects involved in my thesis.

Furthermore, I am grateful for the support and contributions by the co-authors of the Studies involved in this thesis **Patrick, Quinlan, Zeinab Salman, Carl Eckerström, Anders Wallin, David Åberg, Johan Svensson**, and the late **Arto Nordlund**.

The colleagues involved in the Gothenburg MCI study, thank you for helping me going forwards with my research, and always being there to discuss my thoughts, questions and speculations. My fellow, and former PhD mate **Patrick Quinlan**, we have worked alongside each other for many years. You helped me understand so many things throughout this

journey and someone I could always rely on when I got stuck. **Carl Eckerström**, thank you for your patience and trying to teach me various software, for your collaboration and valuable feedback. **Petronella Kettunen**, you have become a true inspiration to me, and I will always cherish our conversations about life and research. **Eva Bringman** for help with technical assistance and for welcoming me to the research group. Lastly, **Niklas Klasson, Mathias Göthlin, Elin Axelsson, Zeinab Salman, Erik Olsson, Jakob Stålhammar, Michael Jonsson** and the late **Arto Nordlund** for all your support, and sharing your expertise with me. This thesis would not have been the same without you.

The **patients and healthy participants** of the Gothenburg MCI study who contributed to this thesis. I am grateful for you letting me become a part of your life and that you let me investigate, reflect, and try to understand various traits you possess. The findings of this thesis will hopefully help pave the way for future research and most importantly, help others who are just like you.

The **memory clinic, Sahlgrenska University Hospital, Mölndal** for letting me perform my research at your offices and for helping me collect data to my thesis. It was often during lunch hours that I got to discuss my research with fellow researchers and other colleagues involved in the Gothenburg MCI study and the memory clinic.

My workplace throughout this journey, **the Sahlgrenska University Hospital**, for their support in me being able to focus on my thesis in tandem with my clinical career. I look forward to continuing this combination in the future.

Lastly, to my dearest **friends** and **family**, thank you for your never-ending support and for providing an environment where I could share my struggles relating to my thesis. Thank you for always believing in me, motivating me, and cheering me up in rather stressful situations. I could not have finished my doctoral studies without you.

REFERENCES

REFERENCES

1. WHO. Dementia, <https://www.who.int/en/news-room/fact-sheets/detail/dementia> (2023).
2. Wimo A, Seeher K, Cataldi R, et al. The worldwide costs of dementia in 2019. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2023; 19: 2865-2873. 20230108. DOI: 10.1002/alz.12901.
3. Wimo A, Guerchet M, Ali GC, et al. The worldwide costs of dementia 2015 and comparisons with 2010. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2017; 13: 1-7. 20160829. DOI: 10.1016/j.jalz.2016.07.150.
4. Skaalvik MW, Norberg A, Normann K, et al. The experience of self and threats to sense of self among relatives caring for people with Alzheimer's disease. *Dementia (London)* 2016; 15: 467-480. 20140217. DOI: 10.1177/1471301214523438.
5. García-Alberca JM, Lara JP and Berthier ML. Anxiety and depression in caregivers are associated with patient and caregiver characteristics in Alzheimer's disease. *Int J Psychiatry Med* 2011; 41: 57-69. DOI: 10.2190/PM.41.1.f.
6. Smith RG, Betancourt L and Sun Y. Molecular endocrinology and physiology of the aging central nervous system. *Endocrine reviews* 2005; 26: 203-250. 20041123. DOI: 10.1210/er.2002-0017.
7. van den Beld AW, Kaufman JM, Zillikens MC, et al. The physiology of endocrine systems with ageing. *Lancet Diabetes Endocrinol* 2018; 6: 647-658. 20180717. DOI: 10.1016/s2213-8587(18)30026-3.
8. Chahal HS and Drake WM. The endocrine system and ageing. *J Pathol* 2007; 211: 173-180. DOI: 10.1002/path.2110.
9. Mazgelytė E, Karčiauskaitė D, Linkevičiūtė A, et al. Association of Hair Cortisol Concentration with Prevalence of Major Cardiovascular Risk Factors and Allostatic Load. *Med Sci Monit* 2019; 25: 3573-3582. 20190514. DOI: 10.12659/msm.913532.
10. Ohlsson C, Vandenput L and Tivesten A. DHEA and mortality: what is the nature of the association? *J Steroid Biochem Mol Biol* 2015; 145: 248-253. 20140402. DOI: 10.1016/j.jsbmb.2014.03.006.
11. Wu FC, Tajar A, Pye SR, et al. Hypothalamic-pituitary-testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: the European Male Aging Study. *J Clin Endocrinol Metab* 2008; 93: 2737-2745. 20080212. DOI: 10.1210/jc.2007-1972.
12. Manolagas SC. From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. *Endocrine reviews* 2010; 31: 266-300. 20100105. DOI: 10.1210/er.2009-0024.

-
13. Ryan AS. Insulin resistance with aging: effects of diet and exercise. *Sports Med* 2000; 30: 327-346. DOI: 10.2165/00007256-200030050-00002.
 14. Svensson J, Carlzon D, Petzold M, et al. Both low and high serum IGF-I levels associate with cancer mortality in older men. *J Clin Endocrinol Metab* 2012; 97: 4623-4630. 20120926. DOI: 10.1210/jc.2012-2329.
 15. Friedrich N, Haring R, Nauck M, et al. Mortality and serum insulin-like growth factor (IGF)-I and IGF binding protein 3 concentrations. *J Clin Endocrinol Metab* 2009; 94: 1732-1739. 20090217. DOI: 10.1210/jc.2008-2138.
 16. Calsolaro V, Bottari M, Coppini G, et al. Endocrine dysfunction and cognitive impairment. *Minerva Endocrinol (Torino)* 2021; 46: 335-349. 20210112. DOI: 10.23736/s2724-6507.20.03295-2.
 17. Quinlan P, Horvath A, Wallin A and Svensson J. Low serum concentration of free triiodothyronine (FT3) is associated with increased risk of Alzheimer's disease. *Psychoneuroendocrinology* 2019; 99: 112-119. 20180905. DOI: 10.1016/j.psyneuen.2018.09.002.
 18. Junnila RK, List EO, Berryman DE, et al. The GH/IGF-1 axis in ageing and longevity. *Nat Rev Endocrinol* 2013; 9: 366-376. 20130416. DOI: 10.1038/nrendo.2013.67.
 19. Nieto-Estévez V, Defterali Ç and Vicario-Abejón C. IGF-I: A Key Growth Factor that Regulates Neurogenesis and Synaptogenesis from Embryonic to Adult Stages of the Brain. *Frontiers in neuroscience* 2016; 10: 52. 2016/03/05. DOI: 10.3389/fnins.2016.00052.
 20. Åberg D. Role of the growth hormone/insulin-like growth factor 1 axis in neurogenesis. *Endocr Dev* 2010; 17: 63-76. 20091124. DOI: 10.1159/000262529.
 21. Russo VC, Gluckman PD, Feldman EL and Werther GA. The insulin-like growth factor system and its pleiotropic functions in brain. *Endocrine reviews* 2005; 26: 916-943. 20050830. DOI: 10.1210/er.2004-0024.
 22. Ohlsson C, Mohan S, Sjogren K, et al. The role of liver-derived insulin-like growth factor-I. *Endocrine reviews* 2009; 30: 494-535. 2009/07/11. DOI: 10.1210/er.2009-0010.
 23. Bondy C, Bach AM and Lee WH. Mapping of brain insulin and insulin-like growth factor receptor gene expression by in situ hybridization. *Neuroprotocols* 1992; 1: 240-249.
 24. Bondy C, Werner H, Roberts CT, Jr. and LeRoith D. Cellular pattern of type-I insulin-like growth factor receptor gene expression during maturation of the rat brain: comparison with insulin-like growth factors I and II. *Neuroscience* 1992; 46: 909-923. DOI: 10.1016/0306-4522(92)90193-6.
 25. Deijen JB, Arwert LI and Drent ML. The GH/IGF-I Axis and Cognitive Changes across a 4-Year Period in Healthy Adults. *ISRN Endocrinol* 2011; 2011: 249421. 2012/03/01. DOI: 10.5402/2011/249421.

-
26. Bellar D, Glickman EL, Juvancic-Heltzel J and Gunstad J. Serum insulin like growth factor-1 is associated with working memory, executive function and selective attention in a sample of healthy, fit older adults. *Neuroscience* 2011; 178: 133-137. 20110120. DOI: 10.1016/j.neuroscience.2010.12.023.
 27. Colao A, Di Somma C, Cascella T, et al. Relationships between serum IGF1 levels, blood pressure, and glucose tolerance: an observational, exploratory study in 404 subjects. *Eur J Endocrinol* 2008; 159: 389-397. 2008/07/08. DOI: 10.1530/eje-08-0201.
 28. Schut AF, Janssen JA, Deinum J, et al. Polymorphism in the promoter region of the insulin-like growth factor I gene is related to carotid intima-media thickness and aortic pulse wave velocity in subjects with hypertension. *Stroke* 2003; 34: 1623-1627. 2003/06/07. DOI: 10.1161/01.Str.0000076013.00240.B0.
 29. Saber H, Himali JJ, Beiser AS, et al. Serum Insulin-Like Growth Factor 1 and the Risk of Ischemic Stroke: The Framingham Study. *Stroke* 2017; 48: 1760-1765. 2017/06/10. DOI: 10.1161/strokeaha.116.016563.
 30. Murman DL. The Impact of Age on Cognition. *Semin Hear* 2015; 36: 111-121. DOI: 10.1055/s-0035-1555115.
 31. Harada CN, Natelson Love MC and Triebel KL. Normal cognitive aging. *Clin Geriatr Med* 2013; 29: 737-752. DOI: 10.1016/j.cger.2013.07.002.
 32. Jessen F, Amariglio RE, Buckley RF, et al. The characterisation of subjective cognitive decline. *Lancet Neurol* 2020; 19: 271-278. 2020/01/21. DOI: 10.1016/s1474-4422(19)30368-0.
 33. Parfenov VA, Zakharov VV, Kabaeva AR and Vakhnina NV. Subjective cognitive decline as a predictor of future cognitive decline: a systematic review. *Dement Neuropsychol* 2020; 14: 248-257. DOI: 10.1590/1980-57642020dn14-030007.
 34. Bradfield NI. Mild Cognitive Impairment: Diagnosis and Subtypes. *Clin EEG Neurosci* 2023; 54: 4-11. 20210922. DOI: 10.1177/15500594211042708.
 35. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders (5th ed.)*. 2013.
 36. Patnode CD, Perdue LA, Rossom RC, et al. U.S. Preventive Services Task Force Evidence Syntheses, formerly Systematic Evidence Reviews. *Screening for Cognitive Impairment in Older Adults: An Evidence Update for the US Preventive Services Task Force*. Rockville (MD): Agency for Healthcare Research and Quality (US), 2020.
 37. Arvanitakis Z, Shah RC and Bennett DA. Diagnosis and Management of Dementia: Review. *Jama* 2019; 322: 1589-1599. 2019/10/23. DOI: 10.1001/jama.2019.4782.
 38. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the

-
- National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011; 7: 270-279. 20110421. DOI: 10.1016/j.jalz.2011.03.008.
39. Mitchell AJ and Shiri-Feshki M. Rate of progression of mild cognitive impairment to dementia--meta-analysis of 41 robust inception cohort studies. *Acta Psychiatr Scand* 2009; 119: 252-265. 20080218. DOI: 10.1111/j.1600-0447.2008.01326.x.
40. Salzman T, Sarquis-Adamson Y, Son S, et al. Associations of Multidomain Interventions With Improvements in Cognition in Mild Cognitive Impairment: A Systematic Review and Meta-analysis. *JAMA Netw Open* 2022; 5: e226744. 20220502. DOI: 10.1001/jamanetworkopen.2022.6744.
41. Tabatabaei-Jafari H, Shaw ME and Cherbuin N. Cerebral atrophy in mild cognitive impairment: A systematic review with meta-analysis. *Alzheimers Dement (Amst)* 2015; 1: 487-504. 2016/05/31. DOI: 10.1016/j.dadm.2015.11.002.
42. Sluimer JD, van der Flier WM, Karas GB, et al. Accelerating regional atrophy rates in the progression from normal aging to Alzheimer's disease. *European radiology* 2009; 19: 2826-2833. 20090718. DOI: 10.1007/s00330-009-1512-5.
43. Dolek N, Saylisoy S, Ozbabalik D and Adapinar B. Comparison of hippocampal volume measured using magnetic resonance imaging in Alzheimer's disease, vascular dementia, mild cognitive impairment and pseudodementia. *J Int Med Res* 2012; 40: 717-725. DOI: 10.1177/147323001204000236.
44. Ma X, Li Z, Jing B, et al. Identify the Atrophy of Alzheimer's Disease, Mild Cognitive Impairment and Normal Aging Using Morphometric MRI Analysis. *Front Aging Neurosci* 2016; 8: 243. 20161018. DOI: 10.3389/fnagi.2016.00243.
45. Visser PJ, Scheltens P, Verhey FR, et al. Medial temporal lobe atrophy and memory dysfunction as predictors for dementia in subjects with mild cognitive impairment. *J Neurol* 1999; 246: 477-485. DOI: 10.1007/s004150050387.
46. Kälin AM, Park MT, Chakravarty MM, et al. Subcortical Shape Changes, Hippocampal Atrophy and Cortical Thinning in Future Alzheimer's Disease Patients. *Front Aging Neurosci* 2017; 9: 38. 20170307. DOI: 10.3389/fnagi.2017.00038.
47. Molinder A, Ziegelitz D, Maier SE and Eckerström C. Validity and reliability of the medial temporal lobe atrophy scale in a memory clinic population. *BMC neurology* 2021; 21: 289. 20210724. DOI: 10.1186/s12883-021-02325-2.
48. Dickerson BC and Sperling RA. Functional abnormalities of the medial temporal lobe memory system in mild cognitive impairment and

-
- Alzheimer's disease: insights from functional MRI studies. *Neuropsychologia* 2008; 46: 1624-1635. 20071208. DOI: 10.1016/j.neuropsychologia.2007.11.030.
49. Abner EL, Kryscio RJ, Schmitt FA, et al. Outcomes after diagnosis of mild cognitive impairment in a large autopsy series. *Ann Neurol* 2017; 81: 549-559. 20170322. DOI: 10.1002/ana.24903.
50. Cover KS, van Schijndel RA, Versteeg A, et al. Reproducibility of hippocampal atrophy rates measured with manual, FreeSurfer, AdaBoost, FSL/FIRST and the MAPS-HBSI methods in Alzheimer's disease. *Psychiatry Res Neuroimaging* 2016; 252: 26-35. 20160511. DOI: 10.1016/j.pscychresns.2016.04.006.
51. Mueller SG, Schuff N, Yaffe K, et al. Hippocampal atrophy patterns in mild cognitive impairment and Alzheimer's disease. *Hum Brain Mapp* 2010; 31: 1339-1347. DOI: 10.1002/hbm.20934.
52. Prestia A, Drago V, Rasser PE, et al. Cortical changes in incipient Alzheimer's disease. *J Alzheimers Dis* 2010; 22: 1339-1349. DOI: 10.3233/jad-2010-101191.
53. de Groot JC, de Leeuw FE, Oudkerk M, et al. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. *Ann Neurol* 2000; 47: 145-151. DOI: 10.1002/1531-8249(200002)47:2<145::aid-ana3>3.3.co;2-g.
54. Teipel SJ, Meindl T, Wagner M, et al. Longitudinal changes in fiber tract integrity in healthy aging and mild cognitive impairment: a DTI follow-up study. *J Alzheimers Dis* 2010; 22: 507-522. DOI: 10.3233/jad-2010-100234.
55. van den Berg E, Geerlings MI, Biessels GJ, et al. White Matter Hyperintensities and Cognition in Mild Cognitive Impairment and Alzheimer's Disease: A Domain-Specific Meta-Analysis. *J Alzheimers Dis* 2018; 63: 515-527. DOI: 10.3233/jad-170573.
56. Assal F. History of Dementia. *Front Neurol Neurosci* 2019; 44: 118-126. 20190430. DOI: 10.1159/000494959.
57. Petretto DR, Carrogu GP, Gaviano L, et al. Dementia and Major Neurocognitive Disorders: Some Lessons Learned One Century after the first Alois Alzheimer's Clinical Notes. *Geriatrics (Basel)* 2021; 6 20210111. DOI: 10.3390/geriatrics6010005.
58. Edemekong P, Boomgars D, Sukumaran S and Schoo C. *Activities of Daily Living*. StatPearls Publishing, 2023.
59. Hippus H and Neundörfer G. The discovery of Alzheimer's disease. *Dialogues Clin Neurosci* 2003; 5: 101-108. DOI: 10.31887/DCNS.2003.5.1/hhippus.
60. Reitz C and Mayeux R. Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. *Biochem Pharmacol* 2014; 88: 640-651. 20140104. DOI: 10.1016/j.bcp.2013.12.024.

-
61. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984; 34: 939-944. 1984/07/01. DOI: 10.1212/wnl.34.7.939.
62. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011; 7: 263-269. 20110421. DOI: 10.1016/j.jalz.2011.03.005.
63. Jack CR, Jr., Albert MS, Knopman DS, et al. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011; 7: 257-262. 20110421. DOI: 10.1016/j.jalz.2011.03.004.
64. Verlinden VJA, van der Geest JN, de Bruijn R, et al. Trajectories of decline in cognition and daily functioning in preclinical dementia. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2016; 12: 144-153. 20150909. DOI: 10.1016/j.jalz.2015.08.001.
65. World Health Organization. *The ICD-10 classification of mental and behavioural disorders clinical descriptions and diagnostic guidelines.*: Geneva: World Health Organization,1992.
66. Mendez MF. Early-Onset Alzheimer Disease. *Neurol Clin* 2017; 35: 263-281. DOI: 10.1016/j.ncl.2017.01.005.
67. Andrade-Guerrero J, Santiago-Balmaseda A, Jeronimo-Aguilar P, et al. Alzheimer's Disease: An Updated Overview of Its Genetics. *Int J Mol Sci* 2023; 24 20230213. DOI: 10.3390/ijms24043754.
68. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993; 261: 921-923. DOI: 10.1126/science.8346443.
69. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *Jama* 1997; 278: 1349-1356.
70. Kanekiyo T, Xu H and Bu G. ApoE and A β in Alzheimer's disease: accidental encounters or partners? *Neuron* 2014; 81: 740-754. DOI: 10.1016/j.neuron.2014.01.045.
71. Liu CC, Liu CC, Kanekiyo T, et al. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nature reviews Neurology* 2013; 9: 106-118. 20130108. DOI: 10.1038/nrneurol.2012.263.

-
72. Ellis RJ, Olichney JM, Thal LJ, et al. Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, Part XV. *Neurology* 1996; 46: 1592-1596. DOI: 10.1212/wnl.46.6.1592.
73. Polvikoski T, Sulkava R, Haltia M, et al. Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein. *N Engl J Med* 1995; 333: 1242-1247. DOI: 10.1056/nejm199511093331902.
74. Fan L, Mao C, Hu X, et al. New Insights Into the Pathogenesis of Alzheimer's Disease. *Front Neurol* 2019; 10: 1312. 20200110. DOI: 10.3389/fneur.2019.01312.
75. Francis PT, Palmer AM, Snape M and Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: a review of progress. *J Neurol Neurosurg Psychiatry* 1999; 66: 137-147. DOI: 10.1136/jnnp.66.2.137.
76. Breijyeh Z and Karaman R. Comprehensive Review on Alzheimer's Disease: Causes and Treatment. *Molecules* 2020; 25 20201208. DOI: 10.3390/molecules25245789.
77. LeBlanc AC, Xue R and Gambetti P. Amyloid precursor protein metabolism in primary cell cultures of neurons, astrocytes, and microglia. *J Neurochem* 1996; 66: 2300-2310. DOI: 10.1046/j.1471-4159.1996.66062300.x.
78. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* 2001; 81: 741-766. DOI: 10.1152/physrev.2001.81.2.741.
79. Van Uden E, Mallory M, Veinbergs I, et al. Increased extracellular amyloid deposition and neurodegeneration in human amyloid precursor protein transgenic mice deficient in receptor-associated protein. *J Neurosci* 2002; 22: 9298-9304. DOI: 10.1523/jneurosci.22-21-09298.2002.
80. DeMattos RB, Bales KR, Cummins DJ, et al. Brain to plasma amyloid-beta efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. *Science* 2002; 295: 2264-2267. DOI: 10.1126/science.1067568.
81. Duering M, Grimm MO, Grimm HS, et al. Mean age of onset in familial Alzheimer's disease is determined by amyloid beta 42. *Neurobiol Aging* 2005; 26: 785-788. DOI: 10.1016/j.neurobiolaging.2004.08.002.
82. Zetterberg H, Blennow K and Hanse E. Amyloid beta and APP as biomarkers for Alzheimer's disease. *Exp Gerontol* 2010; 45: 23-29. 20090819. DOI: 10.1016/j.exger.2009.08.002.
83. Tabaton M and Piccini A. Role of water-soluble amyloid-beta in the pathogenesis of Alzheimer's disease. *Int J Exp Pathol* 2005; 86: 139-145. DOI: 10.1111/j.0959-9673.2005.00428.x.
84. Back MK, Ruggieri S, Jacobi E and von Engelhardt J. Amyloid Beta-Mediated Changes in Synaptic Function and Spine Number of Neocortical Neurons Depend on NMDA Receptors. *Int J Mol Sci* 2021; 22 20210611. DOI: 10.3390/ijms22126298.

-
85. Minter MR, Taylor JM and Crack PJ. The contribution of neuroinflammation to amyloid toxicity in Alzheimer's disease. *J Neurochem* 2016; 136: 457-474. 20151118. DOI: 10.1111/jnc.13411.
86. Leuner K, Schütt T, Kurz C, et al. Mitochondrion-derived reactive oxygen species lead to enhanced amyloid beta formation. *Antioxid Redox Signal* 2012; 16: 1421-1433. 20120228. DOI: 10.1089/ars.2011.4173.
87. Hansson O, Zetterberg H, Buchhave P, et al. Prediction of Alzheimer's disease using the CSF Aβ₄₂/Aβ₄₀ ratio in patients with mild cognitive impairment. *Dement Geriatr Cogn Disord* 2007; 23: 316-320. 20070319. DOI: 10.1159/000100926.
88. Rowe CC, Bourgeat P, Ellis KA, et al. Predicting Alzheimer disease with β-amyloid imaging: results from the Australian imaging, biomarkers, and lifestyle study of ageing. *Ann Neurol* 2013; 74: 905-913. DOI: 10.1002/ana.24040.
89. Morris JC, Roe CM, Grant EA, et al. Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. *Arch Neurol* 2009; 66: 1469-1475. DOI: 10.1001/archneurol.2009.269.
90. Xiong C, Jasielc MS, Weng H, et al. Longitudinal relationships among biomarkers for Alzheimer disease in the Adult Children Study. *Neurology* 2016; 86: 1499-1506. 20160323. DOI: 10.1212/wnl.0000000000002593.
91. Bateman RJ, Xiong C, Benzinger TL, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med* 2012; 367: 795-804. 20120711. DOI: 10.1056/NEJMoa1202753.
92. Andreasen N and Zetterberg H. Amyloid-related biomarkers for Alzheimer's disease. *Curr Med Chem* 2008; 15: 766-771. DOI: 10.2174/092986708783955572.
93. Ferreira D, Perestelo-Pérez L, Westman E, et al. Meta-Review of CSF Core Biomarkers in Alzheimer's Disease: The State-of-the-Art after the New Revised Diagnostic Criteria. *Front Aging Neurosci* 2014; 6: 47. 20140324. DOI: 10.3389/fnagi.2014.00047.
94. Clark CM, Pontecorvo MJ, Beach TG, et al. Cerebral PET with florbetapir compared with neuropathology at autopsy for detection of neuritic amyloid-β plaques: a prospective cohort study. *Lancet Neurol* 2012; 11: 669-678. 20120628. DOI: 10.1016/s1474-4422(12)70142-4.
95. Ruan D and Sun L. Amyloid-β PET in Alzheimer's disease: A systematic review and Bayesian meta-analysis. *Brain Behav* 2023; 13: e2850. 20221227. DOI: 10.1002/brb3.2850.
96. Bennett DA, Schneider JA, Arvanitakis Z, et al. Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology* 2006; 66: 1837-1844. DOI: 10.1212/01.wnl.0000219668.47116.e6.

-
97. Jack CR, Jr., Bennett DA, Blennow K, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2018; 14: 535-562. DOI: 10.1016/j.jalz.2018.02.018.
98. Roberson ED, Scarce-Levie K, Palop JJ, et al. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science* 2007; 316: 750-754. DOI: 10.1126/science.1141736.
99. Herrup K. The case for rejecting the amyloid cascade hypothesis. *Nat Neurosci* 2015; 18: 794-799. DOI: 10.1038/nn.4017.
100. Spittaels K, Van den Haute C, Van Dorpe J, et al. Prominent axonopathy in the brain and spinal cord of transgenic mice overexpressing four-repeat human tau protein. *Am J Pathol* 1999; 155: 2153-2165. DOI: 10.1016/s0002-9440(10)65533-2.
101. Lindwall G and Cole RD. Phosphorylation affects the ability of tau protein to promote microtubule assembly. *J Biol Chem* 1984; 259: 5301-5305.
102. Liu F, Li B, Tung EJ, et al. Site-specific effects of tau phosphorylation on its microtubule assembly activity and self-aggregation. *Eur J Neurosci* 2007; 26: 3429-3436. 20071204. DOI: 10.1111/j.1460-9568.2007.05955.x.
103. Alonso AC, Li B, Grundke-Iqbal I and Iqbal K. Mechanism of tau-induced neurodegeneration in Alzheimer disease and related tauopathies. *Curr Alzheimer Res* 2008; 5: 375-384. DOI: 10.2174/156720508785132307.
104. Grundke-Iqbal I, Iqbal K, Quinlan M, et al. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J Biol Chem* 1986; 261: 6084-6089.
105. Braak H and Braak E. Alzheimer's disease affects limbic nuclei of the thalamus. *Acta Neuropathol* 1991; 81: 261-268. DOI: 10.1007/bf00305867.
106. Braak H and Braak E. On areas of transition between entorhinal allocortex and temporal isocortex in the human brain. Normal morphology and lamina-specific pathology in Alzheimer's disease. *Acta Neuropathol* 1985; 68: 325-332. DOI: 10.1007/bf00690836.
107. Hyman BT, Van Hoesen GW, Damasio AR and Barnes CL. Alzheimer's disease: cell-specific pathology isolates the hippocampal formation. *Science* 1984; 225: 1168-1170. DOI: 10.1126/science.6474172.
108. Lewis DA, Campbell MJ, Terry RD and Morrison JH. Laminal and regional distributions of neurofibrillary tangles and neuritic plaques in Alzheimer's disease: a quantitative study of visual and auditory cortices. *J Neurosci* 1987; 7: 1799-1808. DOI: 10.1523/jneurosci.07-06-01799.1987.
109. Braak H and Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol* 1991; 82: 239-259. DOI: 10.1007/bf00308809.

-
110. Vemuri P, Whitwell JL, Kantarci K, et al. Antemortem MRI based SStructural Abnormality iNDex (STAND)-scores correlate with postmortem Braak neurofibrillary tangle stage. *NeuroImage* 2008; 42: 559-567. 20080520. DOI: 10.1016/j.neuroimage.2008.05.012.
111. Price JL, Davis PB, Morris JC and White DL. The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol Aging* 1991; 12: 295-312. DOI: 10.1016/0197-4580(91)90006-6.
112. Blennow K and Hampel H. CSF markers for incipient Alzheimer's disease. *Lancet Neurol* 2003; 2: 605-613. DOI: 10.1016/s1474-4422(03)00530-1.
113. Hesse C, Rosengren L, Andreasen N, et al. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. *Neuroscience letters* 2001; 297: 187-190. DOI: 10.1016/s0304-3940(00)01697-9.
114. Otto M, Wiltfang J, Cepek L, et al. Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease. *Neurology* 2002; 58: 192-197. DOI: 10.1212/wnl.58.2.192.
115. Blennow K. Cerebrospinal fluid protein biomarkers for Alzheimer's disease. *NeuroRx* 2004; 1: 213-225. DOI: 10.1602/neurorx.1.2.213.
116. Nam E, Lee YB, Moon C and Chang KA. Serum Tau Proteins as Potential Biomarkers for the Assessment of Alzheimer's Disease Progression. *Int J Mol Sci* 2020; 21 20200715. DOI: 10.3390/ijms21145007.
117. Hyman BT, Phelps CH, Beach TG, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2012; 8: 1-13. DOI: 10.1016/j.jalz.2011.10.007.
118. Malpetti M, Joie R and Rabinovici GD. Tau Beats Amyloid in Predicting Brain Atrophy in Alzheimer Disease: Implications for Prognosis and Clinical Trials. *J Nucl Med* 2022; 63: 830-832. DOI: 10.2967/jnumed.121.263694.
119. Guillozet AL, Weintraub S, Mash DC and Mesulam MM. Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. *Arch Neurol* 2003; 60: 729-736. DOI: 10.1001/archneur.60.5.729.
120. Haroutunian V, Purohit DP, Perl DP, et al. Neurofibrillary tangles in nondemented elderly subjects and mild Alzheimer disease. *Arch Neurol* 1999; 56: 713-718. DOI: 10.1001/archneur.56.6.713.
121. Lowe VJ, Bruinsma TJ, Wiste HJ, et al. Cross-sectional associations of tau-PET signal with cognition in cognitively unimpaired adults. *Neurology* 2019; 93: e29-e39. 20190530. DOI: 10.1212/wnl.00000000000007728.

-
122. Ossenkuppele R, Pichet Binette A, Groot C, et al. Amyloid and tau PET-positive cognitively unimpaired individuals are at high risk for future cognitive decline. *Nature medicine* 2022; 28: 2381-2387. 20221110. DOI: 10.1038/s41591-022-02049-x.
123. Bejanin A, Schonhaut DR, La Joie R, et al. Tau pathology and neurodegeneration contribute to cognitive impairment in Alzheimer's disease. *Brain* 2017; 140: 3286-3300. DOI: 10.1093/brain/awx243.
124. Devous MD, Sr., Fleisher AS, Pontecorvo MJ, et al. Relationships Between Cognition and Neuropathological Tau in Alzheimer's Disease Assessed by 18F Flortaucipir PET. *J Alzheimers Dis* 2021; 80: 1091-1104. DOI: 10.3233/jad-200808.
125. Johnson KA, Schultz A, Betensky RA, et al. Tau positron emission tomographic imaging in aging and early Alzheimer disease. *Ann Neurol* 2016; 79: 110-119. 20151215. DOI: 10.1002/ana.24546.
126. Brier MR, Gordon B, Friedrichsen K, et al. Tau and A β imaging, CSF measures, and cognition in Alzheimer's disease. *Sci Transl Med* 2016; 8: 338ra366. DOI: 10.1126/scitranslmed.aaf2362.
127. Jack CR, Jr., Dickson DW, Parisi JE, et al. Antemortem MRI findings correlate with hippocampal neuropathology in typical aging and dementia. *Neurology* 2002; 58: 750-757. DOI: 10.1212/wnl.58.5.750.
128. Du AT, Schuff N, Amend D, et al. Magnetic resonance imaging of the entorhinal cortex and hippocampus in mild cognitive impairment and Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 2001; 71: 441-447. DOI: 10.1136/jnnp.71.4.441.
129. Braak H, Alafuzoff I, Arzberger T, et al. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol* 2006; 112: 389-404. 20060812. DOI: 10.1007/s00401-006-0127-z.
130. Squire LR. Memory systems of the brain: a brief history and current perspective. *Neurobiol Learn Mem* 2004; 82: 171-177. DOI: 10.1016/j.nlm.2004.06.005.
131. Jack CR, Jr., Petersen RC, Xu YC, et al. Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. *Neurology* 1999; 52: 1397-1403. DOI: 10.1212/wnl.52.7.1397.
132. Jack CR, Jr., Petersen RC, Xu Y, et al. Rates of hippocampal atrophy correlate with change in clinical status in aging and AD. *Neurology* 2000; 55: 484-489. DOI: 10.1212/wnl.55.4.484.
133. Scheltens P, Leys D, Barkhof F, et al. Atrophy of medial temporal lobes on MRI in "probable" Alzheimer's disease and normal ageing: diagnostic value and neuropsychological correlates. *J Neurol Neurosurg Psychiatry* 1992; 55: 967-972. DOI: 10.1136/jnnp.55.10.967.
134. Enkirch SJ, Traschütz A, Müller A, et al. The ERICA Score: An MR Imaging-based Visual Scoring System for the Assessment of Entorhinal

-
- Cortex Atrophy in Alzheimer Disease. *Radiology* 2018; 288: 226-333. 20180307. DOI: 10.1148/radiol.2018171888.
135. Rao YL, Ganaraja B, Murlimanju BV, et al. Hippocampus and its involvement in Alzheimer's disease: a review. *3 Biotech* 2022; 12: 55. 20220201. DOI: 10.1007/s13205-022-03123-4.
136. Barnes J, Bartlett JW, van de Pol LA, et al. A meta-analysis of hippocampal atrophy rates in Alzheimer's disease. *Neurobiol Aging* 2009; 30: 1711-1723. 20080317. DOI: 10.1016/j.neurobiolaging.2008.01.010.
137. Fox NC, Warrington EK, Freeborough PA, et al. Presymptomatic hippocampal atrophy in Alzheimer's disease. A longitudinal MRI study. *Brain* 1996; 119 (Pt 6): 2001-2007. DOI: 10.1093/brain/119.6.2001.
138. Eckerström C, Klasson N, Olsson E, et al. Similar pattern of atrophy in early- and late-onset Alzheimer's disease. *Alzheimers Dement (Amst)* 2018; 10: 253-259. 2018/05/22. DOI: 10.1016/j.dadm.2018.02.001.
139. van de Pol L, Gertz HJ, Scheltens P and Wolf H. Hippocampal atrophy in subcortical vascular dementia. *Neurodegener Dis* 2011; 8: 465-469. 20110525. DOI: 10.1159/000326695.
140. van de Pol LA, Hensel A, van der Flier WM, et al. Hippocampal atrophy on MRI in frontotemporal lobar degeneration and Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 2006; 77: 439-442. 20051123. DOI: 10.1136/jnnp.2005.075341.
141. Acosta-Cabronero J, Williams GB, Pengas G and Nestor PJ. Absolute diffusivities define the landscape of white matter degeneration in Alzheimer's disease. *Brain* 2010; 133: 529-539. 20091113. DOI: 10.1093/brain/awp257.
142. Kavcic V, Ni H, Zhu T, et al. White matter integrity linked to functional impairments in aging and early Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2008; 4: 381-389. DOI: 10.1016/j.jalz.2008.07.001.
143. Nir TM, Jahanshad N, Villalon-Reina JE, et al. Effectiveness of regional DTI measures in distinguishing Alzheimer's disease, MCI, and normal aging. *Neuroimage Clin* 2013; 3: 180-195. 20130727. DOI: 10.1016/j.nicl.2013.07.006.
144. Kandiah N, Chander RJ, Ng A, et al. Association between white matter hyperintensity and medial temporal atrophy at various stages of Alzheimer's disease. *Eur J Neurol* 2015; 22: 150-155. 20140821. DOI: 10.1111/ene.12546.
145. Nowrangi MA, Lyketsos CG, Leoutsakos JM, et al. Longitudinal, region-specific course of diffusion tensor imaging measures in mild cognitive impairment and Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2013; 9: 519-528. 20121212. DOI: 10.1016/j.jalz.2012.05.2186.

-
146. Bachman AH, Lee SH, Sidtis JJ and Ardekani BA. Corpus callosum shape and size changes in early Alzheimer's disease: a longitudinal MRI study using the OASIS brain database. *J Alzheimers Dis* 2014; 39: 71-78. 2013/10/15. DOI: 10.3233/jad-131526.
147. Pini L, Pievani M, Bocchetta M, et al. Brain atrophy in Alzheimer's Disease and aging. *Ageing Res Rev* 2016; 30: 25-48. 20160128. DOI: 10.1016/j.arr.2016.01.002.
148. DeTure MA and Dickson DW. The neuropathological diagnosis of Alzheimer's disease. *Mol Neurodegener* 2019; 14: 32. 20190802. DOI: 10.1186/s13024-019-0333-5.
149. Román G. Vascular dementia: a historical background. *International psychogeriatrics* 2003; 15 Suppl 1: 11-13. DOI: 10.1017/s1041610203008901.
150. Korczyn AD, Vakhapova V and Grinberg LT. Vascular dementia. *J Neurol Sci* 2012; 322: 2-10. 20120508. DOI: 10.1016/j.jns.2012.03.027.
151. Román GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology* 1993; 43: 250-260. 1993/02/01. DOI: 10.1212/wnl.43.2.250.
152. Skrobot OA, Black SE, Chen C, et al. Progress toward standardized diagnosis of vascular cognitive impairment: Guidelines from the Vascular Impairment of Cognition Classification Consensus Study. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2018; 14: 280-292. 20171019. DOI: 10.1016/j.jalz.2017.09.007.
153. Khan A, Kalaria RN, Corbett A and Ballard C. Update on Vascular Dementia. *Journal of geriatric psychiatry and neurology* 2016; 29: 281-301. DOI: 10.1177/0891988716654987.
154. Wolters FJ and Ikram MA. Epidemiology of Vascular Dementia. *Arterioscler Thromb Vasc Biol* 2019; 39: 1542-1549. 20190711. DOI: 10.1161/atvbaha.119.311908.
155. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol* 2010; 9: 689-701. DOI: 10.1016/s1474-4422(10)70104-6.
156. Jokinen H, Lipsanen J, Schmidt R, et al. Brain atrophy accelerates cognitive decline in cerebral small vessel disease: the LADIS study. *Neurology* 2012; 78: 1785-1792. 20120516. DOI: 10.1212/WNL.0b013e3182583070.
157. Wardlaw JM. Blood-brain barrier and cerebral small vessel disease. *J Neurol Sci* 2010; 299: 66-71. 20100918. DOI: 10.1016/j.jns.2010.08.042.
158. Caplan LR. Lacunar infarction and small vessel disease: pathology and pathophysiology. *J Stroke* 2015; 17: 2-6. 20150130. DOI: 10.5853/jos.2015.17.1.2.

-
159. Deramecourt V, Slade JY, Oakley AE, et al. Staging and natural history of cerebrovascular pathology in dementia. *Neurology* 2012; 78: 1043-1050. 20120229. DOI: 10.1212/WNL.0b013e31824e8e7f.
160. Nag S. Immunohistochemical localization of extracellular matrix proteins in cerebral vessels in chronic hypertension. *J Neuropathol Exp Neurol* 1996; 55: 381-388. DOI: 10.1097/00005072-199603000-00014.
161. Wang S, Tang C, Liu Y, et al. Impact of impaired cerebral blood flow autoregulation on cognitive impairment. *Front Aging* 2022; 3: 1077302. 20221202. DOI: 10.3389/fragi.2022.1077302.
162. Tian Z, Ji X and Liu J. Neuroinflammation in Vascular Cognitive Impairment and Dementia: Current Evidence, Advances, and Prospects. *Int J Mol Sci* 2022; 23 20220602. DOI: 10.3390/ijms23116224.
163. Iadecola C. Hypertension and dementia. *Hypertension* 2014; 64: 3-5. 20140428. DOI: 10.1161/hypertensionaha.114.03040.
164. Strydom HC, Chandler AB, Dinsmore RE, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arterioscler Thromb Vasc Biol* 1995; 15: 1512-1531. DOI: 10.1161/01.atv.15.9.1512.
165. Grinberg LT and Thal DR. Vascular pathology in the aged human brain. *Acta Neuropathol* 2010; 119: 277-290. 20100214. DOI: 10.1007/s00401-010-0652-7.
166. Holmstedt CA, Turan TN and Chimowitz MI. Atherosclerotic intracranial arterial stenosis: risk factors, diagnosis, and treatment. *Lancet Neurol* 2013; 12: 1106-1114. DOI: 10.1016/s1474-4422(13)70195-9.
167. Barba R, Martínez-Espinosa S, Rodríguez-García E, et al. Poststroke dementia : clinical features and risk factors. *Stroke* 2000; 31: 1494-1501. DOI: 10.1161/01.str.31.7.1494.
168. Rockwood K, Bowler J, Erkinjuntti T, et al. Subtypes of vascular dementia. *Alzheimer Dis Assoc Disord* 1999; 13 Suppl 3: S59-65.
169. Bir SC, Khan MW, Javalkar V, et al. Emerging Concepts in Vascular Dementia: A Review. *J Stroke Cerebrovasc Dis* 2021; 30: 105864. 20210529. DOI: 10.1016/j.jstrokecerebrovasdis.2021.105864.
170. van Dijk EJ, Prins ND, Vrooman HA, et al. Progression of cerebral small vessel disease in relation to risk factors and cognitive consequences: Rotterdam Scan study. *Stroke* 2008; 39: 2712-2719. 20080717. DOI: 10.1161/strokeaha.107.513176.
171. Bornstein NM, Gur AY, Treves TA, et al. Do silent brain infarctions predict the development of dementia after first ischemic stroke? *Stroke* 1996; 27: 904-905. DOI: 10.1161/01.str.27.5.904.
172. Caplan LR. Silent Brain Infarcts. *Cerebrovascular Diseases* 1994; 4: 32-39. DOI: 10.1159/000108559.

-
173. van Veluw SJ, Zwanenburg JJ, Rozemuller AJ, et al. The spectrum of MR detectable cortical microinfarcts: a classification study with 7-tesla postmortem MRI and histopathology. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* 2015; 35: 676-683. 20150331. DOI: 10.1038/jcbfm.2014.258.
174. De Reuck J, Deramecourt V, Auger F, et al. Post-mortem 7.0-tesla magnetic resonance study of cortical microinfarcts in neurodegenerative diseases and vascular dementia with neuropathological correlates. *J Neurol Sci* 2014; 346: 85-89. 20140806. DOI: 10.1016/j.jns.2014.07.061.
175. Fierini F. Mixed dementia: Neglected clinical entity or nosographic artifice? *J Neurol Sci* 2020; 410: 116662. 20191228. DOI: 10.1016/j.jns.2019.116662.
176. Zekry D, Hauw JJ and Gold G. Mixed dementia: epidemiology, diagnosis, and treatment. *J Am Geriatr Soc* 2002; 50: 1431-1438. DOI: 10.1046/j.1532-5415.2002.50367.x.
177. Eckerström C, Eckerström M, Göthlin M, et al. Characteristic Biomarker and Cognitive Profile in Incipient Mixed Dementia. *J Alzheimers Dis* 2020; 73: 597-607. DOI: 10.3233/jad-190651.
178. Magaki S, Yong WH, Khanlou N, et al. Comorbidity in dementia: update of an ongoing autopsy study. *J Am Geriatr Soc* 2014; 62: 1722-1728. 20140715. DOI: 10.1111/jgs.12977.
179. Leech RW, Brumback RA, Poduslo SE, et al. Dementia: the University of Oklahoma autopsy experience. *J Okla State Med Assoc* 2001; 94: 507-511.
180. Brunnström H, Gustafson L, Passant U and Englund E. Prevalence of dementia subtypes: a 30-year retrospective survey of neuropathological reports. *Archives of gerontology and geriatrics* 2009; 49: 146-149. 20080809. DOI: 10.1016/j.archger.2008.06.005.
181. Fu C, Chute DJ, Farag ES, et al. Comorbidity in dementia: an autopsy study. *Arch Pathol Lab Med* 2004; 128: 32-38. DOI: 10.5858/2004-128-32-cid.
182. Jellinger KA and Attems J. Neuropathological evaluation of mixed dementia. *J Neurol Sci* 2007; 257: 80-87. 20070226. DOI: 10.1016/j.jns.2007.01.045.
183. Schneider JA. Neuropathology of Dementia Disorders. *Continuum (Minneapolis)* 2022; 28: 834-851. DOI: 10.1212/con.0000000000001137.
184. Aronson MK, Ooi WL, Geva DL, et al. Dementia. Age-dependent incidence, prevalence, and mortality in the old old. *Arch Intern Med* 1991; 151: 989-992. DOI: 10.1001/archinte.151.5.989.
185. Derby CA, Katz MJ, Lipton RB and Hall CB. Trends in Dementia Incidence in a Birth Cohort Analysis of the Einstein Aging Study. *JAMA Neurol* 2017; 74: 1345-1351. DOI: 10.1001/jamaneurol.2017.1964.

-
186. McVeigh C and Passmore P. Vascular dementia: prevention and treatment. *Clin Interv Aging* 2006; 1: 229-235. DOI: 10.2147/ciia.2006.1.3.229.
187. Gottesman RF, Albert MS, Alonso A, et al. Associations Between Midlife Vascular Risk Factors and 25-Year Incident Dementia in the Atherosclerosis Risk in Communities (ARIC) Cohort. *JAMA Neurol* 2017; 74: 1246-1254. DOI: 10.1001/jamaneurol.2017.1658.
188. Beason-Held LL, Thambisetty M, Deib G, et al. Baseline cardiovascular risk predicts subsequent changes in resting brain function. *Stroke* 2012; 43: 1542-1547. 20120405. DOI: 10.1161/strokeaha.111.638437.
189. Emdin CA, Rothwell PM, Salimi-Khorshidi G, et al. Blood Pressure and Risk of Vascular Dementia: Evidence From a Primary Care Registry and a Cohort Study of Transient Ischemic Attack and Stroke. *Stroke* 2016; 47: 1429-1435. 20160510. DOI: 10.1161/strokeaha.116.012658.
190. Hamer M and Chida Y. Physical activity and risk of neurodegenerative disease: a systematic review of prospective evidence. *Psychol Med* 2009; 39: 3-11. 20080623. DOI: 10.1017/s0033291708003681.
191. Profenno LA, Porsteinsson AP and Faraone SV. Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders. *Biol Psychiatry* 2010; 67: 505-512. 20090409. DOI: 10.1016/j.biopsych.2009.02.013.
192. Biessels GJ, Staekenborg S, Brunner E, et al. Risk of dementia in diabetes mellitus: a systematic review. *Lancet Neurol* 2006; 5: 64-74. DOI: 10.1016/s1474-4422(05)70284-2.
193. Fitzpatrick AL, Kuller LH, Lopez OL, et al. Midlife and late-life obesity and the risk of dementia: cardiovascular health study. *Arch Neurol* 2009; 66: 336-342. DOI: 10.1001/archneurol.2008.582.
194. Luchsinger JA, Tang MX, Shea S and Mayeux R. Hyperinsulinemia and risk of Alzheimer disease. *Neurology* 2004; 63: 1187-1192. DOI: 10.1212/01.wnl.0000140292.04932.87.
195. Norton S, Matthews FE, Barnes DE, et al. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. *Lancet Neurol* 2014; 13: 788-794. DOI: 10.1016/s1474-4422(14)70136-x.
196. Kuiper JS, Zuidersma M, Oude Voshaar RC, et al. Social relationships and risk of dementia: A systematic review and meta-analysis of longitudinal cohort studies. *Ageing Res Rev* 2015; 22: 39-57. 20150505. DOI: 10.1016/j.arr.2015.04.006.
197. Sharp ES and Gatz M. Relationship between education and dementia: an updated systematic review. *Alzheimer Dis Assoc Disord* 2011; 25: 289-304. DOI: 10.1097/WAD.0b013e318211c83c.
198. Stern Y, Chételat G, Habeck C, et al. Mechanisms underlying resilience in ageing. *Nat Rev Neurosci* 2019; 20: 246. DOI: 10.1038/s41583-019-0138-0.

-
199. Steffener J, Reuben A, Rakitin BC and Stern Y. Supporting performance in the face of age-related neural changes: testing mechanistic roles of cognitive reserve. *Brain Imaging Behav* 2011; 5: 212-221. DOI: 10.1007/s11682-011-9125-4.
200. Lee DH, Seo SW, Roh JH, et al. Effects of Cognitive Reserve in Alzheimer's Disease and Cognitively Unimpaired Individuals. *Front Aging Neurosci* 2021; 13: 784054. 20220207. DOI: 10.3389/fnagi.2021.784054.
201. Cohen P. Overview of the IGF-I system. *Hormone research* 2006; 65 Suppl 1: 3-8. 20060302. DOI: 10.1159/000090640.
202. Siddle K. Molecular basis of signaling specificity of insulin and IGF receptors: neglected corners and recent advances. *Front Endocrinol (Lausanne)* 2012; 3: 34. 20120228. DOI: 10.3389/fendo.2012.00034.
203. Haywood NJ, Slater TA, Matthews CJ and Wheatcroft SB. The insulin like growth factor and binding protein family: Novel therapeutic targets in obesity & diabetes. *Molecular metabolism* 2019; 19: 86-96. 20181024. DOI: 10.1016/j.molmet.2018.10.008.
204. LeRoith D, Werner H, Beitner-Johnson D and Roberts CT, Jr. Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocrine reviews* 1995; 16: 143-163. DOI: 10.1210/edrv-16-2-143.
205. Ullrich A, Gray A, Tam AW, et al. Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. *Embo j* 1986; 5: 2503-2512. DOI: 10.1002/j.1460-2075.1986.tb04528.x.
206. Soos MA, Field CE and Siddle K. Purified hybrid insulin/insulin-like growth factor-I receptors bind insulin-like growth factor-I, but not insulin, with high affinity. *Biochem J* 1993; 290 (Pt 2): 419-426. DOI: 10.1042/bj2900419.
207. Heldin CH and Ostman A. Ligand-induced dimerization of growth factor receptors: variations on the theme. *Cytokine Growth Factor Rev* 1996; 7: 3-10. DOI: 10.1016/1359-6101(96)00002-0.
208. Nässel DR, Liu Y and Luo J. Insulin/IGF signaling and its regulation in *Drosophila*. *Gen Comp Endocrinol* 2015; 221: 255-266. 20150120. DOI: 10.1016/j.ygcen.2014.11.021.
209. Shepherd PR, Withers DJ and Siddle K. Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling. *Biochem J* 1998; 333 (Pt 3): 471-490. DOI: 10.1042/bj3330471.
210. Rouse J, Cohen P, Trigon S, et al. A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. *Cell* 1994; 78: 1027-1037. DOI: 10.1016/0092-8674(94)90277-1.
211. Cheng CM, Reinhardt RR, Lee WH, et al. Insulin-like growth factor 1 regulates developing brain glucose metabolism. *Proceedings of the*

-
- National Academy of Sciences of the United States of America* 2000; 97: 10236-10241. DOI: 10.1073/pnas.170008497.
212. Cheng CM, Tseng V, Wang J, et al. Tau is hyperphosphorylated in the insulin-like growth factor-I null brain. *Endocrinology* 2005; 146: 5086-5091. 20050825. DOI: 10.1210/en.2005-0063.
213. Sparkman AM, Schwartz TS, Madden JA, et al. Rates of molecular evolution vary in vertebrates for insulin-like growth factor-1 (IGF-1), a pleiotropic locus that regulates life history traits. *Gen Comp Endocrinol* 2012; 178: 164-173. 20120430. DOI: 10.1016/j.ygcen.2012.04.022.
214. Upton Z, Yandell CA, Degger BG, et al. Evolution of insulin-like growth factor-I (IGF-I) action: in vitro characterization of vertebrate IGF-I proteins. *Comp Biochem Physiol B Biochem Mol Biol* 1998; 121: 35-41. DOI: 10.1016/s0305-0491(98)10111-6.
215. Daza DO, Sundström G, Bergqvist CA, et al. Evolution of the insulin-like growth factor binding protein (IGFBP) family. *Endocrinology* 2011; 152: 2278-2289. 20110419. DOI: 10.1210/en.2011-0047.
216. Kim MS and Lee DY. Insulin-like growth factor (IGF)-I and IGF binding proteins axis in diabetes mellitus. *Ann Pediatr Endocrinol Metab* 2015; 20: 69-73. 20150630. DOI: 10.6065/apem.2015.20.2.69.
217. Baxter RC. Insulin-like growth factor binding proteins in the human circulation: a review. *Hormone research* 1994; 42: 140-144. DOI: 10.1159/000184186.
218. Lamson G, Pham H, Oh Y, et al. Expression of the BRL-3A insulin-like growth factor binding protein (rBP-30) in the rat central nervous system. *Endocrinology* 1989; 125: 1100-1102. DOI: 10.1210/endo-125-2-1100.
219. Janssen JA, van der Lely AJ and Lamberts SW. Circulating free insulin-like growth-factor-I (IGF-I) levels should also be measured to estimate the IGF-I bioactivity. *J Endocrinol Invest* 2003; 26: 588-594. DOI: 10.1007/bf03345225.
220. Guler HP, Zapf J, Schmid C and Froesch ER. Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. *Acta Endocrinol (Copenh)* 1989; 121: 753-758. DOI: 10.1530/acta.0.1210753.
221. Kim H-S, Rosenfeld RG and Oh Y. Biological roles of insulin-like growth factor binding proteins (IGFBPs). *Experimental & Molecular Medicine* 1997; 29: 85-96. DOI: 10.1038/emm.1997.13.
222. Andress DL and Birnbaum RS. Human osteoblast-derived insulin-like growth factor (IGF) binding protein-5 stimulates osteoblast mitogenesis and potentiates IGF action. *J Biol Chem* 1992; 267: 22467-22472.
223. Oh Y, Müller HL, Pham H and Rosenfeld RG. Demonstration of receptors for insulin-like growth factor binding protein-3 on Hs578T human breast cancer cells. *J Biol Chem* 1993; 268: 26045-26048.

-
224. Jones JI, Gockerman A, Busby WH, Jr., et al. Extracellular matrix contains insulin-like growth factor binding protein-5: potentiation of the effects of IGF-I. *J Cell Biol* 1993; 121: 679-687. DOI: 10.1083/jcb.121.3.679.
225. Jones JI and Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. *Endocrine reviews* 1995; 16: 3-34. DOI: 10.1210/edrv-16-1-3.
226. Holman SR and Baxter RC. Insulin-like growth factor binding protein-3: factors affecting binary and ternary complex formation. *Growth Regul* 1996; 6: 42-47.
227. Firth SM and Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. *Endocrine reviews* 2002; 23: 824-854. DOI: 10.1210/er.2001-0033.
228. Zapf J, Waldvogel M and Froesch ER. Binding of nonsuppressible insulinlike activity to human serum. Evidence for a carrier protein. *Arch Biochem Biophys* 1975; 168: 638-645. DOI: 10.1016/0003-9861(75)90296-9.
229. David A, Hwa V, Metherell LA, et al. Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. *Endocrine reviews* 2011; 32: 472-497. 20110427. DOI: 10.1210/er.2010-0023.
230. Murray PG and Clayton PE. Disorders of Growth Hormone in Childhood. In: Feingold KR, Anawalt B, Blackman MR, et al. (eds) *Endotext*. 2000.
231. Hannah-Shmouni F, Trivellin G and Stratakis CA. Genetics of gigantism and acromegaly. *Growth Horm IGF Res* 2016; 30-31: 37-41. 20160810. DOI: 10.1016/j.ghir.2016.08.002.
232. Albertsson-Wikland K, Rosberg S, Karlberg J and Groth T. Analysis of 24-hour growth hormone profiles in healthy boys and girls of normal stature: relation to puberty. *J Clin Endocrinol Metab* 1994; 78: 1195-1201. DOI: 10.1210/jcem.78.5.8175978.
233. Le Roith D, Bondy C, Yakar S, et al. The somatomedin hypothesis: 2001. *Endocrine reviews* 2001; 22: 53-74. DOI: 10.1210/edrv.22.1.0419.
234. Sjögren K, Liu JL, Blad K, et al. Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. *Proceedings of the National Academy of Sciences of the United States of America* 1999; 96: 7088-7092. DOI: 10.1073/pnas.96.12.7088.
235. Sonntag WE, Lynch C, Thornton P, et al. The effects of growth hormone and IGF-1 deficiency on cerebrovascular and brain ageing. *J Anat* 2000; 197 Pt 4: 575-585. 2001/02/24. DOI: 10.1046/j.1469-7580.2000.19740575.x.
236. Lofqvist C, Andersson E, Gelande L, et al. Reference values for insulin-like growth factor-binding protein-3 (IGFBP-3) and the ratio of insulin-like growth factor-I to IGFBP-3 throughout childhood and adolescence. *J Clin*

-
- Endocrinol Metab* 2005; 90: 1420-1427. 2004/12/16. DOI: 10.1210/jc.2004-0812.
237. Bedogni G, Giannone G, Maghnie M, et al. Serum insulin-like growth factor-I (IGF-I) reference ranges for chemiluminescence assay in childhood and adolescence. Data from a population of in- and out-patients. *Growth Horm IGF Res* 2012; 22: 134-138. 2012/05/16. DOI: 10.1016/j.ghir.2012.04.005.
238. Frutos MG, Cacicedo L, Mendez CF, et al. Pituitary alterations involved in the decline of growth hormone gene expression in the pituitary of aging rats. *J Gerontol A Biol Sci Med Sci* 2007; 62: 585-597. 2007/06/28.
239. Muller AP, Fernandez AM, Haas C, et al. Reduced brain insulin-like growth factor I function during aging. *Molecular and cellular neurosciences* 2012; 49: 9-12. 2011/08/03. DOI: 10.1016/j.mcn.2011.07.008.
240. Corpas E, Harman SM and Blackman MR. Human growth hormone and human aging. *Endocrine reviews* 1993; 14: 20-39. DOI: 10.1210/edrv-14-1-20.
241. Lieberman SA, Mitchell AM, Marcus R, et al. The insulin-like growth factor I generation test: resistance to growth hormone with aging and estrogen replacement therapy. *Horm Metab Res* 1994; 26: 229-233. DOI: 10.1055/s-2007-1001671.
242. Blum WF, Alherbish A, Alsagheir A, et al. The growth hormone-insulin-like growth factor-I axis in the diagnosis and treatment of growth disorders. *Endocr Connect* 2018; 7: R212-r222. 20180503. DOI: 10.1530/ec-18-0099.
243. Di Somma C, Brunelli V, Savanelli MC, et al. Somatopause: state of the art. *Minerva Endocrinol* 2011; 36: 243-255.
244. Lieberman SA and Hoffman AR. The somatopause: should growth hormone deficiency in older people Be treated? *Clin Geriatr Med* 1997; 13: 671-684.
245. Vitale G, Pellegrino G, Vollery M and Hofland LJ. ROLE of IGF-1 System in the Modulation of Longevity: Controversies and New Insights From a Centenarians' Perspective. *Front Endocrinol (Lausanne)* 2019; 10: 27. 20190201. DOI: 10.3389/fendo.2019.00027.
246. Novosyadlyy R and Leroith D. Insulin-like growth factors and insulin: at the crossroad between tumor development and longevity. *The journals of gerontology Series A, Biological sciences and medical sciences* 2012; 67: 640-651. 20120315. DOI: 10.1093/gerona/gls065.
247. Key TJ, Appleby PN, Reeves GK and Roddam AW. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. *Lancet Oncol* 2010; 11: 530-542. 20100514. DOI: 10.1016/s1470-2045(10)70095-4.
248. Rinaldi S, Cleveland R, Norat T, et al. Serum levels of IGF-I, IGFBP-3 and colorectal cancer risk: results from the EPIC cohort, plus a meta-

analysis of prospective studies. *Int J Cancer* 2010; 126: 1702-1715. DOI: 10.1002/ijc.24927.

249. Renehan AG, Zwahlen M, Minder C, et al. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. *Lancet (London, England)* 2004; 363: 1346-1353. DOI: 10.1016/s0140-6736(04)16044-3.

250. Wrigley S, Arafa D and Tropea D. Insulin-Like Growth Factor 1: At the Crossroads of Brain Development and Aging. *Front Cell Neurosci* 2017; 11: 14. 20170201. DOI: 10.3389/fncel.2017.00014.

251. Freude S, Leeser U, Müller M, et al. IRS-2 branch of IGF-1 receptor signaling is essential for appropriate timing of myelination. *J Neurochem* 2008; 107: 907-917. 2008/08/23. DOI: 10.1111/j.1471-4159.2008.05631.x.

252. Barres BA, Schmid R, Sendtner M and Raff MC. Multiple extracellular signals are required for long-term oligodendrocyte survival. *Development* 1993; 118: 283-295. 1993/05/01.

253. Ye P, Carson J and D'Ercole AJ. In vivo actions of insulin-like growth factor-I (IGF-I) on brain myelination: studies of IGF-I and IGF binding protein-1 (IGFBP-1) transgenic mice. *J Neurosci* 1995; 15: 7344-7356. 1995/11/01. DOI: 10.1523/jneurosci.15-11-07344.1995.

254. Beck KD, Powell-Braxton L, Widmer HR, et al. Igfl gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons. *Neuron* 1995; 14: 717-730. 1995/04/01. DOI: 10.1016/0896-6273(95)90216-3.

255. Ye P, Popken GJ, Kemper A, et al. Astrocyte-specific overexpression of insulin-like growth factor-I promotes brain overgrowth and glial fibrillary acidic protein expression. *J Neurosci Res* 2004; 78: 472-484. DOI: 10.1002/jnr.20288.

256. Netchine I, Azzi S, Le Bouc Y and Savage MO. IGF1 molecular anomalies demonstrate its critical role in fetal, postnatal growth and brain development. *Best Pract Res Clin Endocrinol Metab* 2011; 25: 181-190. DOI: 10.1016/j.beem.2010.08.005.

257. Woods KA, Camacho-Hübner C, Savage MO and Clark AJ. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *N Engl J Med* 1996; 335: 1363-1367. DOI: 10.1056/nejm199610313351805.

258. Hansen-Pupp I, Hövel H, Hellström A, et al. Postnatal decrease in circulating insulin-like growth factor-I and low brain volumes in very preterm infants. *J Clin Endocrinol Metab* 2011; 96: 1129-1135. 20110202. DOI: 10.1210/jc.2010-2440.

259. Bondy CA and Lee WH. Patterns of insulin-like growth factor and IGF receptor gene expression in the brain. Functional implications. *Ann N Y Acad Sci* 1993; 692: 33-43. DOI: 10.1111/j.1749-6632.1993.tb26203.x.

-
260. Carro E, Trejo JL, Gomez-Isla T, et al. Serum insulin-like growth factor I regulates brain amyloid-beta levels. *Nature medicine* 2002; 8: 1390-1397. 2002/11/05. DOI: 10.1038/nm1202-793.
261. Nishijima T, Piriz J, Duflo S, et al. Neuronal activity drives localized blood-brain-barrier transport of serum insulin-like growth factor-I into the CNS. *Neuron* 2010; 67: 834-846. DOI: 10.1016/j.neuron.2010.08.007.
262. Carro E, Spuch C, Trejo JL, et al. Choroid plexus megalin is involved in neuroprotection by serum insulin-like growth factor I. *J Neurosci* 2005; 25: 10884-10893. 2005/11/25. DOI: 10.1523/jneurosci.2909-05.2005.
263. Ortenlöf N, Vallius S, Karlsson H, et al. Characterization of choroid plexus in the preterm rabbit pup following subcutaneous administration of recombinant human IGF-1/IGFBP-3. *Fluids Barriers CNS* 2023; 20: 59. 20230815. DOI: 10.1186/s12987-023-00460-1.
264. Anderson MF, Aberg MA, Nilsson M and Eriksson PS. Insulin-like growth factor-I and neurogenesis in the adult mammalian brain. *Brain Res Dev Brain Res* 2002; 134: 115-122. DOI: 10.1016/s0165-3806(02)00277-8.
265. Nieto-Bona MP, Garcia-Segura LM and Torres-Alemán I. Transynaptic modulation by insulin-like growth factor I of dendritic spines in Purkinje cells. *Int J Dev Neurosci* 1997; 15: 749-754. DOI: 10.1016/s0736-5748(97)00021-x.
266. Fernandez AM, Fernandez S, Carrero P, et al. Calcineurin in reactive astrocytes plays a key role in the interplay between proinflammatory and anti-inflammatory signals. *J Neurosci* 2007; 27: 8745-8756. DOI: 10.1523/jneurosci.1002-07.2007.
267. Wood TL, Loladze V, Altieri S, et al. Delayed IGF-1 administration rescues oligodendrocyte progenitors from glutamate-induced cell death and hypoxic-ischemic brain damage. *Dev Neurosci* 2007; 29: 302-310. DOI: 10.1159/000105471.
268. Feeney C, Sharp DJ, Hellyer PJ, et al. Serum insulin-like growth factor-I levels are associated with improved white matter recovery after traumatic brain injury. *Ann Neurol* 2017; 82: 30-43. 2017/06/03. DOI: 10.1002/ana.24971.
269. Bondanelli M, Ambrosio MR, Onofri A, et al. Predictive value of circulating insulin-like growth factor I levels in ischemic stroke outcome. *J Clin Endocrinol Metab* 2006; 91: 3928-3934. 20060801. DOI: 10.1210/jc.2006-1040.
270. Åberg D, Jood K, Blomstrand C, et al. Serum IGF-I levels correlate to improvement of functional outcome after ischemic stroke. *J Clin Endocrinol Metab* 2011; 96: E1055-1064. 20110420. DOI: 10.1210/jc.2010-2802.
271. Sonntag WE, Deak F, Ashpole N, et al. Insulin-like growth factor-1 in CNS and cerebrovascular aging. *Front Aging Neurosci* 2013; 5: 27. 20130702. DOI: 10.3389/fnagi.2013.00027.

-
272. Wennberg AMV, Hagen CE, Machulda MM, et al. The association between peripheral total IGF-1, IGFBP-3, and IGF-1/IGFBP-3 and functional and cognitive outcomes in the Mayo Clinic Study of Aging. *Neurobiol Aging* 2018; 66: 68-74. 2018/03/17. DOI: 10.1016/j.neurobiolaging.2017.11.017.
273. Perice L, Barzilai N, Verghese J, et al. Lower circulating insulin-like growth factor-I is associated with better cognition in females with exceptional longevity without compromise to muscle mass and function. *Aging (Albany NY)* 2016; 8: 2414-2424. DOI: 10.18632/aging.101063.
274. Al-Delaimy WK, von Muhlen D and Barrett-Connor E. Insulinlike growth factor-1, insulinlike growth factor binding protein-1, and cognitive function in older men and women. *J Am Geriatr Soc* 2009; 57: 1441-1446. 2009/06/12. DOI: 10.1111/j.1532-5415.2009.02343.x.
275. Doi T, Shimada H, Makizako H, et al. Association of insulin-like growth factor-1 with mild cognitive impairment and slow gait speed. *Neurobiol Aging* 2015; 36: 942-947. 2014/12/04. DOI: 10.1016/j.neurobiolaging.2014.10.035.
276. Vitiello MV, Moe KE, Merriam GR, et al. Growth hormone releasing hormone improves the cognition of healthy older adults. *Neurobiol Aging* 2006; 27: 318-323. 20050323. DOI: 10.1016/j.neurobiolaging.2005.01.010.
277. Baker LD, Barsness SM, Borson S, et al. Effects of growth hormone-releasing hormone on cognitive function in adults with mild cognitive impairment and healthy older adults: results of a controlled trial. *Arch Neurol* 2012; 69: 1420-1429. DOI: 10.1001/archneurol.2012.1970.
278. Maass A, Düzel S, Brigadski T, et al. Relationships of peripheral IGF-1, VEGF and BDNF levels to exercise-related changes in memory, hippocampal perfusion and volumes in older adults. *NeuroImage* 2016; 131: 142-154. 2015/11/08. DOI: 10.1016/j.neuroimage.2015.10.084.
279. Friedlander AL, Butterfield GE, Moynihan S, et al. One year of insulin-like growth factor I treatment does not affect bone density, body composition, or psychological measures in postmenopausal women. *J Clin Endocrinol Metab* 2001; 86: 1496-1503. 2001/04/12. DOI: 10.1210/jcem.86.4.7377.
280. Jack CR, Jr., Petersen RC, Xu Y, et al. Rate of medial temporal lobe atrophy in typical aging and Alzheimer's disease. *Neurology* 1998; 51: 993-999. DOI: 10.1212/wnl.51.4.993.
281. Cao Z, Min J, Tan Q, et al. Circulating insulin-like growth factor-1 and brain health: Evidence from 369,711 participants in the UK Biobank. *Alzheimers Res Ther* 2023; 15: 140. 20230822. DOI: 10.1186/s13195-023-01288-5.
282. Westwood AJ, Beiser A, Decarli C, et al. Insulin-like growth factor-1 and risk of Alzheimer dementia and brain atrophy. *Neurology* 2014; 82: 1613-1619. 2014/04/08. DOI: 10.1212/wnl.0000000000000382.

-
283. Angelini A, Bendini C, Neviani F, et al. Insulin-like growth factor-1 (IGF-1): relation with cognitive functioning and neuroimaging marker of brain damage in a sample of hypertensive elderly subjects. *Archives of gerontology and geriatrics* 2009; 49 Suppl 1: 5-12. 2009/10/27. DOI: 10.1016/j.archger.2009.09.006.
284. Wittfeld K, Raman MR, Conner SC, et al. Insulin-Like Growth Factor, Inflammation, and MRI Markers of Alzheimer's Disease in Predominantly Middle-Aged Adults. *J Alzheimers Dis* 2022; 88: 311-322. 2022/05/24. DOI: 10.3233/jad-220356.
285. Salzmann A, James SN, Williams DM, et al. Investigating the Relationship Between IGF-I, IGF-II, and IGFBP-3 Concentrations and Later-Life Cognition and Brain Volume. *J Clin Endocrinol Metab* 2021; 106: 1617-1629. 2021/02/26. DOI: 10.1210/clinem/dgab121.
286. de Bruijn RF, Janssen JA, Brugts MP, et al. Insulin-like growth factor-I receptor stimulating activity is associated with dementia. *J Alzheimers Dis* 2014; 42: 137-142. 2014/05/14. DOI: 10.3233/jad-140186.
287. Huang YY, Wang HF, Wu BS, et al. Clinical laboratory tests and dementia incidence: A prospective cohort study. *J Affect Disord* 2024; 351: 1-7. 20240128. DOI: 10.1016/j.jad.2024.01.226.
288. Green CJ, Holly JM, Bayer A, et al. The role of IGF-I, IGF-II, and IGFBP-3 in male cognitive aging and dementia risk: the Caerphilly Prospective Study. *J Alzheimers Dis* 2014; 41: 867-875. DOI: 10.3233/jad-132183.
289. Almeida OP, Hankey GJ, Yeap BB, et al. Risk of prevalent and incident dementia associated with insulin-like growth factor and insulin-like growth factor-binding protein 3. *Mol Psychiatry* 2018; 23: 1825-1829. 20170725. DOI: 10.1038/mp.2017.152.
290. Poirier R, Fernandez AM, Torres-Aleman I and Metzger F. Early brain amyloidosis in APP/PS1 mice with serum insulin-like growth factor-I deficiency. *Neuroscience letters* 2012; 509: 101-104. 20111231. DOI: 10.1016/j.neulet.2011.12.048.
291. Carro E, Trejo JL, Gerber A, et al. Therapeutic actions of insulin-like growth factor I on APP/PS2 mice with severe brain amyloidosis. *Neurobiol Aging* 2006; 27: 1250-1257. 20050923. DOI: 10.1016/j.neurobiolaging.2005.06.015.
292. Doré S, Kar S and Quirion R. Insulin-like growth factor I protects and rescues hippocampal neurons against beta-amyloid- and human amylin-induced toxicity. *Proceedings of the National Academy of Sciences of the United States of America* 1997; 94: 4772-4777. DOI: 10.1073/pnas.94.9.4772.
293. Desbois-Mouthon C, Cadoret A, Blivet-Van Eggelpoël MJ, et al. Insulin and IGF-1 stimulate the beta-catenin pathway through two signalling cascades involving GSK-3beta inhibition and Ras activation. *Oncogene* 2001; 20: 252-259. DOI: 10.1038/sj.onc.1204064.

-
294. Schubert M, Brazil DP, Burks DJ, et al. Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation. *J Neurosci* 2003; 23: 7084-7092. DOI: 10.1523/jneurosci.23-18-07084.2003.
295. Galle SA, van der Spek A, Drent ML, et al. Revisiting the Role of Insulin-Like Growth Factor-I Receptor Stimulating Activity and the Apolipoprotein E in Alzheimer's Disease. *Front Aging Neurosci* 2019; 11: 20. 20190212. DOI: 10.3389/fnagi.2019.00020.
296. Wang W, Yu JT, Tan L, et al. Insulin-like growth factor 1 (IGF1) polymorphism is associated with Alzheimer's disease in Han Chinese. *Neuroscience letters* 2012; 531: 20-23. 2012/10/24. DOI: 10.1016/j.neulet.2012.10.015.
297. Vargas T, Martinez-Garcia A, Antequera D, et al. IGF-I gene variability is associated with an increased risk for AD. *Neurobiol Aging* 2011; 32: 556.e553-511. 2010/12/24. DOI: 10.1016/j.neurobiolaging.2010.10.017.
298. Williams DM, Karlsson IK, Pedersen NL and Hagg S. Circulating insulin-like growth factors and Alzheimer disease: A mendelian randomization study. *Neurology* 2018; 90: e291-e297. 2017/12/29. DOI: 10.1212/wnl.0000000000004854.
299. Murialdo G, Barreca A, Nobili F, et al. Relationships between cortisol, dehydroepiandrosterone sulphate and insulin-like growth factor-I system in dementia. *J Endocrinol Invest* 2001; 24: 139-146. DOI: 10.1007/bf03343833.
300. Alvarez A, Cacabelos R, Sanpedro C, et al. Serum TNF-alpha levels are increased and correlate negatively with free IGF-I in Alzheimer disease. *Neurobiol Aging* 2007; 28: 533-536. 2006/03/30. DOI: 10.1016/j.neurobiolaging.2006.02.012.
301. Duron E, Funalot B, Brunel N, et al. Insulin-like growth factor-I and insulin-like growth factor binding protein-3 in Alzheimer's disease. *J Clin Endocrinol Metab* 2012; 97: 4673-4681. 2012/09/28. DOI: 10.1210/jc.2012-2063.
302. Watanabe T, Miyazaki A, Katagiri T, et al. Relationship between serum insulin-like growth factor-1 levels and Alzheimer's disease and vascular dementia. *J Am Geriatr Soc* 2005; 53: 1748-1753. 2005/09/27. DOI: 10.1111/j.1532-5415.2005.53524.x.
303. Hertze J, Nagga K, Minthon L and Hansson O. Changes in cerebrospinal fluid and blood plasma levels of IGF-II and its binding proteins in Alzheimer's disease: an observational study. *BMC neurology* 2014; 14: 64. 2014/04/02. DOI: 10.1186/1471-2377-14-64.
304. Johansson P, Aberg D, Johansson JO, et al. Serum but not cerebrospinal fluid levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 (IGFBP-3) are increased in Alzheimer's disease. *Psychoneuroendocrinology* 2013; 38: 1729-1737. 2013/03/12. DOI: 10.1016/j.psychneuen.2013.02.006.

-
305. Salehi Z, Mashayekhi F and Najji M. Insulin like growth factor-1 and insulin like growth factor binding proteins in the cerebrospinal fluid and serum from patients with Alzheimer's disease. *BioFactors (Oxford, England)* 2008; 33: 99-106. 2008/01/01. DOI: 10.1002/biof.5520330202.
306. Ostrowski PP, Barszczyk A, Forstenpointner J, et al. Meta-Analysis of Serum Insulin-Like Growth Factor 1 in Alzheimer's Disease. *PloS one* 2016; 11: e0155733. 2016/05/27. DOI: 10.1371/journal.pone.0155733.
307. Xu LZ, Li FY, Li BQ, et al. Decreased Levels of Insulin-Like Growth Factor-1 Are Associated with Alzheimer's Disease: A Meta-Analysis. *J Alzheimers Dis* 2021; 82: 1357-1367. 2021/06/22. DOI: 10.3233/jad-210516.
308. Ferguson AC, Tank R, Lyall LM, et al. Alzheimer's Disease Susceptibility Gene Apolipoprotein E (APOE) and Blood Biomarkers in UK Biobank (N=395,769). *J Alzheimers Dis* 2020; 76: 1541-1551. DOI: 10.3233/jad-200338.
309. Trueba-Saiz A, Cavada C, Fernandez AM, et al. Loss of serum IGF-I input to the brain as an early biomarker of disease onset in Alzheimer mice. *Translational psychiatry* 2013; 3: e330. 2013/12/05. DOI: 10.1038/tp.2013.102.
310. Trejo JL, Carro E, Garcia-Galloway E and Torres-Aleman I. Role of insulin-like growth factor I signaling in neurodegenerative diseases. *J Mol Med (Berl)* 2004; 82: 156-162. 20031128. DOI: 10.1007/s00109-003-0499-7.
311. Talbot K, Wang HY, Kazi H, et al. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *The Journal of clinical investigation* 2012; 122: 1316-1338. 2012/04/06. DOI: 10.1172/jci59903.
312. Steen E, Terry BM, Rivera EJ, et al. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease--is this type 3 diabetes? *J Alzheimers Dis* 2005; 7: 63-80. 2005/03/08. DOI: 10.3233/jad-2005-7107.
313. Rivera EJ, Goldin A, Fulmer N, et al. Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine. *J Alzheimers Dis* 2005; 8: 247-268. 2005/12/13. DOI: 10.3233/jad-2005-8304.
314. Dong Y, Yu H, Li X, et al. Hyperphosphorylated tau mediates neuronal death by inducing necroptosis and inflammation in Alzheimer's disease. *J Neuroinflammation* 2022; 19: 205. 20220815. DOI: 10.1186/s12974-022-02567-y.
315. Carro E and Torres-Aleman I. The role of insulin and insulin-like growth factor I in the molecular and cellular mechanisms underlying the pathology of Alzheimer's disease. *European journal of pharmacology* 2004; 490: 127-133. 2004/04/20. DOI: 10.1016/j.ejphar.2004.02.050.

-
316. Vidal JS, Hanon O, Funalot B, et al. Low Serum Insulin-Like Growth Factor-I Predicts Cognitive Decline in Alzheimer's Disease. *J Alzheimers Dis* 2016; 52: 641-649. 2016/04/01. DOI: 10.3233/jad-151162.
317. Tei E, Yamamoto H, Watanabe T, et al. Use of serum insulin-like growth factor-I levels to predict psychiatric non-response to donepezil in patients with Alzheimer's disease. *Growth Horm IGF Res* 2008; 18: 47-54. 20070821. DOI: 10.1016/j.ghir.2007.07.006.
318. Sevigny JJ, Ryan JM, van Dyck CH, et al. Growth hormone secretagogue MK-677: no clinical effect on AD progression in a randomized trial. *Neurology* 2008; 71: 1702-1708. DOI: 10.1212/01.wnl.0000335163.88054.e7.
319. Tarantini S, Tucsek Z, Valcarcel-Ares MN, et al. Circulating IGF-1 deficiency exacerbates hypertension-induced microvascular rarefaction in the mouse hippocampus and retrosplenial cortex: implications for cerebrovascular and brain aging. *Age (Dordr)* 2016; 38: 273-289. 2016/09/11. DOI: 10.1007/s11357-016-9931-0.
320. Toth P, Tarantini S, Ashpole NM, et al. IGF-1 deficiency impairs neurovascular coupling in mice: implications for cerebrovascular aging. *Aging Cell* 2015; 14: 1034-1044. 2015/07/15. DOI: 10.1111/accel.12372.
321. Gong X, Ma M, Fan X, et al. Down-regulation of IGF-1/IGF-1R in hippocampus of rats with vascular dementia. *Neuroscience letters* 2012; 513: 20-24. 2012/02/22. DOI: 10.1016/j.neulet.2012.01.077.
322. Garcia J, Ahmadi A, Wonnacott A, et al. Association of insulin-like growth factor-1 receptor polymorphism in dementia. *Dement Geriatr Cogn Disord* 2006; 22: 439-444. 2006/09/20. DOI: 10.1159/000095803.
323. Ban Y, Watanabe T, Miyazaki A, et al. Impact of increased plasma serotonin levels and carotid atherosclerosis on vascular dementia. *Atherosclerosis* 2007; 195: 153-159. 2006/10/20. DOI: 10.1016/j.atherosclerosis.2006.09.005.
324. Watanabe T, Koba S, Kawamura M, et al. Small dense low-density lipoprotein and carotid atherosclerosis in relation to vascular dementia. *Metabolism: clinical and experimental* 2004; 53: 476-482. 2004/03/27.
325. Wallin A, Nordlund A, Jonsson M, et al. The Gothenburg MCI study: Design and distribution of Alzheimer's disease and subcortical vascular disease diagnoses from baseline to 6-year follow-up. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* 2016; 36: 114-131. 2015/07/16. DOI: 10.1038/jcbfm.2015.147.
326. Reisberg B, Ferris SH, de Leon MJ and Crook T. The Global Deterioration Scale for assessment of primary degenerative dementia. *The American journal of psychiatry* 1982; 139: 1136-1139. 1982/09/01. DOI: 10.1176/ajp.139.9.1136.

-
327. Folstein MF, Folstein SE and McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research* 1975; 12: 189-198. 1975/11/01. DOI: 10.1016/0022-3956(75)90026-6.
328. Wallin A, Edman A, Blennow K, et al. Stepwise comparative status analysis (STEP): a tool for identification of regional brain syndromes in dementia. *Journal of geriatric psychiatry and neurology* 1996; 9: 185-199. 1996/10/01. DOI: 10.1177/089198879600900406.
329. Royall DR, Mahurin RK and Gray KF. Bedside assessment of executive cognitive impairment: the executive interview. *J Am Geriatr Soc* 1992; 40: 1221-1226. 1992/12/01. DOI: 10.1111/j.1532-5415.1992.tb03646.x.
330. Morris JC. Clinical dementia rating: a reliable and valid diagnostic and staging measure for dementia of the Alzheimer type. *International psychogeriatrics* 1997; 9 Suppl 1: 173-176; discussion 177-178. 1997/01/01. DOI: 10.1017/s1041610297004870.
331. Erkinjuntti T, Inzitari D, Pantoni L, et al. Research criteria for subcortical vascular dementia in clinical trials. *J Neural Transm Suppl* 2000; 59: 23-30. 2000/08/29. DOI: 10.1007/978-3-7091-6781-6_4.
332. McKeith IG, Perry EK and Perry RH. Report of the second dementia with Lewy body international workshop: diagnosis and treatment. Consortium on Dementia with Lewy Bodies. *Neurology* 1999; 53: 902-905. DOI: 10.1212/wnl.53.5.902.
333. Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology* 1998; 51: 1546-1554. DOI: 10.1212/wnl.51.6.1546.
334. Gorno-Tempini ML, Hillis AE, Weintraub S, et al. Classification of primary progressive aphasia and its variants. *Neurology* 2011; 76: 1006-1014. 20110216. DOI: 10.1212/WNL.0b013e31821103e6.
335. Bidlingmaier M, Friedrich N, Emeny RT, et al. Reference intervals for insulin-like growth factor-1 (igf-i) from birth to senescence: results from a multicenter study using a new automated chemiluminescence IGF-I immunoassay conforming to recent international recommendations. *J Clin Endocrinol Metab* 2014; 99: 1712-1721. 20140227. DOI: 10.1210/jc.2013-3059.
336. Blum WF. Insulin-Like Growth Factors and Their Binding Proteins. In: Ranke MB (ed): *Diagnostics of Endocrine Function in Children and Adolescents*. 2003: 166-199.
337. Friedewald WT, Levy RI and Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499-502. 1972/06/01.
338. Blennow K, Ricksten A, Prince JA, et al. No association between the alpha2-macroglobulin (A2M) deletion and Alzheimer's disease, and no

-
- change in A2M mRNA, protein, or protein expression. *J Neural Transm (Vienna)* 2000; 107: 1065-1079. 2000/10/21. DOI: 10.1007/s007020070052.
339. Geffen GM, Butterworth P and Geffen LB. Test-retest reliability of a new form of the auditory verbal learning test (AVLT). *Archives of clinical neuropsychology : the official journal of the National Academy of Neuropsychologists* 1994; 9: 303-316. 1994/07/01.
340. Reitan R and Wolfson B. The Halstead-Reitan Neuropsychological Test Battery: Therapy and clinical interpretation. *Neuropsychological Press* 1985.
341. Regard M. Cognitive Rigidity and Flexibility: A Neuropsychological Study. *University of Victoria* 1981.
342. Desikan RS, Ségonne F, Fischl B, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage* 2006; 31: 968-980. 2006/03/15. DOI: 10.1016/j.neuroimage.2006.01.021.
343. Tham A, Nordberg A, Grissom FE, et al. Insulin-like growth factors and insulin-like growth factor binding proteins in cerebrospinal fluid and serum of patients with dementia of the Alzheimer type. *J Neural Transm Park Dis Dement Sect* 1993; 5: 165-176. 1993/01/01. DOI: 10.1007/bf02257671.
344. Yamagata B, Watanabe T, Tomioka H, et al. Preliminary use of insulin-like growth factor-I as a biomarker for sorting high-dose donepezil responders among Japanese patients with Alzheimer's disease. *Regulatory peptides* 2010; 163: 137-142. 2010/05/11. DOI: 10.1016/j.regpep.2010.04.010.
345. Vardy ER, Rice PJ, Bowie PC, et al. Increased circulating insulin-like growth factor-1 in late-onset Alzheimer's disease. *J Alzheimers Dis* 2007; 12: 285-290. 2008/01/17. DOI: 10.3233/jad-2007-12401.
346. Kimoto A, Kasanuki K, Kumagai R, et al. Serum insulin-like growth factor-I and amyloid beta protein in Alzheimer's disease: relationship with cognitive function. *Psychogeriatrics* 2016; 16: 247-254. 2015/10/07. DOI: 10.1111/psyg.12149.
347. Wahlund LO, Barkhof F, Fazekas F, et al. A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke* 2001; 32: 1318-1322. 2001/06/02. DOI: 10.1161/01.str.32.6.1318.
348. Mustafa A, Lannfelt L, Lilius L, et al. Decreased plasma insulin-like growth factor-I level in familial Alzheimer's disease patients carrying the Swedish APP 670/671 mutation. *Dement Geriatr Cogn Disord* 1999; 10: 446-451. 1999/11/24. DOI: 10.1159/000017188.
349. Obermayr RP, Mayerhofer L, Knechtelsdorfer M, et al. The age-related down-regulation of the growth hormone/insulin-like growth factor-1 axis in the elderly male is reversed considerably by donepezil, a drug for

-
- Alzheimer's disease. *Exp Gerontol* 2005; 40: 157-163. 2005/03/15. DOI: 10.1016/j.exger.2004.11.001.
350. de la Monte SM and Wands JR. Alzheimer's disease is type 3 diabetes-evidence reviewed. *J Diabetes Sci Technol* 2008; 2: 1101-1113. DOI: 10.1177/193229680800200619.
351. Schrijvers EM, Witteman JC, Sijbrands EJ, et al. Insulin metabolism and the risk of Alzheimer disease: the Rotterdam Study. *Neurology* 2010; 75: 1982-1987. DOI: 10.1212/WNL.0b013e3181ffe4f6.
352. Nam GE, Park YG, Han K, et al. BMI, Weight Change, and Dementia Risk in Patients With New-Onset Type 2 Diabetes: A Nationwide Cohort Study. *Diabetes Care* 2019; 42: 1217-1224. 20190608. DOI: 10.2337/dc18-1667.
353. Moloney AM, Griffin RJ, Timmons S, et al. Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. *Neurobiol Aging* 2010; 31: 224-243. 2008/05/16. DOI: 10.1016/j.neurobiolaging.2008.04.002.
354. Craft S, Peskind E, Schwartz MW, et al. Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. *Neurology* 1998; 50: 164-168. DOI: 10.1212/wnl.50.1.164.
355. Carro E, Trejo JL, Spuch C, et al. Blockade of the insulin-like growth factor I receptor in the choroid plexus originates Alzheimer's-like neuropathology in rodents: new cues into the human disease? *Neurobiol Aging* 2006; 27: 1618-1631. 2005/11/09. DOI: 10.1016/j.neurobiolaging.2005.09.039.
356. Johansson P, Mattsson N, Hansson O, et al. Cerebrospinal fluid biomarkers for Alzheimer's disease: diagnostic performance in a homogeneous mono-center population. *J Alzheimers Dis* 2011; 24: 537-546. 2011/02/08. DOI: 10.3233/jad-2011-101878.
357. Blennow K, Hampel H, Weiner M and Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nature reviews Neurology* 2010; 6: 131-144. 2010/02/17. DOI: 10.1038/nrneurol.2010.4.
358. Jack CR, Jr., Barkhof F, Bernstein MA, et al. Steps to standardization and validation of hippocampal volumetry as a biomarker in clinical trials and diagnostic criterion for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2011; 7: 474-485.e474. DOI: 10.1016/j.jalz.2011.04.007.
359. Scott SA, DeKosky ST and Scheff SW. Volumetric atrophy of the amygdala in Alzheimer's disease: quantitative serial reconstruction. *Neurology* 1991; 41: 351-356. DOI: 10.1212/wnl.41.3.351.
360. Poulin SP, Dautoff R, Morris JC, et al. Amygdala atrophy is prominent in early Alzheimer's disease and relates to symptom severity.

-
- Psychiatry Res* 2011; 194: 7-13. 20110914. DOI: 10.1016/j.psychres.2011.06.014.
361. Thompson PM, Hayashi KM, de Zubicaray G, et al. Dynamics of gray matter loss in Alzheimer's disease. *J Neurosci* 2003; 23: 994-1005. DOI: 10.1523/jneurosci.23-03-00994.2003.
362. Lerch JP, Pruessner JC, Zijdenbos A, et al. Focal decline of cortical thickness in Alzheimer's disease identified by computational neuroanatomy. *Cereb Cortex* 2005; 15: 995-1001. 20041110. DOI: 10.1093/cercor/bhh200.
363. Wine RN, McPherson CA and Harry GJ. IGF-1 and pAKT signaling promote hippocampal CA1 neuronal survival following injury to dentate granule cells. *Neurotox Res* 2009; 16: 280-292. 20090528. DOI: 10.1007/s12640-009-9060-y.
364. Svensson J, Diez M, Engel J, et al. Endocrine, liver-derived IGF-I is of importance for spatial learning and memory in old mice. *The Journal of endocrinology* 2006; 189: 617-627. 2006/05/30. DOI: 10.1677/joe.1.06631.
365. Calvo D, Gunstad J, Miller LA, et al. Higher serum insulin-like growth factor-1 is associated with better cognitive performance in persons with mild cognitive impairment. *Psychogeriatrics* 2013; 13: 170-174. 2013/09/01. DOI: 10.1111/psyg.12023.
366. Adem A, Jossan SS, d'Argy R, et al. Insulin-like growth factor 1 (IGF-1) receptors in the human brain: quantitative autoradiographic localization. *Brain Res* 1989; 503: 299-303. 1989/12/04. DOI: 10.1016/0006-8993(89)91678-8.
367. Sonntag WE, Lynch CD, Bennett SA, et al. Alterations in insulin-like growth factor-1 gene and protein expression and type 1 insulin-like growth factor receptors in the brains of ageing rats. *Neuroscience* 1999; 88: 269-279. 1999/03/02. DOI: 10.1016/s0306-4522(98)00192-4.
368. Lai M, Hibberd CJ, Gluckman PD and Seckl JR. Reduced expression of insulin-like growth factor 1 messenger RNA in the hippocampus of aged rats. *Neuroscience letters* 2000; 288: 66-70. 2000/06/28. DOI: 10.1016/s0304-3940(00)01170-8.
369. Bouhrara M, Reiter DA, Bergeron CM, et al. Evidence of demyelination in mild cognitive impairment and dementia using a direct and specific magnetic resonance imaging measure of myelin content. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2018; 14: 998-1004. 20180419. DOI: 10.1016/j.jalz.2018.03.007.
370. Scheltens P, Barkhof F, Leys D, et al. Histopathologic correlates of white matter changes on MRI in Alzheimer's disease and normal aging. *Neurology* 1995; 45: 883-888. DOI: 10.1212/wnl.45.5.883.
371. Zeger M, Popken G, Zhang J, et al. Insulin-like growth factor type 1 receptor signaling in the cells of oligodendrocyte lineage is required for normal in vivo oligodendrocyte development and myelination. *Glia* 2007; 55: 400-411. DOI: 10.1002/glia.20469.

-
372. Wallin A, Nordlund A, Jonsson M, et al. Alzheimer's disease--subcortical vascular disease spectrum in a hospital-based setting: Overview of results from the Gothenburg MCI and dementia studies. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* 2016; 36: 95-113. 2015/07/30. DOI: 10.1038/jcbfm.2015.148.
373. Tumati S, Burger H, Martens S, et al. Association between Cognition and Serum Insulin-Like Growth Factor-1 in Middle-Aged & Older Men: An 8 Year Follow-Up Study. *PLoS one* 2016; 11: e0154450. 20160426. DOI: 10.1371/journal.pone.0154450.
374. Huang R, Wang P, Han J, et al. Decreased Serum IGF-1/IGFBP-3 Molar Ratio is Associated with Executive Function Behaviors in Type 2 Diabetic Patients with Mild Cognitive Impairment. *J Alzheimers Dis* 2015; 47: 85-94. DOI: 10.3233/jad-150071.
375. Bayard S, Erkes J and Moroni C. Victoria Stroop Test: normative data in a sample group of older people and the study of their clinical applications in the assessment of inhibition in Alzheimer's disease. *Archives of clinical neuropsychology : the official journal of the National Academy of Neuropsychologists* 2011; 26: 653-661. 20110826. DOI: 10.1093/arclin/acr053.
376. Beauchamp TLCJF. *Principles of biomedical ethics*. New York: Oxford University Press, 2009.
377. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. *Lancet Public Health* 2022; 7: e105-e125. 2022/01/10. DOI: 10.1016/s2468-2667(21)00249-8.