The Role of Insulin and Insulin-like Peptides in Ischemic Stroke and Cognitive Impairment

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Cover illustration: Computed Tomography of 91 year old Swedish woman with cerebral infarction in the right hemisphere that exhibits comparatively limited age-related atrophy of the parenchyma.

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Ineko

För ett grått huvud skall du resa dig upp, och den gamle skall du ära. (3 Mos 19:32)

Om bara ålderdomen kunde vara stark - och ungdomen klok. (Martin Luther King Jr.)

ABSTRACT

Background and aims: Insulin, insulin-like growth factor-I (IGF-I), and the six high-affinity IGF-binding proteins (IGFBPs) play an important role in growth, metabolism and regeneration throughout the entire life span. In contrast, the role of IGF-II in adult life has been unclear. Animal studies have demonstrated that altered brain activity of the insulin/IGF-system is associated with reduced cognitive function and worse outcome after experimentally induced stroke and this is reversed by IGF-I-treatment. The overall aim of this thesis was to determine whether the insulin/IGF-I system is of importance for outcome of ischemic stroke (IS) also in humans and whether insulin and insulin-like peptides are dysregulated in patients with Alzheimer's disease (AD).

Patients and methods: Two well-characterized clinical cohorts were studied. In SAHLSIS (Sahlgrenska Academy Study on Ischemic Stroke; originally 600 IS patients and 600 population-based controls), characterization of patients after IS included serum samples and stroke scales. Furthermore, serum and cerebrospinal fluid (CSF) levels of insulin, IGF-I, and IGF-II were determined in a cross-sectional study of patients (n=60) with AD and other forms of cognitive impairment, and healthy controls (n=20).

Results: In Paper I, high serum IGF-I concentrations were associated with better improvement of functional independence in SAHLSIS. In Paper II, analyses of single-nucleotide polymorphisms (SNPs) in the IGF1 gene showed that the major allele of rs7136446 was associated with favorable post-stroke outcome after 2 years. In Paper III, insulin resistance was associated with functional outcome, especially in patients with cryptogenic stroke. In Paper IV, serum but not CSF levels of IGF-I were increased in patients with AD whereas insulin levels were unchanged both in serum and CSF. In Paper V, CSF IGF-II level was increased in male but not in female patients with AD.

Conclusions: The IGF-I/insulin system is associated with functional outcome after ischemic stroke. Furthermore, levels of IGF-I and IGF-II are dysregulated in Alzheimer's disease.

Keywords: Ischemic Stroke (IS), Alzheimer´s disease (AD), Cognitive Impairment, Dementia, Insulin-like Growth Factor I (IGF-I)

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LIST OF PAPERS

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

I. Åberg D, Jood K, Blomstrand C, Jern C, Nilsson M, Isgaard J, Aberg ND. Serum IGF-I levels correlate to improvement of functional outcome after ischemic stroke. J Clin Endocrinol Metab. 2011:96:E1055-E1064

II. Åberg ND, Olsson S, Åberg D, Jood K, Nilsson M, Blomstrand C, Svensson J, Isgaard J, Jern C. Single nucleotide polymorphisms in the IGF1 gene correlate to outcome but not to risk of ischemic stroke. Eur J Endocrinol. 2013:169:759-765

III. Åberg D, Åberg ND, Jood K, Holmegaard L, Redfors P, Blomstrand C, Isgaard J, Jern C, Svensson J. Insulin resistance and outcome of ischemic stroke. 2016: manuscript.

IV. Johansson P, Åberg D, Johansson J-O, Mattsson N, Hansson O, Ahrén B, Isgaard J, Åberg ND, Blennow K, Zetterberg H, Wallin A, Svensson J. 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

V. Åberg D, Johansson P, Isgaard J, Wallin A, Johansson J-O, Andreasson U, Blennow K, Zetterberg H, Åberg ND, Svensson J. Increased cerebrospinal fluid level of insulin-like growth factor-II (IGF-II) in male patients with Alzheimer's Disease. J Alzheimers Dis. 2015:48:637-646

CONTENTS

ABBREVIATIONS AND ACRONYMS

Aβ	β -amyloid
$A\beta_{1-42}$	β-amyloid$_{1-42}$
AD	Alzheimer´s Disease
BBB	Blood-brain barrier
BMI	Body Mass Index
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CT	Computed Tomography
DLB	Dementia with Lewy Bodies
ELISA	Enzyme-Linked Immuno Sorbent Assay
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Ed
FTD	Frontotemporal Dementia
GH	Growth Hormone
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
ICD-10	International classification of disease, 10th Ed.
IGF-I	Insulin-Like Growth Factor I
IGF-II	Insulin-Like Growth Factor II
IGFBPs	Insulin-Like Growth Factor Binding Proteins
IR	Insulin Resistance
IS	Ischemic Stroke
MCI	Mild Cognitive Impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imaging
NFT	Neuro Fibrillary Tangle
mRS	modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
OCSP	Oxfordshire Community Stroke Project
P-tau	Phosphorylated Tau

SAHLSIS	Sahlgrenska Academy Study on Ischemic Stroke
SMCI	Stable Mild Cognitive Impairment
SNP	Single Nucleotide Polymorphism
SSS	Scandinavian Stroke Scale
TIA	Transient Ischemic Attack
TOAST	Trial of Org 10172 in Acute Stroke Treatment
T-tau	Total tau
VAD	Vascular Dementia

1 INTRODUCTION

Stroke and cognitive impairment are two major causes of morbidity worldwide. Furthermore, the global burden of these conditions increases. Stroke is now the second largest cause of mortality in the world [1], and it is globally the third most common cause of disability-adjusted life year (DALYs) compared to fifth place in 1990 [2]. In Sweden, about 30000 individuals suffer from a stroke annually and stroke is the third cause of death. The mortality rates are slowly declining in Western countries, however, the incidence of stroke is increasing in younger ages [3, 4]. Cognitive impairment including dementia is also a common cause of morbidity, and the burden to the society is increasing with elongated life span. The Swedish Council on Health Technology Assessment, SBU, estimated that the dementia prevalence in Sweden was more than 140000 patients in 2008. Globally, the prevalence of dementia presently exceeds 25 million and is expected to increase 63 million cases in 2030 [5].

1.1 Stroke

1.1.1 Definition, pathology and classification

WHO defines stroke as "rapidly developing clinical signs of focal, and at times global, loss of cerebral function, with symptoms lasting more than 24 hours or leading to death, with no apparent cause other than vascular origin" [6]. If the symptoms remain < 24 hours, they are classified as transient ischemic attack (TIA). Based on the underlying pathology, stroke is classified either as ischemic or hemorrhagic. Although the clinical presentation of stroke may give clues to whether the patient is suffering of ischemic stroke (IS, ≈ 85%) or hemorrhage, accurate classification is done either through computed tomography (CT) or magnetic resonance imaging (MRI). In IS, the common underlying cause is an obstruction of the blood flow in a cerebral blood vessel, which is causing ischemia and subsequent tissue damage. Hemorrhagic stroke is usually caused by a rupture in a cerebral artery with subsequent intracranial bleeding, resulting in distortion and compression of the tissue of the brain.

1.1.2 Classification of ischemic stroke regarding localization

IS can be classified in several ways. One classification is based on localization such as right or left hemisphere, white or grey matter and in

which lobe the lesion is located. The classification proposed by Bamford et al. in Oxfordshire Community Stroke Project (OCSP) [7] divides IS into four subgroups. These groups are 1) large anterior circulation infarcts with both cortical and subcortical involvement (total anterior circulation infarcts, TACI), 2) more restricted and predominantly cortical infarcts (partial anterior circulation infarcts, PACI), 3) infarctions in the vertebrobasilar arterial territory (posterior circulation infarcts, POCI), and 4) infarctions in the deep perforating arteries (lacunar infarcts, LACI).

1.1.3 Classification of ischemic stroke in terms of etiology

The etiological background of IS is complex. Multiple pathophysiological mechanisms and underlying conditions may cause IS, but there are some distinguishable etiological groups that are more common. One way of classifying IS is using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) [8] criteria. TOAST is the most commonly used classification of etiology in both the clinical and scientific context. There is also a high agreement between TOAST and other classifications of etiology like ASCO [9], and increasing evidence indicates that etiology is important for the risk factor profile and prognosis of IS [10-13]. The subtypes in TOAST are large-vessel disease (LVD), small-vessel disease (SVD), cardioembolic (CE) stroke, cryptogenic stroke (i.e. when no cause was identified despite extensive evaluation), other determined cause of stroke, and undetermined stroke.

1.2 Cognitive impairment and dementia

1.2.1 Definition and classification

Dementia is widespread globally, but more common in developed countries [14, 15]. The most important risk factor is age, and as mean lifespan is increasing in the developing countries, the incidence is growing relatively faster in developing regions [5, 14]. The definition of cognitive impairment and the partition into mild cognitive impairment and dementia in some ways differ between the diagnostic instruments used; International Classification of Diseases, tenth Revision (ICD-10), the Diagnostic and Statistical Manual of Mental Disorders, Forth Edition (DSM-IV), and the recently developed DSM-V. Cognitive impairment is a term used for an enduring reduction or barrier in the cognition process which may be a deficit in global intellectual performance and/or a more focal disability concerning specific deficit in a cognitive ability. Mild cognitive impairment (MCI) is the term used for a

milder disorder and dementia for a more severe condition. MCI might progress to Alzheimer´s disease (AD) or other dementia [16], and tests like mini-mental state examination (MMSE) [17] and other cognitive tests that are used for investigating cognitive impairment can be used in predicting AD in MCI [18]. Although the term dementia has been defined a bit differently over time and in different contexts, generally it may be formulated as: the patient has an acquired clinically detectable syndrome with cognitive impairment leading to functional decline with duration over time and involvement of memory deficit and at least one other cognitive domain. There should not be another systemic disease inducing these symptoms and although the etiology differs, organic brain injury is always implied. The state of neurodegeneration regarding the histological features and the localization of these pathological properties differ between the subtypes of dementia. Although there are overlaps, this results in differences in the clinical presentation between dementia forms as well as in the pace of cognitive decline.

1.2.2 Clinical and Neuropathological Diagnosis of AD

The term dementia was introduced by Bayle in 1822 [19], and since the first report in 1906 and subsequent publication by Alois Alzheimer [20], AD has evolved to be the predominant form of dementia; more than 50 % of patients with dementia suffer from AD [14]. In the ICD-10 manual from 1984, AD diagnosis is based on 1) insidious onset with slow deterioration 2) absence of indication of other systemic or brain disease that can induce dementia 3) absence of apoplectic onset or focal neurological signs early in the disease [21]. These criteria are formulated more in detail in National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer´s Disease and Related Disorders Association (NINCDS-ADRDA) [22] and have been revised [23]. In addition, the American Psychiatric Associaton (APA) developed the DSM-IV-criteria, which have been harmonized with ICD-10 [24]. APA in 2013 published the new DSM-V-criteria, where the term dementia has been renamed with major neurocognitive disorder and mild neurocognitive disorder. However, the DSM-5 includes ‘or dementia’ in parentheses [25] and the Alzheimer’s Association outlines different diagnostic criteria for Alzheimer’s disease and retains the use of the word dementia [26].

The unequivocal diagnosis of AD is based on a combination of appropriate clinical data and neuropathological examination [27]. The clinical data for probable AD are defined by NINCDS-ADRDA [22, 23] and in brief include:

• dementia established by clinical examination, e.g. MMSE [17], and confirmed by other neuropsychological testing.
• deficits in two additional areas of cognition.
• progressive deterioration of memory and other cognitive functions.
• no disturbance of consciousness, onset between the ages of 40 and 90, most often after 65.
• absence of systemic disorders or other brain disease that might account for the progressive deficits.

1.2.3 Amyloid Cascade Theory and neurofibrillary tangles

AD is characterized by two neuropathological hallmarks; neurofibrillary tangles (NFT) and amyloid plaques [28]. Neurodegeneration in AD might be caused by deposition of amyloid beta-peptide (Aβ) in brain tissue plaques, and the accumulation of Aβ is a primary event in AD pathogenesis according to the Amyloid Cascade Theory [29]. The further process of the disease, including formation of NFTs containing tau protein, might be caused by an imbalance of Aβ production and Aβ clearance [30]. The 42 amino acid residues-long isoform biomarkers β-amyloid$_{1-42}$ (Aβ_{1-42}), starts a cascade of events, ultimately causing pathology in synaptic function, neuronal loss and brain atrophy [29, 31]. Aβ_{1-42} results from orchestrated β-secretase och γ-secretase cleavages of the large transmembranous amyloid precursor protein (APP). NFTs are tubules of the microtubule-associated protein tau, which are phosphorylated and accumulated intracellularly [32]. This results in disintegration of microtubules [33], causing malfunctions in the chemical signaling and later in apoptosis [34]. The idea that the tau protein abnormalities initiate the disease cascade is called the tau hypothesis, and its role in AD is debated [35].

1.2.4 Cerebrospinal fluid biomarkers for AD

Analyses of biomarkers for AD in the cerebrospinal fluid (CSF) can monitor altered brain metabolism in AD. Several studies have exhibited that increased levels of total tau (T-tau) and phosphorylated tau protein (P-tau) in the CSF in AD reflects axonal degeneration and increased phosphorylation, respectively [36]. In contrast, Aβ_{1-42} decreases in CSF which might be due to peptide sequestration within amyloid plaques [36, 37]. In combining the tau proteins and Aβ_{1-42}, the diagnostic accuracy is high, with a sensitivity and specificity of about 90 % or more for clinically diagnosed AD versus controls [38]. These biomarkers are present early in the AD disease process, and remain stable throughout the entire AD course [39-41].

1.2.5 Other forms of dementia disorders

Although the most widespread etiological subtype of dementia is Alzheimer´s disease (AD), there are several other disorders causing dementia. The second most common form of dementia is vascular dementia (VAD) [42], which can originate from both macrovascular and subcortical small-vessel disease. There are several forms of dementia that are characterized by aggregates of alpha-synuclein proteins: Parkinson´s disease with dementia (PDD) [43], dementia with Lewy bodies (DLB) [44] and multiple system atrophy (MSA). Moreover, progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and frontotemporal dementia (FTD) [45, 46] differ in clinical phenotype, but they share some properties and belong to the taupathies.

1.3 The system of insulin and insulin-like peptides

Insulin plays a crucial role for metabolism and IGF-I plays an important role in growth, metabolism and regeneration in the peripheral tissues and in the CNS nervous system. Since discovery of IGF-I more than half a century ago, initially as the somatomedin hypothesis [47, 48], knowledge of this system of hormones has gradually evolved. Though the level of hormones differ throughout life, they play a significant role in physiology throughout the entire life span and aberrations cause morbidity and counteracts longevity [49, 50].

The insulin and insulin-like peptide family, also often called the IGF-system, is a system that consists of insulin, the insulin-like growth factors (IGFs) IGF-I and IGF-II, and the six high-affinity IGF-binding proteins (IGFBPs). The IGFBPs, which in their turn are regulated by specific proteases, control the activity of the IGFs. IGFBP-3 is the most important IGFBP in the circulation, whereas IGFBP-2 plays an important role in the central nervous system (CNS) [51]. Across species, IGF-I is closely related to insulin. In nematodes (Caenorhabditis elegans) and fruit flies (Drosophila melanogaster), IGF-I and insulin share the same receptor [52]. In mammals including humans, there are separate receptors for IGF-I and insulin, although IGF-I can bind to the insulin receptor with low affinity and vice versa [51, 52]. There is also a crossreactivity between IGF-I and IGF-II and their receptors; IGF-IR binds IGF-I with high affinity and IGF-II with low affinity, and the IGF-IIR binds IGF-II with high affinity and IGF-I with low affinity [51, 53].

IGF-I is to a large extent regulated by growth hormone (GH) from the pituitary gland, but is also influenced by age, physical activity and nutritional status [54]. The system of GH, IGF-I and the IGFBPs plays pleiotropic functions in the brain [51, 55]. Circulating insulin is produced in the pancreas and IGF-I is mainly produced in the liver, but also in other peripheral tissues and in the CNS [54]. IGF-I and IGF-II both pass through the blood brain barrier, and to a higher degree than insulin [56]. IGF-II as well as IGF-I is produced in the CNS [57].

1.3.1 Insulin-like peptides in ischemic stroke and dementia

GH as well as IGF-I decline with age and both low and high levels of IGF-I can cause disease in both the periphery and in the CNS. Deficiency of IGF-I in mice results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule [58] and age-related effects regarding IGF-I have in some ways been reversed by giving IGF-I as intracerebroventricular infusion [59] or subcutaneously [60]. Furthermore, in animal studies, IGF-I participates in the processing of Aβ and may reduce the Aβ-burden [61, 62], at least partly by enhancing the clearance of Aβ at the level of the blood brain barrier (BBB) [62, 63]. In contrast, although important prenatally, the role of IGF-II in adult life has been unclear [64]. However, one study demonstrated that IGF-II, administered in the right time window, have the capacity to consolidate and enhance memory [65].

Animal studies have demonstrated that altered activity of the insulin/IGF-system is associated with impaired brain function and cognitive decline [66]. Furthermore, studies have exhibited that normalization of the activity of insulin or IGF-I [62] and recently also IGF-II [67] can improve memory functions. In experimental studies, GH [68] as well as IGF-I [69] have neuroprotective effects in IS. IGF-I has experimentally produced protective effects in both gray and white matter when given within a few hours after acute ischemic stroke [70] as well as exerted long-term regenerative effects [71, 72].

In humans, adult hypopituitary patients with severe GH deficiency (GHD) display decreased IGF-I and changes in body composition resembling those seen in normal ageing [73, 74]. Furthermore, adult hypopituitarism is associated with increased all-cause and cardiovascular mortality [75]. Administration of recombinant GH, which increases IGF-I in serum [76] and cerebrospinal fluid [77], is nowadays a well-established substitution treatment that improves or normalizes most, but not all, of the features of

untreated adult GHD [78]. A meta-analysis showed impairments of most cognitive domains in adult GHD patients compared with matched controls, and these changes were partly reversible by GH replacement [79].

The body of evidence is growing that GH and IGF-I affect health as well as diseases in humans during adulthood. The level of IGF-I is altered in various brain disorders including traumatic brain injury (TBI) [80], IS and dementia. However, most studies have been relatively small and have shown conflicting results. In IS, a study of 29 elderly patients with acute IS showed increased GH levels was correlated with extensive motor impairment [81] and in another study (n=20, with 8 presumably unmatched controls [82]) decreased levels of circulating IGF-I was observed after IS. Likewise, in AD, circulating levels of IGF-I have been increased [83-85] or decreased [86-88]. Albeit the discrepant results in terms of circulating IGF-I levels, IGF-I could still play a role in the pathophysiology of these diseases. In the relatively small study by Bondanelli et al. (n=42) [89], high IGF-I was associated with better outcome, suggesting that IGF-I could be of importance for the recovery after IS. Furthermore, resistance to insulin and IGF-I signaling have been observed in postmortem brains of AD cases without diabetes mellitus (hereafter: diabetes) [90], and brain resistance to insulin/IGF-I signaling might not result in clear changes in circulating levels of these hormones.

1.3.2 *IGF1* gene locus, serum-IGF-I and ischemic stroke

IGF-I in serum is regulated by GH, age, metabolic state, and physical activity [91]. Furthermore, the serum level of IGF-I is dependent on both the degradation/stability and *de novo* synthesis of the IGF-I protein and the level of IGFBPs [54, 92, 93]. Part of this is determined by transcriptional mechanisms occurring at the *IGF1* gene locus. Known variations at the *IGF1* gene locus include several single nucleotide polymorphisms (SNPs) and a 192 base-pair cytosine-adenine repeat polymorphism (192 bp CA-repeat) in the promoter region. The absence of the 192 bp CA-repeat was associated with lower serum levels of IGF-I [94], as well as an increased risk of IS and a shorter long-term survival after IS [95]. This genetic variant was also associated with an increased risk of myocardial infarction (MI) and diabetes [94]. Another study reported a polymorphism in the IGF-I receptor gene, *IGF-1R,* rs2229765 was associated with a higher risk for IS [96].

1.3.3 Insulin resistance as a risk factor for ischemic stroke and dementia

Lack of insulin and/or insulin resistance, i.e. diabetes type 1 and 2, is rapidly increasing in prevalence. In 2011, 366 million people is estimated to have diabetes globally, and this is expected to rise to 552 million by 2030 [97]. Diabetes is treated with insulin and/or medication against insulin resistance, and is a major risk factor for cardiovascular diseases (CVD) [98, 99]. This increased risk of CVD is related to the degree of hyperglycemia [98, 99]. Moreover, diabetes type 2 is a well-known risk factor for both IS [100, 101] and dementia [102].

Insulin resistance (IR), a hallmark of diabetes type 2, may be present also in patients without manifest diabetes. In diabetic as well as non-diabetic patients, IR is an independent risk factor for CVD including IS [103-105]. Independently of whether diabetes is present, IR and hyperglycemia are commonly seen in response to stressful situations such as critical illness including IS [106, 107]. Although the prognostic importance of treatment is not fully clear [106], efforts are made to maintain glycemic control in patients with severe illness [108].

Diabetes, especially type 2, is a well-known risk factor for dementia [102], and epidemiological studies have found a link between type 2 diabetes/hyperinsulinemia and increased risk of AD [109, 110]. However some studies have failed to confirm insulin resistance as an independent risk factor for AD [111]. Interestingly, in humans, resistance in insulin and IGF-I signaling have been observed in brains of AD cases without diabetes [90], and neurons resistant to insulin receptor or IGF-I receptor signaling might lack trophic signals and therefore degenerate [53, 112].

1.3.4 Insulin resistance and outcome of IS

Diabetes is a risk factor for CVD and IS, and in addition, it has been associated with worse outcome after IS. Stress hyperglycemia and diabetes at admission have been associated with more severe IS or were related to poor functional outcome up to one year after IS [8, 107, 113-116]. Furthermore, hyperglycemia was associated with increased mortality up to 6 years after IS [117], and it was also a risk factor for intracerebral hemorrhage after intravenous thrombolysis [118].

Hyperglycemia (p-glucose >8 mmol/l after acute stroke) in non-diabetic patients was associated with worse short-term outcome (survival time, discharge placement and death 3 months after stroke, n = 750) [119]. In

another study using fasting glucose and oral glucose tolerance tests (OGTTs) (n = 242) [120], diabetes was associated, and prediabetes tended to associate, with a poor early prognosis after acute IS (increase in NIHSS during the first 14 days and or mRS score ≥2 at 30 days) [120]. However, little is known whether IR is associated with long-term functional outcome in non-diabetic IS patients.

2 AIM

2.1 General aim

To determine whether the insulin/IGF-I system is of importance for outcome of ischemic stroke (IS) in humans and whether insulin and insulin-like peptides are dysregulated in human dementia with special focus on Alzheimer's disease (AD).

2.2 Specific aims

2.2.1 Paper I

The primary objective was to evaluate whether serum IGF-I is associated with outcome of ischemic stroke. Secondary objectives were to determine if serum IGF-I is augmented in the acute stage and 3 months after IS and whether serum IGF-I differ between localizations and etiologies of IS.

2.2.2 Paper II

To investigate whether genetic variation at the *IGF1* locus is associated with serum IGF-I levels as well as occurrence, severity, and outcome of IS.

2.2.3 Paper III

To delineate whether HOMA-IR in the acute stage and after 3 months is altered in non-diabetic IS patients. Furthermore, to investigate the association between HOMA-IR and outcome of IS as well as whether HOMA-IR is dependent on the etiology of IS.

2.2.4 Paper IV

To determine whether IGF-I, IGF-binding protein-3 (IGFBP-3), and insulin are altered in serum and CSF in cognitive disorders and if there are associations with CSF AD biomarkers and MMSE score.

2.2.5 Paper V

To investigate whether IGF-II, IGFBP-1, and IGFBP-2 are altered in serum and CSF in cognitive disorders and if there are associations with CSF AD biomarkers and MMSE score.

3 PATIENTS AND METHODS

3.1 General design Papers I-V

Two well-characterized clinical cohorts were studied. In Papers I-III, we used data from SAHLSIS (Sahlgrenska Academy Study on Ischemic Stroke), a study of originally 600 IS patients and 600 population-based controls. Patients in SAHLSIS have been extensively characterized after IS including serum samples, subtyping of IS and stroke scales. In Papers IV-V, we used data from a cross-sectional study in Västra Götaland, where 60 consecutive patients under primary evaluation of cognitive impairment and 20 healthy controls were included. Patients were extensively characterized including subtyping of dementia and serum and cerebrospinal fluid samples were available in all participants.

3.2 Ethical considerations

Regarding Papers I-III as well as Papers IV-V, the study was approved by the ethical committee of University of Gothenburg. All participants gave their written informed consent. However, in Papers I-III, in accordance with the approval of the ethical committee, the next-of-kin consented for a few cases that were unable to communicate. The data handling procedures were approved by the National Computer Data Inspection Board.

3.3 Patients

3.3.1 Patients with ischemic stroke (Papers I-III)

SAHLSIS is a large case-control study that started including patients in 1998. Patients with acute symptoms of first-ever or recurrent acute IS (i.e. CT or MRI without hemorrhage) before the age of 70 years were recruited. Between 1998 and 2003, patients were enrolled consecutively at four Stroke Units in western Sweden (Skövde Hospital, Borås Hospital, Sahlgrenska University Hospital/Östra and Sahlgrenska University Hospital/Sahlgrenska). From 2004 and onwards, patients were only included at Sahlgrenska University Hospital/Sahlgrenska. In these latter patients, data were only available from one occasion (the subacute phase; this dataset only used in Paper II). The controls were randomly selected either from a population-based health survey or the Swedish Population Register to match cases regarding age (+/- 1 year), sex and area of residence. In summary, 600 patients and 600 controls were

followed according to the original design whereas patients recruited after 2004 had limited amount of data. Venous blood samples were collected in the acute phase (at 1-10 days after index stroke; median 4 days), and at 3 month follow-up in IS cases (median 101, range 85–125 days) and once in controls. Blood sampling was performed between 08:30 and 10:30 AM after overnight fasting of > 8 hours. Due to the differences in follow-up and in the availability of blood samples/outcome measures, different numbers of cases from SAHLSIS were included in Papers I-III. The number of IS patients included in the papers originating from SAHLSIS as well as the duration of follow-up is exhibited in Figure 1. In Paper I, only the 407 patients that were first included at Sahlgrenska University Hospital/Sahlgrenska and 40 randomly selected matched controls that had serum for determining IGF-I were included. In Paper II, cases (n=844) and controls (n=668) that were included up to 2008 were included. In Paper III, from the original 600 patients and controls, all participants with diabetes were excluded. Thus, we only included the 441 non-diabetic patients and 560 non-diabetic controls that had adequate blood samples for determination of Homeostatic Model Assessment of IR (HOMA-IR) [121].

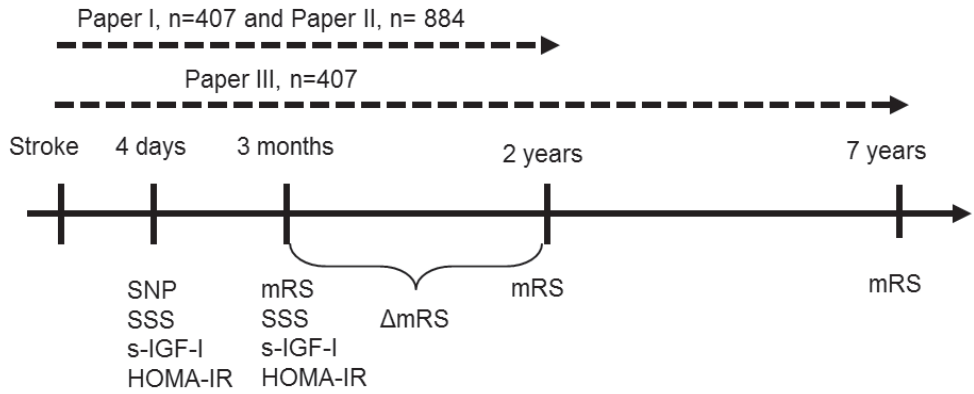

Figure 1. Number of patients included in Paper I-III, outcome data and duration of follow-up.

Stroke etiology was classified using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [8] into the subtypes large-vessel disease (LVD), small-vessel disease (SVD), cardioembolic (CE) stroke, cryptogenic stroke,

i.e. when no cause was identified despite extensive evaluation, other determined cause of stroke, and undetermined stroke [122, 123]. In Paper I we in addition to the above mentioned subtypes, also reported available data of IGF-I regarding arterial dissection (a specific fraction of other determined cause of stroke), localization according to the OCSP-model by Bamford et al [7], right/left hemisphere and cerebellum/brain stem in Table 2 of Paper I. In all patients, initial stroke severity was assessed by the Scandinavian Stroke Scale (SSS) and functional independence using the modified Rankin Scale (mRS). The SSS is similar to the NIHSS [124], with the most important exception that the scales are inverse (i.e. higher values are beneficial in SSS). In Papers I-III, mild IS was classified as SSS = 56-58, moderate IS as SSS = 46-55, and severe IS as SSS = 1-46 [125]. Three months after the index stroke, the assessment by SSS was repeated and functional outcome was evaluated by the mRS score [161-163]. The latter score is being graded 0-6, where 0 is no disability and 6 is death. Evaluation of mRS was also performed after 2 and 7 years, but the data from the 7 year follow-up was not available in Papers I-II. The mRS score is described in detail in Table 1. In the statistical analyses, mRS was dichotomized for poor outcome (death or dependency; mRS $\geq$ 3) versus good outcome (mRS 0-2).

Table 1. Description of the modified Rankin Scale (mRS)

The modified Rankin Scale Description	Score	
No symptoms.	0	Independent
No significant disability. Able to carry out all usual activities and duties, despite some symptoms.	1	
Slight disability. Able to look after own affairs without assistance, but unable to carry out all previous activities.	2	
Moderate disability. Requires some help, but able to walk without assistance	3	Dependent
Moderately severe disability. Unable to attend to own bodily needs without assistance, and unable to walk unassisted.	4	
Severe disability. Requires constant nursing care and attention, bedridden, incontinent.	5	
Dead.	6	

In Papers I-III, after inclusion anthropometric parameters [body mass index (BMI)] was measured and data on hypertension, diabetes and smoking were recorded using a structured questionnaire [122]. Diabetes was defined as receiving diet treatment or medication for diabetes or alternatively, as fasting

plasma glucose ≥ 7.0 mmol/L or fasting blood glucose ≥ 6.1 mmol/L. Smoking history was defined as current versus never or former (cessation of smoking ≥ one year prior to inclusion in the study). Among cases, measurements performed at 3-month follow-up were used for the definition of diabetes and hypertension. Hypertension was defined by pharmacological treatment for hypertension, systolic blood pressure ≥ 160 mmHg, and/or diastolic blood pressure ≥ 90 mmHg. BMI was calculated as kg/m².

3.3.2 Patients with cognitive impairment (Papers IV-V)

Sixty (30 men and 30 women) consecutively recruited Caucasian patients were studied in Papers IV-V. The patients had been admitted by their general practitioner for primary evaluation of cognitive impairment to the memory clinic at Falköping, Sweden. Inclusion criteria, besides being referred for evaluation of suspected dementia, were; age 65-80 years, body mass index (BMI) 20-26 kg/m², and waist:hip ratio 0.65-0.90 in women and 0.70-0.95 in men. Exclusion criteria were serum creatinine > 175 mmol/L, diabetes mellitus, previous myocardial infarction, malignancy including brain tumor, subdural hematoma, ongoing alcohol abuse, medication with cortisone, and previous or present medication with acetylcholine esterase inhibitors. The study also included 20 age-matched healthy controls (10 men and 10 women), recruited from the same geographical area among spouses of the included patients and by advertisements in local newspapers. The control subjects had no subjective symptoms of cognitive dysfunction, and had similar exclusion criteria as the patients. The patients and controls were matched groupwise in terms of age, gender, BMI and waist:hip ratio.

Before the test day, a mini-mental state examination (MMSE) [17] was performed. On the test day morning with the patients in the fasted state, before lumbar puncture was performed, body weight was measured to the nearest 0.1 kg, body height was measured barefoot to the nearest 0.01 m, and body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared. Waist circumference was measured in the standing position with a flexible plastic tape placed midway between the lower rib margin and the iliac crest, and hip girth was measured at the widest part of the hip.

On the test day, all CSF samples were collected by lumbar puncture in the L3/L4 or L4/L5 interspace at the standardized time point 08:30–09:00 h. The first 12 mL of CSF was collected in a polypropylene tube and immediately transported to the local laboratory for centrifugation at 2.000g at +4˚C for 10

minutes. The supernatant was pipetted off, gently mixed to avoid possible gradient effects, and aliquoted in polypropylene tubes that were stored at -80°C pending biochemical analyses, without being thawed and re-frozen. Blood samples were drawn in the morning in the fasted state and serum was prepared by centrifugation after coagulation at room temperature for 15-30 min, aliquoted and stored in cryotubes at -80°C pending biochemical analyses, without being thawed and re-frozen.

An independent physician assessed all diagnoses. Dementia was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), criteria. Patients with dementia were classified as suffering of Alzheimer's disease (AD) [22] or vascular dementia (VAD) [42] according to the requirements by NINDS-AIREN [42] or the guidelines for subcortical VAD [126]. Frontotemporal dementia (FTD) [46], Parkinson disease dementia (PDD), and dementia with Lewy bodies (DLB) [127] were diagnosed according to guidelines and described previously [128].

Mild cognitive impairment (MCI) was diagnosed in the clinical setting according to Petersen [129]. MCI patients were followed at least annually for a median of 3 (range 1-7) years to evaluate whether they later on developed dementia. At primary evaluation, 6 of the 24 AD patients had signs of additional vascular pathology according to brain imaging, but these patients did not differ from the remaining AD patients in terms of CSF levels of the AD biomarkers β-amyloid$_{1-42}$ (Aβ_{1-42}), total-tau (T-tau) or phosphorylated tau protein (P-tau). During the follow-up visits, 13 MCI patients remained in stable cognitive function (SMCI). Others progressed into dementia and the distribution is exhibited in Table 2.

Table 2. Diagnoses of 60 patients with cognitive dysfunction

Diagnoses	numbers	fraction (%)
All Alzheimer´s disease	32	53
MCI that converted to AD	7	12
AD	25	42
All other dementia	15	25
MCI that converted to VAD	3	5
VAD	7	12
MCI that converted to FTD	1	2
DLB	4	7
Stable Mild Cognitve Impairment	13	22

AD, Alzheimer's disease (AD). MCI, Mild cognitive impairment. VAD, vascular dementia. FTD, frontotemporal dementia. DLB, dementia with Lewy bodies.
SMCI, Stable Mild cognitive impairment (MCI)

3.4 Biochemical procedures

3.4.1 Papers I-III

All blood and plasma concentrations of glucose and low-density lipoprotein cholesterol (hereafter LDL) were analyzed using standardized methods at the Department of Clinical Chemistry at the Sahlgrenska University Hospital. Serum levels of high sensitivity C-reactive protein (hsCRP) was analyzed by a solid-phase chemilumniscent immunometric assay on IMMULITE 2000 (Diagnostic Products Corporation, USA) using the manufacturers reagents as directed.

In Paper I and Paper II, serum-IGF-I was measured using an IGF-binding protein-blocked RIA using a commercial kit (Mediagnost, Reutlingen, Germany) at the Department of Clinical Chemistry at the Sahlgrenska University Hospital.

In Paper II, genotyping was performed using the Golden Gate assay (Illumina, Inc. San Diego, Ca, USA) at the SNP&SEQ Technology platform (www.genotyping.se), and the genotyping was performed blinded to case/control status. Eleven single nucleotide polymorphisms (SNPs) were selected in the *IGF1* locus, i.e. the *IGF1* gene, to capture the common variation, based on the data from HapMap project in the CEU.

In Paper III, blood glucose or plasma glucose was analyzed, and blood glucose values were transformed to plasma glucose according to the formula: plasma glucose = blood glucose x 1.11. All blood samples were analyzed using standardized methods at the Department of Clinical Chemistry at the Sahlgrenska University Hospital. Insulin was analyzed by a solid-phase chemilumniscent immunometric assay on IMMULITE 2000 (Diagnostic Products Corporation, USA) using the manufacturers reagents as directed. HOMA-IR was calculated as fasting insulin (microU/L) x fasting glucose (nmol/L) / 22.5 [121].

3.4.2 Papers IV-V

All biochemical analyses regarding biomarkers for AD were performed at the Clinical Neurochemistry Laboratory in Sahlgrenska University Hospital/Mölndal, Sweden, by experienced laboratory technicians with the analyst blinded to the clinical diagnoses and other clinical information. CSF biomarkers have been reported previously [128]. All analyses were performed at one occasion, using the same batch of reagents. CSF Aβ1-42 levels were determined using the INNOTEST® ELISA assay technology

(Innogenetics, Ghent, Belgium). The axonal damage marker CSF T-tau and tau phosphorylated at threonine 181 (P-tau181) were measured using INNOTEST® ELISA assays. Albumin levels were measured by immunonephelometry on a Beckman Immage Immunochemistry system (Beckman Instruments, Beckman Coulter, Brea, CA, USA). All analyses regarding insulin and insulin-like peptides in Paper IV and V were performed with the analyst blinded to the clinical diagnoses and other clinical information.

In Paper IV IGF-I and IGFBP-3 in serum and CSF were determined at the Department of Clinical Chemistry at using at the Sahlgrenska University Hospital/Sahlgrenska using ELISA (Mediagnost, Tübingen, Germany). Insulin was analyzed with an ultrasensitive sandwich immunoassay technique (ELISA) using double monoclonal antibodies highly specific for insulin (Mercodia, Uppsala, Sweden).

In Paper V, IGF-II, IGFBP-1 and IGFBP-2 in serum and CSF was measured with ELISA Mediagnost, Tübingen, Germany) at the Clinical Neurochemistry Laboratory in Sahlgrenska University Hospital/Mölndal.

3.5 Statistical methods (Papers I-V)

All statistical analyses in Papers I-V were performed using SPSS for Windows version 14.0, 16.0, 17.0, 18.0, 19.0, 20.0 and 21.0. In the results $p<0.05$ was regarded as statistically significant, however sometimes $p<0.10$ is also stated. Regarding the tests, we on occasion consulted statistical expertise and chose different methods to adjust for the different situations due to the wide panorama of data; including variables that are categorical and different scales, parametric and non-parametric data. Also, in choosing a suitable statistical analysis, the sample size of Papers I-III was usually larger than that in Papers IV-V. In Papers I-III calculations were made using bivariate correlation Pearson matrices and student´s t-test for pairwise comparison of parametric data and ANOVA followed by Tukey´s post hoc test for multiple comparisons of means between groups and chi-square tests for categorical variables. For comparisons of medians in non-parametric data, we used the Kruskal-Wallis test for multiple groups and the Mann-Whitney U-test for pairwise comparisons.

In Paper I, after consulting statistical expertise, we also used a stepwise multiple linear regression analysis for adjusting for possible confounders and the Cochran-Mantel-Haenszel was used for comparison of the distribution of ordinal data, i.e. mRS, between groups. In Paper II, we also used logistic

regression ta assess the relative frequencies of the major alleles between the groups and regarding association between IGF-I in serum and stroke severity, we executed a linear regression and also a multiplicative genetic model. In Paper III a bivariate logistic regression model was used for examining association between outcome, i.e. good or poor functional outcome, and HOMA-IR.

In Papers IV-V we used the non-parametric Spearman rank order test for correlations. Also, for the smaller number of data that was not evenly distributed, we found it more suitable assessing differences between medians, and not means of the groups. Therefore, we used the Kruskal Wallis test for multiple variables and the Mann-Whitney U test for pairwise comparisons. The descriptive statistical results are given as median (25th-75th percentile).

3.6 Strengths and limitations

To further understand the role of the insulin and insulin-like peptides in IS and cognitive impairment, two well-characterized clinical cohorts were studied. In these cohorts, it was analyzed whether patients with IS or cognitive impairment have altered levels of insulin and insulin-like peptides and whether these levels were associated to outcome. In terms of these analyses, we have some methodological considerations regarding strengths and limitations.

3.6.1 Considerations regarding Papers I-III

In Papers I-III, SAHLSIS is a cohort of relatively young Caucasian patients with IS. In SAHLSIS, imaging (CT or MRI) was used to exclude all primary hemorrhages, which is an advantage when studying etiology of IS in Paper I and genetics in Paper II. However, the homogeneity limits the ability to draw conclusions in a global context as some groups, such as non-Caucasian patients or elderly patients, were not included in SAHLSIS. The SAHLSIS cohort is, compared to other studies of associations between IGF-I/IR and outcome of IS, relatively large and the inclusion of patients is managed by physicians experienced in stroke medicine. Furthermore, strokes are carefully characterized in terms of severity [124, 130], localization [7], and etiology [8], which is beneficial in exploring possible mechanisms of IS in Papers I-III. The patients in SAHLSIS have also been followed for a longer time period than most other studies of IS; in Paper III we were able to use outcome data 7 years after the incident of IS. In Paper III, HOMA-IR was used to estimate insulin resistance (IR) in non-diabetics. Thereby, we used a well validated and convenient method [121], which is correlated with the risk of

cardiovascular morbidity [131]. In contrast to OGTT, the use of HOMA-IR allows inclusion of severely bedridden patients unable to swallow. Finally, in excluding diabetics, we avoided insulin treatment, which interferes with the estimation of HOMA-IR.

The use of the SAHLSIS cohort in Papers I-III has some limitations. Methodological considerations include that SAHLSIS was originally designed to investigate genetic associations and hemostatic risk factors in IS rather than determining the role of insulin and the insulin-like peptides. The first blood sample was taken in the acute phase after median 4 days (range 1-10 days); hence most data in terms of HOMA-IR or IGF-I are not from the admission day. We also lack data from before the admission, such as glycosylated hemoglobin (HbA1c), which would have provided an estimation of the glucose level before the incident of IS. In addition, the controls in SAHLSIS are matched for age and sex but not for metabolic risk factors and CSF samples are lacking both in patients and controls. Thus, although SAHLSIS has several strengths, additional data would have provided a better opportunity of drawing conclusions regarding pathological mechanisms and causality in Papers I-III.

3.6.2 Considerations regarding Papers IV-V

Sixty (30 men and 30 women) consecutively recruited Caucasian patients and 20 matched healthy controls (10 men and 10 women) were studied in Papers IV and V. The patients had been admitted by their general practitioner for primary evaluation of cognitive impairment to the same memory clinic at Falköping, Sweden. Carefully conducted inclusion with predefined inclusion/exclusion criteria was made by one experienced physician, and another independent physician assessed all diagnoses. Inclusion and exclusion criteria were selected in order to minimize the effects of factors known to influence levels of insulin and insulin-like peptides. Inclusion criteria were primary evaluation of cognitive impairment, age 65-80 years, body mass index (BMI) 20-26 kg/m^2, and waist:hip ratio 0.65-0.90 in women and 0.70-0.95 in men. Exclusion criteria were serum creatinine > 175 mmol/L, diabetes mellitus, previous myocardial infarction, malignancy including brain tumor, subdural hematoma, ongoing alcohol abuse, medication with cortisone, and previous or present medication with acetylcholine esterase inhibitors. Thus, patients that fulfilled these criteria were in the early stages of AD or other dementing disorders and relatively free from somatic diseases. In other studies of IGFs and IGFBPs in AD or other forms of dementia, study populations have usually been less homogenous and most often, patients with moderate or severe dementia have

been included. Furthermore, patients with diabetes and patients receiving AD medication have generally not been excluded in other studies, which might confound the results and conclusions of these studies. In Papers IV-V, MCI patients were followed clinically with the aim of having high diagnostic accuracy and all laboratory analyses were performed using established assays in experienced laboratories.

In Papers IV-V, study limitations include the limited number of patients, which might have resulted in lack of statistical power. Therefore, we cannot exclude that some associations could have eluded detection. In addition, the patients were in the early phases of disease but still had clinically detectable symptoms; it is possible that the findings of Papers IV-V are not applicable to very early or late phases of dementing disorders. Furthermore, the cross-sectional design limits our ability to draw conclusions regarding longitudinal changes and causality. Ideally, the study design should have included follow-up of the patients with a consecutive serum and CSF-samples as well as repeated measurements of clinical outcome.

4 RESULTS

4.1 Paper I

4.1.1 High level of serum IGF-I after ischemic stroke

The level of IGF-I in serum was increased in IS patients; mean serum IGF-I was 173.7 ng/ml in the acute phase of IS and 152.6 ng/ml after 3 months compared to 145.4 ng/ml in the controls ($p < 0.001$ and $p < 0.01$ vs. controls, respectively). The level of IGF-I in the acute phase did not differ with respect to stroke severity. The IGF-I levels differed among the different subtypes regarding localization and etiology (Table 2 in Paper I), but this was to a large extent explained by the difference in ages among the subtypes. When we did a post-hoc analysis, and also corrected for age, only the difference that large vessel disease had higher IGF-I than cardioembolic stroke was significant ($p=0.03$).

4.1.2 High level of serum IGF-I is associated with better improvement of functional outcome after ischemic stroke

High serum IGF-I, both in the acute phase and after 3 months, correlated to better improvement of functional outcome between 3 months and 2 years, i.e. ΔmRS. Serum IGF-I is affected by many conditions, and we further adjusted for potential confounding factors. as height, weight, BMI, age, hemoglobin, C-reactive protein, LDL, plasma glucose, high-density lipoprotein, acute fibrinogen, diabetes, and insulin treatment in a stepwise multiple linear regression model. After adjustment the association between both acute IGF-I and 3-month-IGF-I and ΔmRS was retained ($r = 0.134$, $p = 0.017$ and $r = 0.175$, $p = 0.002$).

Another way to investigate the role of serum-IGF-I after stroke is to dichotomize the s-IGF-I levels into above median versus below median in terms of change in distribution of functional outcome between 3 months and 2 years. In the Cochran-Mantel-Haenszel test (see Figure 2) there was a statistically significant improvement in distribution of mRS if serum-IGF was above median after 3 months ($p=0.003$), but this association was absent if serum-IGF was below median.

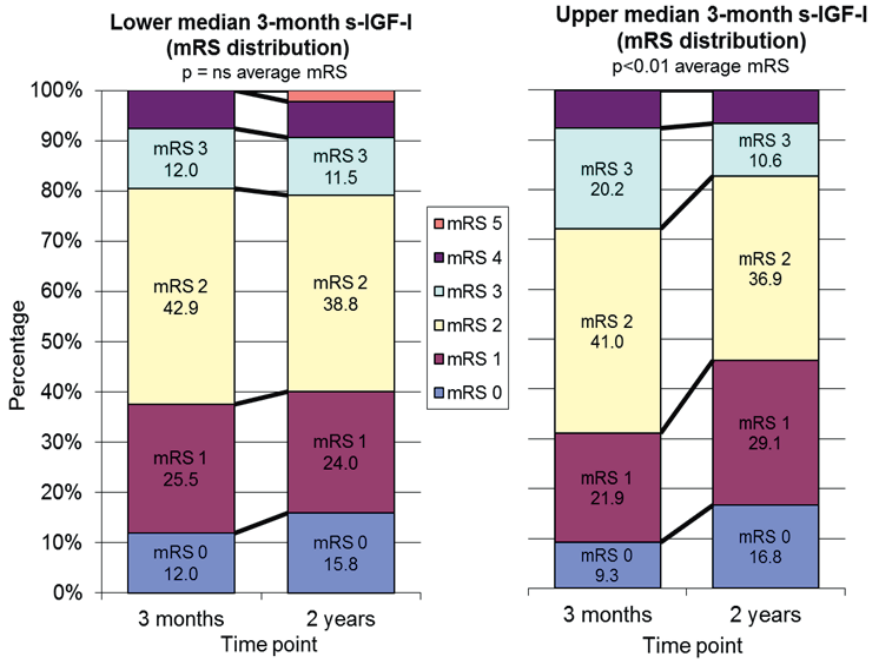

Figure 2. Change of mRS distribution between 3 months and 2 years in lower and higher than median IGF-I in serum 3 months after IS.

4.2 Paper II

4.2.1 Genetic variation at the *IGF1* locus shows association with the level of serum IGF-I and post-stroke outcome

Eleven single nucleotide polymorphisms (SNPs) were selected in the *IGF1* gene to capture the common variation in *IGF1*, i.e. the *IGF1* gene. Analyses of single-nucleotide polymorphisms (SNPs) in the *IGF1* gene showed that the major allele of rs7136446 was associated with serum IGF-I in controls but not in IS. There was no variation correlated to occurrence of IS or stroke severity. However, the major allele of rs7136446 was associated with favorable functional outcome, i.e. mRS, after 24 months (OR 1.46 95 % CI 1.09-1.96, $p < 0.05$). Thus in the same locus, the major allele of rs7136446 was both associated to serum IGF-I and post-stroke outcome.

4.3 Paper III

4.3.1 HOMA-IR in cases and controls

Baseline clinical data are exhibited in Table 3. Controls had less hypertension, smoking and CRP compared to cases. Controls also had prominently lower p-glucose, serum-insulin and HOMA-IR compared to the cases with IS. Especially the level of insulin and HOMA-IR was prominently elevated in the acute phase of IS compared to controls, but the elevation of insulin and HOMA-IR was also to a less degree retained after 3 months. After 3 months, especially the level of glucose had a tendency to even up in cases vs controls. Also, as exhibited in Table 2 in Paper III, the level of acute HOMA-IR in severe stroke, 4.34, was higher than that in mild stroke 3.18 ($p = 0.02$).

Table 3. Baseline clinical data in patients with ischemic stroke and controls.

	Cases			**Controls**			
	N	Mean	SEM	N	Mean	SEM	p-value
SEX (ratio m/f)	441	1.37	0.02	560	1.37	0.02	ns
Age (years)	441	56.1	0.51	560	56.6	0.44	ns
Hypertension (%)	438	58	2.4	559	36	2.0	<0.001
BMI ($kg/m^{2)}$	433	26.1	0.20	559	26.3	0.17	ns
LDL (mmol/l)	395	3.35	0.05	558	3.33	0.04	ns
Current smoking (%)	440	39	2.3	560	18	1.6	<0.001
Acute CRP	433	11.4	0.98	550	5.92	0.22	<0.001
Acute HOMA-IR	441	3.73	0.18	560	1.90	0.09	<0.001
3-month HOMA-IR	429	2.87	0.11	560	1.90	0.09	<0.001
Acute p-glucose	441	5.69	0.06	560	5.09	0.03	<0.001
3-month glucose	429	5.21	0.03	560	5.09	0.03	0.005
Acute insulin	441	14.3	0.63	560	8.26	0.39	<0.001
3-month insulin	429	12.1	0.40	560	8.26	0.39	<0.001

Data are shown as N (i.e. some patients lack specific data), mean and SEM.

P-values case vs controls are stated if < 0.10. ns, not significant;

LDL, low-density lipoprotein, BMI, body mass index, CRP, C-Reactive Protein.

4.3.2 HOMA-IR and long term functional outcome after Ischemic Stroke

For correlations between acute HOMA-IR and poor outcome (mRS 3-6) in the total study population, see Table 3 in Paper III. High acute HOMA-IR was associated with poor outcome after 3 months and after 7 years. After adjusting for covariates in Model A that included well-known cardiovascular confounders, HOMA-IR tended to associate with a poor outcome after 3 months and associated to poor outcome after 7 years. However, after full adjustment for covariates in Model B which also includes stroke severity, only a trend to an association remained for the 7-year outcome (OR 1.75, 95 % CI 0.97-3.16, $p = 0.06$). There was no association between HOMA-IR after 3 months and functional outcome after 2 or 7 years in the total study population.

4.3.3 HOMA-IR and functional outcome in stroke subtypes

For correlations between acute HOMA-IR and poor outcome (mRS 3-6) in the etiological subtypes, see Table 4 in Paper III. In the largest IS subtype, cryptogenic stroke, high HOMA-IR was associated with poor outcome after 3 months (crude OR 3.65, 95 % CI 1.53-8.72; $p < 0.01$) and 2 years (crude OR 4.77, 95 % CI 1.93-11.8; $p < 0.001$). Due to the low number of patients in each IS subtype, we found it proper to adjust for covariates only in this largest subtype, i.e. cryptogenic stroke, and to restrict the number of covariates to two. After adjustment for the two covariates with the largest influence on outcome, i.e. age and acute SSS, only the association with 2-year outcome remained (adjusted OR 2.86, 95 % CI 1.01-8.12; $p < 0.05$).

There was neither any statistically significant association between acute HOMA-IR and 7-year outcome nor any significant associations between HOMA-IR after 3 months and outcome after 2 or 7 years in any IS subtype.

4.4 Paper IV

4.4.1 IGF-I, IGFBP-3 and insulin in serum and CSF in cognitive impairment and AD

For serum levels of IGF-I, IGFBP-3, see Figure 1 and Table 3, Paper IV. Serum IGF-I was increased in AD patients and in patients with other dementias compared to healthy controls. Serum IGFBP-3 concentration was higher in AD and SMCI patients compared to controls. Serum IGF-I/IGFBP-

3 ratio, which is considered to be a marker of bioactive IGF-I, as well as serum insulin level were similar in all study groups. Also CSF levels of insulin, IGF-I and IGFBP-3 as well as the IGF-I/IGFBP-3 ratio in CSF did not differ between groups.

CSF/serum IGF-I ratio was reduced in AD patients and patients with other dementias compared to healthy controls and in patients with other dementias compared to SMCI patients. CSF/serum levels of IGFBP-3 were lower in patients with AD, other dementias, or SMCI compared to controls, whereas CSF/serum insulin ratio was similar in all study groups.

4.4.2 Correlations between IGF-I/insulin and AD biomarkers

A major aim of the project was to investigate whether the IGF-I system in the serum and CSF associated with AD biomarkers. In the total study population (n=80), serum levels of IGF-I and IGFBP-3 both correlated negatively with CSF $A\beta_{1\text{-}42}$ level ($r = -0.29$, $p = 0.01$ and $r = -0.27$, $p = 0.02$). CSF IGF-I level correlated positively with CSF P-tau level. Finally, IGF-I/IGFBP-3 ratio in CSF correlated positively with CSF levels of T-tau and P-tau. MMSE did not correlate with either IGF-I, IGBP-3 or insulin in serum or CSF.

4.5 Paper V

4.5.1 Increased Cerebrospinal Fluid Level of (IGF-II) in Male Patients with Alzheimer's Disease

Levels of IGF-II, IGFBP-1, and IGFBP-2 were similar in all study groups in the total study population. However as exhibited in Figure 1 in Paper V, gender specific analyses showed that in men (n=40), CSF IGF-II level was higher in AD men compared to SMCI men and control men. Furthermore, CSF IGFBP-2 level was increased in AD men vs. SMCI men and tended to be increased vs. control men ($p = 0.09$). There were no between-group differences in women (n=40). As presented in Figure 2 in Paper V; in the total study population (n=80) as well as in men (n=40), CSF levels of IGF-II and IGFBP-2 correlated positively with CSF levels of the AD biomarkers total-tau (T-tau) and phosphorylated tau protein (P-tau).

5 DISCUSSION

5.1 IGF-I is altered and related to outcome of ischemic stroke

In Papers I-III, the IGF-I/insulin system was altered and associated with functional outcome after IS. The role of serum IGF-I was studied in Paper I and in SAHLSIS, circulating IGF-I was augmented in IS patients compared to the controls. This was more predominant in the acute phase of IS and in addition, high acute IGF-I was associated with more severe stroke. Furthermore, in Paper I, acute and 3-month-IGF-I were associated with better improvement of functional outcome. The association seen in Paper I between 3-month-IGF-I and ΔmRS, remained also after adjustment for covariates including stroke severity. This suggests that high serum IGF-I in the recovery phase after IS is favorable for the improvement of functional outcome. In further support of a beneficial effect of high IGF-I, in two previous studies by Bondanelli et al. (n=42 and median age 66.6 years) [89] and Okazaki et al. (n=21, mean age 71.0 years) [132], high subacute IGF-I was related to favorable outcome. However, the results in Paper I (n=407, mean age 55 years) indicate that IGF-I in the rehabilitation phase (after 3 months) might have larger impact on IS recovery than IGF-I in the acute phase of IS.

Other studies have shown discrepant results in terms of the role of circulating IGF-I in IS. In two relatively small studies (n=56, mean age 64.8 years) [133] and (n=46, mean age 61.3 years) [134], with older patients than in SAHLSIS, hypopituitarism (i.e. poor response to releasing hormone testing: GHRH/CRH-testing) was common, but IGF-I was not correlated to stroke outcome in the latter study. In another study (n=255, mean age 68-74 in the various groups) [135], IGF-I levels did not correlate with stroke severity, whereas patients with high IGF-I and IGF-I/IGFBP-3 ratios had better neurological and functional outcome at 3 months [135]. Denti et al. (n=85, mean age 83 years) [136] found that IGF-I, but not IGF-I/IGFBP-3 ratio, was related to outcome, i.e. low acute serum IGF-I was associated to poor outcome 3-6 months after IS. In a study by Tang et al (n=168, mean age 72) [137], serum IGF-1 levels ≤130 ng/mL was as an indicator for unfavorable functional outcome after 3 months. Finally, Ebinger et al (n=100, mean age 64 years) [138] found that low IGFBP-3, but not low IGF-I, was independently associated with poor functional outcome.

The varying results in terms of the association between circulating IGF-I and functional outcome after IS might be explained by differences in the set-ups of studies. In terms of study design, SAHLSIS has several strengths. SAHLSIS consists of a wide range of patients including severely bedridden patients and younger patients (mean age of SAHLSIS is about 55 years). In Paper I, the time of the first blood sample was relatively late in the acute phase (median 4 days). However, our study does include a second IGF-I in the rehabilitation phase in contrast to most other studies of IS. Moreover, in SAHLSIS, we have followed the patients for a longer time period, i.e. years rather than months, compared to other studies. The relation between IGF-I and IS outcome after IS is corroborated by the results of Paper II. In Paper II, it was investigated whether the genetic variation in the *IGF1* locus was associated with circulating IGF-I and IS outcome. The major allele of rs7136446 was associated with higher serum IGF-I in controls and also with favorable functional outcome after 2 years in IS. As genetic variations are present in an individual throughout lifespan, the results of Paper II give clear support for the notion that IGF-I has a role in IS. However, the effect of IGF-I is modest and causality and time window have yet not been fully explored.

5.2 Insulin resistance and outcome of IS

Paper III investigated HOMA-IR in relation to both stroke severity and long-term functional outcome in non-diabetic IS patients. Earlier studies have indicated that patients having diabetes have worse functional outcome of IS compared to non-diabetic patients [113, 114]. IR and hyperglycemia is a common response to critical care illness including stroke [106, 107], and admission hyperglycemia and/or diabetes has been associated with worse outcome up to one year after IS [8, 115, 116]. Furthermore, hyperglycemia in non-diabetic IS patients has been associated with poor short term functional outcome [119] and increased mortality [139]. However, less has been known whether IR is a negative prognostic factor in non-diabetic IS in terms of long-term functional outcome. The relation between IR and stroke severity has also been unclear. Two previous studies reported no correlation [140] or only a weak correlation [120, 141] between IR and stroke severity. Finally, hyperglycemia might influence the risk of hemorrhage after thrombolysis because high HOMA-IR was associated with worse 3-month functional outcome in non-diabetic IS patients that had received intravenous thrombolysis [140, 141].

HOMA-IR in non-diabetic IS patients included in SAHLSIS was elevated both in the acute phase of IS and after 3 months compared to the controls.

Furthermore, in Paper III, HOMA-IR in the acute phase was associated with increased stroke severity. In addition, acute HOMA-IR, but not HOMA-IR at 3 months, was associated with poor functional outcome after 3 months and 7 years. After adjustment for metabolic covariates, the association between acute HOMA-IR and functional outcome after 7 years remained. Stroke severity was then included in the analysis as high HOMA-IR was related to stroke severity, and IR may therefore be viewed either as an effector or mediator of a negative outcome of IS. After inclusion of stroke severity as a covariate in the statistical analyses, also the association between 3-month HOMA-IR and functional outcome after 7 years lost statistical significance. Possibly, this could mean that elevated HOMA-IR is part of the acute morbidity in IS and that the prognostic value of acute HOMA-IR is linked to that of other negative prognostic factors including initial stroke severity. Still, in the largest IS subtype cryptogenic stroke, we observed an association between acute HOMA-IR and poor outcome after 2 years that remained significant even after adjustment for age and stroke severity. The physiological relevance of this association is, however, unclear and needs to be explored in further studies.

The results in Paper I vs. those in Paper III display a difference in terms of when IGF-I and HOMA-IR is of importance for IS outcome. High serum IGF-I after 3 months was associated with improved recovery of IS, suggesting that IGF-I was of most importance in the rehabilitation phase. One explanation for this could be that the anabolic effects of IGF-I enhance the effects of training and rehabilitation. In contrast, in Paper III, HOMA-IR in the acute phase in non-diabetics was associated with long-term functional independence, implying a negative effect of IR in the vulnerable acute phase of IS. This could possibly be explained by glucotoxicity in the penumbra zone [142], which might induce a detrimental effect on the afflicted brain area. Furthermore, IR might be hazardous in the acute phase by increasing the risk of secondary hemorrhage in IS as implicated by the detrimental effect of hyperglycemia during thrombolytic therapy of IS [118, 143]. However, the time aspect is somewhat obscure and our results need to be replicated. There is a need for further studies with collection of blood samples starting from admission at day 1, followed by consecutive blood samples and assessment of outcome data at multiple time points throughout the acute phase and rehabilitation.

5.3 Insulin and Insulin-like Peptides in Cognitive Impairment.

In Paper IV, serum but not CSF levels of IGF-I was increased in patients with AD and other dementias compared to controls, whereas insulin levels were unchanged both in serum and CSF. Several earlier studies have found increased circulating or CSF levels of IGF-I in AD [83-85]. However, decreased [86-88] levels of IGF-I in the circulation or in CSF have also been found in AD patients. The discrepant IGF-I levels in previous studies could to some extent be due to differences in settings and designs of the studies. In the study by Mustafa et al. [86], circulating IGF-I was low in a familial form of AD, and the possibility cannot be excluded that IGF-I levels are different in inherited forms of AD compared to sporadic AD. Furthermore, in the cohort studied in Papers IV-V, strict exclusion criteria were used. The patients did not suffer from e.g. diabetes and did not receive treatment with glucocorticoids or acetylcholine esterase inhibitors. Moreover, patients and controls were comparable in several factors known to influence IGF-I levels such as age, gender, and body composition. In some of the other studies, these factors were not controlled for [83, 88, 90, 144].

The patients studied in Papers IV-V had relatively early AD (median MMSE score = 23). In previous studies, most patients have suffered form more severe AD [87]. Furthermore, as demonstrated by Talbot et al, the AD brain is resistant to insulin receptor and IGF-I receptor signaling [90]. Therefore, the elevated level of IGF-I in Paper IV could reflect a state of IGF-I resistance in early AD. It has been proposed that the early stages of AD is characterized by high IGF-I secondary to resistance to IGF-I action, whereas IGF-I is low in more advanced stages of AD [145]. The reason for a possibly deficiency of IGF-I in late stages of AD is unclear, but underlying mechanisms could speculatively include AD-related changes in the production or clearance of IGF-I or AD-related changes in lifestyle or nutritional intake. However, different IGF-I levels along the progress of the disease might explain the discrepant results in previous studies of AD [83-88].

CSF/serum ratios might reflect the transport through the BBB. In Paper IV, CSF/serum IGF-I ratio was reduced in AD patients and patients with other dementias, possibly suggesting reduced transport of IGF-I through the BBB in these patients. In line with this finding, IGF-I passage through the BBB has been decreased in mouse AD models [63, 146]. Furthermore, in Paper IV, levels of IGF-I in serum or CSF did not correlate with CSF/serum albumin ratio, the most established biomarker for general BBB function [147]. This

argues against a general decrease in the transport of proteins into the CNS in patients with cognitive disorders. Therefore, the results of Paper IV could suggest reduced passage through the BBB that is specific for IGF-I in AD patients and patients with other dementias.

Insulin in serum and CSF was similar in all study groups in Paper IV. The results of epidemiological studies suggest that hyperinsulinemia and type 2 diabetes mellitus are associated with increased risk of AD [109, 110]. Furthermore, resistance to insulin receptor signaling has been observed in brains of AD patients without diabetes [90]. In line with the results of Paper IV, CSF insulin level was unchanged in one study of human AD [148], whereas it was low in another study [149]. The unchanged CSF insulin levels in Paper IV and in the study by Molina et al. [148] do not suggest any major role of insulin in CSF in AD. However, Paper IV was a cross-sectional study and changes over time were not studied. Therefore, it was not possible to evaluate if insulin levels were of importance for the longitudinal progression of AD or other dementing disorders.

In Paper V (median MMSE score = 23), CSF IGF-II level was increased in AD men but not in AD women whereas serum IGF-II level was unchanged. In two studies [83, 144] of more advanced AD (mean MMSE score 15 and 19, respectively), CSF IGF-II was elevated in the total AD group. Tham et al. found increased serum IGF-II in AD [83], whereas serum IGF-II was decreased in the study by Hertze et al. [144]. Therefore, the serum level of IGF-II has been variable between studies whereas elevated CSF IGF-II level seems to be a consistent feature of AD although in early AD, increased CSF IGF-II level was only observed in AD men in Paper V. The possibility cannot be excluded that the elevated IGF-II level in AD is caused by reduced clearance of IGF-II. However, although the IGF-II receptor has the capacity to target IGF-II for degradation [53, 150], reduced expression of the IGF-II receptor in the brain has mainly been detected in advanced AD [57, 151].

In Paper V, serum and CSF levels of IGFBP-1 as well as serum IGFBP-2 level were similar in all study groups. In AD men, CSF IGFBP-2 level was higher than in SMCI men and tended to be higher than in control men. Furthermore, if the SMCI and control groups were merged, CSF IGFBP-2 level was higher in AD men compared to that in men in the merged SMCI and control group. In Paper IV, serum IGFBP-3 level was increased in AD and SMCI patients compared to controls whereas CSF IGFBP-3 level was unchanged. Previous experimental studies demonstrate that IGFBP-3 could inhibit some of the neuroprotective actions of IGF-I [152], and global overexpression of IGFBP-2 reduced brain weight in transgenic mice [51,

153-155]. Thus, the increased CSF IGFBP-2 levels in AD men and the increased serum IGFBP-3 levels in the total AD group could possibly modulate the bioavailability of IGFs, thereby possibly reducing IGF activity.

Correlation analyses were performed in Papers IV-V to investigate whether changes in the IGF-I system were related to the AD biomarkers Aβ1-42, T-tau, and P-tau. In Paper IV, in the total study population (n = 80), serum levels of IGF-I and IGFBP-3 correlated negatively with CSF Aβ1-42 level and in the AD patients (n = 32), CSF/serum IGF-I ratio correlated positively with CSF P-tau level. In Paper V, CSF levels of both IGF-II and IGFBP-2 correlated positively with CSF levels of T-tau and P-tau in the total study population (n= 80) and in the total group of men (n= 40). In all AD patients (n= 32) as well as in AD men (n= 15), only CSF IGFBP-2 level correlated or tended to correlate with CSF levels of T-tau and P-tau. Therefore, although the results were not fully consistent, there were several indications that the studied components of the IGF-I system were associated with markers of AD disease status.

In summary, in Papers IV-V, several components of the IGF-I system (IGF-I, IGF-II, IGFBP-2, and IGFBP-3) were dysregulated in AD patients. Furthermore, IGF-I and IGF-II as well as IGFBPs were correlated with markers of AD disease status. Possibly, the changes in IGF-I and IGF-II could be compensatory mechanisms to protect the AD brain from resistance to insulin-like peptide signaling. However, Papers IV-V had a cross-sectional design, and therefore did not include longitudinal follow-up. Further studies are needed with large study populations that are followed longitudinally for extended time periods.

6 CONCLUSIONS

6.1 Conclusions paper by paper

6.1.1 Paper I

In SAHLSIS, high acute IGF-I in serum was not related with stroke severity, but acute and especially 3-month IGF-I concentrations were associated with improvement in mRS between 3 months and 2 years also after adjusting for confounders. This suggests that high serum IGF-I concentration may be of importance for the functional outcome after ischemic stroke.

6.1.2 Paper II

A variation in one locus of the *IGF1* gene (rs7136446) was associated with post-stroke outcome in IS patients, further illustrating the importance of the IGF-I system for outcome after ischemic stroke.

6.1.3 Paper III

HOMA-IR was elevated in non-diabetic IS patients both in the acute phase and at 3-month follow-up and was associated with increased stroke severity. High acute HOMA-IR was associated with poor functional outcome after 3 months and 7 years, but these associations lost significance after adjustment for multiple covariates. However, in cryptogenic stroke, IR was related to poor outcome after 2 years also after adjustment for age and stroke severity. In contrast to 3-month-IGF-I in Paper I, HOMA-IR after 3 months had no impact on long term functional outcome.

6.1.4 Paper IV

AD patients as well as patients with other dementias had high levels of IGF-I in serum but not in CSF, possibly suggesting reduced passage of IGF-I through the blood-brain barrier. Furthermore, in AD patients, the IGF-I system was associated with biomarkers of AD disease status.

6.1.5 Paper V

AD men, but not AD women, had elevated CSF IGF-II level, and tended to have increased CSF IGFBP-2 level compared to controls of the same gender. Possibly, increased CSF IGF-II level in AD men could be a compensatory mechanism to maintain insulin-like peptide activity in the male AD brain.

6.2 General conclusion

Serum IGF-I was associated with better improvement of functional independence and a locus of the *IGF1* gene (rs7136446) was associated with post-stroke outcome in IS, indicating that IGF-I activity could be of importance for outcome of IS. In addition, insulin resistance was associated with poor outcome of IS especially in cryptogenic stroke. Furthermore, levels of IGF-I, IGF-II, IGFBP-2 and IGFBP-3 were dysregulated in Alzheimer's disease, which strengthens the assumption that insulin-like peptides has a role in cognitive impairment.

7 FUTURE PERSPECTIVES

Experimentally in animals with IS and dementia, treatment with insulin-like peptides have exhibited protective and beneficial effects. In humans, IGF-I has been altered in IS and IR seems to be more prevalent also in non-diabetics with IS, and favorable levels might eventuate in better outcome after IS. Also, IGFs and IGFBPs are altered in cognitive impairment and dementia.

Although these results obviously need to be replicated and more thoroughly studied, it implies that in a certain time frame and subtype of IS and cognitive impairment, pharmacological or non-pharmacological treatment acting on the insulin and insulin-like peptides might be beneficial also in humans.

Pondering data replicate that absence of IR and/or IGFs levels being in the upper physiological range is beneficial, new treatment options can be designed. Randomized controlled studies (RCTs) are warranted for evaluating pharmacological treatment or other measures to reduce IR or raise IGF-I levels, such as diet and training [156]. Regarding pharmaceutical treatment, long-term GH treatment of hypopituitary patients with severe GH deficiency is well-studied and safe [157]. IGF-I might in the future also present a possible way of treatment [158], and treatment is also to some extent already available to induce linear growth in GH insensitivity syndromes. Furthermore, theoretically, sensitizers of the effects of IGF-I in the target organs could be developed. Treatment of IR in type 2 diabetes is well-studied and unquestionable, but less is known about IR in non-diabetics. However, some promising attempts of treating IR in non-diabetics have been made. The recently performed RCT, IRIS (Insulin Resistance Intervention after Stroke), in which non-diabetics with HOMA-IR>3.0 received secondary prevention after IS with pioglitazone, resulted in a significant reduction in stroke and MI risk compared to placebo [159]. However, in shifting the context from long-term secondary prophylaxis of IS, to the context of pharmacological treatment of IR in the dynamic but fragile acute phase of IS, is more risky. In IS, numerous trials of other pharmacological agents have failed to reproduce promising experimental data in full scale clinical trials [160]. Nevertheless, given our results are replicated and reinforced by robust data elucidating causality and the exact time window, RCTs with for example the more recently developed antidiabetic therapies (e.g. GLP-agonists), with low risk of hypoglycemia and other adverse events, might be plausible.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

Stroke kan indelas i hjärninfarkt (ischemisk stroke, IS) och hjärnblödning (hemorrhagisk stroke), varav ca 85 % av alla stroke utgörs av hjärninfarkt. Stroke är en av de vanligaste orsakerna till död och handikapp i Sverige. Lindrig kognitiv störning (mild cognitive impairment, MCI) samt demens utgör också en allt större sjukdomsbörda hos vår allt äldre befolkning. Som en del i den naturliga åldrandeprocessen i kroppen förändras hormonnivåerna. Bland annat minskar halterna av insulinliknade hormoner och även insulinresistens blir vanligare, och detta sistnämnda kan orsaka diabetes som i sin tur är en viktig riskfaktor för att drabbas av stroke såväl som den vanligaste formen av demens: Alzheimers sjukdom (Alzheimer´s disease, AD).

Det finns djurstudier som har visat att förändrade nivåer av insulin och insulinliknande hormoner såsom insulinliknade tillväxfaktor-I (insulin-like growth factor-I, IGF-I) kan leda till sämre utläkning efter hjärninfarkt och även utveckling av förändringar som vid Alzheimers sjukdom. Det finns även djurexperiment som visat att tillförsel av t.ex. IGF-I är gynnsamt för att förbättra återhämtningen efter hjärninfarkt samt att IGF-I också kan motverka utveckling av demensförändringar. Vidare vet man att människor som har diabetes och insulinresistens får sämre utfall efter hjärninfarkt, men det har saknats kunskap huruvida insulinresistens hos icke-diabetiker är kopplat till sämre långtidsutfall efter hjärninfarkt. Det övergripande målet med delprojekten i denna avhandling var att undersöka huruvida insulin/IGF-I systemet är förändrat hos människor som drabbats av hjärninfarkt eller AD och om detta har betydelse för utfallet av dessa sjukdomar.

Vi har gjort detta genom att undersöka två välkaraktäriserade studiekohorter:

1) Studien SAHLSIS (Sahlgrenska Academy Study on Ischemic Stroke) med ursprungligen 600 patienter med hjärninfarkt och 600 friska kontroller. Blodprover har tagits från alla deltagare och patienterna har följts med strokeskalor, bland annat modified Rankin Scale (mRS, ett mått på funktionellt oberoende, d.v.s. om man klarar sig utan hjälp av andra i dagliga aktiviteter), under en flerårig uppföljningstid.

2) En tvärsnittsstudie i Västra Götaland bestående av 60 patienter med kognitiva besvär och 20 friska kontroller. Patienterna med kognitiva

besvär hade lindrig kognitiv störning (MCI) och olika demensformer varav den vanligaste typen var Alzheimers sjukdom. Både patienter och kontroller har utretts med kognitiva test, blodprov och ryggmärgsvätskeprov (kallas ibland även ryggvätska, likvor eller cerebrospinalvätska).

Resultat:

I första delarbetet fann vi en högre nivå av IGF-I i blodprov från patienter med hjärninfarkt jämfört med friska kontroller. En hög nivå av IGF-I var kopplat till större förbättring av funktionellt oberoende, i efterförloppet till hjärninfarkt.

I andra delarbetet analyserades single-nucleotide polymorphisms (SNPs), och vi fann att en variant av *IGF1* genen, rs7136446, var kopplad till funktionellt oberoende två år efter hjärninfarkt.

I tredje delarbetet fann vi att ett mått på insulinresistens, HOMA-IR, var högre hos icke-diabetiker som drabbats av hjärninfarkt jämfört med friska kontroller. Hög nivå av HOMA-IR hos icke-diabetiker var kopplat till sämre långtidsutfall i form av funktionellt oberoende efter hjärninfarkt. Detta samband gällde i synnerhet en variant av hjärninfarkt, kryptogen stroke, där man trots utförlig utredning inte har kunnat hitta någon orsak till hjärninfarkten.

I fjärde delarbetet fann vi att IGF-I-nivån i blodet var högre hos Alzheimers sjukdom och andra demensformer jämfört med friska kontroller, medan nivån av IGF-I i ryggmärgsvätska var oförändrad. Hos patienter med Alzheimers sjukdom var nivån av IGF-I i blodet kopplat till nivån av Alzheimer-relaterade markörer i ryggmärgsvätskan.

I femte delarbetet fann vi att ryggmärgsvätskenivån av IGF-II hos manliga patienter, men inte hos kvinnliga patienter, var högre jämfört med friska kontroller. Däremot så var nivån IGF-II i blod oförändrad hos patienterna jämfört med kontrollerna.

Sammanfattande slutsats: Patienter med hjärninfarkt har förändrade nivåer av hormoner ingående i insulin/IGF-I systemet, och dessa förändringar är relaterade till utfall i form av funktionellt oberoende efter hjärninfarkt. Även vid Alzheimers sjukdom så är nivåerna av IGF-I och IGF-II förändrade.

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