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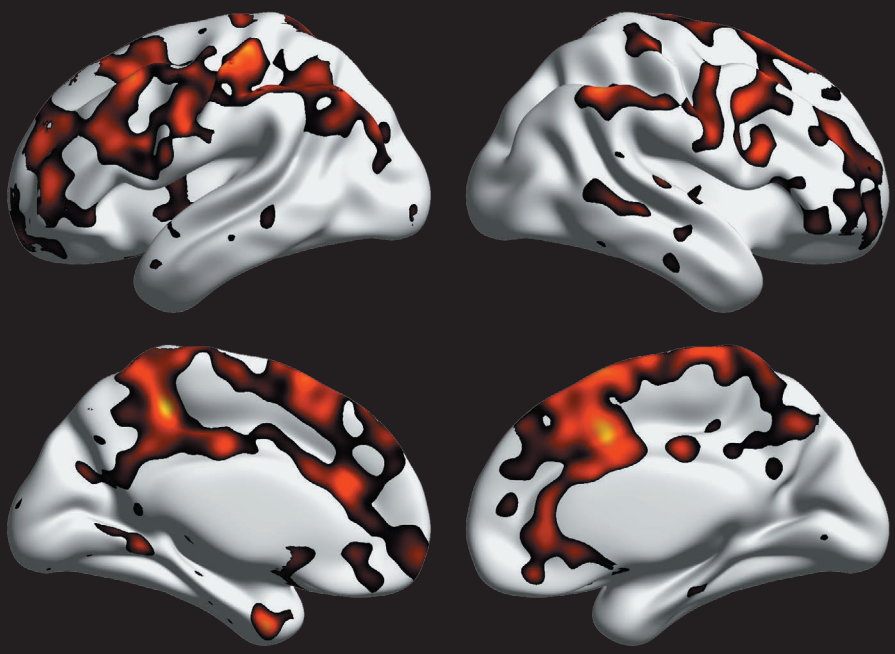

INSULIN RESISTANCE, COGNITION, AND BRAIN AMYLOID ACCUMULATION

An Epidemiological and a Positron Emission Tomography Study

Laura Ekblad

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To Arttu, Otso and Sisu

ABSTRACT

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Insulin resistance is a common phenomenon, closely associated with obesity, and defined as the inability of target tissues to respond normally to insulin. Insulin resistance typically precedes the onset of type 2 diabetes by several years. Type 2 diabetes is a risk factor for dementia and for Alzheimer´s disease (AD), the most common type of dementia. Some epidemiological studies suggest that insulin resistance increases the risk for dementia and AD, even in non-diabetic populations. *In vitro* and animal studies indicate that insulin resistance can contribute to the pathogenesis of AD through multiple different pathways.

This thesis was set out to explore the cross-sectional and longitudinal associations between insulin resistance and cognitive functioning in the Finnish large, nationwide Health 2000 survey, and its follow-up, Health 2011. The possible modulating effects of sex and apolipoprotein E ε4 genotype (*APOE*ε4), the most significant genetic risk factor for sporadic AD, were of specific interest. The aim of this thesis was also to investigate whether midlife insulin resistance increases the risk for brain amyloid accumulation, which is considered an early sign of AD.

Insulin resistance was associated with poorer verbal fluency in women and in non-carriers of *APOE*ε4 cross-sectionally (n=5935). Insulin resistance was an independent predictor of poorer verbal fluency performance after 11 years, and of a steeper decline in verbal fluency during the follow-up in both men and women, and in carriers and non-carriers of *APOE*ε4 (n=3695). Midlife insulin resistance increased the risk for brain amyloid accumulation, measured with positron emission tomography (PET), after a 15-year follow-up (n=60). The risk was similar in both carriers and non-carriers of *APOE*ε4.

These results indicate that midlife insulin resistance is an independent risk factor for cognitive decline, and for late-onset AD.

Keywords: Alzheimer's disease, *APOE*ε4, cognition, HOMA-IR, insulin resistance, [^{11}C]PIB, positron emission tomography, PET, verbal fluency.

TIIVISTELMÄ

Laura Ekblad
INSULIINIRESISTENSSI, KOGNITIO JA AIVOJEN AMYLOIDIKERTYMÄ
Turun yliopisto
Lääketieteellinen tiedekunta
Geriatria
Turun yliopiston kliininen tohtoriohjelma
Valtakunnallinen PET-keskus

Insuliiniresistenssi on tavallinen, keskivartalolihavuuteen liittyvä ilmiö, jolla tarkoitetaan eri kudosten heikentynyttä vastetta insuliinille. Insuliiniresistenssi edeltää tyypillisesti tyypin 2 diabeteksen puhkeamista vuosien ajan. Tyypin 2 diabetes lisää riskiä sairastua vanhuusiän muistisairauteen ja sen yleisimpään muotoon, Alzheimerin tautiin (AT). Joidenkin seurantatutkimusten perusteella insuliiniresistenssi lisäisi riskiä sairastua muistisairauteen myös henkilöillä, joille ei vielä ole kehittynyt tyypin 2 diabetesta. Insuliiniresistenssi voi vaikuttaa AT:lle tyypillisten aivomuutosten kehittymiseen useiden eri mekanismien kautta.

Tämän tutkimuksen tarkoituksena oli selvittää, onko insuliiniresistenssin ja muistin tai muiden tiedonkäsittelytoimintojen välillä yhteyttä väestötasolla, perustuen laajoihin Terveys 2000 ja 2011 -tutkimuksiin. Lisäksi selvitettiin, säätelevätkö sukupuoli ja/tai AT:n tavallisin geneettinen riskitekijä eli apolipoproteiini E -geenin ε4-geenimuoto (*APOE*ε4) insuliiniresistenssin ja tiedonkäsittelytoimintojen välistä yhteyttä. Positroniemissiotomografia (PET)-kuvausten avulla tutkittiin, lisääkö keski-iän insuliiniresistenssi riskiä aivojen amyloidikertymälle, jota pidetään AT:n varhaisena merkkinä.

Tutkimuksessa osoitettiin, että insuliiniresistenssi oli yhteydessä heikompaan suoriutumiseen kielellistä sujuvuutta mittaavassa testissä poikkileikkausaineistossa (n=5935). Tämä yhteys havaittiin vain naisilla, ja vain niillä, jotka eivät kantaneet *APOE*ε4-geenimuotoa. Sen sijaan 11 vuoden seurannassa insuliiniresistenssi ennusti heikompaa suoriutumista ko. testissä sekä naisilla että miehillä, *APOE*ε4-geenimuodosta riippumatta (n=3695). Keski-iän insuliiniresistenssi lisäsi myös riskiä aivojen amyloidikertymälle 15 vuoden seurannassa *APOE*ε4-geenimuodosta riippumatta (n=60).

Löydösten perusteella näyttää siltä, että keski-iän insuliiniresistenssi on itsenäinen tiedonkäsittelytoimintojen heikentymisen ja AT:n riskitekijä.

Avainsanat: Alzheimerin tauti, *APOE*ε4, HOMA-IR, insuliiniresistenssi, kielellinen sujuvuus, [^{11}C]PIB, positroniemissiotomografia, PET.

TABLE OF CONTENTS

ABBREVIATIONS

Aβ	beta-amyloid
AD	Alzheimer's disease
AIBL	Australian Imaging Biomarker and Lifestyle study
*APOE*ε4	Apolipoprotein E ε4 genotype
ARIC	Atherosclerosis Risk in Communities study
BBB	Blood-brain barrier
BDI	Beck´s Depression Inventory
BMI	Body Mass Index
CERAD	Consortium to Establish a Registry for Alzheimer´s Disease
CNS	Central nervous system
CSF	Cerebrospinal fluid
FDG	[^{18}F]fluorodeoxyglucose
hs-CRP	High sensitive C-reactive protein
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
IDE	Insulin degrading enzyme
IR-	HOMA-IR in the lowest tertile of the Health 2000 study population
IR+	HOMA-IR in the highest tertile of the Health 2000 study population
IWG	International Working Group
MCI	Mild cognitive impairment
MetS	Metabolic syndrome
MMSE	Mini-mental State Examination
MRI	Magnetic resonance imaging
NIA-AA	National Institute on Aging-Alzheimer's Association

OGTT	Oral glucose-tolerance test
OR	Odds ratio
PIB	Pittsburgh compound-B
PIB+	Amyloid positive PIB-PET scan
PET	Positron emission tomography
QUICKI	Quantitative Insulin Sensitivity Check Index
ROI	Region of interest
RT	Reaction time
SUVR	Standard uptake value ratio
VaD	Vascular dementia
VC	Visual choice reaction time
VF	Verbal fluency
WHO	World Health Organization
WLDR	Word-list delayed recall
WLL	Word-list learning
WMH	White matter hyperintensity

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications which are referred to in the text by the Roman numerals I–III:

I **Ekblad LL**, Rinne JO, Puukka P, Laine HK, Ahtiluoto SE, Sulkava RO, Viitanen MH, Jula AM (2015) Insulin resistance is associated with poorer verbal fluency performance in women. Diabetologia 58:2545–53.

II **Ekblad LL**, Rinne JO, Puukka P, Laine HK, Ahtiluoto SE, Sulkava RO, Viitanen MH, Jula AM (2017) Insulin resistance predicts cognitive decline – an 11-year follow-up of a nationally representative adult population sample. Diabetes Care 40:751–8.

III **Ekblad LL**, Johansson J, Helin S, Viitanen M, Laine H, Puukka P, Jula A, Rinne JO (2017) Midlife insulin resistance, APOE genotype, and late-life brain amyloid accumulation. In press. Neurology.

 In addition, some unpublished data are presented.

1 INTRODUCTION

Alzheimer´s disease (AD) and other diseases causing dementia have become increasingly important causes of disability in elderly people worldwide (World Health Organization, [WHO] 2003). Dementia is characterized by a progressive cognitive inability that influences activities of daily living, and people with dementia almost inevitably need costly long-term care at the end-stage of the disease. Age is the most important risk factor for dementia. Mainly due to a rise in life-expectancy, the prevalence of dementia is estimated to double during the next 15 years (Prince et al., 2015). Because of the enormous impact of dementia on aging societies, dementia is now considered a public health priority by the WHO (WHO and Alzheimer's Disease International, 2012).

Despite the acknowledgement of the global burden of persons with dementia and intensive research in the field, no curing treatment is yet available for AD or the less common forms of dementia. It has been estimated that if interventions could delay disease progression and onset by approximately one year, there would be roughly nine million fewer individuals with dementia in 2050 (Brookmeyer et al., 2007). The neuropathological changes of AD start to develop years or even decades before the onset of any clinical symptoms (Braak and Braak, 1997; Jack et al., 2010, Villemagne et al., 2013). This finding has led to a shift in the focus of dementia research from treatment to prevention, and from the elderly to the middle-aged.

It is well acknowledged that a low educational level is associated with an increased risk for cognitive decline and dementia. There is epidemiological evidence that cardiovascular risk factors in midlife, such as diabetes (Ott et al., 1999; Cheng et al., 2012), obesity (Kivipelto et al., 2005; Profenno et al., 2010), hypertension (Skoog et al., 1996; Launer et al., 2000; Kivipelto et al., 2005), and high cholesterol (for a review, see Anstey et al., 2017), or the combination of these risk factors (Kivipelto et al., 2005; Kivipelto et al., 2006; Exalto et al., 2014) increase the risk for cognitive decline and dementia. Targeting these modifiable risk factors could potentially decrease the incidence of dementia. In fact, promising evidence has recently been published suggesting that when different generations have been examined with equal criteria for diagnosing dementia, the prevalence of dementia would have stabilized or even declined over the last decades in high-income countries, despite the increase in life-expectancy (Wu et al., 2015). This positive trend is assumed to be caused by better health conditions, prevention and treatment of the aforementioned risk factors, and an increased educational level of successive generations.

Nevertheless, research targeted at finding specific, modifiable risk factors for dementia is needed. The recognition of diabetes as a risk factor for dementia has led to intensive efforts to detect the pathophysiological pathways behind this risk. It seems that insulin resistance would be an important link between diabetes and dementia. As measures of insulin resistance are rarely available from epidemiological studies, the associations among

insulin resistance, cognitive decline, and dementia have not been sufficiently explored. Thus, research focusing on the cognitive and the neuropathological changes associated with insulin resistance is needed to evaluate if insulin resistance is an independent risk factor for cognitive decline and dementia, and more specifically, AD. The epidemiological studies and the positron emission tomography (PET) neuroimaging study presented in this thesis were based on the cross-sectional and the follow-up analyses of the Health 2000 survey, a Finnish nationwide health examination survey. The follow-up nature of these studies rendered it possible to evaluate the effect of midlife insulin resistance on cognition after an 11-year follow-up, and the effect of midlife insulin resistance on brain neuropathology 15 years later *in vivo*.

2 REVIEW OF THE LITERATURE

2.1 Cognitive decline, dementia, and mild cognitive impairment

2.1.1 Definitions and diagnostic criteria

2.1.1.1 Cognitive decline

Cognitive decline is a general term that can be used to describe either a decline in cognitive test scores over time, measured with objective neuropsychological tests, or a subjective complaint of a decline in cognition (compared to the previous cognitive level of the individual). Cognitive decline is neither a clinical nor a diagnostic entity and thus, it does not *per se* suggest a clinically relevant decline in cognitive functioning.

In the general population, a slight decline with age on most cognitive domains is seen from the fourth decade onwards (Salthouse, 2010; Harada et al., 2013). There is, however, a difference between different cognitive domains on the decline associated with aging. In particular, information that is familiar and well-practiced such as vocabulary and general knowledge – a part of cognitive function that is called crystallized intelligence – is well-preserved in healthy aging. Tasks of fluid intelligence which require the processing of new information, executive function, learning and memory, tend to peak in the third decade and thereafter decline steadily with age. (Salthouse, 2012)

Although cognitive performance declines with aging, this decline is subtle in healthy aging, and it does not affect a person's ability to manage everyday activities of daily living independently. The term 'cognitive decline' is often used as a synonym for cognitive impairment or mild cognitive impairment, to imply a decline in cognition that is more severe than the decline seen in normal aging. This should be avoided since, as stated previously, no consensus criteria exist that would determine what is actually meant by cognitive decline. In terms of research, cognitive decline should only be reported if cognitive function has been measured in the same population at more than one time point.

2.1.1.2 Dementia

Dementia is a syndrome caused by a group of illnesses which cause loss of brain cortical functions, such as memory and other cognitive skills, severe enough to impair the ability to maintain activities of daily living (Alzheimer´s Association Report, 2014). In 2015 approximately 47 million people were estimated to have dementia worldwide, and the prevalence of dementia is estimated to nearly double during the next 15 years (Prince et al., 2015). AD is the most common form of dementia, comprising approximately 50–70 % of all dementias (figure 1; Winblad et al., 2016). Other dementia types include vascular dementia (VaD), frontotemporal dementias (degenerations), Lewy body disease, and dementia related to Parkinson´s disease. A combination of any two types of dementia is referred to as mixed dementia. The most common form of mixed dementia is the combination of AD and VaD.

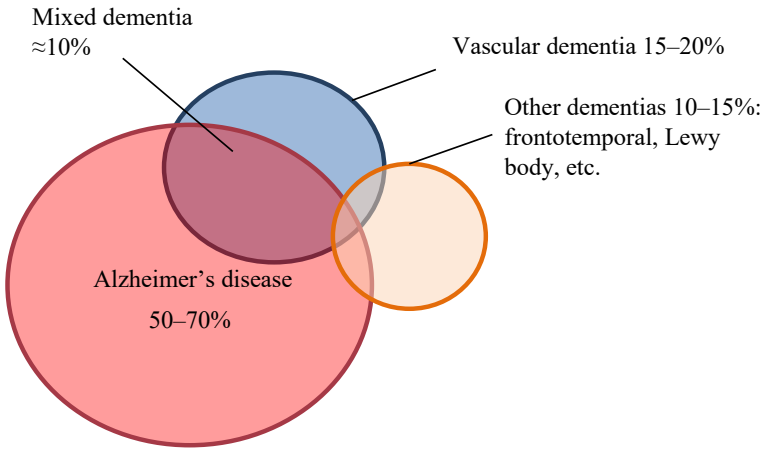


Figure 1. Estimated percentage of different types of dementia according to the Finnish Current Care Guidelines of Memory Disorders (2017).

The most widely used clinical criteria for dementia are currently the ICD-10 criteria (International Statistical Classification of Diseases and Related Health Problems 10th Revision) (World Health Organization, 2010) and the DSM criteria (Diagnostic and Statistical Manual of Mental Disorders) (American Psychiatric Association, 2013). Dementia can be diagnosed when cognitive decline compared to the previous level of cognitive performance is present, and severe enough to disturb activities of daily living. This decline should be measured by neuropsychological testing, or information on cognitive decline should be obtained from a knowledgeable informant. Importantly, cognitive decline should not occur exclu-

sively in the context of a delirium, and cannot be better explained by another mental disorder (for example, by a major depressive disorder or schizophrenia). Both set of criteria encourage to define by which specific disease dementia is caused by after the initial diagnosis of dementia. (World Health Organization, 2010; American Psychiatric Association, 2013). However, in epidemiological studies exploring possible risk factors for dementia, the different diseases causing dementia are rarely specified. Specific diagnoses may not be available from registries, or the prevalence of the less common types of dementia is too small to allow statistical reasoning. Thus, epidemiological studies most often report risk factors for dementia in general, or classify dementia into two categories: Alzheimer´s dementia and "non-Alzheimer´s dementia".

In the newest revision of the DSM criteria, the DSM-5 criteria (American Psychiatric Association, 2013), the term "dementia" is no longer used. Instead, neurocognitive disorders are divided into major and minor neurocognitive disorders. A neurocognitive disorder that has proceeded to the stage of dementia (i.e. a stage where activities of daily living are already impaired) is now classified as a major neurocognitive disorder. The diagnosis of a mild cognitive disorder can be made when there is evidence of modest cognitive decline on one or more cognitive domain, but the activities of daily living are still preserved. This revised approach for diagnosing neurocognitive disorders acknowledges the long time-course of the accumulating neuropathological changes of the different neurodegenerative diseases causing cognitive decline (Dubois et al., 2007); permits earlier diagnosis; and enables early treatment and interventions.

2.1.1.3 Mild cognitive impairment

Mild cognitive impairment (MCI) is a term that is used to describe the interphase between biological aging and dementia. There has been increasing interest to detect this condition, since it is likely that individuals with MCI are either at high risk for later developing dementia, or – according to the DSM-5 criteria and the revised diagnostic criteria for AD (see below) – are at a stage where the diagnostic criteria for a mild neurocognitive disorder are already met.

The 'Mayo criteria' or the 'Petersen criteria' for MCI were introduced in 1999 (Petersen et al., 1999). According to these criteria, mild cognitive impairment is defined as i) the presence of a subjective memory complaint; ii) the preserved ability to perform activities of daily living; iii) memory impairment demonstrated with cognitive testing or by a thorough interview of the patient or of a close relative or friend; iv) normal general cognitive function; v) absence of dementia. These crite-

ria are clinical and they do not specify which type of neuropsychological measurements are to be used. Consequently, no specific cut-off value for cognitive decline is given. For research purposes, however, the most widely used cut-off to define MCI is 1.5 standard deviations (SD) below age-adjusted cognitive test scores (Petersen, 2004; Artero et al., 2006).

The criteria for MCI were later revised by the International Working Group on Mild Cognitive Impairment (Winblad et al., 2004). It was acknowledged that different types of MCI existed, and that these subtypes of MCI could be caused by a variety of underlying conditions, such as a degenerative process, vascular lesions, metabolic disturbances, previous head trauma, or psychiatric conditions (Winblad et al., 2004). Therefore, MCI could be categorized into an amnestic type (amnestic MCI), where memory complaints are present, and to a non-amnestic type (non-amnestic MCI), where other forms of cognitive deficits are detected, but memory function is preserved. (Petersen, 2004; Winblad et al., 2004).

A meta-analysis on MCI showed that there is substantial variation in the annual conversion rates from MCI to dementia, depending on the criteria used, and if the study population came from a specialist clinic or from a community setting. Although not all, and in fact, not most individuals with MCI proceeded to develop dementia in the meta-analysis, the relative risk of progression to dementia, when compared to healthy individuals, was from 6.2 (non-amnestic MCI) to 15.9 (amnestic MCI). (Mitchell and Shiri-Feshki, 2009). A more recent review of longitudinal studies on MCI conversion to dementia reported annual conversion rates ranging from 7.5–16.5% in specialist settings and 5.4–11.5% in community settings (Ward et al., 2013).

In conclusion, these studies show that individuals with MCI constitute a risk group for dementia, but the majority of individuals with MCI will either recover or will not proceed to develop dementia during their lifetime. Of the subtypes of MCI, amnestic MCI seems to represent a risk for developing AD, whilst individuals with non-amnestic MCI will more probably develop another form of dementia. In terms of dementia and especially Alzheimer's dementia prevention trials the wide variation in the annual conversion rate from MCI to dementia has proved problematic, since treating individuals with MCI has meant that treatment will be given to many individuals who would never have proceeded to dementia, and this is likely to dilute possible effects of a preventive treatment (Dubois et al., 2007). Thus, other methods to detect preclinical AD – mainly based on biomarkers reflecting underlying neuropathology – are now often being utilized in dementia prevention trials and in implementation of early treatment.

This literature review will now focus on AD, and on the common risk factors for cognitive decline, dementia, and AD.

2.2 Alzheimer´s disease

AD is a progressive neurodegenerative disease, characterized neuropathologically by amyloid plaques, mainly consisting of misfolded beta-amyloid (Aβ), and by neurofibrillary tangles consisting of hyperphosphorylated tau-protein. (Scheltens et al., 2016). The typical symptoms of severe AD and the neuropathological changes typical for AD were first described by Alois Alzheimer, based on the case of Auguste D, a 51-year old woman presenting with memory impairment, reduced comprehension, aphasia, disorientation, and psychosocial impairment (Maurer et al., 1997).

There are two forms of AD. The rare, autosomally dominantly inherited familial type typically begins before the age of 65 years (early-onset AD or familial AD) and accounts for <1% of AD cases. The vast majority of AD cases are due to the sporadic form that begins after 65 years of age (late-onset AD). (Alzheimer´s Association 2014). Both types share similar neuropathological and clinical features, but it appears that the pathogenesis of especially late-onset AD is a complex process of which the details are not yet fully understood, despite intensive research efforts. (Scheltens et al., 2016).

2.2.1 Pathogenesis

The most widely recognized hypothesis for the pathogenesis of AD is the "amyloid cascade" hypothesis (Hardy and Higgins, 1992). According to this hypothesis, Aβ deposition would trigger a cascade of events that would lead to neuronal dysfunction, the formation of neurofibrillary tangles, and ultimately, neuronal death. The amyloid cascade hypothesis has later been revised (Hardy and Selkoe, 2002), and it has been acknowledged that short oligomers of Aβ may be more neurotoxic than the longer forms of Aβ that formulate amyloid plaques (Ballard et al., 2011). The hypothesis has also received criticism stating that Aβ might be necessary, but not sufficient for the development of AD. In particular, the amyloid cascade hypothesis seems to apply better for the rare early-onset cases of AD than for sporadic AD. (Musiek and Holzman, 2015). Other proposed hypotheses include the "mitochondrial cascade" hypothesis, claiming that mitochondrial dysfunction would precede Aβ and be the driver of both Aβ and neurofibrillary tangle formation (Swerdlow and Khan 2004). The "dual-pathway hypothesis" suggests, in accord with the mitochondrial cascade hypothesis, that there might be a common upstream driver for both Aβ and tau (Small and Duff, 2008). At present, the pathogenesis of sporadic i.e. late-onset AD is considered a multi-factorial process, where the accumulation

of Aβ is an early feature, but where other factors – including lifetime vascular risk factors – also play an important role (Scheltens et al., 2016).

2.2.2 Diagnostic criteria

The most widely used diagnostic criteria for AD are the NINCDS-ADRDA criteria (McKhann et al., 1984). These criteria are based on a clinical examination and they categorize individuals as having possible or probable AD. The diagnosis of probable AD is based on the detection of steadily progressive memory loss that starts gradually. A diagnosis of definite AD according to the NINCDS-ADRDA criteria can only be made post mortem, based on the neuropathological changes typical for AD.

Since these consensus criteria were published, new biomarkers to detect the pathological hallmarks of AD *in vivo* have become available. These methods include direct biomarkers of AD pathology such as $A\beta_{1–42}$, phosphorylated tau (P-tau) and total tau (T-tau) measured from cerebrospinal fluid (CSF), and neuroimaging with positron emission tomography (PET) utilizing radioligands that bind to amyloid or tau protein. Indirect biomarkers of AD include the detection of medial temporal lobe and hippocampus atrophy on magnetic resonance imaging (MRI), and cerebrocortical hypometabolism on [^{18}F]fluorodeoxyglucose (FDG) PET imaging. (Dubois et al. 2014). AD is now considered a continuum from an at-risk asymptomatic state (where the pathology of AD already exists, but no cognitive symptoms are present) to Alzheimer´s dementia, where neurodegeneration has proceeded to a stage where cognitive symptoms are evident, and activities of daily living compromised (Dubois et al., 2014; Jack et al., 2010; figure 2).

Therapeutic trials for the disease-modifying treatment of AD have been targeted at individuals with clinical AD i.e. Alzheimer´s dementia. In the last decade, all of these trials have, to a great disappointment in the field, failed. In retrospect, it has been argued that treatments targeted at the pathological hallmarks of AD, such as Aβ, have been aimed at a wrong patient population. In Alzheimer´s dementia there has already been substantial neuronal loss, which is probably the reason why patients with Alzheimer´s dementia have not profited from treatment with anti-amyloid drugs. (Herrup et al., 2013).

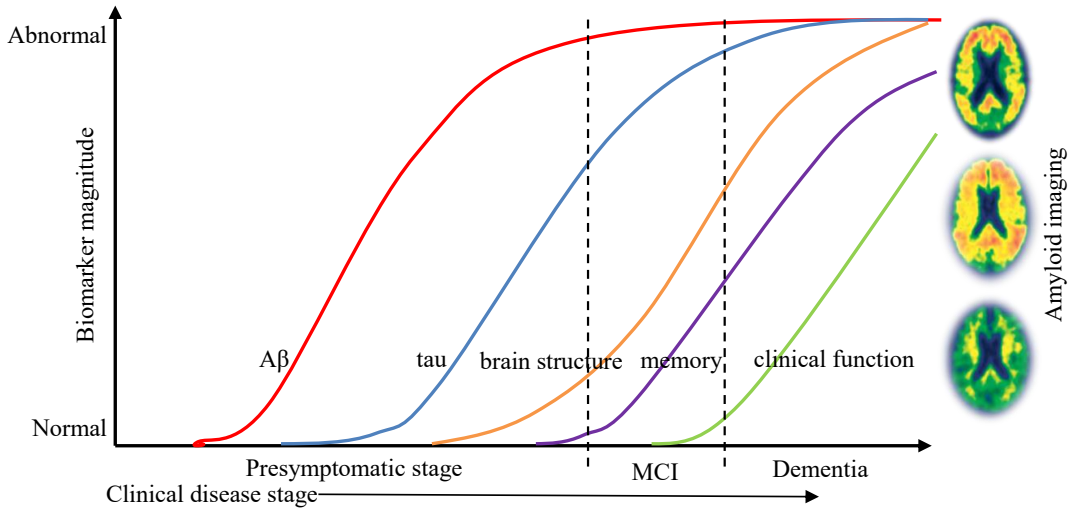


Figure 2. Hypothetical model of the temporal association of the biomarkers and the symptoms of AD, according to disease stage. Modified from Jack et al., 2010.

The acknowledgement of AD pathology developing years or even decades before the clinical onset of the disease (Morris, 2005; Jack et al. 2010; Jack et al., 2013; Villemagne et al., 2013) has led to the development of new criteria for AD for research purposes. The aim of these criteria is to detect individuals with AD neuropathology who are still symptom-free, and thus, could potentially benefit from preventive, disease-modifying treatment.

The research criteria for early detection of preclinical AD include the National Institute on Aging-Alzheimer's Association (NIA-AA) criteria (Sperling et al., 2011) and the International Working Group (IWG) criteria, which were originally published in 2007 (Dubois et al., 2007), and have since been revised in 2010 (Dubois et al., 2010) and in 2014 (Dubois et al., 2014). These criteria have introduced term 'asymptomatic, at risk for AD' (IWG criteria), and 'preclinical AD' (NIA-AA criteria), which refer to an individual who has *no sign of cognitive decline*, but who is *biomarker positive for AD neuropathology* (decreased $A\beta_{1–42}$ together with increased T-tau or P-tau in CSF or increased retention on fibrillar amyloid PET). The authors of the aforementioned articles propose that interventions targeted at modifying the pathologic cascade of AD should be aimed at this patient population.

Although the aforementioned revised criteria have been developed mainly for research purposes, they can be used also in the clinical setting, as neuroimaging with

especially MRI has become widely available in many developed countries. In Finland, the Current Care Guidelines for Memory Disorders (2017) recommend the IWG-1 criteria (Dubois et al., 2007) for diagnosing AD. According to these criteria, AD can be diagnosed when i) there is evidence of early and significant episodic memory impairment that is gradual and progressive for over six months (reported by the patient or an informant), and ii) this episodic memory impairment is objectively detected with appropriate cognitive tests. The episodic memory impairment can be isolated or associated with other cognitive changes. In addition to cognitive impairment, the diagnosis of AD requires the presence of one or more of the following biomarker criteria (the three first biomarkers are presented in the order in which they are recommended to be performed according to the Finnish Current Care Guidelines): i) presence of medial temporal lobe atrophy on MRI scan; ii) abnormal cerebrospinal fluid biomarker (decreased $A\beta_{1–42}$, increased T-tau or P-tau); iii) specific pattern on functional neuroimaging with PET (reduced glucose metabolism in bilateral temporal parietal regions on FDG-PET or amyloid positive PET scan); or iv) proven AD autosomal dominant mutation within the immediate family. (Dubois et al., 2007; Memory Disorders: Current Care Guidelines, 2017).

2.2.3 PET imaging in Alzheimer´s Disease

Neuroimaging with PET can be utilized to detect the early neuropathological features of AD, such as Aβ and tau protein. In addition to these direct biomarkers reflecting underlying pathology, FDG-PET imaging can be used to detect regional cerebral hypometabolism typical for AD. (Dubois et al., 2014). Most patients presenting with symptoms of AD – typically gradually progressive episodic memory impairment – do not require PET imaging for the diagnosis of AD. In the clinical setting, PET imaging is reserved for use in cases with diagnostic uncertainty; for patients presenting with progressive symptoms under the age of 65 years; or for patients in whom the clinical course of the disease is not typical (Minoshima et al., 2016).

2.2.3.1 Principles of positron emission tomography

Positron emission tomography (PET) is a molecular imaging method which can be used to examine the function of different organs *in vivo*. PET is based on the binding and distribution of specific molecules that have been labeled with a radioactive isotope (most commonly ^{11}C, ^{15}O, and ^{18}F) in the body. These radiolabeled molecules are called radioligands. When administered into the body (most often intravenously, but on some occasions also inhalation is used) the radioligand either

mimics the function of a molecule in the body (such as in the case of [^{18}F]FDG mimicking glucose) or binds to specific molecules such as receptors (for example, [^{11}C]raclopride that binds to dopamine receptors in the brain). (Turkington, 2001).

The radioactive isotopes are produced in cyclotrons. During the process a stable atom is accelerated until the nucleus receives one extra proton and becomes unstable, i.e. radioactive. The radioactive nuclei undergo decay via positron emission. As a result of this decay, the nuclei emit one positron and one neutrino. The positron can travel only a small distance in matter (approximately 1 mm) before losing its energy through ionization and excitation of nearby atoms and molecules. After losing its energy the positron will annihilate with an electron. This annihilation results in the emission of two photons with the energy of 511 keV that travel into opposite directions. (figure 3; Turkington, 2001).

The principle of the PET scanner is the possibility to detect both of the two photons that have been emitted by the annihilation of the positron and a nearby electron. During the course of the PET scan the scanner counts how many times a pair of detectors on opposite sides of the scanned object is receiving information from photons from the same line of response (LOR). The LOR is the straight line between the two photons that are travelling into opposite directions (figure 3). Because of the specific distribution of the radioligand in the body, most photons will be emitted from regions where the radioligand binds to during the scan. The distribution of the areas where the photons have been emitted from can be represented by grouping together parallel LORs to form the raw data of the PET scan. (Turkington, 2001).

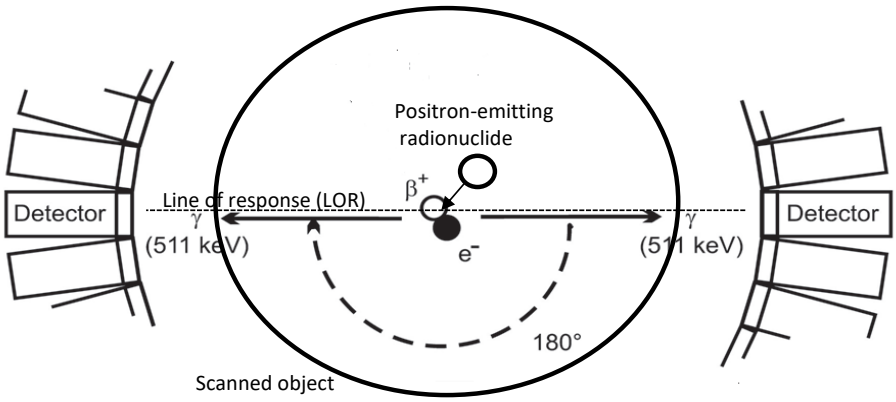
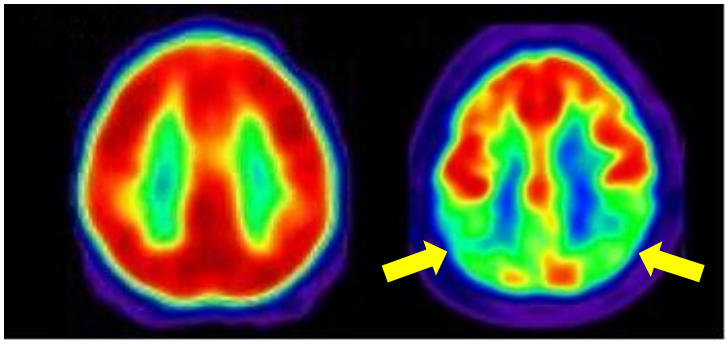


Figure 3. A schematic illustration of positron emission inside a scanned object, annihilation, and detection of two photons travelling in opposite directions along the line of response (LOR). Modified from Verel et al., 2005.

2.2.3.2 [^{18}F]Fluorodeoxyglucose

[^{18}F]FDG is a glucose analog where the normal hydroxyl group at the C-2 position in the glucose molecule has been substituted with positron-emitting radionuclide fluorine-18. FDG enters the brain through the same transport systems as glucose, and it provides information on glucose metabolism *in vivo*. The first report describing the usage of FDG for measuring local cerebral glucose utilization in humans with PET was published in 1979. (Reivich et al., 1979). Since then, distinct patterns of regional cerebral hypometabolism have been shown to occur in different neurodegenerative diseases. As glucose is the primary substrate of the synaptic function, regional cerebral hypometabolism is thought to reflect the loss of neurons in the region where hypometabolism is seen. (Foster et al., 2007; Garibotto et al., 2017).

In AD, FDG-PET typically shows a pattern of regional hypometabolism in the posterior cingulate cortex at early stages of the disease, and bilaterally in the temporoparietal regions at more advanced stages (figure 4). Metabolism in the occipital cortex is most often well-preserved. The frontal areas show reduced glucose metabolism typically only in advanced cases of AD, or in atypical cases presenting with dysexecutive function or behavioral disturbancies early in the disease progression. These regional patterns allow the differential diagnosis between AD and frontotemporal degenerations (where hypometabolism is found in frontotemporal regions), and between AD and Lewy body disease (where occipital hypometabolism, often extending to the primary visual cortex, is found) (Garibotto et al., 2017). A meta-analysis concluded that FDG-PET has the pooled sensitivity of 86%, and a specificity of 84% to differentiate AD patients from healthy controls. The ability to detect AD at the MCI stage showed a sensitivity of 76%, and a specificity of 74% (Frisoni et al., 2013). Thus, FDG imaging can be used to strengthen the diagnosis of AD in uncertain cases, and to differentiate between AD and other neurodegenerative diseases. For detecting AD in the MCI stage or even before any memory impairment, however, amyloid imaging has shown to be more sensitive than FDG-PET (Zhang et al., 2012).



A B

Figure 4. A typical pattern of cerebral glucose metabolism on FDG-PET in healthy controls (A) and in AD (B). The arrows point at the bilateral temporoparietal hypometabolism typical for AD. Image acquired and modified from http://www.alzforum.org/print-series/256356.

2.2.3.3 Amyloid imaging

Until approximately a decade ago the neuritic plaques typical for AD neuropathology could be detected only in *post mortem* examinations of the brain tissue. The introduction of radioligands that bind to Aβ have made it possible to evaluate the accumulation of Aβ *in vivo*, and to examine the temporal course of Aβ accumulation from a cognitively normal stage to the advanced stages of AD. (Villemagne et al., 2017).

In 2003 Mathis et al. (2003) introduced a new radioligand, [^{11}C]6-OH-BTA-1 which they named Pittsburgh compound-B (PIB). [^{11}C]PIB was shown to bind specifically to amyloid plaques in the brain tissue *in vitro*, and to readily enter the brains of mice and baboons (Mathis et al., 2003). The initial study in humans showed that [^{11}C]PIB retention was greater in AD patients when compared to healthy controls in the brain regions which were known to contain large amounts of neuritic plaques in AD based on neuropathological examinations, such as the frontal, parietal and temporal cortices (Klunk et al., 2004). After these preliminary findings, a large body of evidence has shown that Aβ accumulation starts already years before the any clinical symptoms of AD are present (figure 2; Jack et al., 2010; Jack et al., 2013; Villemagne et al., 2013), and that Aβ burden measured with PET imaging has good predictive value in individuals who are presymptomatic, or at the prodromal stages of AD. In MCI patients, the positive predictive value of cortical Aβ accumulation on converting to AD is over 80 % (Rowe et al.,

2013). Consequently, Aβ imaging is being used in the clinical setting to strengthen the diagnosis of AD for example in patients presenting with progressive symptoms under the age of 65 years, or in patients in whom the clinical course of the disease is not typical (Minoshima et al., 2016). However, there is increasing interest also to identify individuals in the presymptomatic stage of AD who could profit from, for example, anti-amyloid treatments (Villemagne et al., 2017). In clinical investigations and clinical drug trials amyloid imaging is already being used also for this purpose.

Because of the short half-life of 20 minutes of ^{11}C the use of [^{11}C]PIB is limited to centers with their own cyclotrons. Thus, radioligands labeled with ^{18}F have been developed to allow wider utilization of amyloid imaging. Of these, [^{18}F]florbetabir, [^{18}F]florbetaben, and [^{18}F]flutemetamol have been approved in the United States and in Europe by the respective drug administration authorities for clinical use, and are available for purchase and distribution commercially. (Villemagne et al., 2017).

Amyloid PET scans can be analyzed quantitatively or qualitatively. In the clinic the scans are rated visually on a binary scale as “amyloid positive” or “amyloid negative”, based on whether or not there is radiotracer uptake in the cerebral cortex i.e. the gray matter (figure 5). Amyloid negative PET scans normally show only nonspecific radiotracer binding in the white matter. (Minoshima et al., 2016). Quantitative, continuous measures of radiotracer uptake can be based on laborious methods involving up to 90 minutes of scanning time, arterial blood sampling and Logan analysis; or by using the carotid artery for estimated image based arterial input. A more simple method for quantitative analysis is to use the cortex of the cerebellum as a reference region for cortical radiotracer uptake, and to use a shorter PET scan duration for the image analyses. This simplified image analysis method has been shown to correlate well with the more laborious methods. (Lopresti et al. 2005). In scientific studies, different cut-offs for defining an amyloid positive PET scan according to the quantitative analysis of radioligand uptake have been reported for different radioligands, and for different study populations. For [^{11}C]PIB PET imaging, many groups have validated [^{11}C]PIB tissue-to-cerebellar cortex ratio >1.5 as a cut-off for amyloid positivity in healthy elderly controls. (Jack et al., 2008; Bourgeat et al., 2010; Rowe et al., 2010; Villemagne et al., 2013).

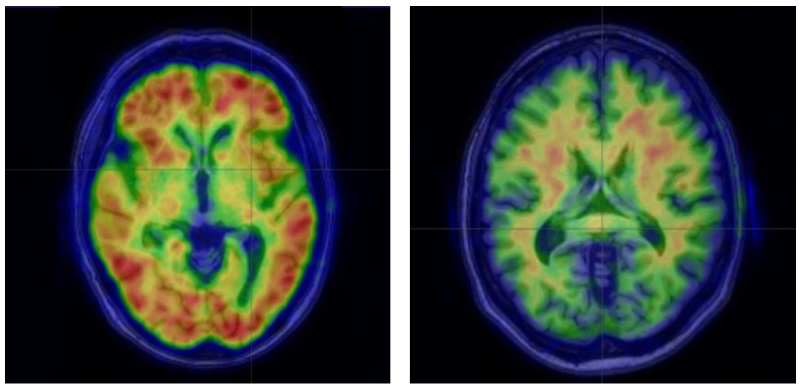


Figure 5. An example of an "amyloid positive" (A) and an "amyloid negative" (B) [^{11}C]PIB-PET scan of two participants in Study III.

2.2.3.4 Tau imaging

Neurofibrillary tangles consisting tau-protein are, in addition to neuritic plaques, the second neuropathological hallmark of AD. Neurofibrillary tangles are initially found in the transentorhinal cortex from where they spread to the hippocampus, and finally the other parts of the neocortex, reflecting different neuropathological stages (Braak and Braak, 1991). In contrast to Aβ accumulation, which reaches a plateau early in the disease process, the distribution of neurofibrillary tangles correlates with AD symptoms and severity (figure 2; Arriagada et al., 1992; Nelson et al., 2012). In recent years, radioligands that bind to tau-protein have been developed. The most widely used of these new radioligands is [^{18}F]AV-1541 (Chien et al., 2013). Tau imaging is not yet suitable for clinical use, but already numerous studies have reported differences in tau tracer retention between cognitively normal elderly and AD patients (Cho et al., 2016; Scholl et al., 2016; Wang et al., 2016; Johnson et al., 2017). In addition, preliminary findings suggest that tau tracer retention correlates with AD symptoms and cortical atrophy (Xia et al., 2017). There are still some issues limiting the interpretation of the results of studies performed with the presently available tau tracers – concerning for example the off-target binding of these tracers to the basal ganglia and the choroid plexus. Thus, "second generation" tau tracers that show no or less off-target binding to the aforementioned regions are under development. (Villemagne, 2017). Most probably, tau imaging will offer a possibility to evaluate the progression of AD neuropathology in relation to symptom progression *in vivo*. A combination of different AD biomarkers will probably provide better diagnostic accuracy in preclinical AD than any single biomarker alone.

2.3 Risk factors for cognitive decline, dementia, and Alzheimer´s disease

2.3.1 Genetic risk factors

There are three known dominantly inherited genetic mutations that cause the rare, familial, early-onset form of AD. Patients with these mutations typically develop symptoms of AD before the age of 65, and at times as early as at the age of 30 years. The mutations in presenilin 1, presenilin 2, and the amyloid precursor protein (APP) genes directly interfere with the processing and cleavage of APP in the brain, leading to overproduction of amyloidogenic Aβ. (Hardy and Selkoe, 2002; Alzheimer Disease and Frontotemporal Dementia Mutation Database, 2017)

The most important genetic risk factor for the most common form of AD, sporadic or late-onset AD, is the ε4 allelle of the apolipoprotein E gene (*APOE*) located in chromosome 19 (Corder et al., 1993, Saunders and Roses, 1993). Individuals who carry two copies of the ε4 allelle have a 50% risk of developing AD by the age of 85 years. In individuals who carry one ε4 and one ε3 allelle the risk for AD by the age of 85 years is 23% in men and 30% in women, and in the general population 11% for men and 14% for women. This suggests that *APOE* is a moderately penetrant gene with semi-dominant inheritance. (Genin et al., 2011). Human studies show that *APOE*ε4 i) increases the rate of cognitive decline in healthy *APOE*ε4 carriers (Caselli et al., 2004; Caselli et al., 2007); ii) increases the rate of conversion from MCI to Alzheimer´s dementia (Elias-Sonnenschein et al., 2011); iii) is associated with greater medial temporal lobe atrophy in individuals with MCI (Korf et al., 2004) or AD (Hashimoto et al., 2001); and iv) increases the risk for cerebral amyloid accumulation in cognitively normal *APOE*ε4 carriers (Jansen et al., 2015), when compared to non-carriers of *APOE*ε4.

The mechanisms by which *APOE*ε4 contributes to the pathophysiology of AD are multifaceted, and the evidence for these mechanisms mainly comes from rodent studies with transgenic mice, and *in vitro* studies (Liu et al., 2013). *APOE* codes the different isoforms of Apolipoprotein E (Apo-E): Apo-E2, Apo-E3 and Apo-E4. Studies on transgenic mice show that Apo-E4 impairs Aβ clearance in the brain, when compared to the Apo-E3 isoform (Castellano et al., 2011). Also, Aβ aggregation might be modulated through the different isoforms of Apo-E (Liu et al., 2013). Apo-E4 increases neurotoxicity triggered by for example Aβ or other neuronal stress factors (Huang, 2010).

A fairly recent finding is that the polymorphism of *TOMM40*, a gene located closely to the *APOE* gene on chromosome 19, can be used to improve the prognosis of the age of onset of late-onset AD, especially in individuals who carry the *APOE*ε3 allele. The *TOMM40* gene codes Tom40, the main channel on the mitochondrial outer membrane through which proteins pass to the mitochondria (Roses et al., 2010, Roses et al., 2016).

Other genes that have been associated with late-onset AD in large genome-wide association studies include the *CLU*, *BIN1*, *PICALM*, *CR1*, *ABCA7*, *MS4A6A*, *MS4A4E*, *CD33*, and *CD2AP* genes (Bertram et al., 2007). However, the added value of single-nucleotide polymorphisms on these genes on predicting AD has been modest, when compared to the *APOE* and the *TOMM40* genes (Roses et al., 2016).

The potential genetic risk factors for sporadic VaD are currently not well established. Most elderly individuals with clinically diagnosed AD also have signs of cerebrovascular disease on neuroimaging or in neuropathological examinations. Thus, it seems that the risk genes for AD also increase the risk for mixed dementia. (Perneczky et al., 2016).

2.3.2 Metabolic and vascular risk factors

It has been well-established that midlife vascular and metabolic risk factors, such as hypertension, obesity, high total cholesterol, and diabetes increase the risk for cognitive decline and dementia later in life. (Kivipelto et al. 2006; Exalto et al. 2014; Norton et al., 2014; Winblad et al., 2016; figure 6). Although findings from individual studies have been conflicting, large meta-analyses of individual risk factors suggest that the increased risk for dementia associated with obesity is approximately 1.59 (Profenno et al., 2010), for total cholesterol 2.14 (Anstey et al., 2017), and for diabetes 1.51 (Cheng et al., 2012). A meta-analysis of studies on hypertension and dementia yielded negative results (Guan et al., 2011) which might also reflect the association between anti-hypertensive medication and a reduced risk for dementia (Tully et al., 2016).

In addition to evaluating single vascular risk factors and risk for cognitive decline, also the combination of vascular risk factors have been evaluated in studies assessing the association between the metabolic syndrome (MetS) and cognition. A meta-analysis of longitudinal studies on MetS and cognition concluded that MetS was associated with cognitive decline in individuals younger than 70 years of age, but not in elderly individuals (Siervo et al., 2014).

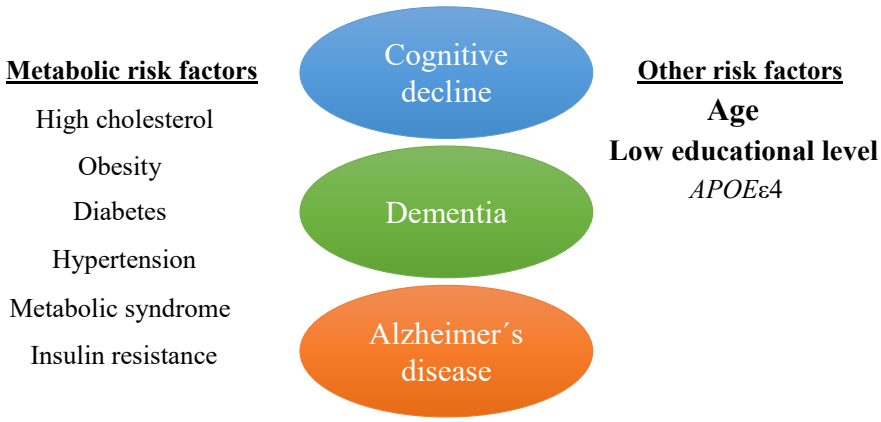


Figure 6. Common risk factors for cognitive decline, dementia, and Alzheimer´s disease.

The importance of measuring vascular risk factors already in midlife is emphasized by studies such as the aforementioned study on MetS and cognition (Siervo et al., 2014), and the meta-analysis of total cholesterol and risk for dementia (Anstey et al., 2017) that indicate that the increased risk for dementia associated with vascular risk factors seems to apply only for midlife, and not late-life risk factors. In fact, the onset of clinical dementia might be preceded by weight loss (Profenno et al., 2010), and a subsequent decrease in other cardiovascular risk factors.

Potentially, better prevention, treatment and management of the risk factors for dementia should, over time, decrease the age-dependent incidence of dementia. Based on data from the Rotterdam Study, a 30% reduction in dementia cases could be achievable if the most common modifiable risk factors (obesity and overweight, hypertension, diabetes, unfavorable cholesterol values, and a low educational level) could be eliminated (de Bruijn et al., 2015). Accordingly, a decline in the prevalence of dementia (when equal criteria diagnostic criteria for dementia have been used over the entire course of the study) has been reported from longitudinal studies conducted in Sweden, Spain, the Netherlands and the UK (Wu et al., 2015). This decline over the last decades is likely the result of improved education, healthier life-styles, and better management of vascular risk factors.

Although a consensus exists of an association between midlife vascular risk factors, cognitive decline, and dementia, the mechanisms by which these risk factors contribute to the pathophysiology of AD and other types of dementia are not yet sufficiently established. Some studies suggest that the risk between midlife vascular risk factors and AD would be, at least partly, mediated through brain vascular lesions (Hughes and Craft, 2016), and that in addition to Aβ and tau, brain vascular lesions might be necessary or a “second-hit” to the clinical manifestation of AD

(Provenzano et al., 2013). Thus, the prevalence of mixed dementia with features from both AD and VaD neuropathology would be greater than often acknowledged, with the majority of late-onset AD cases presenting features from both AD and VaD (Hughes and Craft, 2016; Perneczky et al., 2016; Scheltens et al., 2016).

The expanding evidence of an association between diabetes and dementia, and between MetS and a decline in cognition, has led to an intensive search for a link between diabetes, MetS, and the pathogenesis of AD. It has become evident that type 2 diabetes and AD share many common pathological pathways, which has led to some researchers even proposing that AD could be called "type 3 diabetes" (Steen et al., 2005; de la Monte, 2014). This finding has resulted in an increased interest towards insulin resistance, the hallmark of type 2 diabetes and MetS. Next, the associations among of insulin resistance, cognition and dementia will be further discussed.

2.4 Insulin resistance

2.4.1 Definition and measurements

Insulin resistance is commonly defined as the resistance to insulin-stimulated glucose uptake (Reaven 1988), or the inability of target tissues to respond to normal circulating concentrations of insulin (Goldstein 2002). Insulin resistance is the core feature of MetS and type 2 diabetes (Reaven 1988; Goldstein 2002; Kahn et al., 2014). There are some conditions, such as pregnancy and puberty, where insulin resistance can be regarded physiological (Wallace and Matthews 2002). However, in most cases insulin resistance is a pathologic condition with numerous harmful effects in the body (Reaven 1988).

Insulin resistance and hyperinsulinemia typically precede the onset of type 2 diabetes by years or even decades (Reaven 1988; Goldstein 2002; Kahn et al., 2014). In this prediabetic stage blood glucose levels are maintained normal or only slightly elevated by the increased secretion of insulin from pancreatic beta-cells. Although fasting plasma glucose and even oral glucose tolerance test levels may be normal, hyperinsulinemia and insulin resistance *per se* have been shown to associate with cardiovascular risk factors, such as hypertension, hypertriglyceridemia and low HDL cholesterol values. A combination of these risk factors was first named "Syndrome X" (Reaven 1988), and has later been renamed as the "insulin resistance syndrome" (Reaven 2004) or MetS (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001). In addition to the aforementioned risk factors, insulin resistance increases the likelihood of

many different abnormalities, such as endothelial dysfunction, procoagulant factors, hemodynamic changes, increased inflammatory markers, and abnormal uric acid metabolism, thereby associating with an increased risk of not only type 2 diabetes and cardiovascular disease, but also hypertension, polycystic ovary syndrome, nonalcoholic fatty liver disease, sleep apnea and certain forms of cancer (Reaven 2004).

The different methods to assess insulin resistance have been reviewed by Matthews et al. (Wallace and Matthews 2002; Wallace et. al., 2004) and, more recently, by Cersosimo et al. (2014). The golden standard for measuring insulin resistance is the euglycemic insulin clamp (DeFronzo et al., 1979). However, this method is rarely used in the clinical setting or in large studies, due to its time-consuming and fairly invasive nature. Instead, more simple methods such as the Homeostatic Model Assessment of insulin resistance (HOMA-IR) (Matthews et al., 1985) or the Quantitative Insulin Sensitivity Check Index (QUICKI) (Katz et al., 2000), have been developed to evaluate insulin sensitivity based on venous blood sampling. The other methods used to measure insulin resistance (the hyperglycemic clamp, the intravenous glucose tolerance test with minimal model, the insulin sensitivity test, the short insulin sensitivity test, the Matsuda Index, and the oral glucose tolerance test, OGTT) are beyond the scope of this review.

The hyperinsulinemic-euglycemic clamp is based on the assumption that hepatic glucose production is completely suppressed at high insulin-infusion doses. At this steady-state the rate of infused glucose is thought to equal whole-body glucose disposal, and thus, the clamp method can be used to measure insulin resistance. The smaller the amount of glucose needed to maintain the predetermined blood glucose concentration is, the greater is the degree of insulin resistance. During the clamp glucose is maintained at a predetermined concentration by adjusting a glucose infusion according to blood glucose concentrations measured at 3 to 5 minute intervals. Simultaneously, a fixed-rate insulin infusion, calculated as a dose per unit of body surface area of the study volunteer, is administered. (Wallace and Matthews, 2002; Charmaine et al., 2012)

In contrast to the euglycemic clamp method which measures stimulated insulin resistance, the HOMA-IR method estimates basal insulin resistance after an overnight fast. HOMA-IR is a mathematical model which is based on experimental data from humans and animals on the rate of insulin production, hepatic glucose- and insulin dependent glucose efflux and uptake, and insulin decay, i.a. (Wallace et al., 2004). HOMA-IR has been reported to correlate well with the euglycemic insulin clamp ($R=0.88$, $p<0.0001$, Matthews et al., 1985; $R=0.85$, $p<0.0001$, Bonora et al., 2000). HOMA-IR can be counted simply by the equation fasting plasma glucose times fasting plasma insulin, divided by 22.5. Ideally, since the secretion

of insulin is pulsatile, the mean value of three measures of fasting insulin, taken at five-minute intervals should be used. However, larger studies have commonly used only a single measurement of insulin. An updated, computer-based version of HOMA-IR was published in 1998 (Levy et al., 1998), to provide non-linear modelling, and to account for variations in hepatic and peripheral glucose resistance.

To date, there is no defined cut-off value for defining insulin resistance according to HOMA-IR that would apply to different populations. HOMA-IR varies according to age, sex, and ethnicity, and it is probable that different cut-off values will be needed for different populations. WHO encourages the cut-off being set at the 75th percentile of a non-diabetic population (Alberti and Zimmet, 1998). According to this recommendation, the cut-off for HOMA-IR has varied from 2.0 in a Swedish population (Hedblad et al., 2000) to 3.8 in a French population (Marques-Vidal et al., 2002). Many studies have used other cut-offs than the 75th percentile, for example the highest tertile (Summer et al., 2008) or the 90th percentile (Geloneze et al., 2006; Timóteo et al., 2014) of the populations´ HOMA-IR values.

The QUICKI, similarly to HOMA-IR, is based on fasting insulin and glucose measurements. QUICKI is counted by the equation QUICKI=1/[(log(fasting plasma insulin) + 1 log(fasting plasma glucose)], and it has been shown to correlate well with the euglycemic clamp method (Katz et al., 2000). However, as pointed out by Wallace et al. (2004), QUICKI is actually the logarithmic transformation of the original HOMA-IR equation, and thus the same advantages and disadvantages apply to the usage of QUICKI as to the original HOMA-IR equation.

2.4.2 Insulin, insulin resistance, and the central nervous system

Insulin, a peptide secreted by pancreatic beta-cells, has many important functions in the central nervous system (CNS). Insulin is actively transported through the blood-brain barrier (BBB) by a transporter in a saturable manner. In addition to transporting insulin from the blood flow to the CNS, the insulin-binding sites act as receptors and activate intra-cellular signaling cascades that alter the functions of the BBB cells in numerous ways. (Banks et al., 2012).

The main behavioral actions of insulin in the CNS consist of regulation of cognition and feeding. Insulin receptors exist throughout the brain. They have been found in the synapses of both astrocytes and neurons, and they are particularly abundant in the olfactory bulb, the cerebral cortex, and in the hippocampus. Insulin signaling contributes to synaptogenesis and synaptic remodeling. (Abbott et al., 1999, Cholerton et al., 2013). In addition to these receptor-site actions, insulin is thought to have an effect on long-term potentiation (an essential part of memory

formation) (Skeberdis et al., 2001). The traditional view has been that circulating insulin levels would not affect cerebral utilization of glucose. However, a FDG-PET study showed that basal insulin levels increase cortical cerebral glucose metabolism in humans, compared to level a where insulin production was suppressed with a somatostatin infusion (Bingham et al., 2002). Animal studies suggest that in contrast to peripheral (peripheral is used here to refer to organs outside the CNS) insulin, CNS insulin elevates blood glucose levels, decreases feeding and body weight, and has even been shown to decrease blood insulin levels (Banks et al., 2012).

Insulin resistance has traditionally thought to occur mainly in the muscles, the liver, and in fat cells (Reaven 1988, Goldstein 2002). In recent years, however, evidence of insulin resistance occurring also in the CNS has started to accumulate. In obese rodents the binding of insulin to the endothelium of the BBB was reduced when compared to lean animals, and plasma insulin levels correlated negatively to specific insulin binding in the BBB. A similar inverse relationship was found between higher plasma insulin and a lower number of insulin receptors in the liver, suggesting that peripheral hyperinsulinemia downregulates the expression of insulin receptors at the BBB in a similar way as in the peripheral tissues. (Schwartz et al., 1990). A magnetoencephalographic study showed that in obese humans the cerebrocortical response to infused insulin during a hyperinsulinemic euglycemic clamp was reduced when compared to lean individuals (Tschritter et al., 2006). These results indicate that *peripheral hyperinsulinemia* leads to a *reduced* insulin response in the brain, probably due to lower insulin concentrations in the CNS as a result of insulin transporter down-regulation at the BBB.

2.4.3 Insulin resistance and the risk for cognitive decline and dementia

2.4.3.1 Possible pathogenic mechanisms

As explained above, persistent peripheral hyperinsulinemia can lead to an insulin-resistant brain state, where CNS insulin levels are reduced.

It seems that reduced levels of insulin in the CNS can directly contribute to the neuropathology of AD in multiple ways. The interplay of insulin and Aβ, one of the neuropathologic hallmarks of AD, appears to be complex (for a recent and thorough review, see Mullins et al., 2017). In human and rodent cell cultures insulin reduces the phosphorylation of amyloid precursor protein, and increases the level of antiamyloigenic proteins, such as the insulin degrading enzyme (IDE) and β-secretase (Pandini et al., 2013). In addition to insulin, IDE degrades even Aβ in

the brain. Insulin is needed also for the transportation of intracellular Aβ to the plasma membrane and further to the extracellular space, where Aβ clearance is thought to occur (Cholerton et al., 2013). Thus, *reduced* insulin levels in the CNS can lead to reduced levels of antiamylogenic proteins, and both the overproduction and an impaired clearance of Aβ (figure 7). Furthermore, CNS insulin prevents the soluble oligomeric form of Aβ (which is thought to play a pivotal role in neuronal loss in the AD brain due to its synaptotoxicity) from binding to synapses, thereby preserving the normal function of synapses. Thus, reduced insulin levels can even promote the actions of toxic Aβ oligomers (De Felice et al., 2009).

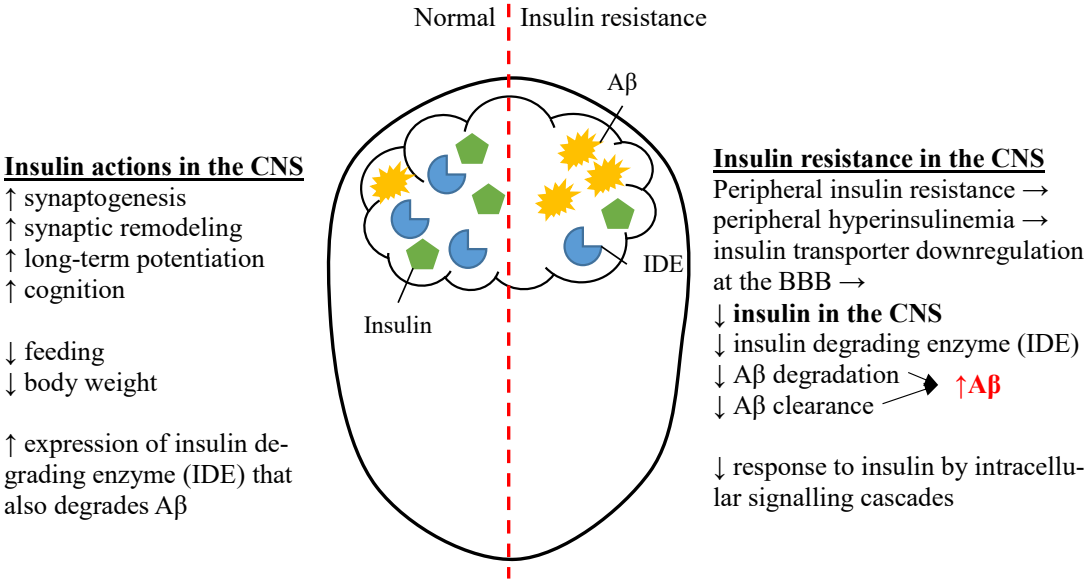


Figure 7. Insulin actions in the central nervous system (CNS), and proposed consequences of insulin resistance in the CNS. Based on Cholerton et al., 2013. IDE: insulin degrading enzyme.

Other insulin-related mechanisms in AD neuropathology include the formation of neurofibrillary tangles from hyperphosphorylated tau-protein. Insulin inhibits the phosphorylation of tau, and again, when CNS insulin levels are reduced, the level of hyperphosphorylated tau is elevated (Cholerton et al., 2013, Mullins et al., 2017).

Insulin resistance has also been shown to occur in the AD brain at cellular level. The response of intracellular insulin signaling cascades – triggered by the activation of insulin receptors at the cell membrane – to insulin incubation was lower in the brain tissues of AD patients than in those with MCI, and lower in MCI patients than cognitively normal individuals, regardless of diagnosis of diabetes during life.

(Talbot et al., 2012). Thus, in addition to the influence of reduced CNS levels of insulin to AD neuropathology, also the attenuated response of insulin receptors to insulin can play an important role in the AD brain.

One possible pathological link between insulin resistance, type 2 diabetes, and AD could be the accumulation of amylin deposits in the pancreas and in the brain (Jackson et al., 2013). Amylin (also known as islet amyloid polypeptide, IAPP) is a hormone that is secreted by the pancreatic beta cells simultaneously with insulin. In hyperinsulinemia also amylin secretion is elevated. Excess amounts of amylin in the blood flow causes oligomerization of amylin (Westermark et al., 2011) and deposit formation in a similar way as with Aβ in the brains of AD patients. Jackson et al. (2013) showed that amylin plaques can be detected *post mortem* in the brain temporal lobe gray matter of AD patients, regardless of whether they had been diagnosed with diabetes during their lifetime.

In addition to the direct effects of insulin resistance on AD neuropathology, insulin resistance has negative effects on cerebrovascular function (reviewed by Hughes and Craft, 2016). MetS – of which insulin resistance is the key feature – is associated with an increased risk of stroke (Li et al., 2017) and brain white matter lesions (Segura et al., 2009). The brain is highly dependent of adequate microvascular blood flow, which is maintained by vascular reactivity, and mediated by nitric oxide and endothelial function. Individuals with insulin resistance have been shown to have lower cerebral blood flow in the cortex than normal controls (Rusinek et al., 2015). The vascular injuries associated with insulin resistance might promote the neuropathological changes of AD by, for example, disturbing Aβ transportation between the CNS and periphery. In turn, Aβ deposits in the blood vessel wall can induce inflammation thereby damaging the endothelium (Bell and Zlokovic, 2009). To summarize, insulin resistance is linked to vascular cognitive impairment through brain vascular lesions which, in turn, might be a “second hit” that contributes to the clinical symptoms of AD in the presence of AD neuropathological changes, i.e. Aβ and neurofibrillary tangles (figure 8; Provenzano et al., 2013).

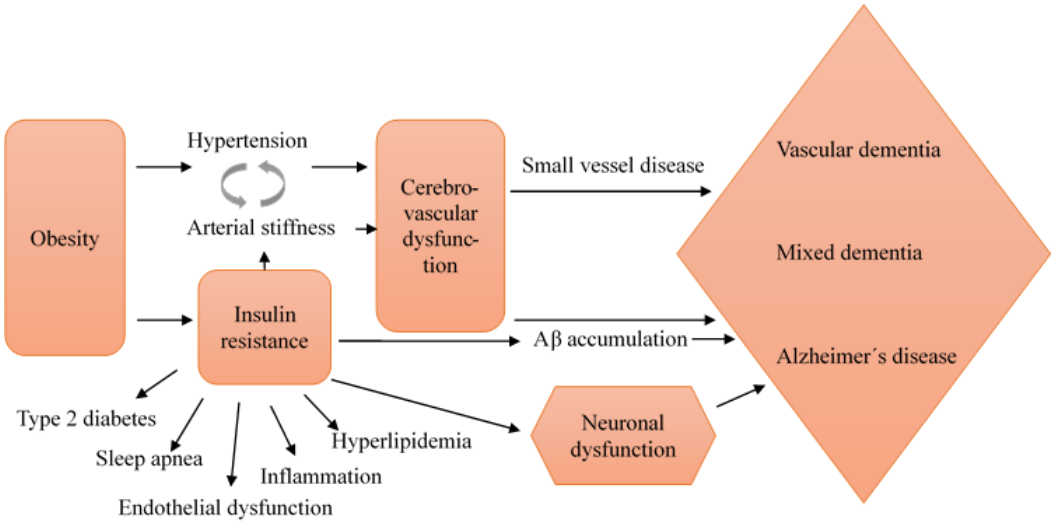


Figure 8. Schematic drawing of the complex interplay of vascular risk factors, cerebrovascular disease, and the neuropathology of AD, VaD and mixed dementia. Modified from Hughes and Craft, 2016.

2.4.3.2 Epidemiological studies

There are a number of cross-sectional epidemiological studies associating insulin resistance with poorer cognitive functioning in the elderly (for a summary of cross-sectional studies, see Table 1.). Studies on middle-aged populations are less frequent (Schuur et al., 2010; Tan et al., 2011; Sanz et al., 2013; Backeström et al., 2015). Table 2 summarizes the longitudinal studies showing an association between insulin resistance and cognitive decline. The cross-sectional and longitudinal studies indicate that insulin resistance is most often associated with lower scores on tests measuring executive functions, such as verbal fluency performance, and less frequently with tasks measuring memory.

A few studies have assessed the risk between insulin resistance and dementia. The first large, epidemiological study linking hyperinsulinemia with AD was conducted in Kuopio, Finland. In the cross-sectional study of 980 randomly selected elderly individuals hyperinsulinemia was associated with AD (Kuusisto et al., 1997). Higher levels of insulin were associated with a two-fold risk of AD in non-demented elderly individuals during a mean follow-up time of 5.4 years in the population-based Northern Manhattan study (n=683) (Luchsinger et al., 2004). In the Honolulu-Asia study both low and high levels of insulin were associated with an increased risk for dementia in elderly men (n=2568), during a follow-up of approximately 5 years (Peila et al., 2004). Insulin resistance, measured with HOMA-

IR, was associated with an increased risk for dementia in a 32-year follow-up study of Swedish men (n=2322) (Rönnemaa et al., 2008). In the Rotterdam study (n=3139) higher HOMA-IR associated with an increased risk for AD within three years from baseline, but not during a longer follow-up (Schrijvers et al., 2010).

Observational studies on metformin, an insulin-sensitizing and the most widely used oral antidiabetic agent, have shown that metformin usage was associated with a decreased risk for cognitive impairment in individuals with diabetes (Ng et al., 2014), and that in patients with newly diagnosed diabetes those taking metformin had a smaller risk of developing dementia during a mean follow-up of 2.4 years than patients taking other oral antidiabetic agents (Cheng et al., 2014).

2.4.3.3 Neuropathological studies

Only two studies have, thus far, assessed the risk of AD neuropathology associated with insulin resistance in post mortem studies, and the results are inconsistent. The Hisayama study showed that measures of glucose intolerance, fasting insulin and HOMA-IR, assessed 10–15 years before death, were associated with an increased risk for neuritic i.e. Aβ plaques. No association was found between the aforementioned measurements and neurofibrillary tangles. (Matsuzaki et al., 2010). In contrast, no association was found between repeated measures of insulin resistance and glucose intolerance during lifetime over a 20-year-period, and AD neuropathology in the Baltimore Longitudinal Study of Ageing (Thambisetty et al., 2013b).

2.4.3.4 Cerebrospinal fluid studies

A few cross-sectional studies on insulin resistance and CSF biomarkers have been published. In a study from the Wisconsin Alzheimer's Research Center (n=70) higher HOMA-IR levels associated cross-sectionally with higher levels of CSF AD biomarkers (Hoscheidt et al., 2016). In a subpopulation (n=58) of the Metabolic Syndrome in Men Study from Kuopio, Finland, no difference was found between insulin resistant and non-insulin resistant men in CSF AD biomarkers, but plasma insulin levels correlated with CSF Aβ/tau ratio in the entire study population (Westwood et al., 2017). Very recently, the Australian Imaging Biomarker and Lifestyle (AIBL) study reported that higher HOMA-IR associated with elevated CSF T-tau and P-tau in cognitively normal elderly individuals (n=36) (Laws et al., 2017). To date, no longitudinal studies have been published on insulin resistance and CSF AD biomarkers.

Table 1. Summary of cross-sectional studies on insulin resistance and cognition.

Reference	N	Mean age or range (years)	Measures of insulin resistance	Cognitive tests	Results associated with insulin resistance	Sex difference	*APOE* difference
Kuusisto et al., 1993	744*	73	fP-insulin	neuropsychological test battery	↓ semantic memory, language, problem solving	not reported	not reported
Kalminj et al., 1995	462 (only men)*	69 to 89	fP- insulin, euglycemic hyperinsulinemic clamp	MMSE	↓ MMSE	not reported	not reported
Stolk et al., 1997	5510*	~68	2 h OGTT insulin and insulin-to-glucose ratio	MMSE	↓ MMSE	YES, only in women	not reported
Kilander et al., 1998	999 (only men)*	69 to 75	fP-insulin, euglycemic hyperinsulinemic clamp	MMSE, TMT-A and TMT-B	↓ cognitive z-scores	not reported	not reported
Abbatecola et al., 2004	597*	65 to 93	HOMA-IR	MMSE, TMT-A and TMT-B	↓ TMT-A and TMT-B, but not MMSE	not reported	not reported
Geroldi et al., 2005	523*	70 to 90	HOMA-IR, QUICKI	MMSE	↓ executive function	not reported	not reported
Schuur et al., 2010	1898†	18 to 86	HOMA-IR	neuropsychological test battery	↓ executive function	YES, only in women	not reported
Tan et al., 2011	2439*	62	HOMA-IR, fP-insulin	neuropsychological test battery	↓ executive function and visuospatial memory	not reported	not reported
Benedict et al., 2012	285*	75	HOMA-IR	7 MS	↓ verbal fluency	not reported	not reported
Zhong et al., 2012	328‡	~79	HOMA-IR, fP-insulin	neuropsychological test battery	↓ MMSE, orientation, delayed memory, attention	not reported	not reported
Sanz et al., 2013	1172*	35 to 64	HOMA-IR	4 cognitive tests	↓ Stroop, not significant in adjusted analyses	NO	not reported
Backeström et al., 2015	291*	51	HOMA-IR, fP-glucose	episodic and semantic memory tests	No association	NO	not reported
Laws et al., 2017	905§	71	HOMA-IR	neuropsychological test battery	↓ episodic memory, executive function, global cognition	YES, only in women	not reported

*Population-based study. †Family-based cohort. ‡Sample of patients from a geriatric hospital. §Volunteer-based sample.

2.4.3.5 Neuroimaging studies

There is a growing number of MRI studies linking insulin resistance with alterations in brain structure in regions that control memory performance, and that show atrophy in AD, such as the medial temporal lobe and, more specifically, the hippocampus. HOMA-IR has been associated with lower total brain volume (Tan et al., 2011), and lower temporal lobe gray matter volume (Benedict et al., 2012) in cross-sectional studies. In addition, higher HOMA-IR was associated with progressive atrophy of medial temporal lobe gray matter during a follow-up of four years (Willette et al., 2013). Higher levels of HOMA-IR have been associated with lower total hippocampal volume in late middle-aged women (Rasgon et al., 2011). More recently, higher HOMA-IR was cross-sectionally associated with alterations in brain white matter integrity (Ryu et al., 2014), and with reduced functional activity, assessed with functional MRI, during an episodic memory test in the hippocampus, the angular gyrus, and the prefrontal cortex (Cheke et al., 2017).

The association between brain function and insulin resistance has been studied also with PET imaging (Table 3.). Baker et al. (2011), and Willette et al. (2015a) have found that in cognitively normal older adults (Baker et al.) and in late middle-aged adults at risk for AD (Willette et al.) higher HOMA-IR associated with reduced brain glucose metabolism in frontal, parietotemporal and cingulate regions, assessed with FDG-PET. These are regions that show reduced metabolic activity also in AD. Interesting findings regarding the temporal association of insulin resistance and brain glucose metabolism according to disease progression come from a FDG-PET study that included cognitively normal controls, individuals with MCI that progressed to AD during two years of follow-up, individuals with MCI that remained stable, and individuals with AD. In AD patients higher HOMA-IR associated with cerebral hypometabolism, whilst in MCI progressors the correlation between HOMA-IR and glucose metabolism was positive, i.e. higher HOMA-IR levels associated with hypermetabolism in the medial temporal lobe and in the hippocampus (Willette et al., 2015b). Regional glucose *hypermetabolism* has previously been shown to correlate with amyloid accumulation in MCI patients, whilst in AD patients cortical amyloid burden correlated with cerebral *hypometabolism* (Cohen et al., 2009). Thus, hypermetabolism might be an early compensatory reaction to Aβ accumulation.

Table 2. Summary of longitudinal studies on insulin resistance and cognition, including studies published after Study II.

Reference	N	Follow-up time (years)	Age at baseline (years)	Measures of insulin resistance	Cognitive tests	Results associated with insulin resistance	Sex difference	*APOE* difference
Kilander et al., 1998	999 (only men)*	20	50	fP-insulin	TMT-A and TMT-B	↓ cognitive z-score at follow-up	not reported	not reported
Okereke et al., 2005	718 (only women)*	10	61 to 69	C-peptide	Telephone interview test battery	↓ Global cognition and verbal memory	not reported	not reported
Young et al., 2006	7148*	9	45 to 64	fP-insulin and HOMA-IR	Delayed recall, DSS, verbal fluency	↓ cognitive test scored at follow-up and a decline in cognition	not reported	not reported
Willette et al., 2013	121*	4	57.7	HOMA-IR	Rey Auditory Verbal Learning Test	↓ cognition at follow-up	not reported	not reported
Fava et al., 2017	477†	6.8	66	HOMA-IR	MMSE, ADAS-Cog, ADAS-ADL	↓ MMSE, ADAS-Cog, but not ADAS-ADL	not reported	not reported
Hughes et al., 2017	4392*	10	45 to 84	fP-insulin and HOMA-IR	CASI, Digit Span, Digit Symbol coding	Change in HOMA-IR: ↓ cognition at follow-up	not reported	association stronger in ε4 negative
Lutski et al., 2017	489‡	~20	57.7	HOMA-IR	Computerized test battery	↓ cognition, executive function and memory	not reported	not reported
Neergaard et al., 2017	1759 (only women)*	15	68	HOMA-IR	Short blessed test, verbal fluency	↓ performance on both tests, ↑ odds for cognitive dysfunction	not reported	not reported
Tortelli et al., 2017	797*	20	53.3	HOMA-IR	MMSE	↓ MMSE at follow-up	not reported	not reported

*Population-based study. †Case-control study (diabetics vs non-diabetics). ‡Patients with cardiovascular disease at baseline.

To date, the only longitudinal study evaluating impaired glucose tolerance and change in brain function with repeated PET scans used radio-labeled water (^{15}O-water) to evaluate regional cerebral blood flow in cognitively normal individuals during eight years with annual PET scans. The results showed that in the 15 individuals with impaired glucose tolerance 12 years before the first of eight PET scans regional cerebral blood flow declined during the follow-up time in frontal, parietal and temporal cortices, when compared to the 49 individuals with normal glucose tolerance. Measures of insulin resistance were not reported in the study. (Thambisetty et al., 2013a).

To date, three studies have examined the association between insulin resistance and Aβ accumulation *in vivo* with PET imaging. In a cross-sectional study of 186 late middle-aged, cognitively normal adults at risk for AD higher HOMA-IR was associated with higher [^{11}C]Pittsburgh-compound B (PIB) uptake (reflecting higher Aβ accumulation) in frontal and temporal regions in individuals who were normoglycemic, but not in those with hyperglycemia (Willette et al. 2015c). In contrast, in the Baltimore Longitudinal Study of Aging repeated measures of glucose intolerance and insulin resistance during 20 years showed no association with [^{11}C]PIB uptake in a study sample of 53 individuals at a mean age of 79 years (Thambisetty et al., 2013b). Recently, no association was found between HOMA-IR and brain amyloid accumulation in a cross-sectional study of cognitively normal elderly volunteers (n=262) in the AIBL study (Laws et al., 2017).

The Atherosclerosis Risk in Communities (ARIC) study offered indirect evidence for an association between midlife insulin resistance and late-life brain amyloid accumulation. In the ARIC study (n=322) the association of midlife vascular risk factors (obesity, diabetes, smoking, hypertension, and hypercholesterolemia) and late-life amyloid accumulation were evaluated (Gottesman et al., 2017). The study found that midlife, but not late-life risk factors were associated with an increased risk for an amyloid positive PET scan. Of the five vascular risk factors evaluated in the study only obesity independently predicted an amyloid positive PET scan at follow-up. Measures of insulin resistance were not reported.

Table 3. Summary of neuroimaging studies utilizing PET to evaluate the association between insulin resistance and cerebral alterations.

Reference	N	Age at baseline (years)	Follow-up	Measures of insulin resistance	radioligand	What was measured?	Results associated with insulin resistance	Sex difference	*APOE* difference
Baker et al., 2011	29	74	NO	HOMA-IR	[^{18}F]FDG	Cerebral glucose metabolism	↓ in frontal, parietemporal and cingulate regions	not reported	not reported
Thambisetty et al., 2013a	64	57.2	Up to 8 annual scans from 69.6 years	2 h OGTT	[^{15}O]water	Regional cerebral blood flow	↓ in frontal, parietal and temporal cortices	not reported	not reported
Thambisetty et al., 2013b	53	53	26 years	2h OGTT, HOMA-IR	[^{11}C]PIB	Brain amyloid load	No association found	not reported	*APOE* not assessed
Willette et al., 2015a	150	60.7	NO	HOMA-IR	[^{18}F]FDG	Cerebral glucose metabolism	↓ in frontal, parietal and temporal cortices	not reported	not reported
Willette et al., 2015b	280	75	NO	HOMA-IR, QUICKI	[^{18}F]FDG	Cerebral glucose metabolism	↓ in AD patients ↑ in MCI progressors	not reported	not reported
Willette et al., 2015c	186	60.4	NO	HOMA-IR	[^{11}C]PIB	Brain amyloid load	↑ amyloid burden in frontal and temporal areas	not reported	not reported
Gottesman et al., 2017	322	45–64	23.5 years	BMI, diabetes	[^{18}F]Florbetapir	Brain amyloid load	↑ midlife BMI predicted ↑ amyloid burden	not reported	No interaction found
Laws et al., 2017	262	71	NO	HOMA-IR	[^{11}C]PIB, [^{18}F]Florbetapir, [^{18}F]Flutemetamol	Brain amyloid load	No association found	not found	not reported

2.4.3.6 Insulin-related therapies targeting cognitive decline

To date, several studies utilizing insulin-related therapies to treat cognitive impairment and/or AD have been published. Treatment of patients with MCI or mild to moderate AD with intranasal insulin for four months improved memory (Craft et al., 2012; Craft et al., 2017), and was associated with preserved volume on MRI and with reduction in CSF tau-P/Aβ42 ratio (Craft et al., 2017). However, these were small studies with 12 (Craft et al., 2017) or 30–38 (Craft et al., 2012) patients per group.

In addition to intranasal insulin treatment, treatment of MCI and AD with insulin-sensitizing oral antidiabetic agents have been studied. A phase III, 24 week clinical trial (n=693) on rosiglitazone in mild-to-moderate AD reported no significant difference between the treatment and control groups in cognitive test scores (Gold et al., 2010). In a pilot study on metformin 80 obese or overweight, non-diabetic individuals with amnestic MCI were treated either with placebo or with metformin. The treatment group performed slightly better on a test measuring total recall than the control group after 12 months of treatment (Luchsinger et al., 2016). The influence of metformin treatment on preventing cognitive decline has also been evaluated. Data from a recent, large randomized trial (n=2280), the Diabetes Prevention Program, showed that treatment of individuals at risk for developing diabetes with metformin decreased the risk for diabetes, but had no effect on cognitive performance at 8 and 10 years after the intervention (Luchsinger et al., 2017).

Glucagon-like peptide-1 (GLP-1) analogs such as liraglutide and exenatide are a group of injectable drugs for the treatment of type 2 diabetes. GLP-1 receptor activation improves learning and memory in mice models (During et al., 2013), and reduces cognitive decline in transgenic AD mice (Hansen et al., 2015). In humans, one randomized, placebo-controlled, double-blind study (n=38) evaluated the effect of liraglutide treatment on cerebral glucose metabolism and amyloid accumulation with PET, and cognition in AD patients over six months. Cerebral glucose metabolism was preserved or slightly increased in the treatment group, whilst the placebo group showed a decline in cerebral glucose metabolism. No difference in cognitive performance or amyloid accumulation was found between the groups. (Gejl et al., 2016). DPP-4 inhibitors (another group of drugs for treatment of type 2 diabetes) inhibit the enzyme dipeptidyl peptidase-4 (DPP-4) that metabolizes GLP-1. Thus, treatment with DPP-4 inhibitors increases the half-life of endogenous GLP-1, retaining its functions. (Deeks 2012). Animal studies have reported that treatment with DPP-4 inhibitors can improve cognition and insulin sensitivity (Pipatpiboon et al., 2013; Gault et al., 2015). Randomized, controlled trials on DPP-4 inhibitors and cognitive function in humans are lacking.

Potentially, targeting life-style risk factors as early as in midlife could also improve cognition and prevent cognitive impairment and dementia, especially in obese individuals with impaired glucose tolerance and insulin resistance. Disappointingly, two large intervention studies aimed at preventing diabetes have shown that life-style interventions, such as a healthier diet and regular exercise, in midlife had no effect on cognitive outcomes after a follow-up of 8–10 years (Luchsinger et al., 2015; Luchsinger et al., 2017).

2.4.3.7 Sex and APOEε4 genotype as modulators of insulin resistance and cognition

Some of the studies linking insulin resistance with AD have suggested an interaction between insulin or insulin resistance and *APOE*ε4 genotype and risk for AD (Kuusisto et al., 1997; Rönnemaa et al., 2008). A few studies have found a sex difference in the association between insulin resistance and cognition (Stolk et al., 1997; Schuur et al., 2010; Laws et al., 2017). One longitudinal study on the metabolic syndrome and cognition found that the metabolic syndrome predicted cognitive decline over a follow-up of 16 years only in women (McEvoy et al., 2012). However, in most epidemiological studies on insulin resistance and cognition the possible interactions between insulin resistance and *APOE*ε4 genotype, and insulin resistance and sex have not been reported (see Tables 1 and 2 for a summary). Interestingly, the AIBL study found that the association between higher HOMA-IR and higher levels of CSF P-tau and T-tau (reflecting AD neuropathology) was significant in women only (Laws et al., 2017).

Findings by Craft et al. (1998 and 1999) have shown that the pattern of insulin metabolism seems to be different in AD patients with and without the *APOE*ε4 genotype, and possibly different between men and women with AD. A reduced CSF-to-plasma insulin ratio is thought to reflect reduced brain insulin uptake in the presence of peripheral hyperinsulinemia (see chapter 2.4.3.1). AD patients who were ε4/ε4 homozygotes did not differ from healthy controls in terms of CSF-to-plasma insulin ratio. In contrast, AD patients who were not homozygous for the ε4 allele had a reduced CSF-to-plasma insulin ratio when compared to healthy adults and AD patients with two copies of the ε4 (Craft et al., 1998). Furthermore, AD patients who did not carry the *APOE*ε4 genotype had lower insulin sensitivity than AD patients with one or two ε4 alleles, or normal controls who were *APOE*ε4 non-carriers. In the same study women with AD had lower insulin sensitivity than men. (Craft et al. 1999).

A recent preclinical study explored the possible mechanisms between *APOE*ε4 genotype and insulin signaling in primary neurons in vitro, and in apoE4-targeted

replacement (TR) mice, in which murine *APOE* is replaced by human *APOE* (Zhao et al., 2017). It was shown that aged *APOE*ε4-TR mice developed cerebral insulin resistance, which was further accelerated in mice with a high fat diet that caused peripheral insulin resistance. Cerebral insulin resistance was not found in APOEε3-TR mice. Moreover, the experiments with primary neurons showed that Apo-E4 lipoprotein particles bound to the insulin receptor at the cell surface of primary neurons more strongly than Apo-E3 lipoprotein particles. Also, Apo-E4 reduced the binding between insulin and insulin receptor, when compared to Apo-E3. In addition, Apo-E4, but not Apo-E3, significantly downregulated the amount of insulin receptors at the cell surface, providing further evidence for an association between Apo-E4 and impaired insulin signaling. (Zhao et al., 2017). These findings provide a mechanistic background for the possible interactions between APOEε4 and insulin on cognition.

Studies on insulin treatment and cognition have also reported sex and *APOE*ε4 differences in treatment outcomes. The effect of both intravenously and intranasally administered insulin on memory and cognitive performance has been studied in cognitively normal individuals, and in patients with MCI and AD. Benedict et al. (2008) found that memory was improved after administration of intranasal insulin in women, but not in men. In AD and MCI patients only *APOE*ε4 negative individuals benefited from intranasal insulin in terms of cognitive performance (Reger et al. 2006). The results regarding a sex difference in response to intranasal insulin in MCI and AD patients are less clear, and might be dose-dependent. After four months of treatment with either 20 IU or 40 IU intranasal insulin both men and women showed improvement on delayed-story memory on the 20 IU dose, but only men improved with the higher dose of 40 IU (Claxton et al. 2013).

In the Honolulu-Asia study diabetes was an additive risk factor for amyloid accumulation in carriers, but not in non-carriers of *APOE*ε4, suggesting an interaction for *APOE*ε4 genotype and diabetes on brain Aβ accumulation (Peila et al., 2002). The possible interactions of *APOE*ε4 genotype and insulin resistance on the neuropathology of AD have not yet been extensively studied.

Taken together the findings reviewed here, there is preliminary evidence that the effects of insulin and insulin resistance on cognition and the risk for AD vary by sex and *APOE*ε4 genotype. Women and non-carriers of *APOE*ε4 seem to be more susceptible to the harmful effects of persistent peripheral insulin resistance on the CNS than men and *APOE*ε4 carriers.

2.5 Summary of the literature

Altogether, there is a body of evidence suggesting that insulin has important effects on brain functioning and, conversely, that the effects of insulin resistance in the CNS are detrimental. However, there are still many open questions in the field. It seems that sex and *APOE*ε4 genotype might modulate the effects of insulin and insulin resistance on cognitive functioning, but the mechanisms behind these findings remain unclear. Epidemiological studies have shown that vascular risk factors in midlife increase the risk for late-life dementia and the most common form of dementia, AD. As the neuropathology of AD starts to develop years or even decades prior to the clinical symptoms of the disease, it seems plausible to assume that midlife vascular risk factors could contribute to the neuropathological hallmarks of AD. The evidence of a link between insulin resistance and Aβ accumulation gained from *in vitro* and animal studies seems convincing. Nevertheless, studies examining the effect of midlife insulin resistance on late-life AD neuropathology in humans are scarce. Thus, research focusing on midlife insulin resistance and its effects on cognitive functioning, and on cerebral changes over time is needed to better understand the link between insulin resistance and dementia.

3 OBJECTIVES OF THE STUDY

This thesis was set out to investigate the possible associations among insulin resistance, cognitive functioning, and brain amyloid accumulation in the Finnish adult population. In addition, the possible interactions between insulin resistance and *APOE*ε4 genotype, and between insulin resistance and sex on cognitive functioning were studied.

The specific objectives of this thesis are:

I To evaluate if insulin resistance associates with cognitive functioning in the Finnish adult population, and if this association is modulated by sex or *APOE*ε4 genotype.

II To evaluate if insulin resistance predicts cognitive functioning and cognitive decline during 11 years, and if sex or *APOE*ε4 genotype modulate these longitudinal associations.

III To evaluate if midlife insulin resistance is a risk factor for brain amyloid accumulation, measured 15 years after the baseline measurement of insulin resistance, and if this risk would be different in carriers and non-carriers of the *APOE*ε4 genotype.

Note: The roman numerals refer to the original publications.

4 MATERIALS AND METHODS

The materials and methods for Studies I–III are covered in the following sections. Study I was based on the Health 2000 health examination survey – a representative sample of the Finnish adult population aged 30 years or more – and study II was based on the Health 2000 survey and its follow-up, the Health 2011 survey. Study III was based on a study sample of 60 volunteers, drawn from the Health 2000 study population. All participants gave written informed consent before agreeing to participate in the studies. The Health 2000 and the Health 2011 surveys were approved by the ethical committee of the Hospital District of Helsinki and Uusimaa, and Study III was approved by the ethical committee of the Hospital District of Southwest Finland. All studies were conducted according to the declaration of Helsinki.

4.1 Overall study design

The study design of the Health 2000 and the Health 2011 surveys has been previously thoroughly described in the respective methodology reports of the studies (Aromaa and Koskinen, 2004; Lundqvist and Mäki-Opas 2016). Simplified schematic drawings of the study protocols of Studies I, II, and III are provided below in figures 9, 10 and 11.

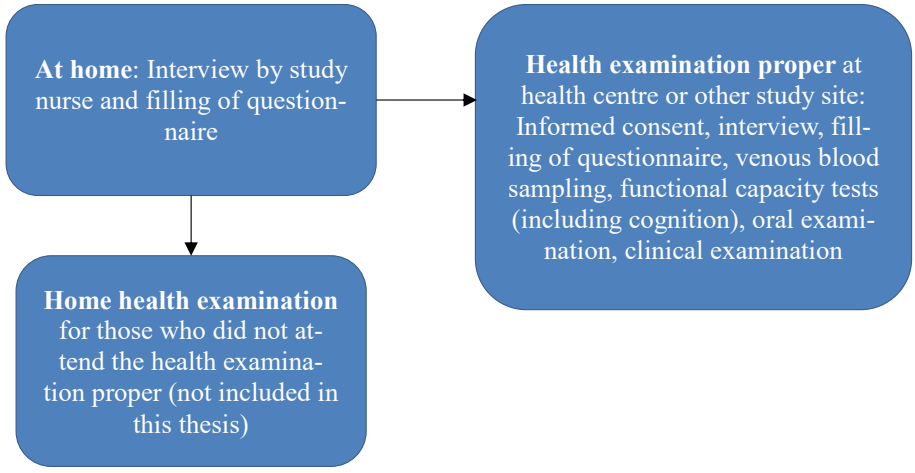


Figure 9. Health 2000 survey protocol (Modified from Aromaa and Koskinen, 2004).

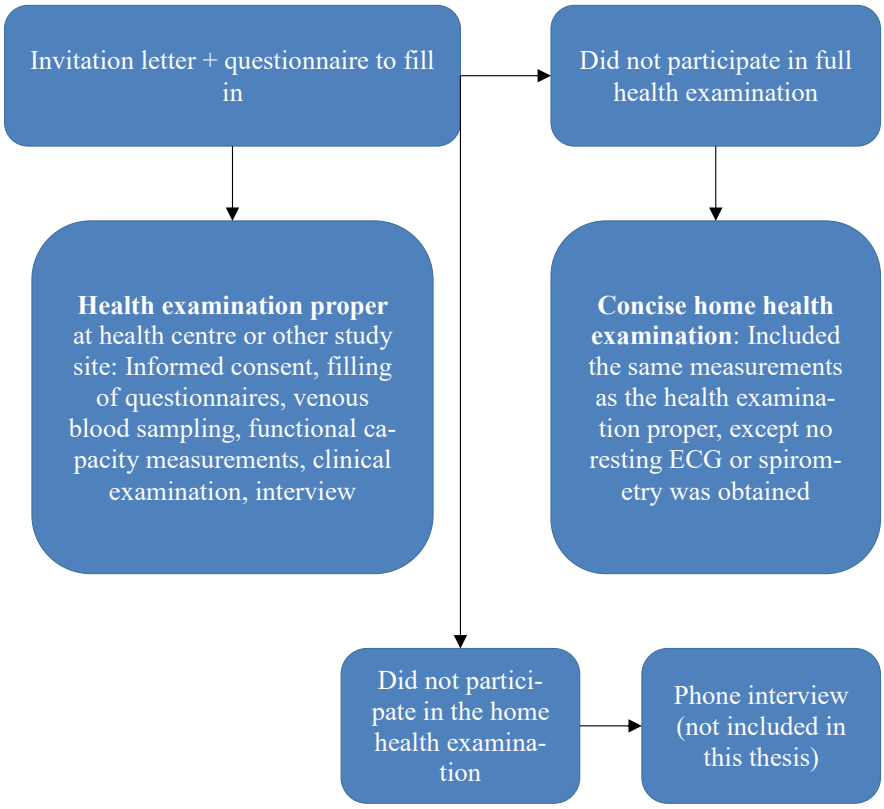


Figure 10. Health 2011 survey protocol (Modified from Lundqvist and Mäki-Opas, 2016).

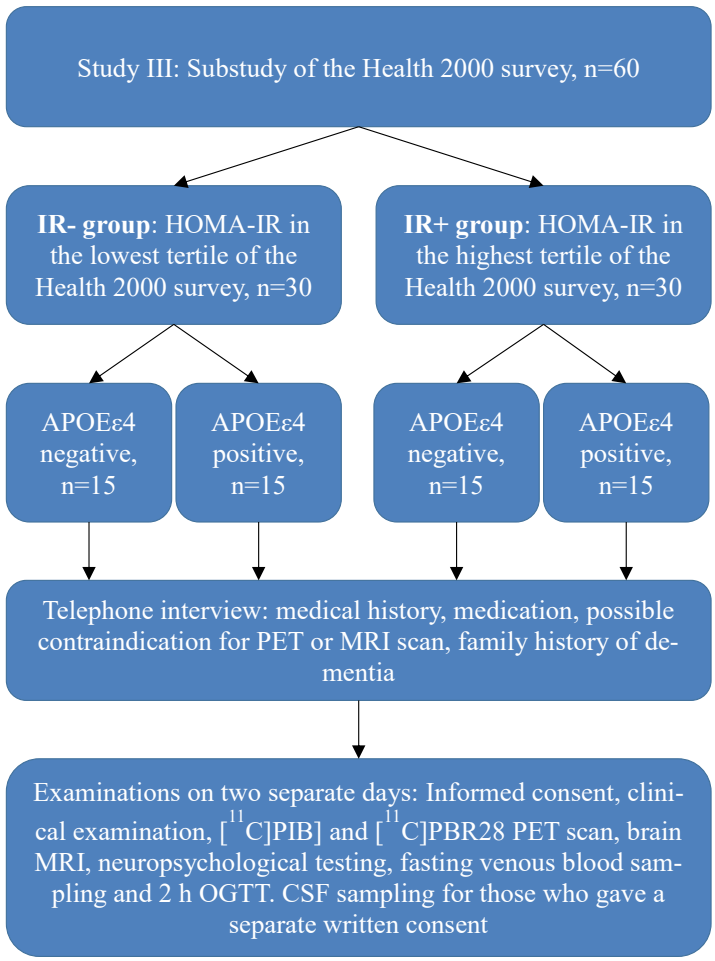


Figure 11. Study protocol of Study III.

4.2 Study populations

The study population of Study I was based on the Finnish nationwide, comprehensive health examination survey, the Health 2000 survey, which was conducted in Finland in 2000–2001 by the Finnish National Institute for Health and Welfare. The Health 2000 survey was a representative sample of the Finnish adult population. In the survey 8028 individuals aged 30 years or more were randomly selected from the Finnish population register from 80 health service districts throughout Finland using a two-stage stratified cluster sampling procedure. 79% (n=6354) of the study population attended the health examination proper. (Aromaa and Koskinen, 2004). Study I included 5935 individuals who had attended the health examination proper; fasted for four hours or longer prior to venous blood sampling;

who were not treated with insulin; who had plasma insulin and glucose values available; and who had completed most of the cognitive tests (figure 12).

The Health 2011 survey, carried out in the year 2011, was a follow-up examination of the Health 2000 survey. In 2011 all the individuals who had been invited to participate in the original Health 2000 survey, who were alive, and still lived in Finland, and who had not refused to participate in the Health 2000 survey, were invited to participate in the Health 2011 follow-up survey (Koskinen et al., 2012). The study population of Study II consisted of the 3695 individuals who, in addition to being included in Study I, had participated in the health examination proper or the home health examination in 2011 and thus had cognitive test scores available from both time points (figure 12).

Study III was based on a subsample of 60 volunteers from the Health 2000 study population. The number of participants in Study III was planned according to the power calculations of test-retest analyses of [^{11}C]PIB-PET scans, which indicate that for a 90% power to obtain statistically significant difference between groups, five persons per group would be needed to detect a 15% difference in [^{11}C]PIB accumulation in the frontal cortex (Aalto et al., 2009). The study was designed to assess a difference in [^{11}C]PIB uptake between individuals with normal (IR- group, n=30) and elevated (IR+ group, n=30) levels of insulin resistance in midlife. To evaluate the possible modulating effect of *APOE*ε4 genotype, both study groups were enriched for *APOE*ε4 carriers, resulting in 50% (n=15) *APOE*ε4 carriers in both groups.

The volunteers for Study III were recruited by a recruitment letter sent by the National Institute for Health and Welfare, and based on the following criteria: i) birth year 1934–1949 (age at time of PET scan 65–80 years), ii) HOMA-IR in the year 2000 in the highest tertile of the Health 2000 study population (HOMA-IR>2.17, IR+ group) or HOMA-IR in the lowest tertile of the Health 2000 study population (HOMA-IR<1.25, IR- group), and iii) *APOE*ε4 genotype carriership status. The IR+ and IR- groups both consisted of 30 individuals, and half of the participants in both groups (n=15) were *APOE*ε4 carriers (ε4/ε4 or ε4/ε3 genotype, individuals with both a risk allele and a protective allele i.e. ε4/ε2 were not included in the study). Exclusion criteria were a contraindication for PET or MRI scan (such as claustrophobia), history of major stroke, diagnosis of dementia, type 2 diabetes in 2000 and, for the IR- group, diagnosis of diabetes after the year 2000.

A flow chart of the study populations in studies I–III is provided in figure 12.

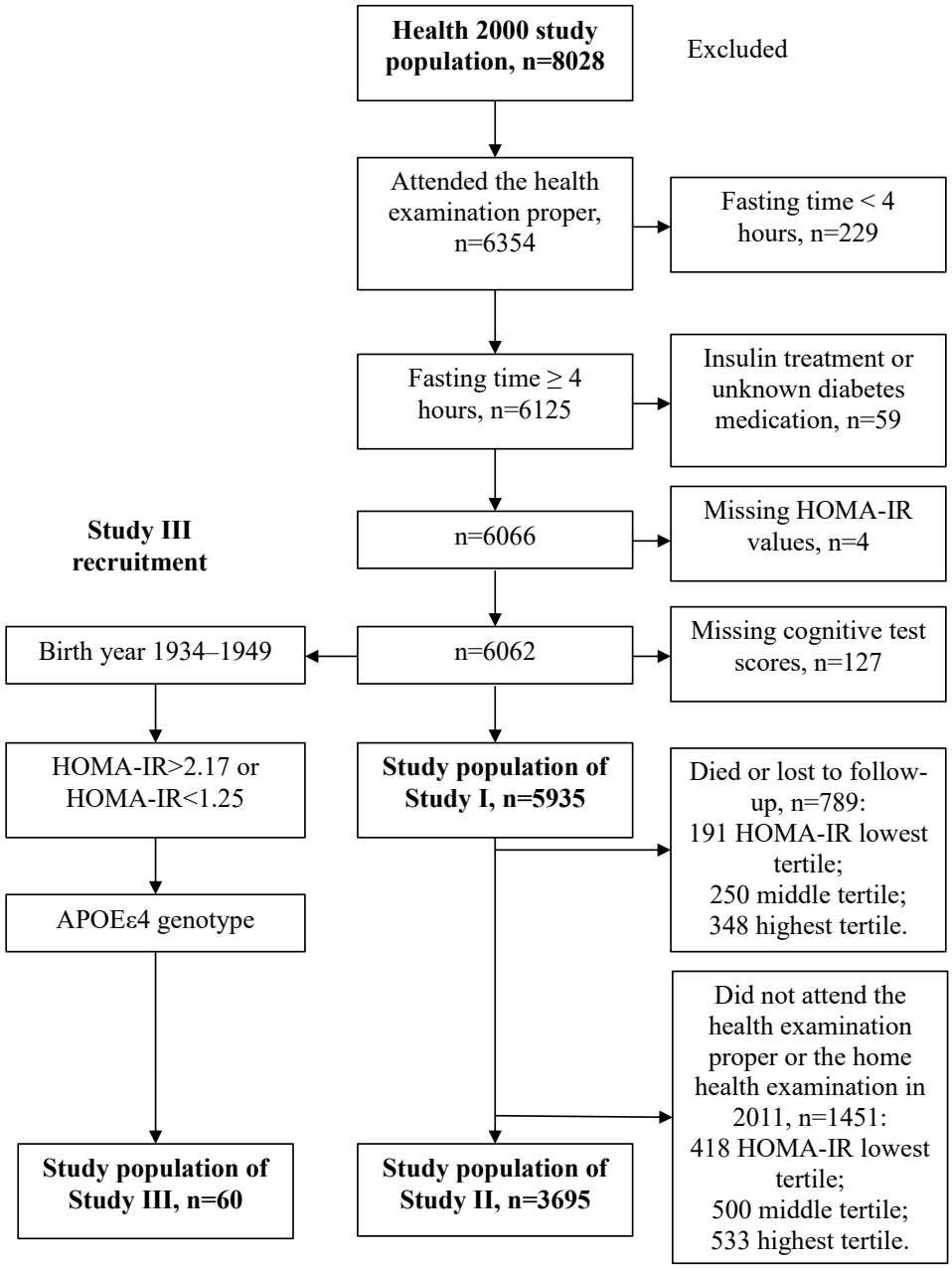


Figure 12. Flow chart of the study populations in studies I–III.

4.3 Methods

4.3.1 Demographic data

In the Health 2000 survey blood pressure was measured in sitting position from the right arm with a standard mercury manometer (Mercuro 300; Speidel & Keller, Jungingen, Germany). Before the measurements the participants had sat still for at least five minutes. The measurement was repeated twice. The average of the two measurements was used for Studies I and II. In study III hypertension was classified as systolic blood pressure ≥140 or diastolic blood pressure ≥90 or use of antihypertensive medication. Height was measured using a wall-mounted stadiometer (Person-Check, Medizintechnik, KaWe, Kirchner & Wilhelm, Germany). Weight was measured simultaneously with measuring bioimpedance with an eight-polar tactile-electrode impedance meter (InBody 3.0, Biospace, Soul, South Korea). Body mass index (BMI) was counted as weight (kg) divided by the square of height (in meters). (Heistaro, 2008).

Information on years of formal education, frequency of exercise (Studies I and II), level of alcohol consumption (Study II) and smoking (Studies II and III) was obtained by a questionnaire. Smoking was classified as current use of tobacco products (yes or no). Excessive alcohol consumption was classified as >24/16 doses of alcohol (12 g of alcohol per dose) per week (for men/women). Self-reported physical activity was assessed by asking the participants how often, in their free time, they exercised for at least 30 minutes and vigorously enough to cause sweating and breathlessness to a mild extent. The results were classified as: 1 = a few times a year or more seldom, 2 = 2-3 times a month, 3 = once a week, 4 = 2-3 times a week, 5 = 4-6 times a week, 6 = daily. (Heistaro, 2008).

Depressive symptoms were evaluated with Beck´s depression inventory (BDI) (Beck et al., 1996). The maximum score of the inventory is 63 points. In studies I and II the BDI score was analyzed as a continuous variable.

4.3.2 Laboratory assessments and APOE genotyping

In the Health 2000 survey the participants were asked to arrive at the study site after a minimum fasting time of four hours, but preferably an overnight fast. The fasting times of the participants were recorded, and venous blood samples were drawn. The samples were stored at -70°C until analyzed. Serum cholesterol values were determined by a CHOD PAP test (Olympus system reagent, Hamburg, Ger-

many), HDL-cholesterol by a HDL-C Plus test (Roche Diagnostics GmbH, Mannheim, Germany), triglycerides by a GPO PAP test (Olympus System Reagent, Germany), and glucose by a hexokinase test (Olympus System Reagent, Germany). Serum insulin was determined by a microparticle enzyme immunoassay (Abbott Laboratories, Dainabot, Tokyo, Japan). HbA_{1c} was determined with an immunoturbidimetric method (Hemoglobin A1c assay, Abbott Laboratories). Serum high sensitive C-reactive protein (hs-CRP) was analyzed by an automated analyzer (Optima, Thermo Electron Oy, Vantaa, Finland) and an ultrasensitive immunoturbidimetric test (Ultrasensitive CRP, Orion Diagnostica, Espoo, Finland). (Heistaro, 2008). Non-HDL-cholesterol was counted as total cholesterol minus HDL-cholesterol. HOMA-IR was by the equation fasting insulin (μU/ml) times fasting glucose (mmol/l) divided by 22.5 (Matthews et al., 1985).

APOE genotyping was performed in all individuals who gave their written consent for DNA sampling (95.9% in Study I, and 93.8% in Study II). *APOE* genotyping was performed with the MassARRAY system (Sequenom, San Diego, California) with a modified protocol which has been described elsewhere (Jänis et al., 2004). *APOE*ε4 genotype was considered positive for subjects with one or two ε4 alleles.

During Study III venous blood samples were drawn after an overnight fast (minimum 10 hours). Fasting insulin and glucose, HbA_{1c}, total cholesterol and triglycerides were determined from the samples. After the initial blood samples the participants were given 75 g of glucose orally. Plasma glucose was measured from venous blood samples after two hours (2 h OGTT). The blood samples were analyzed at the laboratory of Turku University Hospital (Tykslab). Insulin was determined by ECLIA (electrochemi-luminescence immunoassay) with Cobas e602 immunochemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany), and glucose by enzymatic photometry with a Cobas c702 chemistry analyzer (Roche Diagnostics GmbH, Germany). HbA_{1c} was determined with an immunochemical method with Cobas c501 analyzer (Roche Diagnostics GmbH, Germany).

4.3.3 Cognitive tests

In the Health 2000 and the Health 2011 surveys the participants were tested for verbal fluency and encoding and retaining verbal material according to the Finnish version (Hänninen et al., 1999) of the CERAD test battery (The Consortium to Establish a Registry for Alzheimer´s Disease) (Morris et al., 1989). In the verbal fluency test the participants were asked to list as many animals as possible during one minute. In the word-list learning test ten words were shown to the participants. The participants were asked to read the words aloud, to memorize them, and to repeat the words they remembered within 90 seconds. This was repeated twice

more. The score of the word-list learning test was the sum score of the words learned after 3 rounds. The range in this test is 0–30 points. In the Health 2000 survey, if the participant remembered all ten words after the first round the score was counted as full 30 points, and the two repetition rounds were left out, because of the tight schedule of the study procedures in 2000. In the Health 2011 survey all three rounds were repeated for all participants, regardless of whether they learned all ten words after one round or not, according to the instructions of the original CERAD test battery. On both occasions, the participants were asked to recall all ten words after five minutes (word-list delayed recall test) (Heistaro, 2008; Lundqvist and Mäki-Opas 2016).

Psychomotor speed and executive functions were examined in the Health 2000 survey with a reaction time test. This test was not included in the health examination of the Health 2011 survey. The test used in 2000–2001 was a computer program by Good Response, Metitur Co., Jyväskylä, Finland. The participants were told to react as quickly as possible to a light that appeared on the panel by shifting the index finger of their writing hand from the waiting switch to the switch that had lit up. The 7 target buttons were located at an even distance of 100 mm from the waiting switch. The participants performed two different tests: a simple reaction time test, and a visual choice reaction time test. In the simple reaction time test the same switch lit up 12 times at random intervals. In the visual choice test the lights lit up 12 times at different parts of the panel at random intervals. The participants were asked to touch the switch which lit up as quickly as possible. There were programmed time limits for both tests (reaction time < 100 ms for both simple and visual choice tests; reaction time>1500 ms for the simple test; reaction time > 2500 ms for the visual choice test). If the participant did not respond within these time limits the results were disqualified. In the visual choice test touching the wrong switch was counted as a slow response (>2500 ms). (Heistaro, 2008; Era et al., 2011).

In Study II the change in cognitive test scores during the follow-up was counted as cognitive test score at baseline in 2000 minus cognitive test score at follow-up in 2011. As the reaction time tests were not performed in the Health 2011 survey, the longitudinal results were only available for the verbal fluency, the word-list learning, and the word-list delayed recall tests.

All individuals who participated in Study III underwent a thorough neuropsychological exam, which was conducted by psychology students who had received separate training to perform the exam. In this thesis only the results of the CERAD examination in Study III are reported. All parts of the CERAD examination were performed as previously instructed (Morris et al., 1989; Hänninen et al., 1999). A CERAD total score was counted as proposed by Chandler et al. (2005).

4.3.4 [^{11}C]PIB PET (Study III)

4.3.4.1 Radiotracer synthesis

[^{11}C]PIB ([^{11}C]6-OH-BTA-1) was manufactured at the Turku PET Centre in high molar radioactivity (mean 680 MBq/nmol [SD 240] at the time of injection) utilizing in-target produced [^{11}C]methane as previously described (Snellman et al. 2017).

4.3.4.2 Scanning protocol

The study volunteers were scanned with a brain-dedicated high-resolution PET scanner, the ECAT HRRT (Siemens Medical Solutions, Knoxville, TN, USA). The volunteers were positioned in a supine position, and an individually shaped thermoplastic mask was placed on the face of each study volunteer to minimize head movement. An external position detector (Polaris Vicra, Northern Digital, Waterloo, Ontario, Canada) was used to monitor possible movements of the head. First, a 6-minute transmission scan was obtained. Then, [^{11}C]PIB (mean dose 489 MBq [SD 42]) was injected intravenously and flushed with saline. A dynamic, 90-minute PET scan was performed. During the scan altogether 28 frames were obtained (4 x 30 s, 9 x 60 s, 3 x 180 s, 10 x 300 s, 2 x 600 s).

4.3.4.3 MRI

All participants of Study III underwent a 3 Tesla brain MRI scan with Philips Ingenuity TF PET-MR device (Philips Healthcare, Eindhoven, The Netherlands) to obtain anatomic reference for the PET scans, and to exclude structural abnormalities.

4.4 Statistical analysis

The statistical analyses were performed with SAS 9.3. (SAS Institute, Cary, N.C., U.S.A.) (Study I), and with JMP Pro, versions 11.0 and 12.0 (SAS Institute, Cary, N.C., U.S.A.) (Studies I–III). In Study III voxel-by-voxel differences between two groups were assessed with Statistical Parametrical Mapping (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK). Before all analyses the normality of distribution of all variables was evaluated by visually inspecting the histograms.

The distributions that were skewed to the right were corrected by a logarithmic transformation (natural logarithm), when appropriate. Statistical significance was set at $p<0.05$, except for interaction testing, where statistical significance was set at $p<0.1$ (Studies I and II).

4.4.1 Demographic data (Studies I–III)

Differences between the demographics between Studies I and II, and Studies I and III were assessed with Student´s *t*-test for continuous variables, and with Pearson´s ChiSquare test for categorical variables. In Study I the demographic data was presented separately for men and women. Differences in demographic data between men and women were assessed with Student´s two-sample *t*-test for continuous variables with a normal distribution, and with the non-parametrical Wilcoxon test for variables that were skewed. Sex differences between categorical variables were assessed with Pearson´s Chi-Square test. The differences in HOMA-IR values according to fasting time were tested with the Kruskal-Wallis test, and each pair were compared with the Steel-Dwass method.

In Study II the demographic data was presented according to baseline HOMA-IR tertiles. Differences among the tertiles of HOMA-IR were assessed first with ANOVA, and then age and sex adjusted differences were examined with analysis of covariance for continuous variables, and with logistic regression analysis for categorical variables.

In Study III the demographic data was presented according to the study groups IR- and IR+, which were based on baseline HOMA-IR measurements. Group differences were assessed with Student´s two-sample *t*-test for continuous variables, and with Pearson´s Chi-Square test for categorical variables.

4.4.2 Cross-sectional associations between insulin resistance and cognitive test scores (Study I)

Cross-sectional correlations of HOMA-IR and other cognitive risk factors with cognitive test scores were assessed with Pearson´s correlation coefficient for normally distributed variables, and with Spearman´s rank order correlation coefficient for variables that were skewed.

Multivariate linear regression analysis was used to determine which risk factors were independently associated with cognitive test scores. Before the analyses the skewed distributions of HOMA-IR, triglycerides, BDI score and the simple and

visual choice reaction time tests were corrected with a logarithmic transformation ($\log_e$). First, the analyses were adjusted for age, sex and years of education (model 1). Then, further adjustments for metabolic risk factors and *APOE*ε4 genotype were made to evaluate if HOMA-IR would associate with cognitive performance independently of these previously reported risk factors for cognitive decline. In addition to age, sex and education, model 2 was further adjusted for *APOE*ε4 genotype, BMI, systolic blood pressure, HDL and non-HDL cholesterol, and triglycerides. Finally, since depression (Ismail et al., 2014) and physical activity (Behrman and Ebmeier, 2014) have been associated with cognitive decline, adjustments were made even for depressive symptoms (BDI score) and level of self-reported physical activity in model 3. The fully adjusted multivariate linear regression model thus included age, sex, years of education, *APOE*ε4 genotype, systolic blood pressure, HDL and non-HDL cholesterol, triglycerides, BMI, BDI score, and level of physical activity.

Interactions between HOMA-IR and sex, and HOMA-IR and *APOE*ε4 genotype on cognitive test scored were also analyzed with linear regression analysis, and sex and *APOE*ε4 genotype stratified results were presented when the interactions of these variables with HOMA-IR reached statistical significance ($p<0.1$) in the fully adjusted model.

4.4.3 Longitudinal associations between insulin resistance and cognitive test scores (Study II)

In Study II the associations between baseline HOMA-IR, insulin, glucose, HbA_{1c}, and hs-CRP and cognitive test scores (verbal fluency, word-list learning, and word-list delayed recall) at follow-up in 2011, and the change in cognitive test scores from 2000 to 2011 were evaluated with multivariate linear regression analysis. Because of skewed distributions, a logarithmic transformation ($\log_e$) was used of HOMA-IR, insulin, glucose, HbA_{1c}, and hs-CRP in the analyses.

First, the associations between each of the aforementioned variables and the cognitive test scores were analyzed in separate linear regression models, adjusted for age, sex, and years of education (model 1). Then, further adjustments were made for baseline metabolic risk factors (BMI, type 2 diabetes, systolic blood pressure, HDL and non-HDL cholesterol, and triglycerides), and *APOE*ε4 genotype (model 2).

In additional analyses, adjustments were made even for smoking, excessive alcohol consumption, depressive symptoms (BDI score), and level of physical activity (model 3). Again, HOMA-IR, insulin, glucose, HbA_{1c}, and hs-CRP were analyzed

in separate models, adjusted for the covariates as explained above. All analyses considering the change in cognitive test scores were adjusted even for baseline cognitive test scores.

Interactions between HOMA-IR and *APOE*ε4 genotype, HOMA-IR and sex, and HOMA-IR and type 2 diabetes were tested in the linear regression model adjusted for *APOE*ε4 genotype and metabolic risk factors (model 2). Stratified analyses were performed only if the interactions reached statistical significance ($p<0.1$).

4.4.4 [^{11}C]PIB analysis (Study III)

Voxel-by-voxel [^{11}C]PIB standardized uptake values (SUV) were calculated using imaging data from 60 to 90 min after tracer injection (frames 25–28). Automated region-of-interest (ROI) generation was conducted using FreeSurfer software (version 5.3.0, http://freesurfer.net/) and individual T1 weighted MRI data as input, yielding six ROIs (parietal cortex, prefrontal cortex, anterior cingulum, posterior cingulum, precuneus, and lateral temporal cortex) and cerebellar cortex. SUV ratios (SUVRs) were then obtained by using the cerebellar cortex as a reference region (Lopresti et al., 2005). The cerebellum contains very little fibrillary amyloid in the AD brain (Joachim et al., 1989), and similarly, [^{11}C]PIB binding to the cerebellum in post mortem studies is low both in brains of individuals who had AD and those who were cognitively normal (Klunk et al., 2004). Thus, as [^{11}C]PIB retention is not evident in the cerebellar cortex, the cerebellar cortex can be used as a reference region when analyzing cortical uptake of [^{11}C]PIB (Lopresti et al., 2005).

Voxel-by-voxel differences in PIB score between the IR- and the IR+ groups were assessed using Statistical Parametric Mapping (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK), with two-sample Student's *t*-test. The test was regarded statistically significant at $p<0.025$ (uncorrected for multiple comparisons). ROI-based analysis was conducted in the six ROIs mentioned above using the regional average PIB SUVR. A composite PIB score was calculated as the average PIB SUVR over all six ROIs. The [^{11}C]PIB-PET scan was considered amyloid positive (PIB+), when the PIB composite score was >1.5, a cut-off which has previously been validated in healthy, elderly populations (Pike et al., 2007; Jack et al., 2008; Bourgeat et al., 2010; Rowe et al., 2010; Villemagne et al., 2013).

4.4.5 Association between midlife insulin resistance and [^{11}C]PIB uptake 15 years later (Study III)

The odds ratio (OR) for an increased risk of having a PIB+ PET scan was first evaluated with logistic regression analysis in an unadjusted model, and then in model 1, adjusted for baseline age, time from baseline to PIB-PET scan, sex, and years of education. Model 2 further adjusted for baseline BMI and hypertension, and Model 3 for HDL cholesterol and triglycerides. Because the IR+ and IR- groups contained an equal number of *APOE*ε4 carriers, *APOE* genotype was not added as a covariate in these models.

The trend for an increasing risk for having a PIB+ PET scan according to IR group and *APOE*ε4 genotype status was assessed with Pearson´s ChiSquare test. To evaluate if *APOE*ε4 genotype would modulate the risk between IR group and amyloid accumulation, interactions between 'IR group × *APOE*ε4' and PIB+ PET scan (logistic regression), and continuous PIB composite score (linear regression) were analyzed in Model 1.

The differences between the IR- and IR+ groups on [^{11}C]PIB composite score, and [^{11}C]PIB uptake (SUVR) in all the six regions of interest were first assessed with Student´s *t*-test. Then, multivariate adjusted analyses were performed with linear regression analysis first adjusted for age at baseline, time from baseline measurements to PET scan, sex, and years of education (model 1). Further adjustments were made for baseline BMI and hypertension (model 2), and finally even for HDL cholesterol and triglycerides (model 3). To evaluate if higher levels of IR at baseline would correlate with higher PIB composite score, analyses where baseline HOMA-IR was treated as a continuous variable were performed with linear regression analysis and adjusted for the covariates as mentioned above, but also including *APOE*ε4 genotype in the analyses. Additional cross-sectional analyses were performed between continuous HOMA-IR at follow-up and PIB composite score in Model 1.

Statistical significance was set at $p<0.05$ for all analyses.

5 RESULTS

5.1 Demographic data (Studies I–III)

The demographics of studies I–III have been presented in the respective articles, and a summary of the demographics in each study is presented in Table 4. The participants in Study II were younger than in Study I, and the volunteers who participated in Study III were older than the participants of Studies I and II, in accordance with the recruitment criteria of Study III. Overall, the subpopulation in Study III represented the Health 2000 study population well, and the participants of Study II were healthier than the participants of the Health 2000 survey.

Table 4. Baseline demographics of Studies I–III.

Baseline demographics	***Study I***	***Study II***	***Study III***
n	5935	3695	60
women (n/%)	3262 / 55.0	2050 / 55.5	33 / 55.0
age at baseline (years)	52.5 (14.7)	49.3 (12.0)***	55.4 (3.3)***
education (years)	11.2 (4.1)	12.0 (3.9)***	12.0 (4.1)
APOEε4 genotype (n/%)	1781 / 32.1	1120/ 32.3	30 / 50.0**
HOMA-IR	2.22 (4.26)	1.98 (1.73)***	1.97 (1.23)
BMI (kg/m^2)	26.9 (4.6)	26.7 (4.5)*	27.5 (4.0)
HbA_{1c} (%, mmol/mol)	5.3 (0.5)	5.2 (0.5)***	5.2 (0.3)
Insulin(mU/l)	8.7 (11.0)	8.0 (5.6)***	8.0 (4.7)
Glucose (mmol/l)	5.5 (0.9)	5.4 (0.8)***	5.4 (0.5)
Systolic blood pressure (mmHg)	135 (21)	132 (20)***	133 (20)
Total cholesterol (mmol/l)	5.9 (1.1)	5.9 (1.1)	6.2 (1.0)
LDL cholesterol (mmol/l)	3.8 (1.2)	3.8 (1.1)	4.1 (1.1)
HDL cholesterol (mmol/l)	1.33 (0.37)	1.35 (0.37)*	1.39 (0.39)
Triglycerides (mmol/l)	1.57 (1.02)	1.50 (0.92)***	1.49 (0.78)
BDI score	7.1 (6.9)	6.5 (6.6)***	7.4 (7.4)
Current smoking (n/%)	1269 / 21.5	699 / 19.0**	9 / 15.0

The demographics are presented as mean (SD), unless otherwise stated. *APOE*ε4 genotype refers to an individual carrying one or two ε4 alleles. *P<0.05, **P<0.01, ***P<0.001. P-values for a difference between Study I and study II, and between Study I and Study III, assessed with Student´s *t*-test for continuous variables, and with Pearson´s ChiSquare test for categorical variables. A logarithmic transformation is used of HOMA-IR, insulin, glucose, HbA_{1c}, triglycerides, and BDI score in the analyses.

5.2 Cognitive test scores in 2000 and 2011

Table 5. Cognitive test scores of the participants in Studies I–III at baseline in 2000, at follow-up in 2011, and the change in cognitive test scores from baseline to follow-up.

Cognitive test score in 2000	***Study I***	***Study II***	***Study III***
Verbal fluency (VF)	23.7 (7.2)	25.0 (7.0)***	26.1 (6.3)**
Word-list learning (WLL)	20.6 (4.6)	21.6 (3.9)***	21.4 (3.5)
Word-list delayed recall (WLDR)	7.0 (2.0)	7.4 (1.8)***	7.1 (1.6)
Simple reaction time (s) (RT)	0.33 (0.09)	0.32 (0.07)***	0.32 (0.07)
Visual choice reaction time (s) VC)	0.48 (0.13)	0.46 (0.11)***	0.48 (0.16)
Cognitive test score in 2011			
Verbal fluency	N/A	24.2 (7.4)	25.4 (6.5)
Word-list learning	N/A	21.2 (4.5)	21.4 (4.0)
Word-list delayed recall	N/A	7.2 (2.1)	7.2 (1.8)
Change in cognitive test score from 2000 to 2011			
Verbal fluency	N/A	-0.86 (6.2)	-0.49 (6.1)
Word-list learning	N/A	-0.40 (3.6)	-0.02 (3.9)
Word-list delayed recall	N/A	-0.22 (1.72)	0.12 (1.5)

The results are shown as mean (SD). *P<0.05, **P<0.01, ***P<0.001. *P*-values for a difference between Studies I and II, and between Studies I and III, assessed with Student´s *t*-test. A logarithmic transformation is used of the reaction time tests in the analyses.

The cognitive test scores declined only very little during the 11-year follow up from 2000 to 2011 in studies II and III. In the subpopulation of Study III there was virtually no decline on the word-list learning test, and the study volunteers performed better on word-list delayed recall at the follow-up than at baseline. (Table 5.). The means of the cognitive test scores in Studies I–III were well above the cut-off scores used in Finland for screening for AD (cut-off for verbal fluency 17 p.; for word-list learning 17 p.; for word-list delayed recall 5 p.) (Sotaniemi et al., 2012). The individuals who attended the 11-year follow-up of Study I, the Health 2011 Survey, had higher scores on all cognitive tests at baseline when compared to the original Health 2000 study cohort. The participants of Study III performed better on the verbal fluency test than the participants of Study I (p=0.01). There were no differences between the participants of Study I and Study III on the other cognitive tests. (Table 5.)

5.3 Cross-sectional associations between insulin resistance and cognitive test scores (Study I)

Higher logarithmic HOMA-IR values were strongly correlated with lower scores on all five cognitive tests in the unadjusted analyses in the Health 2000 survey (p-values for all cognitive tests <0.0001). When adjustments were made for age, sex and years of education (model 1), the associations remained significant for HOMA-IR and the categorical verbal fluency test (VF) ($p<0.0001$), and for both reaction time tests (simple reaction time [RT], $p<0.0001$, visual choice reaction time [VC], $p=0.02$) (Table 6.).

Adding *APOE*ε4 genotype, BMI, systolic blood pressure, HDL and non-HDL cholesterol, and triglycerides (model 2) to the regression model improved the explanatory value (R^2) of the analyses slightly for all other tests except the RT test (Table 3.). In model 2 higher levels of HOMA-IR associated with poorer verbal fluency performance ($p<0.0001$), and with a slower reaction time on the RT test ($p=0.0006$). (Table 6.).

In the final model of adjustment (Model 3, adjusted even for BDI score and level of physical activity) higher HOMA-IR was associated with a lower verbal fluency score ($p=0.0003$), and with a slower response on the RT test ($p=0.02$). No association was found between HOMA-IR and the word-list learning test (WLL) ($p=0.73$), the word-list delayed recall test (WLDR) ($p=0.81$), or the visual choice reaction time test ($p=0.44$). (Table 6.)

Since the objective of Study I was to evaluate the possible modulating effects of sex and *APOE*ε4 genotype on the associations between HOMA-IR and cognition, interactions for 'sex × HOMA-IR' and '*APOE*ε4 genotype × HOMA-IR' on each cognitive test were analyzed in the fully adjusted model 3. There was a significant 'sex × HOMA-IR' interaction for the VF test ($p=0.099$), and for the WLL test ($p=0.07$). The interaction of '*APOE*ε4 genotype × HOMA-IR' was significant for the VF test ($p=0.093$), and for the WLDR test ($p=0.097$). Sex and *APOE*ε4 genotype stratified analyses were performed according to these interaction analyses (Table 7.).

Table 6. Cross-sectional univariate and multivariate correlations of HOMA-IR with cognitive test scores.

	VF	***WLL***	***WLDR***	***RT***	***VC***
HOMA-IR	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)
Unadjusted	-1.49 (0.14)***	-1.05 (0.09)***	-0.41 (0.04)***	0.04 (0.004)***	0.04 (0.005)***
R^2*(%)*	2.0	2.4	1.9	1.4	1.3
Model 1	-0.58 (0.13)***	-0.10 (0.07)	-0.01 (0.03)	0.02 (0.004)***	0.01 (0.004)*
R^2*(%)*	19.7	37.7	33.9	14.7	25.9
Model 2	-0.71 (0.17)***	-0.09 (0.09)	-0.02 (0.04)	0.02(0.005)***	0.009 (0.006)
R^2*(%)*	19.8	38.2	34.1	14.5	26.4
Model 3	-0.66 (0.18)***	-0.03 (0.10)	-0.01 (0.05)	0.01 (0.006)*	0.005 (0.006)
R^2*(%)*	18.6	37.3	32.4	14.5	25.7

The results are shown as β (SE), and the analyses were performed with linear regression. The unadjusted correlations of HOMA-IR with cognitive test scores are shown on the first row. Model 1: adjusted for age, sex and years of education. Model 2: further adjusted for *APOE*ε4 genotype, BMI, systolic blood pressure, HDL and non-HDL cholesterol, and triglycerides. Model 3: adjusted even for BDI and physical activity score. R^2 shows the adjusted explanatory value of each model. A logarithmic transformation is used of HOMA-IR, triglycerides, BDI score, and the RT and VC tests in the analyses. *P<0.05, **P<0.01, ***P<0.001.

The sex stratified analyses showed that higher HOMA-IR independently associated with poorer verbal fluency performance in all models of adjustment in women ($p<0.0001$ in the fully adjusted model 3), but not in men ($p=0.56$ in model 3). In addition, higher HOMA-IR associated with a lower word-list learning score in women in model 1 ($p=0.002$), and in model 2 ($p=0.04$), but not in the fully adjusted model 3 ($p=0.25$). HOMA-IR did not associate with WLL score in men in any of the models (all p-values > 0.24) (Table 7A.).

In analyses stratified for *APOE*ε4 genotype higher HOMA-IR was associated with lower verbal fluency scores in individuals who did not carry an *APOE*ε4 allelle (model 3, $p=0.0003$), but not in *APOE*ε4 carriers (model 3, $p=0.28$). There was no significant association between HOMA-IR and the WLDR test in carriers ($p=0.26$) or non-carriers of *APOE*ε4 ($p=0.60$) (Table 7B.).

Table 7. Sex and *APOE* genotype stratified associations among HOMA-IR and verbal fluency, word-list learning, and word-list delayed recall scores.

	VF		***WLL***	
	men	women	men	women
HOMA-IR	β (SE)	β (SE)	β (SE)	β (SE)
Unadjusted	-0.90 (0.21)***	-2.00 (0.15)***	-0.48 (0.12)***	-1.38 (0.12)***
R^2*(%)*	0.7	3.5	5.8	4.0
Model 1	-0.30 (0.19)	-0.83 (0.17)***	0.12 (0.10)	-0.32 (0.10)**
Model 2	-0.35 (0.26)	-1.04 (0.22)***	0.10 (0.13)	-0.27 (0.13)*
Model 3	-0.17 (0.29)	-1.02 (0.24)***	0.11 (0.15)	-0.16 (0.14)
R^2*(%)*	15.7	20.9	34.7	36.0

A

	VF		***WLDR***	
	*APOE*ε4 carrier	*APOE*ε4 non-carrier	*APOE*ε4 carrier	*APOE*ε4 non-carrier
HOMA-IR	β (SE)	β (SE)	β (SE)	β (SE)
Unadjusted	-1.15 (0.25)***	-1.68 (0.17)***	-0.45 (0.07)***	-0.42 (0.05)***
R^2*(%)*	1.1	2.5	2.1	2.0
Model 1	-0.34 (0.23)	-0.72 (0.16)***	-0.08 (0.06)	-0.009 (0.04)
Model 2	-0.61 (0.30)*	-0.76 (0.20)***	-0.10 (0.08)	0.02 (0.05)
Model 3	-0.35 (0.32)	-0.81 (0.22)***	-0.09 (0.08)	0.03 (0.06)
R^2*(%)*	17.6	18.9	33.0	32.2

B

The results of the sex (Table A) and *APOE*ε4 stratified (Table B) analyses are shown as β (SE), and the analyses were performed with linear regression. The unadjusted correlations of HOMA-IR with cognitive test scores are shown on the first row. Model 1: adjusted for age, sex (Table B) and years of education. Model 2: further adjusted for *APOE*ε4 genotype (Table A), BMI, systolic blood pressure, HDL and non-HDL cholesterol, and triglycerides. Model 3: adjusted even for BDI and physical activity score. R^2 shows the adjusted explanatory value of HOMA-IR alone, and the fully adjusted Model 3. A logarithmic transformation is used of HOMA-IR, triglycerides, BDI score, and the RT and VC tests in the analyses. *P<0.05, **P<0.01, ***P<0.001.

5.4 Longitudinal associations between insulin resistance and cognitive test scores (Study II)

In Study II the associations among HOMA-IR, insulin, glucose, HbA_{1c}, and hs-CRP at baseline in 2000 and the cognitive test scores at follow-up in 2011 were evaluated. In addition, the associations among baseline HOMA-IR, insulin, glucose, HbA_{1c}, and hs-CRP, and the change in cognitive test scores from 2000 to 2011 were assessed. Here, the results of the analyses between HOMA-IR and the cognitive test scores will be presented.

The mean cognitive test scores at baseline and at follow-up, adjusted for age, sex and education, are presented in figure 13. Similarly to the cross-sectional analyses, higher HOMA-IR at baseline was associated with poorer verbal fluency performance at follow up (model 2, p=0.0002; model 3, p=0.01) (Table 8.), and with a steeper decline in verbal fluency from 2000 to 2011 (model 2: p=0.004; model 3: p=0.051) (Table 9.). There was no significant association between baseline HOMA-IR and word-list learning or word-list delayed recall at follow-up (model 2: p=0.60 and p=0.38, respectively; model 3 p=0.92 and p=0.40, respectively), or with the change in word-list learning (model 2: p=0.55; model 3, p=0.88), or word-list delayed recall from baseline to follow-up (model 2: p=0.96; model 3, p=0.98).

Table 8. Associations between baseline HOMA-IR and cognitive test scores at follow-up in 2011.

	VF	***WLL***	***WLDR***
HOMA-IR	β (SE)	β (SE)	β (SE)
Unadjusted	-1.76 (0.19)***	-1.09(0.11)***	-0.42(0.05)***
R^2*(%)*	2.4	2.5	1.6
Model 1	-0.81 (0.17)***	-0.14 (0.09)	0.01 (0.05)
R^2*(%)*	20.3	35.9	32.5
Model 2	-0.86 (0.23)***	-0.07 (0.12)	0.05 (0.06)
R^2*(%)*	21.0	36.2	32.9
Model 3	-0.62 (0.25)*	0.01 (0.14)	0.06 (0.07)
R^2*(%)*	21.9	36.9	33.4

The analyses were performed with linear regression. Unadjusted correlations of HOMA-IR with cognitive test scores are shown on the first row. Model 1: adjusted for age, sex, and years of education. Model 2: further adjusted for *APOE*ε4 genotype, BMI, systolic blood pressure, HDL and non-HDL cholesterol, triglycerides, and type 2 diabetes. Model 3: adjusted even for smoking, excessive alcohol intake, BDI, and physical activity score. R^2 shows the adjusted explanatory value of each model. A logarithmic transformation is used of HOMA-IR, triglycerides, and BDI score in the analyses. *P<0.05, **P<0.01, ***P<0.001.

Table 9. Associations between baseline HOMA-IR and change in cognitive test scores from baseline in 2000 to follow-up in 2011.

	VF	***WLL***	***WLDR***
HOMA-IR	β (SE)	β (SE)	β (SE)
Unadjusted	0.92 (0.15)***	0.50 (0.09)***	0.22 (0.04)
R^2*(%)*	13.9	7.8	6.8
Model 1	0.41 (0.14)**	0.09 (0.08)	0.005 (0.04)
R^2*(%)*	23.3	23.1	21.3
Model 2	0.55 (0.19)**	0.07 (0.11)	0.002 (0.05)
R^2*(%)*	24.1	23.5	22.0
Model 3	0.40 (0.21)	0.02 (0.12)	-0.002 (0.06)
R^2*(%)*	24.9	24.2	22.6

The analyses were performed with linear regression. Model 1: adjusted for age, sex, and years of education. Model 2: further adjusted for *APOE*ε4 genotype, BMI, systolic blood pressure, HDL and non-HDL cholesterol, triglycerides, and type 2 diabetes. Model 3: adjusted even for smoking, excessive alcohol intake, BDI, and physical activity score. Note that all analyses are adjusted even for baseline cognitive test scores, and that a positive estimate indicates a greater decline during the follow-up. R^2 shows the adjusted explanatory value of each model. A logarithmic transformation is used of HOMA-IR, triglycerides, and BDI score in the analyses. *P<0.05, **P<0.01, ***P<0.001.

There was no significant interaction between HOMA-IR and *APOE*ε4 genotype on cognitive test scores (model 2, all p-values>0.26), nor between HOMA-IR and type 2 diabetes (model 2, all p-values>0.48). The interaction between HOMA-IR and sex was significant for word-list delayed recall at follow-up (p=0.06). No other interactions were found for 'HOMA-IR×sex' (all other p-values>0.29).Thus, sex-stratified analyses were performed only for HOMA-IR and this cognitive test. In stratified analyses HOMA-IR did not predict word-list learning performance in men (p=0.26) or in women (p=0.99) (data not shown).

In conclusion, higher baseline HOMA-IR values predicted lower verbal fluency test scores after a follow-up of 11 years, and a steeper decline in verbal fluency from 2000 to 2011 in the Finnish adult population. Baseline HOMA-IR values were not associated with word-list learning or word-list delayed recall.

Baseline glucose or hs-CRP values were not associated with cognitive performance at follow-up, or with a decline in cognition from 2000 to 2011 (original publication II).

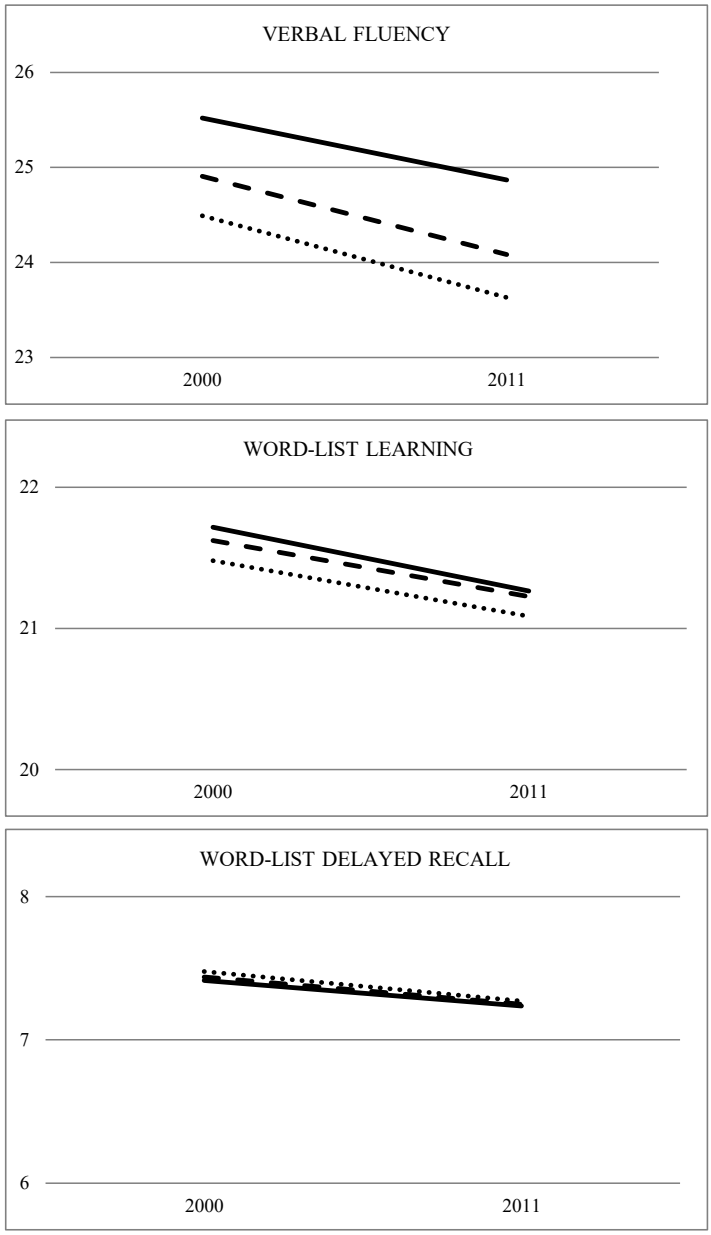


Figure 13. Age, sex, and education adjusted means of cognitive test scores at baseline in 2000 and at follow-up in 2011 according to baseline HOMA-IR tertiles. —— Lowest tertile; ------ Middle tertile; ····· Highest tertile. Modified from Figure 1. in Study II. N=3965.

5.5 Association between midlife insulin resistance and [^{11}C]PIB uptake 15 years later (Study III)

Insulin resistance in midlife predicted higher cortical [^{11}C]PIB uptake, reflecting greater cortical amyloid accumulation, at a mean age of 71 years. In addition, midlife insulin resistance was a risk factor for an amyloid positive PET scan 15 years after the baseline measurements of HOMA-IR.

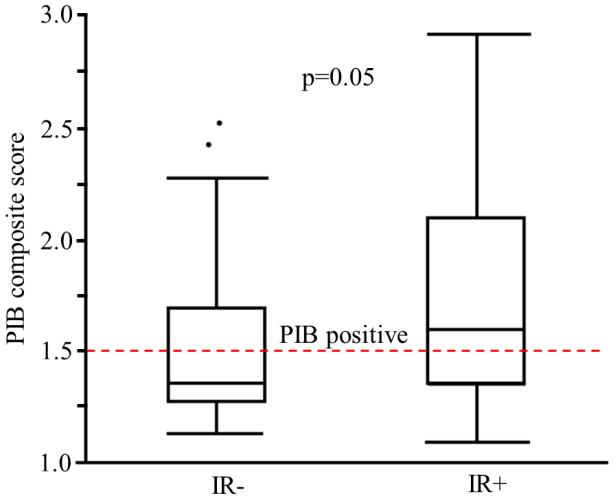


Figure 14. PIB composite score according to IR group. P-value for unadjusted difference in PIB composite score between the IR groups, assessed with Student´s *t*-test. N=30 in both groups.

There was a significant difference between the IR+ and the IR- groups on PIB composite score in unadjusted analyses (p=0.05) (figure 14), and in analyses adjusted for baseline age, sex, years of education, and time from baseline measurements to PET scan (p=0.02) (Table 10.). In addition, [^{11}C]PIB uptake (SUVR) was greater in the IR+ group than in the IR- group in all the six ROIs analyzed (Table 10.). The ROIs were chosen based on the brain regions where amyloid accumulation is typically seen in AD (Braak and Braak, 1997). Baseline continuous HOMA-IR correlated with a greater PIB composite score (r=0.25, p=0.05; figure 15).

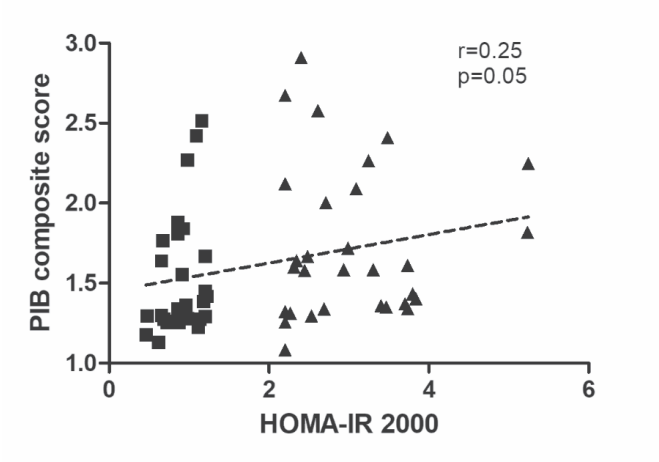


Figure 15. Correlation of baseline HOMA-IR with PIB composite score at follow-up. Black squares represent individuals in the IR- group and black triangles represent individuals in the IR+ group. R and *p*-values assessed with Pearson's correlation. N=60.

Table 10. PIB standardized uptake value ratios (SUVRs) ROIs typical for amyloid accumulation in AD, according to baseline IR groups.

Region of interest	*IR-*	*IR+*	*p-value*[a]	*p-value*[b]
PIB composite score	1.51 (0.37)	1.73 (0.48)	0.050	0.04
Parietal cortex	1.55 (0.39)	1.78 (0.49)	0.046	0.05
Prefrontal cortex	1.53 (0.39)	1.75 (0.50)	0.056	0.03
Lateral temporal cortex	1.34 (0.27)	1.48 (0.41)	0.10	0.10
Cingulum anterior	1.69 (0.38)	1.97 (0.50)	0.02	0.01
Cingulum posterior	1.76 (0.46)	2.02 (0.52)	0.04	0.04
Precuneus	1.73 (0.50)	2.06 (0.65)	0.03	0.04

The results are shown as unadjusted mean SUVR (SD). [a]*P*-values for unadjusted differences between individuals with and without insulin resistance at baseline in 2000, assessed with Student´s *t*-test. [b] adjusted for baseline age, time from baseline to PIB scan, sex, and years of education, assessed with linear regression analysis.

Table 11. Multivariate baseline predictors of brain amyloid accumulation (PIB composite SUVR score) 15 years later.

	Model 1	*Model 2*	*Model 3*
Predictors	β (SE)	β (SE)	β (SE)
HOMA-IR	0.08 (0.04)*	0.13 (0.05)*	0.11 (0.05)*
age	0.02 (0.01)	0.03 (0.02)	0.03 (0.02)
time from baseline to [^{11}C]PIB scan	0.09 (0.07)	0.09 (0.07)	0.06 (0.07)
years of education	0.004 (0.01)	-0.0003 (0.01)	0.000003 (0.01)
sex	-0.008 (0.10)	-0.04 (0.10)	0.03 (0.11)
APOE ε4	0.46 (0.09)***	0.44 (0.09)***	0.45 (0.10)***
hypertension		-0.02 (0.10)	-0.03 (0.11)
BMI		-0.02 (0.02)	-0.03 (0.02)
HDL cholesterol			-0.07 (0.17)
triglycerides			-0.27 (0.18)
R^2 (%)	34.9	35.2	35.6

The results are shown as estimate (standard error). Model 1: adjusted for age, time from baseline to PIB scan, sex, years of formal education and APOEε4 genotype; Model 2: further adjusted for hypertension and BMI; Model 3: adjusted even for HDL cholesterol and triglycerides. HOMA-IR, age, time from baseline to [^{11}C]PIB scan, BMI, HDL cholesterol, and triglycerides were analyzed as continuous variables. A logarithmic transformation ($\log_e$) was used of triglycerides. The analyses were performed with multivariate linear regression. R^2 shows the adjusted explanatory value of each model of adjustment. *p<0.05, ***p<0.001.

In multivariate regression analyses adjusted for age at baseline, time from baseline measurements to PET scan, years of education, sex, *APOE*ε4 genotype, baseline BMI, hypertension, HDL cholesterol and triglycerides (model 3) higher continuous baseline HOMA-IR independently predicted a greater PIB composite score (β=0.11, 95% confidence interval [CI] 0.003–0.21, p=0.04) (Table 11). In contrast, continuous HOMA-IR measured at follow-up was not associated with PIB composite score in unadjusted analyses (β=0.12, 95% CI -0.03–0.26, p=0.11), or in analyses adjusted for model 1 (β=0.10, 95% CI -0.03–0.23, p=0.14) (data not shown).

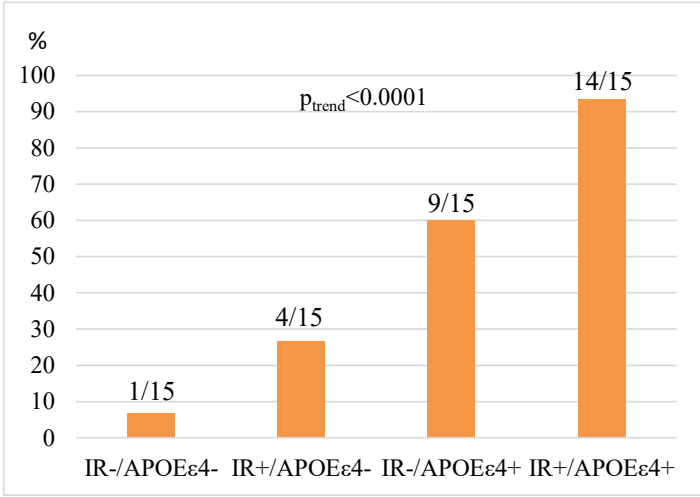


Figure 16. Percentage and number of individuals with an amyloid positive PIB-PET scan (PIB composite score >1.5), according to IR group and *APOE*ε4 genotype. P_{trend} for an increasing prevalence of an amyloid positive PET scan according to IR and *APOE* genotype, assessed with Pearson's ChiSquare test. Modified from Fig 1. in Study III.

60% (18/30) of individuals in the IR+ group, and 33.3% (10/30) of the IR- group had a PIB+ PET scan at follow up (unadjusted OR 3.0, 95% CI 1.1–8.9, p=0.04, Model 1: OR 4.4, CI 1.3–17.1, p=0.02; Model 2: OR 12.2, CI 2.2–95.0, p=0.003; Model 3: OR 11.1, CI 1.9–91.5, p=0.007).

In *APOE*ε4 genotype stratified analyses, the percentage of individuals with a PIB+ PET scan was 6.7% (1/15) in the IR-/*APOE*ε4- group; 26.7% (4/15) in the IR+/*APOE*ε4- group; 60.0% (9/15) in the IR-/*APOE*ε4+ group, and 93.3% (14/15) in the IR+/*APOE*ε4+ group (p_{trend}<0.0001) (figure 16). There was no significant interaction for 'IR group × *APOE*ε4', on being PIB+ (p=0.78) or PIB composite score (p=0.30) and thus, no further *APOE*ε4 stratified analyses were performed.

The SPM analysis that detected voxel-by-voxel differences between the IR+ and IR- groups showed that [^{11}C]PIB uptake was greater in the IR+ group (uncorrected for multiple comparisons) in wide regions of the frontal and the parietal cortices and in the lateral temporal cortex, reflecting a pattern of amyloid accumulation that is typically found in early AD (Braak and Braak, 1997) (figure 17).

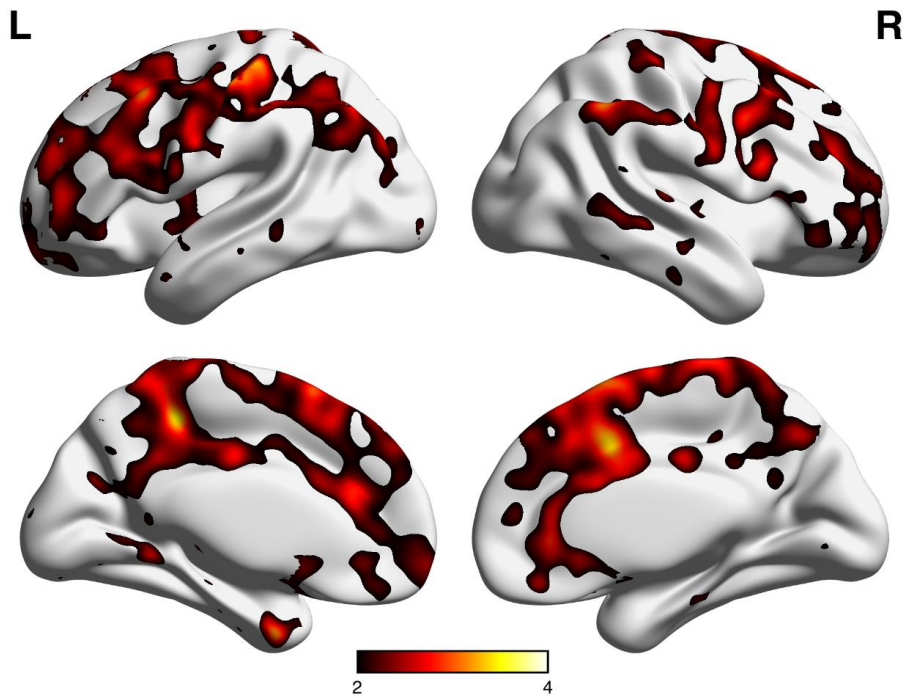


Figure 17. Voxel-by-voxel SPM analysis of [^{11}C]PIB uptake showing regions where individuals with insulin resistance 15 years before the PET scans had higher [^{11}C]PIB uptake than the control group. The color scale starts from the height threshold (T) 2.0, derived from SPM analysis adjusted for age, time from baseline to PIB scan, sex and years of education, and indicating difference between IR- and IR+ groups for all regions shown in color in the image, yellow being most significant ($p<0.025$ when $T=2.0$, uncorrected for multiple comparisons). N=60. (Original publication III).

6 DISCUSSION

6.1 Insulin resistance and cognitive functioning

6.1.1. Cross-sectional findings

The main finding of Study I was that insulin resistance was associated with poorer verbal fluency performance in women, but not in men, in a large, population-based sample of the Finnish adult population. The adjusted explanatory value (R^2) of HOMA-IR in predicting verbal fluency was greater in women than in men (3.5 vs. 0.7 %, Table 7.), and the explanatory of the fully adjusted model in Study I was 20.9% in women and 15.9% in men (Table 7.), suggesting that other underlying factors might explain verbal fluency performance in men. In addition, insulin resistance associated with word-list learning in women, but not in men, although this association was no longer significant in the fully adjusted model. There was also an interaction for HOMA-IR and *APOE*ε4 genotype on verbal fluency performance. In *APOE*ε4 stratified analyses the association between higher HOMA-IR and lower verbal fluency scores was significant only in non-carriers of *APOE*ε4. Higher HOMA-IR levels were associated with a slower reaction time, reflecting slower psychomotor speed in both men and women. No association was found between HOMA-IR and word-list delayed recall or visual choice reaction time in adjusted analyses.

These findings are line with previous studies that have shown an association between higher levels of insulin resistance and poorer cognitive performance (Table 1.), and more specifically, poorer executive function (Abbatecola et al., 2004; Geroldi et al., 2005; Schuur et al., 2010, Tan et al., 2011, Sanz et al., 2013). Of the previous studies summarized in Table 1., only four reported results from middle-aged populations (Schuur et al., 2010; Tan et al., 2011, Sanz et al., 2013 and Backeström et al., 2015). Five studies assessed sex differences in the association between insulin resistance and cognition (Stolk et al., 1997; Schuur et al., 2010; Sanz et al. 2013; Backeström et al., 2015). Three of these studies found that insulin resistance was associated with poorer cognition in women only (Stolk et al., 1997; Schuur et al., 2010; Laws et al., 2017), whilst the other two studies (Sanz et al. 2013; Backeström et al., 2015) found no interaction between insulin resistance and sex. Study I was the first nationwide, population-based study including early middle-aged individuals to report a sex difference in the association between HOMA-IR and cognitive function.

Study I was also the first epidemiological study indicating an interaction between insulin resistance and *APOE*ε4 genotype on cognitive performance. This finding is supported by epidemiological studies on insulin resistance and the prevalence of AD. In a cross-sectional study by Kuusisto et al. (1997) hyperinsulinemia was associated with AD only in non-carries of *APOE*ε4. A similar finding was reported from the Uppsala Longitudinal Study of Adult Men, where the association between impaired insulin secretion and cumulative AD risk was stronger in non-carriers than in carriers of *APOE*ε4 (Rönnemaa et al., 2008). In line with these findings, a Finnish study reported that in individuals with impaired glucose tolerance (type 2 diabetes or abnormal OGTT) carriers of the *APOE*ε2 allele performed worse on cognitive tests than carriers of *APOE*ε4 (Helkala et al., 2002).

6.1.2. Longitudinal findings

In Study II, the 11-year follow-up of Study I, insulin resistance was associated with poorer verbal fluency performance at follow-up, and with a greater decline in verbal fluency scores from baseline to follow-up. These associations remained statistically significant after adjusting for previously reported risk factors for cognitive decline (age, sex, education, *APOE*ε4) and metabolic risk factors (BMI, systolic blood pressure, HDL and non-HDL cholesterol, triglycerides, and type 2 diabetes). Insulin resistance did not predict word-list learning or word-list delayed recall scores. In contrast to the findings in Study I regarding sex and *APOE*ε4 differences, no interactions between insulin resistance and sex, nor between insulin resistance and *APOE*ε4 genotype on cognitive functioning were found in Study II.

Before Study II only one large, population-based cohort study, the Atherosclerosis Risk in Communities (ARIC) study, had examined the effects of insulin resistance and cognitive decline over time (Young et al., 2006; Table 2.). In the ARIC study higher baseline levels of HOMA-IR were associated with lower scores on delayed word recall, the digit-symbol subtest, and with first-letter verbal fluency at follow-up. Three smaller studies (Kilander et al., 1998; Okereke et al., 2005; Willette et al., 2013; Table 2.) reported similar results. In contrast to the ARIC study, no association was found between insulin resistance and delayed recall in Study II.

Recently, five new longitudinal studies on insulin resistance and cognition, with a follow-up time up to 20 years, have been published (Table 2: Fava et al., 2017; Hughes et al., 2017; Lutski et al., 2017; Neergaard et al., 2017; Tortelli et al., 2017). In line with the findings in Study II, all other longitudinal studies published thus far except the Multi-Ethnic Study of Atherosclerosis (MESA) (Hughes et al., 2017) suggest that midlife insulin resistance is an independent risk factor for cognitive decline. Although no significant association was found between baseline

HOMA-IR and cognitive functioning at follow-up in the MESA study, the change in HOMA-IR during the 10-year follow-up i.e. a rise in HOMA-IR levels over the follow-up time was an independent predictor of poorer cognition, and this association was evident only in non-carriers of *APOE*ε4 (Hughes et al., 2017).

6.2 Insulin resistance as a risk factor for brain amyloid accumulation

Study III showed that midlife insulin resistance was an additive risk factor for late-life brain amyloid accumulation in carriers and non-carriers of *APOE*ε4. As expected, *APOE*ε4 genotype was a stronger risk factor for amyloid accumulation than insulin resistance. The risk for an amyloid positive PET scan rose linearly from *APOE*ε4 non-carriers with normal midlife insulin sensitivity, to *APOE*ε4 non-carriers with midlife insulin resistance, to *APOE*ε4 carriers with normal midlife insulin sensitivity, to *APOE*ε4 carriers with midlife insulin resistance (figure 16). The prevalence of amyloid positivity was 93.3% at the age of 71 years in non-demented *APOE*ε4 carriers with midlife insulin resistance, indicating that these individuals are at very high risk for developing AD. Moreover, the pattern of amyloid accumulation in the cerebral cortex of insulin resistant individuals was similar to the pattern seen in early AD (figure 17; Braak and Braak, 1997). Late-life insulin resistance was not associated with brain amyloid accumulation.

Study III was the first study to provide evidence for an association between midlife insulin resistance and late-life *in vivo* biomarkers of AD, a finding supported by *in vitro* and animal studies, and by one neuropathological study (Matsuzaki et al., 2010). The results of the only previous study on midlife insulin resistance and late-life amyloid accumulation found no association between midlife insulin resistance and amyloid accumulation with neither [^{11}C]PIB-PET imaging nor in neuropathological assessments (the BLSA study, Thambisetty et al., 2013b). However, the participants of the previous study were older than the participants in Study III (mean age at time of PET scan 79 vs. 71 years), and the prevalence of *APOE*ε4 genotype was not reported, nor controlled for in the BLSA study, which might have interfered with the results.

The results of Study III are in line with the previous cross-sectional study on HOMA-IR and amyloid accumulation from the Wisconsin Registry for Alzheimer's Prevention study in late middle-aged individuals (Willette et al., 2015c), which found that higher levels of HOMA-IR associated with higher [^{11}C]PIB uptake in normoglycemic, but not in hyperglycemic individuals. Similarly, the results of Study III are supported by the cross-sectional findings of an association between insulin resistance and CSF biomarkers of AD (Hoscheidt et al., 2016; Westwood

et al., 2017; Laws et al., 2017). To date, no longitudinal studies have been published on insulin resistance and CSF AD biomarkers.

Indirectly, the findings of Study III are supported by the ARIC study that measured midlife vascular risk factors and late-life amyloid burden with PET. In the ARIC study obesity, but not the other measured vascular risk factors (diabetes, hypercholesterolemia, hypertension and smoking), independently predicted a higher brain amyloid load (Gottesman et al., 2017). Since obesity is closely associated with insulin resistance (Ferrannini et al., 1997) this association could reflect the underlying effects of insulin resistance on amyloid accumulation. Similarly to our findings, there was no interaction between *APOE*ε4 genotype and the vascular risk factors on amyloid accumulation in the ARIC study.

In accordance to the findings in Study III, the ARIC study also showed that midlife, but not late-life vascular risk factors associated with late-life amyloid accumulation (Gottesman et al., 2017). Similarly to the results of Study III, HOMA-IR was not associated with brain amyloid accumulation in the cross-sectional AIBL study on cognitively normal elderly individuals (Laws et al., 2017).

These findings indicate that – consistent with Aβ accumulation starting even decades before the onset of Alzheimer´s dementia – midlife insulin resistance, but apparently not late-life insulin resistance, would play an important role in the early pathogenesis of AD.

6.3 Methodological considerations

6.3.1 Study populations

The main strength of Studies I and II was the large, epidemiological study design of the Health 2000 health examination survey, and its follow-up, Health 2011. The study population in the Health 2000 survey was a nationally representative sample of the Finnish adult population, aged 30 years or more. The inclusion of middle-aged individuals made it possible to evaluate early effects of insulin resistance on cognition, before the onset of cognitive impairment or dementia. The follow-up nature of these studies rendered it possible to examine the effects of midlife insulin resistance on cognitive decline during 11 years.

The study population in Study III was a relatively small subsample (n=60, n=30/group) of the Health 2000 study population. The study sample was powered to detect a 15% difference in [^{11}C]PIB accumulation in the frontal cortex between

the control (non-insulin resistant in midlife) and the exposure group (insulin resistant in midlife) (Aalto et al., 2009). In longitudinal, observational studies there is a risk of change in risk factors from baseline to follow-up, which might dilute the differences between to exposure and the control groups. In an attempt to control for this risk, the recruitment criteria of Study III excluded individuals who had been diagnosed with type 2 diabetes from the control group. Also, the difference in HOMA-IR values between the control and the exposure group were evaluated also at the follow-up visit. Individuals with midlife insulin resistance had higher HOMA-IR ($p<0.0001$), fasting glucose ($p<0.0001$) and fasting insulin ($p<0.0001$) levels also at follow-up, allowing the assumption that the exposure and the control group differed in terms of insulin resistance throughout the follow-up time. In addition, the results of volunteer-based studies such as Study III might be biased. Individuals with a family history of dementia, or those suffering from subjective memory complaints might be more eager to volunteer to participate in studies involving neuroimaging. However, we have no reason to assume this selection bias would have been different between the IR- and the IR+ groups.

6.3.2 Cognitive tests

One strength of Study I was that five different cognitive tests were used to assess cognitive functioning, allowing the evaluation of the associations between insulin resistance and different cognitive domains. Some of the previous cross-sectional studies (Kalminj et al., 1995; Stolk et al., 1997) assessed cognitive functioning only with the Mini-mental State Examination (MMSE), which is a crude screening test for dementia mainly used to follow the progression of dementia in individuals with diagnosis of dementia (Folstein et al., 1975), or with a combination of the MMSE and the trail-making tests A and B (TMT-A and TMT-B) (Abbatecola et al., 2004; Geroldi et al., 2005).

The cognitive tests used in Studies I and II are part of the Finnish version of the CERAD test battery (Morris et al., 1989). The word-list learning test was used as an assessment of verbal learning and memory. The word-list delayed recall test was used to evaluate episodic memory (the ability to recall 10 words after a five minute delay). The categorical verbal fluency test was used to assess language skills and executive functions. These tests have been validated in Finland for screening for mild AD in an elderly population (Sotaniemi et al., 2012). It is possible that the lack of an association between insulin resistance and delayed recall in studies I and II could be explained by the short delay between the word-list learning and the word-list delayed recall tests. Possibly, in a middle-aged population, a test with a longer delay before word recall would have been more sensitive

in detecting a subtle decline in delayed recall. The word-list learning test was performed slightly differently in Studies I and II, which might have influenced the results of both the word-list learning and the word-list delayed recall tests. However, only 17 participants had received full 30 points on the word-list learning test in 2000 (and thus had read the word list only once) and excluding these individuals did not affect the results of change in cognition in Study II.

In Study I reaction time and processing speed were assessed with a computer program. Unfortunately, this test was not included in the Health 2011 survey protocol, which is why the longitudinal associations between insulin resistance and reaction time could not be evaluated. The reaction time tests in Study I were apparently difficult to complete, as data were missing for 277 individuals for the reaction time test, and for 438 for the visual choice reaction time test. This could be the explanation for a lack of an association between insulin resistance and the visual choice reaction time test, which, in addition to the verbal fluency test, was used to assess executive functions in Study I.

The participants of Study III underwent a comprehensive neuropsychological examination in 2014–2016. However, as the focus of Study III was on amyloid accumulation and not cognitive functioning, only the results of the CERAD total score (Chandler et al., 2005) according to study group were presented to demonstrate that the study groups did not differ in cognitive functioning.

6.3.3 Definition of insulin resistance

HOMA-IR (Matthews et al., 1985) was used as an assessment of insulin resistance in Studies I–III. The baseline examinations in the Health 2000 survey included single measurements of insulin and glucose. Thus, an index of insulin sensitivity that could be assessed by the measurements available had to be used. There are more sophisticated and more accurate methods to estimate insulin resistance, most notably the hyperinsulinemic euglycemic clamp. However, such laborious methods are not feasible for use in large epidemiological studies such as the Health 2000 survey.

An obvious limitation of Studies I–III is the variation of fasting times of the participants. The participants of the Health 2000 survey were asked fast for a minimum of four hours before attending the health examination proper. The health examinations were conducted either in the morning or in the afternoon. Thus, not all participants had fasted overnight before the blood samples were drawn, and not all blood samples were drawn in the morning. The variation in baseline fasting times in Studies I and II are shown in figure 18.

HOMA-IR has been validated for usage after an overnight fast (Matthews et al., 1985). As HOMA-IR is calculated based on glucose and insulin values, the possible effects of fasting time on glucose and insulin levels need to be considered to evaluate the influence of fasting time in Studies I–III.

The secretion of insulin is pulsatile, and it is largely dependent on blood glucose values. There also seems to be a circadian rhythm in insulin secretion, at least under monitored clamp conditions. According to a 48-h hyperglycemic clamp study, serum insulin levels tended to be lowest during the night, peak in the early morning and stay elevated throughout the afternoon. This rhythm was disrupted in type 2 diabetes. (Boden et al., 1999). A large epidemiological study evaluated the impact of fasting duration on glucose levels. The study found no significant differences in blood glucose levels after a fasting time of >3 hours, when compared to the commonly used >8 hour fast, and concluded that a 3 hour fast would be sufficient before measuring blood glucose (Moebus et al., 2011).

Before deciding to use 4 hours as a minimum fasting time in Study I, the HOMA-IR values according to the participants´ fasting time were compared. There was no significant difference between HOMA-IR values among individuals who had fasted for 4–6 h, 6–8 h or 8–10 h before the blood samples were drawn (differences were assessed with the non-parametrical Kruskal-Wallis test, and each pair was compared by the Steel-Dwass method). Individuals who had fasted overnight, i.e. for longer than 10 hours had significantly higher HOMA-IR values than the other groups (possibly reflecting either the circadian rhythm of insulin secretion, or that glucose levels were higher in the morning), which allowed the assumption that including individuals with a fasting time 4–10 h in the analyses would not result in falsely greater HOMA-IR values.

In Study II additional analyses were conducted for the proportion of the study population that had fasted for longer than 10 hours (35.8%, n=1321, original publication II). In these analyses the associations between insulin resistance and cognitive functioning were similar to the analyses of the whole study population.

Considering the evidence presented above, it is probable that the variation in fasting times in our study population would have diluted, rather than strengthened the associations between insulin resistance and cognition. Thus, it seems unlikely that including individuals with a fasting time between 4 and 10 hours would have resulted in false associations between insulin resistance and cognitive test scores, or between midlife insulin resistance and late-life brain amyloid accumulation.

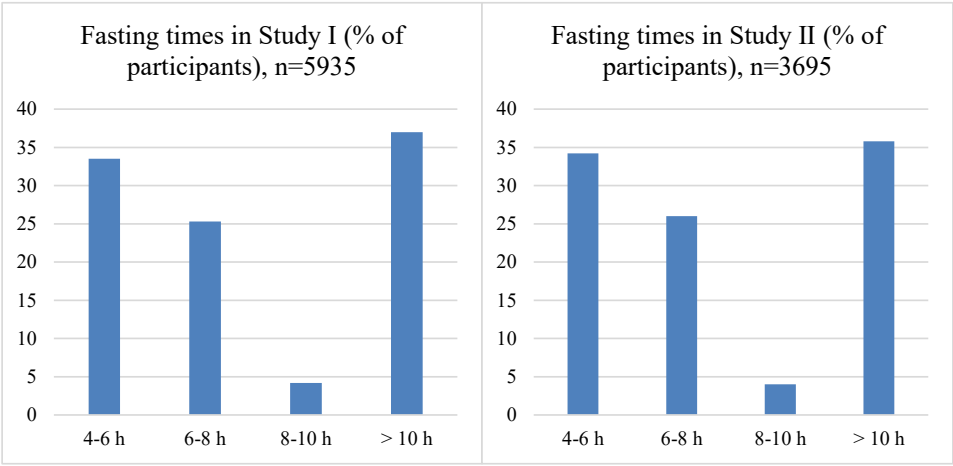


Figure 18. Variation in fasting time in Studies I and II.

6.3.4 PET imaging and data analysis

In Study III the [^{11}C]PIB-PET images were analyzed quantitatively as standardized uptake value ratios (SUVRs). In an article comparing different methods to quantitate [^{11}C]PIB uptake, late-scan (from 40 to 90 minutes) SUVR showed lowest test-retest variability, and was slightly better than the other methods evaluated (methods based on arterial blood sampling or arterial input) in distinguishing AD patients from normal controls (Lopresti et al., 2005).

In Study III, a [^{11}C]PIB SUVR composite score greater than 1.5 was chosen to represent amyloid positivity. There is no standard cut-off value to define amyloid positivity, based on [^{11}C]PIB uptake. The optimal cut-off varies depending on the age and disease stage of the study populations. In healthy, elderly controls a cut-off of 1.5 SUVR composite score has been validated in numerous studies (Pike et al., 2007; Jack et al., 2008; Bourgeat et al., 2010; Rowe et al., 2010; Villemagne et al., 2013). In the study by Villemagne et al. (2013) the [^{11}C]PIB SUVRs of 145 healthy controls aged 59–89 years were evaluated with hierarchical cluster analysis. The cut-off of 1.5 SUVR provided a sensitivity of 99% and an accuracy of 78% for distinguishing healthy controls from patients with AD.

6.4 Clinical implications

There are several possible pathways to explain our findings on an inverse association between the level of insulin resistance and verbal fluency performance. Categorical verbal fluency is thought to represent the function of both the prefrontal and the temporal cortex (Gourovitch et al., 2000); to reflect verbal ability, language skills and executive function (Shao et al., 2014); and to be closely related to fluid intelligence (Roca et al., 2012), thus declining with age (Salthouse, 2012). Consistent with the localization of verbal fluency in brain frontal and temporal regions, insulin resistance has been shown to associate with lesser gray matter volume in the temporal lobe (Benedict et al., 2012); and with a reduced glucose metabolic rate in the frontal and parietotemporal regions (Baker et al., 2011; Willette et al., 2015a; Willette et al., 2015b) in different populations.

There was no association between insulin resistance and word-list delayed recall or word-list learning in Studies I and II, in analyses adjusted for potential confounding factors. Both of these tests have been shown to discriminate cognitively normal elderly from individuals with early AD (Sotaniemi et al., 2012). The lack of an association between insulin resistance and these tests measuring memory could be due to the relatively young study populations in Studies I and II. Late-onset Alzheimer´s disease begins after 65 years of age, and the mean age in Study I was 52.5 years, and in Study II 49.3 years at baseline. The symptoms of Alzheimer´s disease typically begin with a decline in episodic memory, but this decline is only clinically evident close to the onset of the disease (Twamley et al., 2006; Villemagne et al., 2013). However, subtle cognitive decrements, including a slight decline in executive function, can be detected as early as 18 years before the diagnostic criteria of AD are met (Rajan et al., 2015).

The accumulation of amyloid in the cerebral cortex is a slow process that precedes the diagnosis of AD by approximately two decades (Villemagne et al., 2013; figure 2). Possibly, the decline in verbal fluency associated with insulin resistance in Study II could be an early sign of pathologic changes in the brain, later leading to AD. This hypothesis is supported by the results of Study III, which showed that although there was no significant difference in CERAD total score between the study groups, individuals with midlife insulin resistance had a greater level of amyloid in the cerebral cortex, when compared to non-insulin resistant individuals.

Study III showed that midlife insulin resistance increases the risk for an amyloid positive PET scan. The participants of Study III were well-functioning, dementia-free volunteers, with a mean age of 71 years. According to Villemagne et al. (2013), the threshold for amyloid positivity will be reached approximately 17 years before the onset of dementia. Thus, not all individuals with an amyloid positive

PET scan in Study III will develop dementia during their lifetime. However, according to the present research criteria for AD, these individuals would be classified as 'asymptomatic, at risk for AD' (IWG criteria) (Dubois et al., 2014), or as having 'preclinical AD' (NIA-AA criteria) (Sperling et al., 2011), indicating that if they lived long enough they would most probably proceed to develop Alzheimer´s dementia at a later stage in life.

As for the underlying mechanisms of the interactions between insulin resistance and sex, and insulin resistance and *APOE*ε4 on cognition, only hypotheses can be presented. These interactions were found only in the cross-sectional analyses in Study I. It is possible that the acute effects of insulin resistance on cognitive functioning are different from the long-term effects. Studies on intranasal insulin treatment have reported sex and *APOE*ε4 interactions (Reger et al., 2006; Benedict et al., 2008; Claxton et al., 2013). The acute effects of insulin administered intranasally might be mediated through changes in cellular insulin signaling, whilst the long-term effects of insulin resistance on brain functioning could depend on vascular mechanisms (Hughes and Craft, 2016), and possibly also on the accumulation of Aβ (Study III).

Sex differences in cerebrovascular lesions, such as white matter hyperintensities (WMHs) seen on MRI scans have been reported. Women seem to have more WMHs than men (Sachdev et al., 2009), and insulin resistance seems to correlate with WHMs (Katsumata et al., 2010). WMHs have been associated with poorer verbal fluency performance (Makino et al., 2014). These findings might explain the results regarding interactions between insulin resistance and sex found in Study I and in other cross-sectional studies on insulin resistance and cognition (Stolk et al., 1997; Schuur et al., 2010, Laws et al., 2017). However, the AIBL study also suggested an interaction between insulin resistance and sex on CSF biomarkers of AD, showing that the positive association between HOMA-IR and CSF P-tau and T-tau was found only in women (Laws et al., 2017). Thus, there are probably several different mechanisms that could render women more susceptible to the harmful effects of insulin resistance on cognition than men.

The individuals that were included in Study I, but were not included in Study II (n=2240) (figure 12), were older than the participants of Study II, which might be one explanation for that the sex differences were no longer found in Study II. Study III was not powered to detect sex differences, and thus the possible differences between men and women in amyloid accumulation associated with insulin resistance were not evaluated in Study III.

The possible mechanisms explaining the interactions between insulin resistance and *APOE*ε4 in Study I and in one longitudinal study thus far (Hughes et al., 2017) could be explained by *APOE* mediated changes in insulin signaling in the CNS,

and in insulin receptor density at the cellular surface of neurons, as demonstrated in *in vitro* and mice studies (Zhao et al., 2017). However, further preclinical research focusing on the potential mechanisms explaining the interactions between *APOE* genotype and insulin in the CNS is needed.

The differences in verbal fluency scores between the lowest and highest tertile of HOMA-IR in Study II were modest (≈1 point) when adjusted for age, sex and education (Figure 13) and most probably not of clinical significance at individual level. However, the difference between the highest and lowest tertile corresponded to a degree of cognitive decline during approximately six years of aging in Study II. In line with the findings from Studies I and II, modest decrements in cognitive test scores, in the degree of 0.3–0.5 SD units below the non-diabetic population, have been reported in patients with diabetes throughout the lifespan (Biessels et al., 2014; figure 19). According to Biessels et al. (2014) these subtle decrements in cognitive functioning most likely do not represent early dementia, but it is possible that they can lower the threshold for cognitive impairment and dementia later in life.

The results of this thesis suggest that individuals with insulin resistance represent a similar "at-risk" group for cognitive impairment and AD as individuals with diabetes (figure 19). Moreover, the results of Study III support the hypothesis, based on animal and *in vitro* findings, that midlife insulin resistance would have an important role in the early neuropathological process of AD, i.e. the accumulation of Aβ in the cerebral cortex.

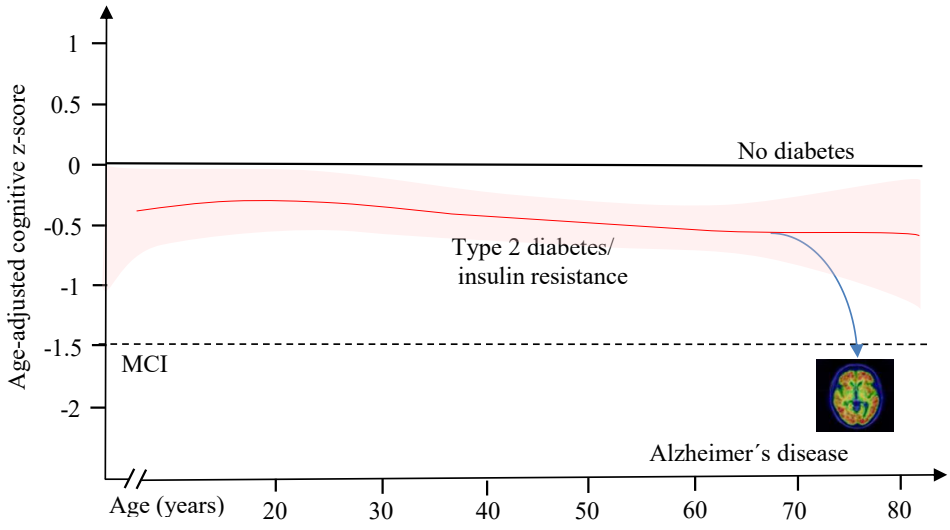


Figure 19. Estimated cognitive test scores in individuals with diabetes and/or insulin resistance across different age groups, compared to non-diabetic individuals (modified from Biessels et al., 2014).

One of the few randomized controlled trials reporting a positive effect on cognitive functioning was the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER). The study recruited elderly individuals who had an elevated risk for developing dementia according to the CAIDE dementia risk score (Kivipelto et al., 2006). In the study the treatment group that received a multidomain intervention (including diet, exercise, cognitive training and monitoring of vascular risk factors) was shown to outperform the control group on z-scores of a neuropsychological test battery after two years (Ngandu et al., 2015). These results suggest that in addition to traditional life-style interventions, also cognitive training might be needed to reduce cognitive impairment in elderly individuals.

At present, there is substantial evidence of midlife vascular risk factors influencing late-life risk for dementia and AD. Thus, health advice given in for example primary care should not only focus on risk for cardiovascular disease, but also on the risk for developing dementia. This risk can be evaluated with the CAIDE dementia risk score that evaluates a person´s individual risk for developing dementia during the following 20 years based on age, sex, educational level, BMI, systolic blood pressure, serum total cholesterol values, and physical activity (Kivipelto et al., 2006). The CAIDE dementia risk score also allows adding *APOE*ε4 genotype to the model to predict dementia risk, although, at present, the assessment of *APOE*ε4 genotype to evaluate the risk for AD in symptom-free individuals is not recommended, since no preventative treatment for AD or amyloid accumulation is yet available. However, as genetic testing is becoming more widely commercially available, doctors will most probably need to answer questions concerning for example the risk for AD associated with *APOE*ε4 genotype. Based on previous research, life-style interventions combined with cognitive training is effective for prevention of cognitive decline (Ngandu et al., 2015). Based on findings from this thesis, individuals with insulin resistance in midlife – and especially *APOE*ε4 carriers with insulin resistance – could benefit from life-style interventions that influence insulin resistance, and the previously reported vascular risk factors associated with insulin resistance, to decrease their risk for the neuropathology of AD, i.e. amyloid accumulation.

6.5 Future prospects

The cerebrovascular alterations that have been suggested to associate with insulin resistance (Hughes and Craft, 2017) were not evaluated in this thesis. Thus, future studies should include evaluation of brain vascular lesions such as WMHs, and the evaluation of the temporal course of amyloid accumulation and vascular lesions

associated with insulin resistance with follow-up studies, to determine if amyloid accumulation is preceded by cerebrovascular dysfunction. In addition, as insulin has been proposed to influence the hyperphosphorylation of tau, and thus the formation of neurofibrillary tangles (Cholerton et al., 2013; Mullins et al., 2017), neuroimaging studies utilizing radioligands that bind to tau-protein and CSF studies measuring P-tau and T-tau should be conducted. Another interesting line of research would be the association of insulin resistance with neuroinflammation that seems to play a role in the pathogenesis of AD (Kreisl et al., 2013), and the relationship of neuroinflammation with amyloid and tau accumulation, and cognitive functioning.

The cross-sectional interactions between insulin resistance and sex, and insulin resistance and *APOE*ε4 in Study I strengthen the hypothesis that personalized interventions might be useful in treatment and prevention of dementia. Treatment strategies targeted at specific risk populations are more likely to be effective than interventions in the general population (Hanson et al., 2015). Thus, future observational and treatment studies on insulin resistance and AD should assess possible sex and *APOE*ε4 interactions.

Considering the global epidemic of obesity and type 2 diabetes, enormous challenges to implement lifestyle interventions that would decrease the prevalence of dementia are faced. The long time course of amyloid accumulation (Villemagne et al., 2013), and the negative results of the lifestyle intervention studies (Luchsinger et al., 2015; Luchsinger et al., 2017) and the study on metformin (Luchsinger et al., 2017) on prediabetic individuals in midlife indicate that interventions to prevent insulin resistance and obesity might have to be started even earlier than in midlife. As none of the aforementioned intervention studies (Luchsinger et al., 2015; Luchsinger et al., 2017) included functional brain imaging, long-term intervention studies utilizing either imaging or CSF biomarkers of AD as end-points should be conducted.

Although the evidence for a link between insulin resistance and cognitive decline seems strong, no intervention targeted at midlife insulin resistance thus far has succeeded in preventing cognitive decline. However, treatment of patients with MCI or mild-to-moderate AD with insulin-related therapies show promising results. The findings from Study III suggest a very high risk for brain amyloid accumulation in individuals with midlife insulin resistance and *APOE*ε4 genotype, but this finding needs to be confirmed in larger studies. If these results were to be confirmed, future preventative treatments targeted at the neuropathological hallmarks of AD, such as anti-amyloid treatment, could be applied to this patient population, providing that future treatments would have been shown to be safe.

7 CONCLUSIONS

The following main conclusions of the work presented in this thesis can be made:

1. In the Finnish adult population, insulin resistance was associated cross-sectionally with poorer performance on categorical verbal fluency, a test measuring language skills and executive functions. This association was found only in women. In addition, the association between higher levels of insulin resistance and a lower verbal fluency score was found in non-carriers, but not in carriers of the *APOE*ε4 genotype. Insulin resistance was not associated with assessments of memory, i.e. word-list learning or delayed recall.

2. Insulin resistance was an independent predictor of poorer performance on the categorical verbal fluency test 11 years after the baseline measurements of insulin resistance. In addition, higher baseline insulin resistance predicted a greater decline in verbal fluency scores from baseline to follow-up. These associations were similar in men and women, and in carriers and non-carriers of the *APOE*ε4 genotype. No longitudinal associations were found among insulin resistance, word-list learning or word-list delayed recall.

3. Midlife insulin resistance was a risk factor for an amyloid positive PET scan in non-demented elderly individuals. Insulin resistance was an additive risk factor for brain amyloid accumulation in both carriers and non-carriers of the *APOE*ε4 genotype, suggesting that midlife insulin resistance is an independent risk factor for sporadic AD. 93.3% of the individuals who were both insulin resistant in midlife and *APOE*ε4 carriers had an amyloid positive PET scan at a mean age of 71 years, indicating that these individuals are at high risk for later developing Alzheimer´s dementia.

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Laura Ekblad

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