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To my family

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ABSTRACT

Insulin resistance is a common abnormal metabolic state related to obesity and many other health problems where target organs cannot respond normally to insulin. Insulin resistance and the dysfunction of the pancreatic insulin-secreting β-cells are the basic features of type 2 diabetes and its preceding stages, i.e. prediabetes. Diabetes is a risk factor for cognitive decline and progressive memory disorders, of which Alzheimer's disease (AD) is the most common. Presumably, the risk for cognitive decline begins increasing at the prediabetic stages.

This thesis aimed to clarify the associations between midlife insulin resistance, late-life cognitive performance, and neuroimaging changes related to AD among individuals with and without insulin resistance in midlife. Vascular changes were assessed with magnetic resonance imaging (MRI), and neuroinflammation and beta-amyloid (Aβ) deposition, which is an early marker of AD pathology, with positron emission tomography (PET) (n=60). This thesis also evaluated if measures from an oral glucose tolerance test (OGTT) can predict cognitive decline in the Finnish nationwide Health 2000 survey and its follow-up, the Health 2011 study (n=961).

Midlife insulin resistance was associated with poorer executive functions and slower processing speed but not with brain vascular changes after 15 years. Aβ deposits, but not vascular lesions, were related to slower processing speed. Aβ was associated with neuroinflammation among those with only small amounts of Aβ accumulation. Insulin resistance, low-grade systemic inflammation, and a higher body mass index (BMI) were associated with higher levels of neuroinflammation in brain regions where Aβ is first detected in AD. Higher 2-hour glucose (reflecting impaired glucose tolerance) and a decreased early insulin response (an indicator of β-cell function) in an OGTT predicted poorer performance and greater decline in a test of episodic memory after ten years.

These results indicate that midlife insulin resistance, impaired glucose tolerance, and decreased early insulin secretion – all related to prediabetic stages – are risk factors for cognitive decline. These results also suggest that early amyloid accumulation is associated with neuroinflammation.

KEYWORDS: beta-amyloid, cognitive decline, insulin resistance, magnetic resonance imaging, memory disorders, neuroinflammation, oral glucose tolerance test, [^{11}C]PBR28, [^{11}C]PiB, positron emission tomography, prediabetes

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TIIVISTELMÄ

Insuliiniresistenssillä tarkoitetaan tavallista, lihavuuteen ja moniin muihin terveysongelmiin kytkeytyvää metabolista häiriötilaa, jossa insuliinin vaste sen kohde-elimissä on poikkeava. Insuliiniresistenssi sekä haiman insuliinia erittävien beetasolujen heikentynyt toiminta ovat tyypin 2 diabeteksen ja sen esiasteiden tunnusomaisia piirteitä. Diabetes on riskitekijä kognition heikkenemiselle ja muistisairauksille, joista Alzheimerin tauti (AT) on yleisin. Kognition heikkenemisen riski kasvaa todennäköisesti jo tyypin 2 diabeteksen esiastevaiheissa.

Tämän tutkimuksen tavoitteena oli selventää keski-iän insuliiniresistenssin, myöhemmän iän kognition sekä AT:iin liittyvien kuvantamismuutosten välisiä yhteyksiä. Aivoja kuvannettiin magneettikuvantamisella (verenkiertoperäiset muutokset) ja positroniemissiotomografialla (PET) (AT:n patologiaan liittyvät amyloidikertymät (Aβ) sekä neuroinflammaatio) (n=60). Lisäksi laajojen Terveys 2000 ja 2011 tutkimusten avulla selvitettiin, voiko kahden tunnin glukoosirasituskoe ennustaa myöhempää kognition heikkenemistä (n=961).

Keski-iän insuliiniresistenssi oli yhteydessä heikompaan suoriutumiseen eksekutiivisia toimintoja ja prosessointinopeutta mittaavissa testeissä, mutta ei aivojen verenkiertoperäisiin muutoksiin 15 vuotta myöhemmin. Aβ-kertymät, toisin kuin verenkiertoperäiset muutokset, olivat yhteydessä hitaampaan prosessointinopeuteen. Aβ-kertymät olivat yhteydessä myös neuroinflammaatioon niillä tutkittavilla, joilla oli vain vähäisiä määriä aivojen Aβ-kertymiä. Insuliiniresistenssi, matala-asteinen tulehdus ja korkeampi painoindeksi olivat yhteydessä neuroinflammaatioon niillä aivoalueilla, jonne Aβ kertyy ensimmäisenä AT:ssa. Korkeampi kahden tunnin glukoosiarvo sekä matalampi varhainen insuliinivaste glukoosirasituskokeessa olivat yhteydessä heikompaan suoriutumiseen episodisen muistin testissä 10 vuoden seurannan jälkeen.

Tulokset viittaavat siihen, että keski-iän insuliiniresistenssi, heikentynyt glukoosinsieto ja alentunut ensivaiheen insuliinin eritys, jotka kaikki liittyvät diabeteksen esiasteisiin, ovat kognition laskun riskitekijöitä. Lisäksi näyttää siltä, että aivojen varhaiset Aβ-kertymät ovat yhteydessä neuroinflammaatioon.

AVAINSANAT: beeta-amyloidi, glukoosirasituskoe, insuliiniresistenssi, kognition lasku, magneettikuvantaminen, muistisairaudet, neuroinflammaatio, [^{11}C]PBR28, [^{11}C]PiB, positroniemissiotomografia, prediabetes

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Abbreviations

Aβ	Beta-amyloid
AD	Alzheimer's disease
ADA	The American Diabetes Association
APOE ε4	Apolipoprotein E ε4 genotype
APP	Amyloid precursor protein
BDI	Beck depression inventory
BMI	Body mass index
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CSF	Cerebrospinal fluid
CT	Computed tomography
DVR	Distribution volume ratio
EIR	Early insulin response
FDG	[^{18}F]fluorodeoxyglucose
FLAIR	Fluid Attenuation Inversion Recovery
HbA1c	Glycated hemoglobin
HDL	High-density lipoprotein
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
IAPP	Islet amyloid polypeptide
IDE	Insulin degrading enzyme
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IWG	The International Working Group
hs-CRP	High sensitivity C-reactive protein
MCI	Mild cognitive impairment
MetS	Metabolic syndrome
MMSE	The Mini-Mental State Examination
MRI	Magnetic resonance imaging
NIA-AA	US National Institute on Aging and Alzheimer's Association
NPH	Normal pressure hydrocephalus
NSAID	Nonsteroidal anti-inflammatory drug
OGTT	Oral glucose tolerance test
PET	Positron emission tomography
PBR	Peripheral benzodiazepine receptor

PiB	[^{11}C]Pittsburgh compound-B
ROI	Region of interest
SUV	Standardized uptake value
SUVR	Standardized uptake value ratio
TREM2	Triggering receptor expressed on myeloid cells 2
TSPO	Translocator protein 18 kDa
V_T	Distribution volume
WHO	World Health Organization
WMH	White matter hyperintensity
YKL-40	Chitinase 3-like protein 1

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I **Sini Toppala**, Laura L. Ekblad, Jyrki Lötjönen, Semi Helin, Saija Hurme, Jarkko Johansson, Antti Jula, Mira Karrasch, Juha Koikkalainen, Hanna Laine, Riitta Parkkola, Matti Viitanen, Juha O. Rinne. Midlife insulin resistance as a predictor for late-life cognitive function and cerebrovascular lesions. *J Alzheimers Dis*. 2019;72:215–228.

II **Sini Toppala**, Laura L. Ekblad, Jouni Tuisku, Semi Helin, Jarkko Johansson, Hanna Laine, Eliisa Löyttyniemi, Päivi Marjamäki, Kaj Blennow, Henrik Zetterberg, Antti Jula, Matti Viitanen, Juha O. Rinne. Association of Early Beta-amyloid Accumulation and Neuroinflammation Measured with [^{11}C]PBR28 in Elderly Individuals Without Dementia. *Neurology,* 2021;96:e1608–e1619.

III **Sini Toppala**, Laura L. Ekblad, Matti Viitanen, Juha O. Rinne, Antti Jula. Oral Glucose Tolerance Test Predicts Episodic Memory Decline: A 10-Year Population-Based Follow-up Study. *Diabetes Care,* 2021;44:2435–2437.

IV **Sini Toppala**, Laura L. Ekblad, Matti Viitanen, Juha O. Rinne, Antti Jula. Lower early insulin response to glucose load predicts episodic memory decline – a 10-year follow-up study. Manuscript submitted for publication.

1 Introduction

The prevalence of overweight and obesity has increased rapidly during the past few decades, and obesity has become a major health problem worldwide. (Ng et al., 2014) Obesity is strongly linked to numerous health problems such as insulin resistance, prediabetes, and type 2 diabetes. (Lingvay et al., 2022) These conditions have also become increasingly common globally. In the United States, the prevalence of prediabetes is estimated to be 38%. (Menke et al., 2015)

Insulin resistance, i.e. decreased insulin sensitivity, is when target tissues cannot respond normally to insulin. Consequently, insulin secretion from the pancreatic β-cells first increases, leading to elevated blood insulin levels, i.e. hyperinsulinemia. However, over time, β-cell failure occurs, and the blood glucose values increase. (Freeman & Pennings, 2022). Prediabetes is a term used to describe the condition where the blood glucose values of an individual are above normal but below diabetes thresholds. People with prediabetes are at high risk for diabetes, but not everyone with prediabetes will develop diabetes. (Perreault, 2019; Tabák et al., 2012) Previous epidemiological studies have indicated that type 2 diabetes is an independent risk factor for cognitive decline and dementia. (Cheng et al., 2012; Zhang et al., 2017) Further, it has been suggested that the cognitive impairment related to type 2 diabetes would begin developing at the prediabetic stages. (Biessels et al., 2014).

Memory and also other cognitive functions decline in progressive memory disorders (i.e. diseases causing dementia). Alzheimer's disease (AD) is the most common dementia-causing disease. Other common illnesses causing dementia include vascular cognitive impairment (vascular dementia), Lewy body disease, dementia related to Parkinson's disease, and frontotemporal lobar degeneration. Mixed dementia (with both AD and vascular pathology) is common, particularly among the elderly. (O'Brien & Thomas, 2015; Winblad et al., 2016) Most dementia-causing diseases develop slowly but progressively. In AD, the underlying pathological process has typically evolved for years before clinical symptoms emerge. Thus, the development of AD is regarded as a continuum with normal cognition at one end and dementia at the other end of the continuum. (Sperling et al., 2011). Neuroimaging and fluid biomarkers have made detecting pathological brain

changes related to memory disorders possible, even before symptoms are present. Regarding AD, this stage is often called preclinical AD.

Currently, no cure for dementia-causing diseases exists. Previous studies suggest that managing the modifiable risk factors for cognitive decline and dementia could postpone progressive memory disorders. (Ngandu et al., 2015; Norton et al., 2014) Thus, identifying those with a heightened risk for cognitive decline is important to target interventions.

The underlying pathological processes linking type 2 diabetes to cognitive decline and dementia are not yet fully understood, but presumably, multiple and possibly overlapping mechanisms behind the association exist. (Biessels et al., 2014; Srikanth et al., 2020) One possible hypothesis is that impaired insulin signaling or inflammation would link type 2 diabetes to cognitive decline since they are common molecular nominators both in type 2 diabetes and AD. (de Felice & Ferreira, 2014)

Neuroimaging Studies I and II of this thesis aim to clarify the associations among midlife insulin resistance, late-life cognitive decline, and neuroimaging changes related to memory disorders (including vascular changes, neuroinflammation, and beta-amyloid [Aβ] accumulation), while the epidemiological Studies III and IV based on the Health 2000 and 2011 surveys evaluate if measures from an oral glucose tolerance test (OGTT), a commonly used test to detect prediabetes and diabetes, can predict future cognitive decline.

2 Review of the Literature

2.1 Overview

This literature review will discuss disturbances of glucose metabolism, review the spectrum from normal cognitive aging to dementia, focus on the associations between glucose metabolism and cognitive decline, review neuroinflammation in cognitive disorders, and finally discuss the neuroimaging of patients with cognitive decline.

2.2 Disturbances of glucose metabolism

Among healthy people, glucose homeostasis is strictly regulated by several mechanisms. Normal fasting and postprandial blood glucose levels depend on normal insulin sensitivity of the target tissues and the expected function of the pancreatic insulin-secreting β-cells. Here, the concept of 'disturbances of glucose metabolism' is used as a general term to describe alterations in these functions and what they lead to (prediabetes and diabetes). Next, the concepts of insulin resistance and prediabetes will be discussed in more detail. Since this thesis focuses not on type 2 diabetes but the stages preceding its onset, type 2 diabetes will be reviewed less thoroughly.

2.2.1 Insulin resistance

Insulin resistance is a pathological state where target tissues – primarily skeletal muscle, the liver, and adipose tissue – cannot respond normally to insulin. Under normal conditions, insulin promotes glucose utilization (for example in skeletal muscle and adipose tissue) and suppresses glucose production in the liver from amino acids and other intermediates of metabolism (i.e. gluconeogenesis) and glycogen (i.e. glycogenolysis) as well as adipose tissue lipolysis. Insulin is the only hormone that lowers blood glucose levels through these mechanisms. Insulin resistance, in turn, impairs these processes. Thus, glucose disposal and the suppression of hepatic glucose production and lipolysis are impaired. The impairment of glucose disposal further leads to a compensatory increase in

pancreatic insulin secretion and consequently elevated insulin levels. (Figure 1). (Freeman & Pennings, 2022; Lam & LeRoith, 2019

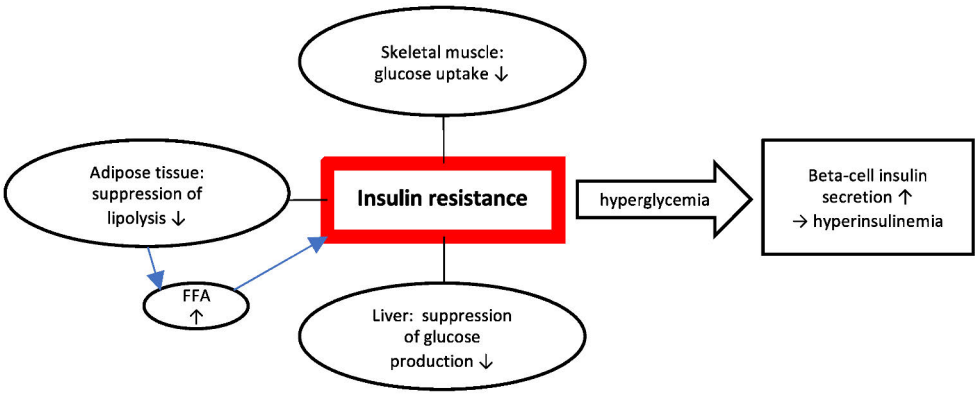

Figure 1. Schematic drawing of the influences of insulin resistance in skeletal muscle, adipose tissue, and the liver, as well as the compensatory increase of pancreatic β-cell insulin secretion. FFA = free fatty acids.

Insulin resistance typically occurs as a component of a cluster of metabolic disturbances originally called "Syndrome X" (Reaven, 1988), and then named "the insulin resistance syndrome" (Reaven, 2004) or "**the metabolic syndrome**" (MetS). According to Reaven, the syndrome is featured by insulin resistance, impaired glucose tolerance, hyperinsulinemia, increased triglycerides, decreased high-density lipoprotein (HDL) cholesterol, and hypertension. Reaven suggested that insulin resistance is the basic abnormality in the syndrome, whereas all the other changes are secondary to insulin resistance. (Reaven, 1988) Since then, multiple definitions and diagnostic criteria of MetS have been proposed. The main differences have concerned the measure for central obesity. In 2009, several major organizations gave a Joint Statement to harmonize the criteria for MetS. According to these criteria, the presence of any three of the following five risk factors constitutes a diagnosis of MetS. (K.G.M.M. Alberti et al., 2009)

- Elevated waist circumference (population and country-specific definitions)
- Elevated triglycerides (or drug treatments for elevated triglycerides)
- Reduced HDL (or drug treatment for reduced HDL)
- Elevated blood pressure (or antihypertensive drug treatment)
- Elevated fasting glucose (or drug treatment of elevated glucose)

Pathological conditions commonly associated with insulin resistance are demonstrated in Figure 2. Insulin resistance tightly relates to major public health problems such as obesity (especially visceral adiposity), hypertension, and cardiovascular disease. (Freeman & Pennings, 2022; Muniyappa et al., 2021) Ectopic lipid deposition, i.e. lipid accumulation in non-adipose tissues, is closely associated with insulin resistance and type 2 diabetes. (Trouwborst et al., 2018) It has been suggested that type 2 diabetes could result from excess fat in the liver and pancreas. (Taylor, 2021) Lifestyle modification, including nutritional intervention with calorie and high glycemic index carbohydrate reduction, decreased saturated and increased unsaturated fatty acid intakes, and increased physical activity, is the primary focus of treating insulin resistance. However, medications that improve insulin sensitivity or contribute to weight loss might be useful. (Freeman & Pennings, 2022; Jula et al., 2002; Pahkala et al., 2020; Vessby et al., 2001) Recent studies show that achieving remission, even in type 2 diabetes, is possible through weight reduction. The likelihood of remission after weight loss seems to depend mainly on the duration of diabetes. (Taylor, 2021)

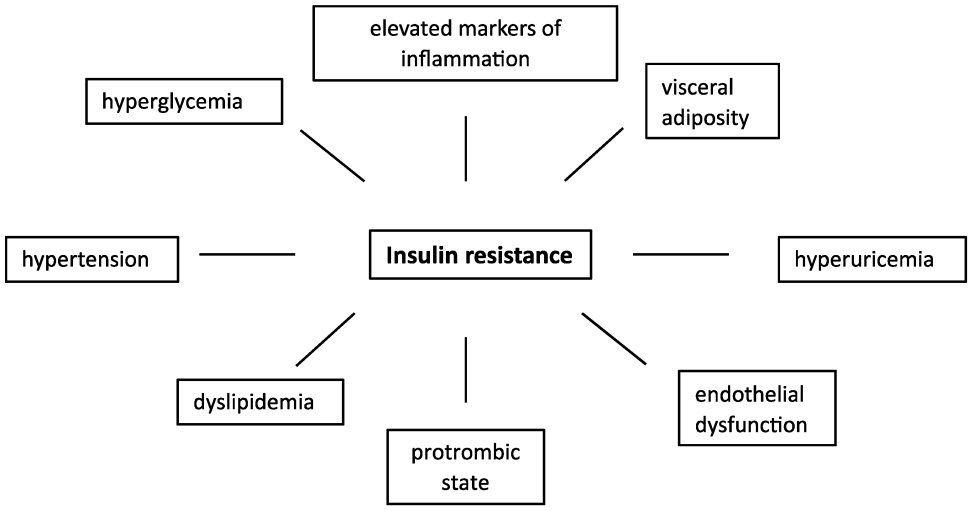

Figure 2. Manifestations of insulin resistance. (Freeman & Pennings, 2022; Muniyappa et al., 2021)

Several methods to evaluate insulin sensitivity/resistance have been developed – ranging from simple tests of fasting blood samples to complex, invasive, and time-consuming procedures. The gold standard method to assess insulin resistance is the **euglycemic-hyperinsulinemic clamp**. The test is performed after an overnight fast. Insulin is infused constantly, and plasma glucose is maintained at a steady level by

intravenous glucose infusion. The euglycemic-hyperinsulinemic clamp provides a steady-state estimate of insulin sensitivity/resistance where hepatic glucose production is assumed to be completely suppressed. The rate of infused glucose required reflects whole-body glucose disposal. Insulin sensitivity/resistance is calculated based on glucose disposal and body size. (DeFronzo et al., 1979; Freeman & Pennings, 2022) The **hyperglycemic clamp**, in turn, evaluates the β-cell activity of the pancreas. Plasma glucose concentration is raised acutely by glucose infusion and then held constant. During sustained hyperglycemia, the insulin response is biphasic: There is an early insulin response during the first six minutes, then a gradual increase in plasma insulin concentration. (DeFronzo et al., 1979) However, the clamp is laborious (two intravenous lines are required), time-consuming, and unusable in extensive epidemiological studies. Thus, more straightforward surrogate methods to quantify insulin sensitivity/resistance have been developed.

Homeostatic model assessment (HOMA) is used to evaluate the β-cell function and insulin sensitivity/resistance from fasting blood samples (glucose and insulin or C-peptide concentrations). HOMA was originally described by Matthews et al. in 1985. (Matthews et al., 1985) HOMA is derived from a mathematical evaluation of the interaction between β-cell function and insulin resistance based on experimental data on humans and animals. An estimate for insulin resistance (**HOMA-IR**) is calculated with an equation '(fasting insulin times fasting glucose) divided by 22.5'. HOMA-IR correlates well with measures derived from the euglycemic clamp. The model makes no distinction between hepatic or peripheral insulin sensitivity. (Mather et al., 2001; Matthews et al., 1985; Wallace et al., 2004; Wallace & Matthews, 2002) However, HOMA-IR is suggested to reflect above all insulin resistance in the liver. (Abdul-Ghani, 2006) No cut-off values for HOMA-IR exist, limiting the classifying of individuals as insulin sensitive or insulin resistant.

Assessing β-cell function (**HOMA-%B**) is calculated by an equation '(20 times fasting insulin) divided by (fasting glucose minus 3.5)'. The corrected nonlinear **HOMA2** computer model is an updated version of HOMA, which can be used to estimate insulin sensitivity/resistance and β-cell function from paired fasting plasma glucose and insulin or C-peptide concentrations. Like the original HOMA, HOMA2 assumes a feedback loop between the liver and the pancreatic β-cell. Glucose concentration in the basal state is regulated by (insulin dependent) glucose output, whereas insulin concentration depends on the β-cell response to glucose. HOMA2 considers variations in hepatic and peripheral glucose resistance, i.e. the reduction in the suppression of hepatic glucose output (by hyperglycemia) and the reduction of peripheral glucose stimulated glucose uptake. HOMA2 is suggested to be used when comparing HOMA with other models. (Matthews et al., 1985; Wallace et al., 2004)

The Quantitative Insulin Sensitivity Check Index (QUICKI) is another surrogate method of evaluating insulin sensitivity/resistance. QUICKI is a mathematical transformation of fasting blood glucose and plasma insulin concentrations. (Katz et al., 2000; Muniyappa et al., 2021)

HOMA and clamps provide steady-state measures of insulin sensitivity/resistance and insulin secretion, whereas the **intravenous glucose tolerance test (IVGTT)** and the OGTT provide measures of dynamic (non-steady-state) insulin sensitivity and insulin secretion. (Ferrannini & Mari, 1998) During the IVGTT, a rapid bolus of dextrose is given intravenously, and glucose and insulin concentrations are measured. Bergman et al.'s **minimal model** of glucose kinetics calculates insulin-mediated glucose disposal from the IVGTT. (Bergman et al., 1981) The minimal model is slightly less complicated to perform than the euglycemic-hyperinsulinemic clamp, since intravenous infusions requiring constant adjustment are unneeded. Nevertheless, the minimal model is laborious because intravenous lines and multiple blood sampling are required. (Muniyappa et al., 2021)

A standard **OGTT** is performed after an overnight fast of 8–14 hours. In OGTT, patients are given a glucose solution of 300 ml containing 75 g of glucose and water. Blood samples are drawn before and 120 minutes after the glucose ingestion. (Alberti & Zimmet, 1998) In research, insulin is commonly determined together with glucose and blood samples are often drawn at other time points as well (often at 0, 30, 60, and 120 min). OGTT reflects the disposal of glucose after an oral glucose load. (Muniyappa et al., 2021) Compared to steady-state measures, OGTT attempts to examine a more physiological state where the glucose load mimics a meal. None of the single glucose or insulin measures of the OGTT directly provide a good measure of insulin sensitivity/resistance, but individuals with higher glucose increments during the OGTT have shown to be more insulin resistant than those whose glucose values remain lower. (Ferrannini et al., 2011; Matsuda & DeFronzo, 1999) However, OGTT-derived methods utilizing insulin and glucose values, the **Matsuda** index, for example, have been developed to evaluate insulin sensitivity/resistance. (Matsuda & DeFronzo, 1999) Insulin secretion can also be estimated from the OGTT. The **early insulin response** (EIR) to oral glucose load can be calculated by the equation 'the increment above basal insulin or C-peptide divided by the increment in glucose in the same time interval' or as the increase in insulin or C-peptide above the basal up to 30 minutes after glucose ingestion. (Phillips et al., 1994)

Some surrogate measures of insulin sensitivity/resistance are calculated from triglycerides alone or triglycerides in relation to HDL cholesterol. However, these methods are outside the scope of this review. (Freeman & Pennings, 2022)

2.2.2 Insulin secretion

The islet **β-cells** of the pancreas secrete insulin. The ability of β-cells to respond to glycemic changes by increasing or decreasing insulin secretion is essential to glycemic control. An increase in circulating glucose concentration stimulates insulin secretion, promoting glucose uptake and suppressing hepatic glucose production. Although insulin's main function is maintaining glucose homeostasis, it has additional functions. Insulin is an anabolic hormone that stimulates numerous cellular responses and is involved in fat and amino-acid metabolism, and cardiovascular, kidney, and brain function. A severe lack of insulin results in extreme hyperglycemia, protein wasting, keto-acidosis, and ultimately death. (Ferrannini & Mari, 1998; Muniyappa et al., 2021) Conversely, excessive insulin is detrimental as it increases the risk of obesity and cardiovascular disease. (Kolb et al., 2020)

Insulin resistance leads to increased pancreatic insulin secretion, **hyperinsulinemia,** as an attempt to maintain euglycemia. (Figure 1). (Goldstein, 2002; Reaven, 1988) Hyperinsulinemia promotes weight gain, further increasing insulin resistance. This vicious cycle proceeds until **β-cell failure** occurs when the pancreatic β-cells can no longer fully compensate for the increased need for insulin; thus, glucose values begin increasing. (Freeman & Pennings, 2022) Also, an alternative theory has been proposed: hyperinsulinemia, *per se*, would contribute to insulin resistance. (Shanik et al., 2008) Hyperinsulinemia could be at least partly due to decreased insulin clearance. (Bergman et al., 2019) In turn, insulin resistance could be seen as a protective mechanism maintaining glucose homeostasis by preventing excess glucose transport despite hyperinsulinemia. (Kolb et al., 2020)

Although insulin's role is crucial in transporting glucose into the cells, previous studies have shown a non-insulin-dependent uptake of glucose into the cells, mediated by glucose. The ability of glucose *per se* to suppress endogenous glucose production and stimulate peripheral glucose uptake is called **glucose effectiveness** and can be estimated from the IVGTT. Glucose effectiveness is impaired for example in type 2 diabetes and obesity. (Ahrén & Pacini, 2021; Alford et al., 2018)

Previous studies have demonstrated that insulin resistance and pancreatic β-cell dysfunction start years before the clinical onset of diabetes. (Figure 3.) (Abdul-Ghani, 2006; Gastaldelli et al., 2004; Lillioja et al., 1993; Tabák et al., 2009; Zethelius et al., 2004) Insulin resistance and pancreatic β-cell dysfunction are considered hallmarks of prediabetes and type 2 diabetes (Kahn, 2001; Tabák et al., 2012) which will be discussed in the following chapters.

2.2.3 Prediabetes

Individuals with glycemic parameters above normal but below diabetes thresholds are considered to have "prediabetes" or "intermediate hyperglycemia"; the latter

term is used by the World Health Organization (WHO). (Alberti & Zimmet, 1998) The prevalence of prediabetes is increasing worldwide. Over 470 million people are estimated to have prediabetes by 2030. Individuals with prediabetes have an increased risk for diabetes with an annual conversion rate of 5–10%. However, not everyone with prediabetes will ever progress to diabetes. (D. H. Morris et al., 2013; Perreault, 2019; Tabák et al., 2012) People with prediabetes can be identified by measuring fasting glucose, glycated hemoglobin (HbA1c), or performing an OGTT. (Alberti & Zimmet, 1998; American Diabetes Association, 2018)

Two distinct types of prediabetes are **impaired fasting glucose (IFG)** and **impaired glucose tolerance (IGT)**. According to the WHO, IFG is defined as a fasting plasma glucose of 6.1–6.9 mmol/L (in the absence of IGT) and IGT as OGTT 2-hour glucose of 7.8–11.0 mmol/L. (Alberti & Zimmet, 1998) The American Diabetes Association (ADA) uses a lower cut-off value for IFG (5.6–6.9 mmol/L). ADA has also included HbA1c in the criteria: individuals with HbA1c between 39 and 47 mmol/mol (5.7–6.4%) are considered to have prediabetes. (American Diabetes Association, 2018). (Table 1)

Insulin resistance is present both in individuals with IFG and IGT, but the site of insulin resistance is different. IFG is especially associated with hepatic insulin resistance, while IGT is associated with insulin resistance, particularly in muscles. (Abdul-Ghani, 2006; Abdul-Ghani et al., 2006; Ferrannini et al., 2011) β-cell dysfunction is also present in both conditions. Individuals with IFG have impaired EIR during OGTT. However, their insulin secretion improves during the later phase of the OGTT, whereas individuals with IGT have impaired early and late phase insulin secretion. (Kanat et al., 2012; Tabák et al., 2012)

Table 1. The diagnostic criteria for IFG, IGT, and diabetes according to WHO and ADA. Modified from Alberti & Zimmet, 1998 and American Diabetes Association, 2018.

	Normal	**IFG**	**IGT**	**Diabetes**
fasting glucose (mmol/l)	≤ 6.0 (WHO) ≤ 5.5 (ADA)	6.1–6.9 (WHO) 5.6–6.9 (ADA)		≥ 7.0
OGTT 2-hour glucose (mmol/l)	< 7.8		7.8–11.0	> 11.0
Random glucose value of a patient with symptoms (mmol/l)				> 11.0
glycated hemoglobin (HbA1c)	< 39 mmol/ml (5.7 %) (ADA)			≥ 48 mmol/mol (6.5 %)

2.2.4 Type 2 diabetes

Type 2 diabetes is generally characterized by insulin resistance and a relative deficiency of insulin secretion leading to elevated blood glucose concentration. (Chatterjee et al., 2017; Solis-Herrera et al., 2018) The diagnostic criteria for diabetes are fasting glucose ≥ 7.0 mmol/l, 2-hour glucose in the OGTT ≥ 11.1 mmol/l, or HbA1c ≥ 48 mmol/mol (6.5%). A second test is recommended for confirmation unless a clear clinical diagnosis (i.e. classic symptoms of hyperglycemia and a random plasma glucose ≥ 11.1 mmol/L) exists. (Alberti & Zimmet, 1998; American Diabetes Association, 2018; Syed IA, 2011)

Type 2 diabetes is a heterogeneous disease with different subtypes. A recent study suggested that adult-onset diabetes could be classified into five subtypes with different underlying disease mechanisms, disease progression, and risk of complications. (Ahlqvist et al., 2018)

Insulin resistance has been shown to begin years before diabetes emerges. The development of type 2 diabetes from normal glucose tolerance is considered a continuous process with several stages. The first phase is a long compensatory period: Insulin resistance is present, but insulin secretion is increased as a compensatory mechanism. The second stage is considered a stable adaptation period: The pancreas can no longer fully compensate for the increased insulin resistance, so glucose values (fasting and post-load) are increased, although they remain within the normal range. In the next stage, glucose levels start rising because of β-cell dysfunction. (Tabák et al., 2012) For example, in the longitudinal British Whitehall II study, among individuals who developed diabetes, fasting glucose and post-load glucose values were higher over ten years before diabetes was diagnosed than in those who did not develop diabetes. A fast elevation of the glucose values was seen 3-6 years before the diabetes diagnosis. Insulin sensitivity had declined 13 years before the diabetes diagnosis; a steeper reduction was seen five years before the diagnosis. Among those who developed diabetes, insulin secretion was elevated throughout the 13-year follow-up, with a greater increase 3-4 years before diagnosis, followed by a steep decrease at diagnosis. (Tabák et al., 2009) Figure 3 demonstrates the pathophysiology of the diabetes continuum.

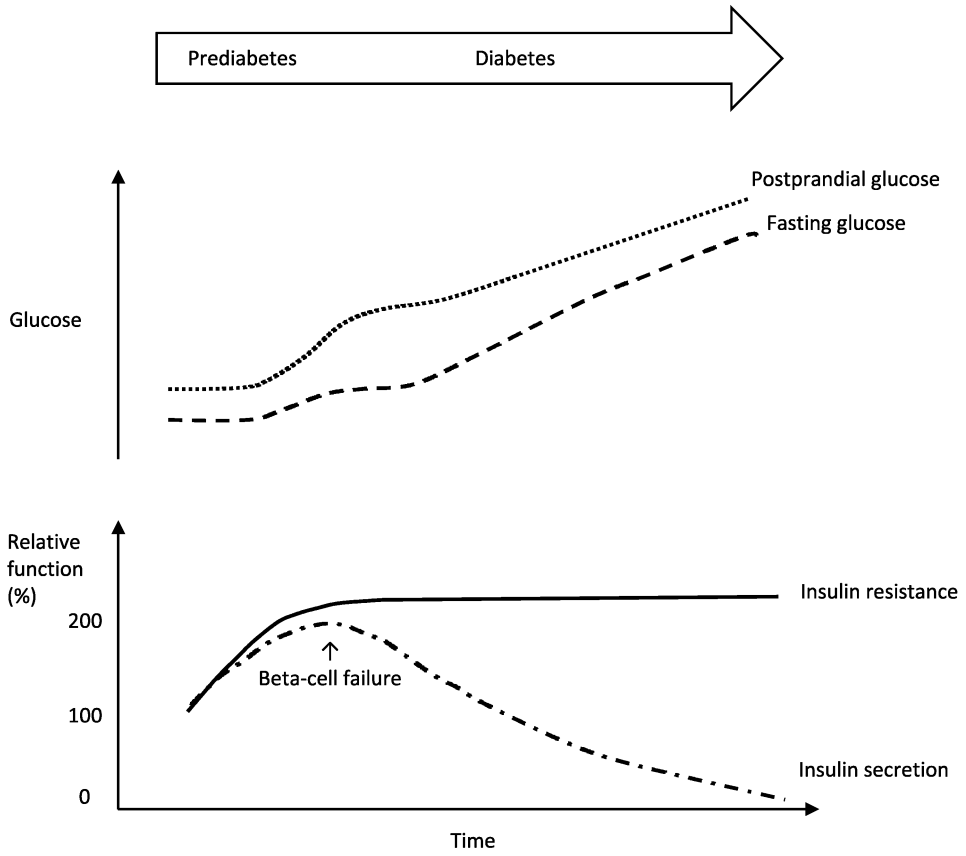

Figure 3. A schematic illustration of the pathophysiology of prediabetes and diabetes. Modified from Perreault, 2019.

2.3 A continuum from normal cognitive function to dementia

Memory and other cognitive functions decline in pathological conditions such as AD or other diseases causing dementia. Even among healthy elderly individuals, some cognitive functions – especially those dependent on cognitive processing speed and efficiency – are declined from earlier. Working memory and executive functions are considered the most vulnerable cognitive domains to ageing. However, the cognitive decline related to ageing is subtle and does not disturb an individual's ability to manage activities of daily living. (Cohen et al., 2019)

The development of memory disorders (especially AD) can be seen as a continuum with normal cognition at one end and dementia at the other end of the continuum. The point at which one transitions from the asymptomatic phase to the symptomatic predementia phase – or from the symptomatic predementia phase to the

onset of dementia – is difficult to identify. Further, the differential diagnostics of the illnesses causing cognitive decline and dementia may be complex. Cognitive and functional assessments are required and metabolic or structural etiologies responsible for symptoms should be ruled out. Traditionally, neuropathological confirmation has been needed for a definitive diagnosis. However, the development of biomarkers has aided the diagnostics of the AD continuum.

The next chapter will review the evaluation of cognitive function and the concepts of mild cognitive impairment (MCI) and dementia, after which AD – the most common disease leading to dementia – and AD biomarkers will be discussed.

2.3.1 Assessment of cognitive function

Cognitive performance is often characterized and classified by referring to domains of cognitive function. Domains such as memory and executive functions are hierarchical and not independent of each other. Typically, subdomains exist within each domain. (Harvey, 2019) Comprehensive neuropsychological test batteries consisting of several tests assess performance in different cognitive domains (e.g. attention and concentration, memory, executive functioning, language skills, and visuospatial abilities). Neuropsychological tests are sensitive to early and subtle cognitive changes. (Masur et al., 1994) However, a neuropsychological evaluation is time-consuming and unavailable for every patient with memory complaints.

The Mini-Mental State Examination (**MMSE**) (Folstein et al., 1975), The consortium to establish a registry for Alzheimer's disease (**CERAD**) test battery (J. C. Morris et al., 1989), and the Montreal Cognitive Assessment (**MoCA**) (Nasreddine et al., 2005) are cognitive tests commonly used in clinical practice. MMSE is a brief test for cognitive screening; however, is imprecise for detecting early cognitive decline. MoCA was created as an alternative screening method for MMSE, with a greater sensitivity to detect MCI. (Nasreddine et al., 2005) CERAD is a more comprehensive test including measures of cognitive functions where AD-related impairments initially occur. The Finnish version of CERAD (including verbal fluency; item naming; MMSE; word-list learning, recall and recognition; constructional praxis and recall; and clock drawing) is recommended as a screening tool for memory disorders among elderly patients in basic health care. (Hänninen et al., 1999)

2.3.2 Mild cognitive impairment

Mild cognitive impairment (MCI) describes the heterogeneous group of people with impaired cognitive function that is not severe enough to cause significantly impaired daily function. MCI occurs between a continuum with normal cognition at one end and dementia at the other. However, not everyone with MCI will ever develop

dementia – some may even recover. AD or any other disease affecting cognitive functioning can cause MCI. MCI's prevalence increases with age. Individuals with MCI are at high risk of developing dementia. Cumulative dementia incidence for MCI individuals older than 65 years, followed for two years, has been reported to be 15%. (Petersen et al., 2018)

The first diagnostic criteria of MCI focused on amnestic cognitive impairment, considered the prodromal stage of AD. (Petersen et al., 1997, 1999) Later, it became clear that underlying causes other than AD can lead to MCI, and a broader classification was needed. The International Working Group on MCI revised the MCI criteria in 2004. These modified criteria characterized MCI as a broader condition with multiple clinical profiles due to different etiologies. The general criteria for MCI are as follows: 1) considering cognitive measures, a person is abnormal and not demented, 2) a person's functional activities are mainly preserved, and 3) evidence of cognitive decline exists, measured by self and/or an informant report in conjunction with deficits on an objective cognitive evaluation and/or evidence of decline over time on an objective cognitive evaluation. (Winblad et al., 2004) The diagnostic criteria of MCI due to AD will be discussed in chapter 2.3.6.

2.3.3 Dementia

Dementia is a heterogeneous syndrome characterized by a significant decline in cognition severe enough to interfere with independent daily functioning. The loss of independence is the primary feature differentiating dementia from MCI. A group of diseases can cause dementia: **AD** is the most common disease causing dementia, accounting for 50–70% of cases. (Sacuiu, 2016; Winblad et al., 2016) **Vascular dementia** (vascular cognitive impairment) is the second most common dementia form, causing around 15% of cases. Vascular cognitive impairment can be classified into many subtypes depending on the pathological changes. (O'Brien & Thomas, 2015) Some other common neurodegenerative diseases causing dementia are **Lewy body disease**, **dementia related to Parkinson's disease**, and **frontotemporal lobar degeneration**. With ageing, the presence of various brain pathologies increases. It has been stated that among individuals over 80, **mixed dementia** (with vascular and AD pathology) is the norm, not the exception. (O'Brien & Thomas, 2015).

AD is characterized by a slow but progressive loss of cognitive function. AD typically begins with a decline in episodic memory, but behavioural, visuospatial, or language symptoms can be the main symptoms in less common variants of AD. The most common form of AD is the late-onset sporadic AD (from 65 years of age), accounting for about 95% of all cases. (Winblad et al., 2016) A substantial part of the early-onset AD cases (onset before age 65) are familial, caused by mutations in the amyloid precursor protein (APP), presenilin 1 (PSEN1), or presenilin 2 (PSEN2)

genes. (Bettens et al., 2013) Clinical stages of AD range from cognitively normal to MCI and dementia (Figure 4). Thus, dementia is considered the result of a long-time presence of AD pathology. (Scheltens et al., 2021)

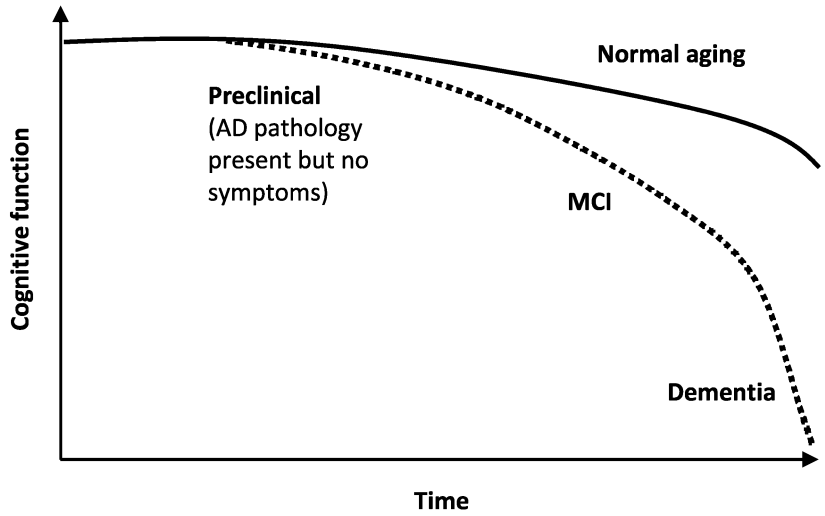

Figure 4. Hypothetical model of the continuum of AD. Modified from Sperling et al., 2011.

2.3.4 Basics of Alzheimer's disease pathology

The exact causes and pathogenesis of AD are not yet fully understood. However, AD is a multifactorial disorder where genetic and environmental factors and their interaction over the lifespan are thought to contribute to the disease's pathological processes and clinical manifestation. (Winblad et al., 2016) AD's pathological process is slow; AD pathology can be detected up to over 20 years before the onset of symptoms. (Jack et al., 2010; Jansen et al., 2015; J. C. Morris, 2005)

Brain **atrophy** (medial temporal lobe and cortical atrophy) is characteristic of AD. There is also a progressive loss of synaptic connections between neurons and a loss of neurons. Neuropathological hallmarks of AD are the accumulation of **extracellular plaques consisting of Aβ** and **intraneuronal neurofibrillary tangles consisting of hyperphosphorylated tau**. (Castellani et al., 2010; Hyman et al., 2012) In a recent post-mortem study on an elderly sample, most of the late-onset AD subjects did not have pure AD pathology. Instead, they had also DNA-binding protein 43 (TDP-43) (associated with frontotemporal lobe degeneration and amyotrophic lateral sclerosis [ALS]), α-synuclein (characteristic of Lewy body dementia and dementia related to Parkinson's disease) depositions, and hippocampal sclerosis and microvascular changes. (James et al., 2016)

2.3.5 Biomarkers in Alzheimer's disease continuum

The latest diagnostic criteria for AD have included using biomarkers in diagnosing AD. (Dubois et al., 2007, 2014; Jack et al., 2011, 2018; McKhann et al., 2011) Fluid biomarkers currently used in clinical practice, and the criteria emphasizing using biomarkers are reviewed briefly in the following sections. However, imaging biomarkers (temporomesial atrophy on MRI, posterior cingulate and temporoparietal hypometabolism on FDG-PET, and cortical Aβ accumulation on amyloid-PET) will be discussed in chapter 2.6.

A **biomarker** is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". (Atkinson et al., 2001) Biomarkers of AD are measurable indicators of specific AD-related pathology along the disease continuum. Biomarkers provide an opportunity to detect AD at the predementia stage. Early diagnosis is vital in clinical practice and in research. (Hane et al., 2017; Jack et al., 2010) Biomarkers are useful also in the differential diagnostics of illnesses causing dementia.

During the past three decades, three fluid biomarkers of AD measured from the cerebrospinal fluid (CSF) have been identified and evaluated in numerous studies. These core CSF biomarkers are the 42-aminoacid form of Aβ (**Aβ42**), total tau (**T-tau**), and phosphorylated tau (**P-tau**). Decreased levels of Aβ42 (reflecting cortical Aβ deposition) and increased T-tau (reflecting neurodegeneration) and P-tau (reflecting cortical tangle formation) have been shown to associate strongly with MCI due to AD and AD dementia. Also, a decreased Aβ42 level seems to predict AD dementia in the disease's preclinical stage. (Blennow & Zetterberg, 2018; Olsson et al., 2016) Each core biomarker differentiates AD patients from healthy controls with a sensitivity and specificity of around 80–90%. (Blennow et al., 2010) Besides Aβ42, there are several other Aβ species in the human CSF, such as Aβ40. Aβ40 alone does not identify AD but the ratio **Aβ42/Aβ40** detects AD better than Aβ42 alone. CSF Aβ42 and the Aβ42/Aβ40 ratio are the AD (CSF) biomarkers that become positive earliest during the AD continuum. (Blennow & Zetterberg, 2018)

2.3.6 Biomarker-based criteria for MCI and AD

In 2007, The International Working Group (**IWG**) revised The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (**NINCDS-ADRDA)** criteria for AD since modern imaging techniques and CSF AD-biomarker analyses had been developed. The IWG aimed to include the prodromal stages of AD and integrate biomarkers as supportive features in the criteria. The criteria were further updated in 2014. (Dubois et al., 2007, 2014) The US National Institute on Aging and

Alzheimer's Association (**NIA-AA**) workgroup defined updated **diagnostic and research criteria for the AD continuum** in 2011. The goal was to ensure that the revised criteria could be used in general healthcare lacking access to neuropsychological testing, advanced imaging, and CSF sampling, as well as in research with access to these measures. According to the workgroup, AD can be divided into three periods: **preclinical AD**; the earliest symptomatic stage i.e., **MCI**; and AD **dementia**. The core clinical criteria for MCI due to AD and AD dementia are intended to guide diagnosis in the clinical setting, whereas the criteria for preclinical AD are designed for research. (Albert et al., 2011; Jack et al., 2011; McKhann et al., 2011; Sperling et al., 2011)

The NIA-AA core clinical criteria for MCI include 1) concern about a change in cognition compared to the person's previous level, 2) impairment in one or more cognitive domains, 3) preservation of independence in functional abilities, and 4) the cognitive changes are sufficiently mild so that there is no significant impairment in social or occupational functioning. Also, other conditions that could account for the decline in cognition should be excluded. In the proposed research criteria, a positive biomarker of Aβ deposition and/or a positive biomarker reflecting neuronal injury are used to estimate the likelihood of MCI due to AD. (Albert et al., 2011)

If the clinical criteria for probable AD dementia are met, **biomarker evidence** may increase the certainty of AD's pathophysiological process, although using AD biomarkers for diagnostic purposes is not advocated routinely. However, using biomarkers to enhance the certainty of the AD patholophysiological process may be useful in investigational studies, clinical trials, and as optional clinical tools if they are available and when considered appropriate. (McKhann et al., 2011)

The NIA-AA research criteria for preclinical AD include three stages: Stage 1 is asymptomatic with cerebral Aβ; stage 2 is asymptomatic with cerebral Aβ and evidence of synaptic dysfunction, early neurodegeneration, or both (elevated CSF tau or P-tau, hypometabolism in an AD-like pattern on FDG-PET, or medial temporal lobe and/or cortical atrophy in a specific anatomic distribution on MRI); stage 3 contains cerebral Aβ, evidence of neurodegeneration, and subtle cognitive or behavioral decline. The workgroup emphasizes that also preclinical AD represents a **continuum**, and not all with preclinical AD will ever progress beyond the stage of Aβ accumulation. (Sperling et al., 2011) In 2018, the NIA-AA guidelines were updated for observational and interventional research use. These criteria focus on the diagnosis of AD with biomarkers. In the **AT(N) classification** system, biomarkers are categorized into three groups: Aβ deposition (A), pathologic tau (T), and neurodegeneration (N). (Jack et al., 2018)

2.4 Disturbances of glucose metabolism and the risk for cognitive decline

Several extensive studies have shown that diabetes is an independent risk factor for cognitive decline. According to previous meta-analyses evaluating longitudinal studies, individuals with diabetes have a higher risk for MCI, AD, vascular dementia, and any dementia than those without diabetes. (Cheng et al., 2012; Zhang et al., 2017). However, the cognitive impairment related to type 2 diabetes most probably begins to develop at the prediabetic stages and evolves slowly thereafter. (Biessels et al., 2014) The following chapters will focus on previous studies evaluating the association between prediabetes or conditions related to prediabetes and cognitive decline or cerebral alterations.

2.4.1 Evidence from epidemiological studies

According to previous longitudinal studies, **prediabetes** (defined by HbA1c) might be a risk factor for cognitive decline. The Atherosclerosis Risk in Communities Neurocognitive Study (n=13,351), the English Longitudinal Study of Aging (n=5189), and The Swedish National Study on Aging and Care-Kungsholmen (n=2746) all found that not only diabetes but prediabetes (defined by HbA1c levels) as well was associated with accelerated cognitive decline. (Marseglia et al., 2019; Rawlings et al., 2014; Zheng et al., 2018)

Several of the cross-sectional studies assessing **glucose tolerance test** results and cognitive function, summarized in Table 2, have suggested an association between a higher **2-hour glucose** level in the OGTT and poorer cognitive performance. For example, in a Swedish population-based study (n=2994), 2-hour glucose as well as fasting glucose were associated with a worse MMSE total score among people with and without diabetes. (Dybjer et al., 2018) Another study (n=411) showed a negative association between fasting and 2-hour OGTT glucose and episodic memory in women but not in men. (Rolandsson et al., 2008)

The previous longitudinal studies that examined the association between OGTT results and the risk for cognitive decline are summarized in Table 3, with conflicting results. (Kanaya et al., 2004; Kumari & Marmot, 2005; Lamport et al., 2009; Rönnemaa et al., 2009; Tuligenga et al., 2014; Vanhanen et al., 1998). Some studies have found that **IGT** (defined by OGTT 2-hour values) or a higher **2-hour glucose** concentration (as a continuous variable without a cut-off point) in the OGTT increases the risk of cognitive decline. (Kanaya et al., 2004; Rönnemaa et al., 2009; Vanhanen et al., 1998) Conversely, others have not found an association between IGT (defined by OGTT 2-hour values) and cognitive decline. (Kumari & Marmot, 2005; Tuligenga et al., 2014)

Table 2. Cross-sectional studies evaluating the association between glucose tolerance test results and cognitive function.

Study	N	Age (years)	Glucose tolerance test (time points of blood sampling [minutes])	Cognitive tests	Association between glucose tolerance test and cognition.
Vanhanen et al., 1997	108*	65	OGTT (glucose and insulin at 00, 60, 120)	neuropsychological test battery	Those with increased 2-h glucose and insulin values performed worse in cognitive testing than those with lower values.
Stolk et al., 1997	5510†	69	OGTT (insulin at 120)	MMSE	Higher post-load insulin was associated with a lower cognitive function in women.
Helkala et al., 2001	528 men§	54 or 60	OGTT (glucose and insulin at 00, 120)	word-list learning, TMT-A, TMT-B	No independent association between abnormal glucose tolerance and cognitive function.
Hiltunen et al., 2001	379†	76	OGTT (glucose at 00, 120)	MMSE	Impaired cognitive function was more frequent among those with abnormal than those with normal glucose tolerance.
Messier et al., 2003	57‡	55–84	OGTT (glucose and insulin at 00, 60, 120)	neuropsychological test battery	1-hour glucose and evoked glucose (60 min minus 00), but not insulin values correlated with cognitive performance.
Convit et al., 2003	30‡	68.6	IVGTT	Wechsler paragraphs recall tests, MMSE	Fasting and 2-h glucose, and glucose AUC were inversely associated with the Wechsler paragraph recall test scores.
Rolandsson et al., 2008	411†	51	OGTT (glucose at 00, 120)	recall, recognition, knowledge, fluency	Fasting and 2-h glucose were negatively associated with episodic memory in women but not in men.
Wright et al., 2015	172‡	64	OGTT (glucose at 0, 30, 60, 90, 120)	neuropsychological test battery	No association between 2-h glucose and cognitive performance after adjustments.
Dybjer et al., 2018	2994†	72	OGTT (glucose at 00, 120)	MMSE, AQT	Fasting and 2-hour glucose were associated with a worse cognitive performance.

*Participants of a study examining risk factors for myocardial infarction. †Population-based study. §Subpopulation of a population-based study. ‡Volunteers.

Table 3. Longitudinal studies evaluating the association between glucose tolerance test results and cognitive decline.

Study	N	Age (years)	Follow-up (years)	Glucose tolerance test (time points of blood sampling [minutes])	Cognitive test	Association between glucose tolerance test and cognition.
Vanhanen et al., 1998	586*	73	3.5	OGTT (glucose and insulin at 00, 60, 120)	neuropsychological test battery	Persistent IGT was associated with weaker cognitive function. Fasting and 2-h insulin were associated with lower MMSE in the persistent IGT -group.
Kanaya et al., 2004	999*	42–89	4	OGTT (glucose at 00, 120)	MMSE, verbal fluency, TMT-B	IGT was associated with impaired verbal fluency but only when the 25th percentile cut-off for cognitive decline was used.
Kumari & Marmot, 2005	5647†	56 at follow-up	2.5–6	OGTT (glucose at 00, 120)	neuropsychological test battery	IGT was unrelated to any measure of cognition.
Rönnemaa et al., 2009	1125 men*	71	12	OGTT (glucose and insulin at 00, 30, 120)	MMSE, TMT-A, TMT-B, 7-min screening test	2-hour glucose was associated with an increased risk of cognitive impairment and dementia. A low EIR, but not low insulin sensitivity, was associated with increased risk for AD.
Thambisetty et al., 2013	64§	57	12	OGTT (glucose at 0, 20, 40, 60, 80, 100, 120)	neuropsychological test battery	No differences between IGT and normal glucose tolerance groups in cognitive performance.
Tuligenga et al., 2014	5653†	54	10	OGTT (glucose at 00, 120)	neuropsychological test battery	Participants with IFG or IGT had similar rates of cognitive decline to those with normoglycemia.
Willmann et al., 2020	160§	65	4	OGTT (glucose and insulin at 00, 30, 60, 90, 120)	extended version of CERAD	Lower insulin sensitivity was associated with a steeper cognitive decline. No association between insulin secretion and cognition existed.

*Population-based study. †Occupational cohort study. §Cohort of elderly participants with prodromal markers of neurodegeneration.

Insulin resistance as a risk factor for cognitive decline has received increasing interest in recent years because it is one of the common molecular denominators in prediabetes, type 2 diabetes, and AD. (de Felice & Ferreira, 2014; Tabák et al., 2012) Further, obesity and MetS – associated with prediabetes and insulin resistance – have been linked to cognitive decline, AD, and all-cause dementia. (Schrijvers et al., 2010; Whitmer et al., 2008; Yaffe et al., 2004) Thus, insulin resistance is a potential factor linking type 2 diabetes and the increased risk for cognitive decline. Epidemiological studies, some of which will be reviewed in the next paragraph, have suggested an association between insulin resistance and declined cognitive function.

Several cross-sectional studies have found an association between insulin resistance or **hyperinsulinemia**, and poorer cognitive performance or AD. (Ekblad et al., 2015; Kuusisto et al., 1993, 1997; Laws et al., 2017; Tan et al., 2011) In a study of 744 nondiabetic individuals, Kuusisto et al. showed that those with hyperinsulinemia and hypertension had worse cognitive performance than those with normal insulin levels and hypertension. (Kuusisto et al., 1993) A few years later, the same study group reported that hyperinsulinemia was associated with AD in a cross-sectional study (n=980). (Kuusisto et al., 1997)

Similarly, in a longitudinal study of 683 persons aged 65 years and older (follow-up of 5.4 years), hyperinsulinemia was associated with a decline in memory-related cognitive scores and also with a higher risk for AD. (Luchsinger et al., 2004) Interestingly, a study of elderly men (n=2568) with a follow-up of over five years showed that both low and high levels of insulin were associated with an increased risk of dementia in a U-shaped functional form (the association between insulin and cognitive function was uninvestigated). (Peila et al., 2004) However, a recent study of a female population followed for over 34 years (n=1212) found that individuals with low but not with high fasting insulin values had an increased risk of dementia. (Mehlig et al., 2018)

Some longitudinal studies have assessed insulin resistance measured with **HOMA-IR** as a risk factor for cognitive decline or dementia. For example, the Atherosclerosis Risk in Communities (ARIC) study (n=7148) found that higher fasting insulin and HOMA-IR were associated with greater cognitive decline in delayed word recall and word fluency over six years. (Young et al., 2006) Similarly, Ekblad et al. showed in a population-based study (n=3695) that HOMA-IR was associated with poorer verbal fluency and a greater decline in verbal fluency after 11 years. (Ekblad et al., 2017) A recent study found an association between insulin resistance and cognitive decline (n=1299) but not with AD CSF biomarkers (n=212) in a sample of middle-aged and older adults without dementia at baseline. (Ennis et al., 2021) Hughes et al. showed that change in HOMA-IR was associated with lower cognitive function among APOEε4-negative individuals but not among APOEε4-carriers (n=4392, follow-up ten years). (Hughes et al., 2017) In the Rotterdam study

(n=3139), higher HOMA-IR was associated with a higher risk of AD within three years of baseline, but the risk did not increase thereafter. (Schrijvers et al., 2010)

The discrepancies across the studies are presumably explained by the heterogeneity of the study populations (age, sex, different exclusion criteria), the use of different measures of cognitive functioning and insulin resistance, and the heterogeneity in the confounding factors included in the analyses.

Changes in **insulin secretion** during an OGTT have also been linked to cognitive decline and dementia. A study that examined 1125 elderly Swedish men with a follow-up of 12 years showed that a lower early insulin response (EIR) to oral glucose load was associated with an increased risk for AD. (Rönnemaa et al., 2009) The same study group also showed that impaired insulin secretion at midlife, assessed with the intravenous glucose tolerance test (IVGTT), was associated with an increased risk for cognitive decline, AD, and any dementia after 32 years in a sample of 1792 men. (Rönnemaa et al., 2008) Vanhanen et al. found that higher 2-hour insulin levels in the OGTT were associated with lower MMSE scores among participants with IGT but not among participants with normal glucose tolerance. (Vanhanen et al., 1998) In contrast, a small cross-sectional study investigating 57 nondiabetic participants (aged 55–84) found no correlation between the insulin values measured at 00, 60, and 120 minutes or HOMA-IR and the cognitive test results. (Messier et al., 2003)

2.4.2 Evidence from neuroimaging and neuropathological studies

Insulin resistance seems to be associated with **brain glucose metabolism**, **brain atrophy**, and potentially with **brain vascular** and **Aβ pathology** already in the prediabetic stages. (Dearborn et al., 2015; Ekblad et al., 2018; Lee et al., 2016; Matsuzaki et al., 2010; Tan et al., 2011; Willette, Johnson, et al., 2015) Baker et al. demonstrated that HOMA-IR was associated with lower glucose metabolism in brain regions susceptible to AD in cognitively healthy elderly adults with prediabetes or newly diagnosed type 2 diabetes (n=23, mean age 74 years). (Baker et al., 2011) Accordingly, Willette et al. showed similar results with a larger study sample (n=150). (Willette, Bendlin, et al., 2015)

The longitudinal Framingham Offspring study (n=2439) demonstrated that insulin resistance was associated with a lower total brain volume. (Tan et al., 2011) Aligning with the Framingham Offspring study, Willette et al. showed in a cohort of asymptomatic, late middle-aged individuals that higher HOMA-IR predicted a lower brain volume in regions that are affected early in AD. (Willette et al., 2013)

According to the Atherosclerosis Risk in Communities study (n=934), MetS and insulin resistance were associated with incident lacunar infarcts but not with white

matter hyperintensity (WMH) progression. (Dearborn et al., 2015) MetS has been linked to cerebrovascular changes also in other studies. (H. M. Kwon et al., 2006; Portet et al., 2012; Viscogliosi et al., 2015)

A few previous PET studies have shown an association between insulin resistance and Aβ accumulation (Ekblad et al., 2018; Willette, Johnson, et al., 2015), while others (Laws et al., 2017; Pekkala et al., 2020; Thambisetty et al., 2013) have not. In a recent longitudinal study, insulin resistance did not predict change in AD biomarkers (CSF markers or Aβ chronicity measure with Aβ-PET). (Ennis et al., 2021) Presumably, the discrepancy is explained by differences in the study designs (cross-sectional or longitudinal) and the variation in the ages of the study populations.

A neuropathological study showed that higher 2-hour glucose in the OGTT, fasting insulin, and HOMA-IR ten years antemortem were all associated with an increased risk for Aβ accumulation postmortem. (Matsuzaki et al., 2010) However, another study found no significant association among HOMA-IR values, lifetime glucose intolerance (evaluated by OGTT), or diabetes and brain Aβ detected by PET imaging or post-mortem AD pathology. (Thambisetty et al., 2013)

2.4.3 Possible pathological mechanisms

Multiple etiologies presumably determine cognitive decline in diabetes. (Biessels et al., 2014; Srikanth et al., 2020) Hyperglycemia might influence brain function negatively via several mechanisms such as glucose neurotoxicity, vascular mechanisms, and accumulation of advanced glycation end products. (Kellar & Craft, 2020) Insulin resistance, inflammation, and mitochondrial dysfunction are common features in type 2 diabetes and AD. (de Felice & Ferreira, 2014; Pugazhenthi et al., 2017) Here, the possible insulin-related mechanisms linking disturbances of glucose metabolism and cognitive decline will be discussed. The topic has recently been reviewed comprehensively by Ribe and Lovestone, Kellar and Craft, and Biessels et al. (Biessels et al., 2020; Kellar & Craft, 2020; Ribe & Lovestone, 2016) Inflammation will also be discussed briefly.

Insulin was first identified in the rat brain in the late 70s. (Havrankova et al., 1978) Since then, increasing evidence has emerged about **insulin and insulin-related signaling in the brain**. Insulin, secreted by the pancreas, is transported into the brain through the blood-brain barrier via a receptor-mediated transport process in a saturable manner. (Banks et al., 2012) Insulin receptors are ubiquitously expressed in the brain and densely expressed for example in the hippocampus and prefrontal cortex. (Arnold et al., 2018; Kullmann et al., 2020) Cerebral insulin action is associated with multiple behavioral and metabolic consequences; disturbances of

brain insulin signalling seem linked to ageing, dementia, obesity, and type 2 diabetes. (Kullmann et al., 2016)

Insulin influences **the formation and maintenance of synapses**. Insulin regulates synaptic plasticity for example in the hippocampus – a crucial brain region for memory function. **Brain insulin resistance**, in turn, is associated with impaired synaptic integrity. (Arnold et al., 2018; Kellar & Craft, 2020) It has been proposed that central insulin resistance might occur independently of glycemic control and peripheral insulin resistance and that brain insulin resistance increases with ageing. (Fadel & Reagan, 2016; Ribe & Lovestone, 2016) Insulin resistance occurs at a cellular level in the brains of patients with AD compared to individuals with MCI or normal cognition. In a previous study, higher cerebral insulin resistance post-mortem was associated with poorer antemortem episodic and working memory test results, independently of AD pathology and APOE genotype, suggesting that cerebral insulin resistance, and insulin-related signalling in the brain, could directly influence cognitive functioning. (Ribe & Lovestone, 2016; Talbot et al., 2012)

Intranasal insulin administration, in which insulin bypasses the blood–brain barrier along the olfactory nerve channels and the trigeminal perivascular channels, has been investigated as a potential treatment for cognitive disorders, and small trials have provided some initial promising results. (Kellar & Craft, 2020). For instance, in a study investigating patients with early AD or amnestic MCI, those receiving intranasal insulin had better cognitive test results in word-list delayed recall and attention after three weeks of treatment than the placebo group. (Reger et al., 2008) However, further studies with larger study samples and longer follow-up times are needed to assess the benefit of intranasal insulin for cognitive function.

Acute peripheral hyperinsulinemia increases brain and CSF insulin levels and seems to positively influence cognitive function. **Chronic peripheral hyperinsulinemia**, however, downregulates blood–brain barrier insulin receptors possibly leading to **reduced transport of insulin into the brain** and resulting in reduced insulin signaling and impairment of neuronal function and synaptogenesis. Thus, chronic hyperinsulinemia is considered detrimental to cognitive function. However, whether the impaired brain insulin action is a consequence of the reduced insulin transport across the blood–brain barrier or due to cerebral insulin resistance – or both – is unclear. (Heni et al., 2014; Ribe & Lovestone, 2016; Schwartz et al., 1990)

Peripheral insulin resistance is linked to endothelial dysfunction (Freeman & Pennings, 2022); with other cardiovascular risk factors (e.g. hypertension), insulin resistance increases the risk for cerebrovascular disease and subsequent cognitive dysfunction (vascular cognitive impairment). (Winblad et al., 2016) Insulin influences cerebral perfusion. At normal concentrations, insulin acts as a vasodilator, but during hyperinsulinemia, it is a vasoconstrictor. Thus, insulin resistance

promotes vasoconstriction, leading to elevated blood pressure and decreased cerebral perfusion. (Kellar & Craft, 2020)

Another potential link between insulin and cognitive decline is **amylin** i.e. islet amyloid polypeptide (IAPP), secreted by the pancreatic β-cells along with insulin. In hyperinsulinemia, amylin levels also increase. Amylin can cross the blood–brain barrier, and amylin plaques have been found postmortem in the brains of AD patients. (Enoki et al., 1992; Jackson et al., 2013)

Insulin might also influence the clearance of **Aβ** (and thus the accumulation of Aβ) and the **phosphorylation of tau** – the hallmarks of AD pathology. Some animal studies with mice have suggested that dysregulation of insulin signaling in the brain would promote tau hyperphosphorylation and synaptic dysfunction. (Arnold et al., 2014; Kothari et al., 2017; Liu et al., 2015) In a preclinical study, insulin increased APP secretion by a glucose-independent mechanism. (Solano et al., 2000) Furthermore, insulin, amylin, and Aβ are all substrates for a common degrading enzyme in the brain: **the insulin degrading enzyme (IDE**). (Cholerton et al., 2013; Qiu & Folstein, 2006) In an animal study with mice, IDE deficiency resulted in an over 50% decrease in Aβ degradation. (Farris et al., 2003) IDE is downregulated in mice with insulin resistance, resulting in Aβ accumulation in the brain. (Mullins et al., 2017)

Brain insulin resistance has also been linked to disturbances in peripheral metabolism. Functional MRI, electroencephalography (EEG), and magnetoencephalography (MEG) studies have shown that peripheral insulin resistance is associated with a reduced response to intranasal insulin among people with insulin resistance -related disorders such as obesity and type 2 diabetes. (Heni et al., 2015) It has been proposed that the hypothalamus is the brain region controlling the whole-body energy homeostasis. Boosting brain insulin action could possibly modulate peripheral metabolism by increasing insulin sensitivity and by suppressing hepatic glucose production. (Kullmann et al., 2020)

Inflammation

Chronic low-grade inflammation and the overproduction of proinflammatory cytokines, such as tumor necrosis factor alpha and interleukin 6, typically accompany obesity and obesity-related metabolic disorders such as insulin resistance and type 2 diabetes. Whether inflammation is the cause or the consequence of these states is still unclear. However, it has been proposed that inflammation could lead to insulin resistance and disruption of energy homeostasis. An inflammatory program is activated early in the adipose tissue expansion, and during chronic obesity, the immune system is shifted to a proinflammatory phenotype. (Esser et al., 2014; Hotamisligil, 2006; Saltiel & Olefsky, 2017)

It has been hypothesized that also inflammation could be the link between disturbances of glucose metabolism and cognitive decline. Chronic low-grade inflammation is associated with cognitive decline and an increased risk of AD. (Singh-Manoux et al., 2014; Tan et al., 2007; Tao et al., 2018; Walker et al., 2019; Yaffe et al., 2004) Several epidemiological studies have also shown that using nonsteroidal anti-inflammatory drugs (**NSAIDs**) could reduce the risk of AD, however, most randomized controlled trials assessing NSAIDs for treating AD have not shown the efficacy of NSAIDs. (Jaturapatporn et al., 2012; Vlad et al., 2008; Wang et al., 2015) Further, systemic immune cells and secreted signaling proteins seem to communicate with the brain, and they are suggested to associate with neuroinflammation and neurodegeneration. (Czirr & Wyss-Coray, 2012) The inflammatory mediators can cross the blood–brain barrier and they are suggested to influence brain microglia and astrocytes resulting in neuroinflammation. (Ribe & Lovestone, 2016) Neuroinflammation's role in diseases causing cognitive decline will be discussed in chapter 2.5.

2.4.4 Other risk and protective factors for cognitive decline and Alzheimer's disease

Known risk and protective factors for cognitive decline and dementia are represented in Figure 5. **Life-style-related metabolic and vascular risk factors in midlife** increase the risk for cognitive decline. (Kivipelto et al., 2005, 2006; Livingston et al., 2020; Norton et al., 2014; Winblad et al., 2016) Midlife hypertension, hypercholesterolemia, obesity, and smoking have been linked to increased risk for cognitive decline or AD. (Kivipelto et al., 2005; Livingston et al., 2020; Whitmer et al., 2008) However, in older age, higher weight is associated with decreased risk of dementia. (Danat et al., 2019) Considering weight is known to decrease in later life in those with or developing dementia, lower weight among the elderly might indicate illness. (Singh-Manoux et al., 2018)

High **education** early in life is associated with a decreased risk of dementia. (Meng & D'Arcy, 2012) Performing cognitively or mentally stimulating activities and maintaining a rich social network have also been associated with a reduced risk of dementia, whereas depression and hearing loss seem to increase the risk for cognitive decline and dementia. (Diniz et al., 2013; Livingston et al., 2020; Slade et al., 2020; Winblad et al., 2016)

It has been suggested that "**cognitive reserve**" and "**brain reserve**" explain the differences between individuals' susceptibility to pathological brain changes. (Stern, 2012) Cognitive reserve is thought to represent the ability to engage alternative brain networks or cognitive strategies to manage the pathological changes. Brain reserve refers to the brain's capacity to withstand the pathological changes, possibly due to

a greater synaptic density or a larger number of healthy neurons. Further, psychosocial factors might also modify the association between neuropathology and cognitive function. (Bennett et al., 2006, 2014)

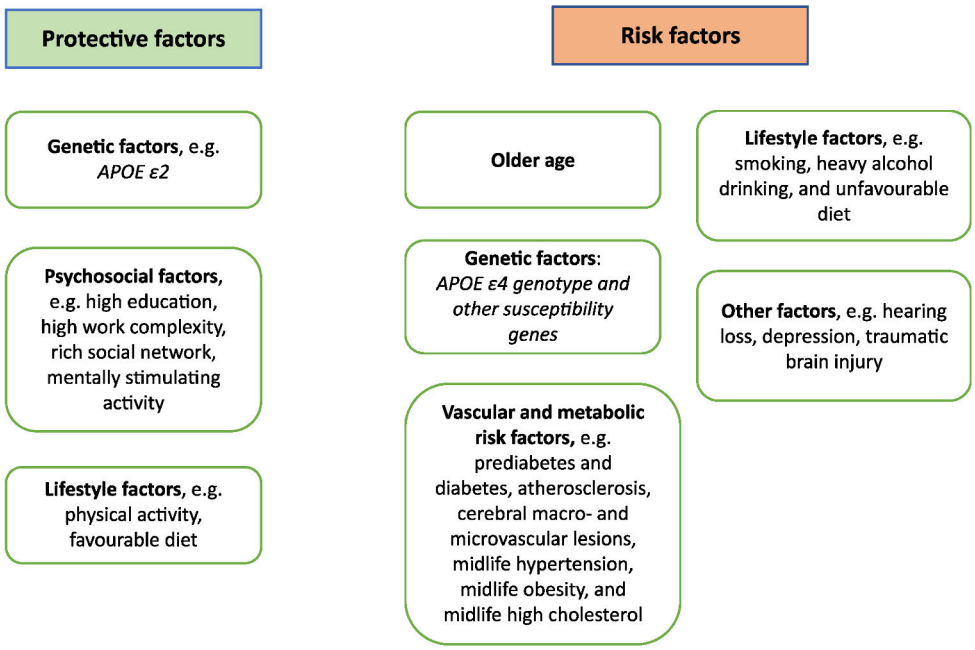

Figure 5. Putative protective factors and risk factors for cognitive decline or late-onset dementia. Modified from Winblad et al., 2016.

The apolipoprotein E (APOE) ε4 allele is the most well-established genetic risk factor for late-onset AD. (Bettens et al., 2013; Corder et al., 1993) Carrying one ***APOE ε4*** allele increases the risk of developing AD by approximately 3.7 times compared to the most common *APOE ε3/ε3* genotype, whereas carrying one or two *APOE ε2* alleles reduces the risk of AD. Being homozygous for the *APOE ε4* allele increases the risk up to 12 times. (Reiman et al., 2020; Serrano-Pozo et al., 2021) *APOE ε4* carriers have cerebral Aβ load at an earlier age on average and faster Aβ accumulation than non-carriers. (Mishra et al., 2018) During the last decade, technological development has enabled more comprehensive research on the genetic background of diseases leading to dementia, especially with genome-wide association studies and sequencing studies. Up to 50 genes/loci have been linked to the increased risk of AD, even if some might be false-positive findings. ***TREM2*** is the most studied genetic risk factor of the microglia-dependent pathophysiological process in AD. (Bellenguez et al., 2020; Sims et al., 2020) Previously, *TREM2* had been associated with Nasu-Hakola disease. (Paloneva et

al., 2002) However, recent studies have shown that rare variants of *TREM2* increase the risk for late-onset AD, with an odds ratio like that of the *APOE ε4* allele. (Jiang et al., 2013; Jonsson et al., 2013; Neumann & Daly, 2013) *TREM2* will be reviewed in the following chapter.

2.5 Neuroinflammation in cognitive disorders

Neuroinflammation, i.e. the reactivity of microglia and astrocytes, the immune cells of the brain and elevated levels of inflammatory mediators, respectively, is suggested to play a crucial role in the pathogenesis of AD and other neurodegenerative diseases. (Calsolaro & Edison, 2016; Heneka et al., 2015; McGeer & McGeer, 2013; van Eldik et al., 2016) Inflammatory markers such as cytokines and complement factors have been found in the CSF and Aβ plaques in patients with AD. (Aktas et al., 2007)

However, the time-course and pathogenic relevance of neuroinflammation across the AD continuum and other diseases causing dementia is not yet fully understood. It has been suggested that neuroinflammation would be beneficial and detrimental, depending on the stage of the disease pathogenesis. (Edison & Brooks, 2018) Neuroinflammation's consequences depend on the molecular mediators the brain cells produce. (Czirr & Wyss-Coray, 2012; Dá Mesquita et al., 2016) The initial microglial activation is possibly protective, but the inflammatory process might become unfavorable with disease progression. (Hamelin et al., 2016; van Eldik et al., 2016) Individuals with defective microglial functioning might develop cognitive impairment at an earlier stage of the pathological process of AD than those with normal microglial function (Figure 6). (Leng & Edison, 2021)

Several AD risk genes, such as *TREM2* relate to microglial response pathways, highlighting the potential role of neuroinflammation in the pathogenesis of AD. The *TREM2* gene encodes triggering receptor expressed on myeloid cells 2 (**TREM2**). TREM2 is expressed on macrophages, dendritic cells, osteoclasts, and microglia. (Jiang et al., 2013) In the CNS, TREM2 is selectively expressed by microglia. In the normal brain, TREM2 is distributed at high levels in the white matter, hippocampus, and neocortex but at low levels in the cerebellum – partly consistent with the Aβ accumulation pattern. (Guerreiro et al., 2013) TREM2 has been suggested to suppress the proinflammatory response of microglial cells and may participate in regulating phagocytosis of Aβ and damaged neurons. Thus, the loss of function of TREM2 might result in inflammation and Aβ accumulation. (Jiang et al., 2013) However, the mechanisms underlying the link between neuroinflammation and cognitive disorders and between TREM2 and AD must be elucidated in the future.

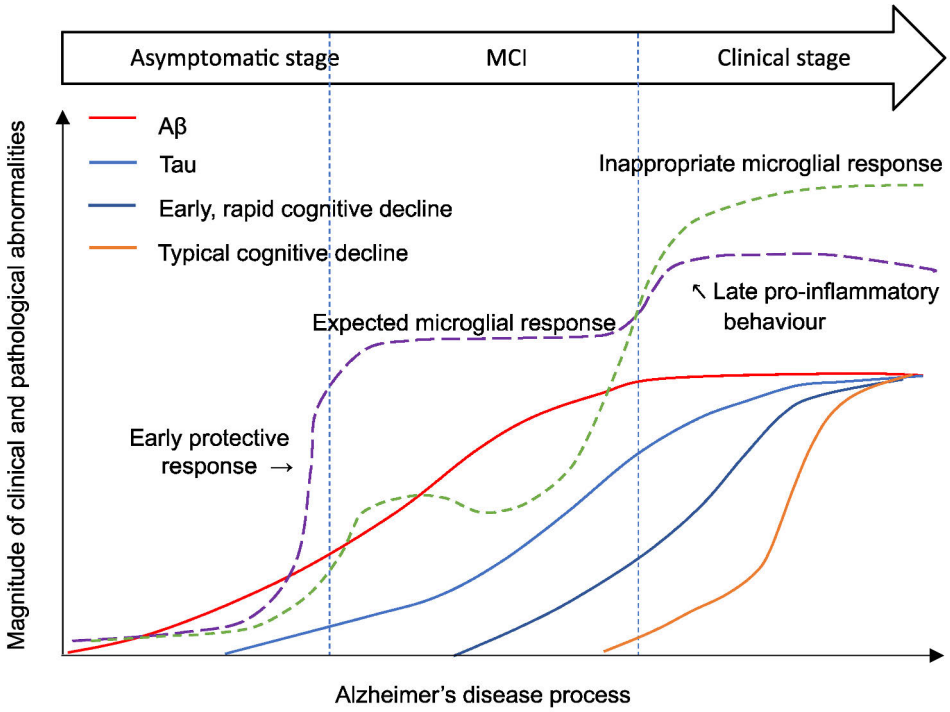

Figure 6. Hypothetical model of the microglial reactivity in relation to AD pathology and symptoms. Modified from Leng & Edison, 2021.

2.5.1 Microglia and astrocytes

Reactive microglia and astrocytes are the main inflammatory mediators in the CNS. Microglia are ubiquitously found in the brain and are the resident phagocytes of the CNS. Microglia also contribute to protecting and remodelling synapses. (Heneka et al., 2015) Astrocytes, the most abundant cell type in the brain, were long regarded as passive neuronal support cells only. However, they are now known to have an active role in maintaining physiological homeostasis. (Oksanen et al., 2019)

Traditionally, the activation of microglia and astrocytes have been categorized as neurotoxic (pro-inflammatory) or neuroprotective, depending on their activation status. M1-phenotype of microglia and A1-phenotype of astrocytes have been considered neurotoxic, while M2-phenotype of microglia and A2-phenotype of astrocytes have been considered neuroprotective. However, recent data have revealed that microglia and astrocytes have multiple reactive phenotypes, and the phenotypic distribution can change along the continuum of neurodegenerative diseases. Thus, the balance between pro-inflammatory and neuroprotective microglia and astrocytes might be crucial in the progression of neurodegenerative diseases. (Heneka et al., 2015; H. S. Kwon & Koh, 2020)

Microglia and astrocytes seem to interact with Aβ, and dysfunctions in the metabolism of microglia and astrocytes might lead to the deposition of Aβ. It has also been suggested that the pro-inflammatory microglia would increase the phosphorylation of tau. (Cai et al., 2014; H. S. Kwon & Koh, 2020)

Microglial reactivity related to cognitive disorders can be evaluated in vivo using fluid biomarkers, reviewed in the next chapter, or by PET imaging, discussed in chapter 2.6.6.

2.5.2 Fluid biomarkers of neuroinflammation

Many studies have investigated levels of pro- and anti-inflammatory cytokines in the CSF of MCI and AD dementia patients, but the results have conflicted. (Brosseron et al., 2014) Presumably, different stages of the disease at the time of CSF sampling can explain the conflicts. (Heneka et al., 2015)

YKL-40 – chitinase 3-like protein 1 (CHI3L1) – is a glycoprotein expressed by reactive astrocytes and microglia in the CNS. (Cantó et al., 2015) Because it can be measured from the CSF, it might be a biomarker to detect neuroinflammation in AD. (Muszyński et al., 2017) The soluble variant of TREM2 (sTREM2) can be measured in the CSF, thus this variant may serve as a marker of microglial activity. (Suárez-Calvet et al., 2016) Previous studies have suggested that CSF sTREM2 and YKL-40 levels are already increased at AD's early stages. (Janelidze et al., 2018; Nordengen et al., 2019; Suárez-Calvet et al., 2016) According to a previous meta-analysis, CSF sTREM2 and YKL-40 levels are higher in MCI and AD patients than in healthy controls. (Shen et al., 2019) Another meta-analysis showed that CSF YKL-40 is associated with AD, but the relationship seems to be notably weaker than that of AD's core biomarkers (Aβ42, T-tau, P-tau). (Olsson et al., 2016)

2.6 Neuroimaging of early changes related to cognitive disorders

Neuroimaging is increasingly used to help diagnose diseases causing cognitive decline. Magnetic resonance imaging (MRI) and computed tomography (CT) are used for brain structural imaging in patients with cognitive complaints. PET imaging enables quantitative measuring and functional imaging of biological processes and metabolism in a living subject. In clinical practice, PET imaging with fluorodeoxyglucose (FDG) is used in the (differential) diagnostics of memory disorders, but Aβ imaging can also be performed. In research, imaging of neuroinflammation and tau is possible. The following chapters will review the use of MRI in individuals with cognitive decline, Aβ scanning, and imaging of neuroinflammation. Additionally, FDG-PET and tau imaging are discussed briefly.

2.6.1 Brain MRI

MRI is the preferred brain structural imaging for patients with cognitive decline. (Scheltens et al., 2002; Vanninen et al., 2011) Evaluating the degree of the brain changes that are characteristic of diseases leading to dementia is more reliable with MRI than CT. However, especially among older patients or those with already severe cognitive decline, CT suffice. (Vanninen et al., 2011; Wattjes et al., 2009)

The sequences in the brain MRI of patients with cognitive complaints include T2 axial and fluid-attenuated inversion recovery (FLAIR) (with which vascular and other focal changes are seen) as well as T1-weighted 3D-imaging to evaluate global atrophy and medial temporal lobe atrophy. Susceptibility weighted imaging (SWI) or hemosiderin sensitive T2* may be included if previous haemorrhage, other vascular insults, or amyloid angiopathy are suspected. Diffusion sequence is used to evaluate recent ischemic lesions. If a tumour is suspected, a contrast agent is used. (Vanninen et al., 2011)

Traditionally, brain cortical **atrophy** and the localization of atrophy are evaluated visually. Hippocampus atrophy can be evaluated by the classification composed by **Scheltens** et al. (Scheltens et al., 1992) **WMH**s are typically graded with the **Fazekas** classification. (Fazekas et al., 1987) Recently, automatic image quantification tools, which can quantify brain volumes and vascular changes, have been developed. (J. Koikkalainen et al., 2016)

In AD, the typical findings on MRI are hippocampus atrophy, shrinkage of the entorhinal cortex, and global central and cortical atrophy, whereas brain changes in the white matter do not differ from those seen in healthy ageing. (Masdeu, 2020; Vanninen et al., 2011) Several previous studies have indicated that atrophy in the medial temporal lobe is present already in the preclinical stage of AD, but subtle changes are challenging to detect with the human eye. (Leandrou et al., 2018)

Confluent T2 WMHs, lacunar infarcts, cortical-subcortical infarcts, and possibly central atrophy can be seen in vascular cognitive impairment. Patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) often have white matter changes at the pre-symptomatic phase. In frontotemporal dementia, symmetrical and bilateral frontal lobe and temporal lobe atrophy are often detected on MRI. Atrophy in hippocampi is minor compared to the atrophy seen in AD. In Lewy body disease, no specific findings on MRI exist, but patients show global cortical atrophy without significant white matter changes. Medial temporal lobe atrophy is less prominent than in AD. In dementia related to Parkinson's disease, there are no specific MRI findings either. Hippocampi are typically preserved, but if hippocampus atrophy is seen, coincident dementia related to Parkinson's disease and AD is possible. (Masdeu, 2020; Vanninen et al., 2011)

MRI is also vital in excluding possibly treatable conditions such as tumors, subdural haemorrhage, and normal pressure hydrocephalus (NPH). Enlarged lateral and third ventricles out of proportion to the cortical sulci characterize NPH.

However, radiologically distinguishing central atrophy from NPH might be difficult. (Masdeu, 2020; Vanninen et al., 2011)

2.6.2 Basics of PET imaging

PET imaging is a method with various clinical and research applications. It utilizes **tracers** labelled with positron-emitting radioactive isotopes (most commonly ^{18}F, ^{11}C, ^{15}O, or ^{68}Ga). PET is a very sensitive imaging method enabling three-dimensional mapping of radioactive tracers (**radioligands**) without causing any relevant physiological or pharmacological effects. It provides molecular-level information of processes or metabolic changes (e.g. due to cancer) even before any changes are visible in structural imaging. PET-tracers bind to specific molecules (receptors) in target organs or mimic a compound the body normally uses. A tracer is most often administered intravenously, after which it accumulates into the target tissues. The tracer's emitted radiation is then detected with a PET camera. The PET data is typically combined with CT or MRI to acquire an accurate image of body structures. In the clinic, PET imaging has become more common during the past two decades. It is used in oncology, as well as in cardiology and in diagnosing dementia, epilepsy, and infectious and inflammatory diseases. In neuroimaging, PET imaging is performed, depending on the tracer used, to assess cerebral blood flow and metabolism, accumulation of certain proteins such as Aβ, or the function of neurotransmitters. (Janatuinen & Kemppainen, 2020; Kokkonen et al., 2020; Lameka et al., 2016)

The radioactive isotopes are manufactured in **cyclotrons**, often close to the PET-imaging sites, due to the short half-lives of most of the positron-emitting radioisotopes (half time for ^{18}F is 109 min; for ^{11}C, 20 min; for ^{15}O, 2 min; and for ^{68}Ga, 68 min). Radiotracers labelled with ^{18}F can be produced at offsite locations and transported to the PET imaging site because the half-life of ^{18}F is long enough. PET scanning typically starts 10-60 minutes after tracer injection, depending on the radiotracer. (Janatuinen & Kemppainen, 2020)

In the **positron emission decay process** of the unstable radioactive nuclei, a positron (a positively charged antiparticle of the electron) and a neutrino (a chargeless and almost massless particle) are emitted. The emitted positron rapidly unites with a nearby electron, resulting in the **annihilation** event, in which two photons (γ-rays), each with an energy of 511 keV, are emitted. The photons travel in opposite directions. PET-scanners can detect both photons as they hit detectors opposite to each other (almost) simultaneously, within the coincidence time window. The **line of response** is the line connecting two opposed detectors, along which the coincidence events occur. The detectors record this information, and the raw data is formed, after which image reconstruction and several corrections (such as for for attenuation, scatter, and random coincidence) are performed. (Lameka et al., 2016)

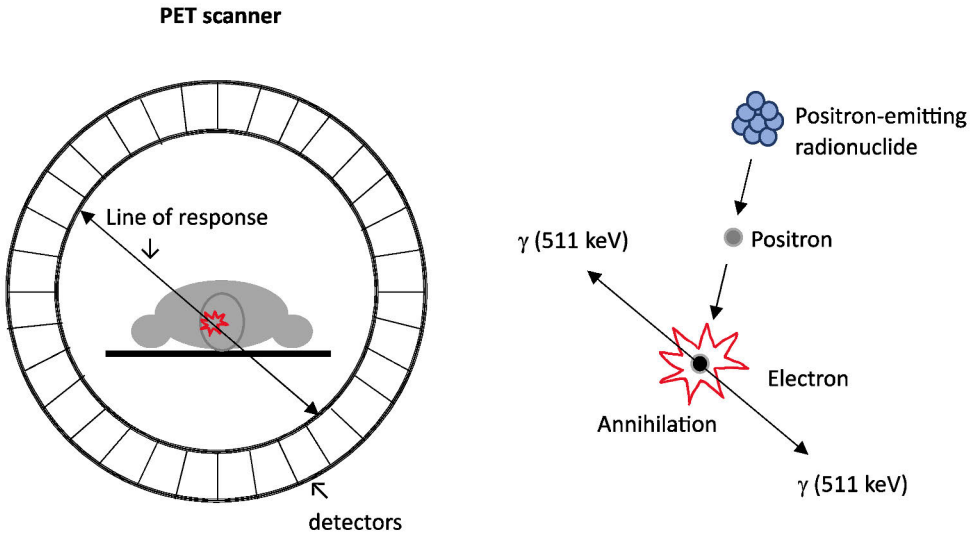

Figure 7. A schematic illustration of a PET scanner and the annihilation event. The gamma-ray detectors opposite each other detect the emitted photons. Modified from van der Veldt et al., 2013.

Determining a fully quantitative measure of a tracer concentration requires dynamic PET scanning and arterial blood sampling. A tracer's **distribution volume (V_T)** is a quantitative outcome measure, defined as the ratio of the radioligand concentration in tissue to that in plasma. Calculating VT requires arterial blood sampling. **Standard uptake value (SUV)** is the most common semi-quantitative method for measuring tracer uptake – easily calculated as the ratio of the activity concentration in the tissue and the injected activity divided by body weight. It does not require blood sampling. However, SUV is prone to biases because of biological and technical factors. (Brendle et al., 2015; Oikonen, n.d.) **SUV ratio** is the ratio of a tissue SUV to the SUV of a reference region (such as the cerebellum in PiB-PET imaging). (Oikonen, n.d.)

2.6.3 FDG-PET

Glucose is the main metabolic substrate in the brain, and thus, a PET-tracer [^{18}F]-fluorodeoxyglucose ([^{18}F]FDG) – a glucose analog – can assess metabolism in the brain. Cells with higher metabolic activity uptake more FDG than cells with lower metabolic activity. Diseases leading to dementia manifest a loss of neurons and decreased synaptic connections. FDG-PET can show distinct patterns of **cortical hypometabolism** in the damaged brain areas. In the clinic, FDG-PET is useful in the differential diagnostics of different dementia-causing diseases. (Dave et al., 2020; Ricci et al., 2020)

Reduced glucose utilization and deficient energy metabolism already occur in individuals with MCI. (Minoshima et al., 1997; Mistur et al., 2009). Hypometabolism in FDG-PET is typically seen in the posterior cingulate cortex in the early stages of AD. At a more advanced stage, hypometabolism is also seen in the precuneus and the parietal and posterior temporal lobes. Frontal hypometabolism may be seen in moderate to severe stages of AD. Bilateral, asymmetric hypometabolism in the frontal and anterior temporal lobes is characteristic of frontotemporal lobar degeneration. In dementia with Lewy bodies, hypometabolism is found in the occipital cortex (and also in parietal and posterior temporal lobes). (Dave et al., 2020; Lameka et al., 2016)

2.6.4 Amyloid imaging with PET

Aβ plaques are in the cortical grey matter in patients with AD, most abundantly in the frontal cortex; cingulate gyrus; precuneus; and lateral parietal and temporal regions. Also, 50–70% of patients who have dementia with Lewy bodies have Aβ deposition, which is generally lower and more variable than in patients with AD. In contrast, Aβ accumulation is not found in frontotemporal lobar degeneration or pure vascular dementia. (Braak & Braak, 1997; Rowe et al., 2010; Rowe & Villemagne, 2011)

PET imaging has made assessing the accumulation of Aβ in vivo possible, and several Aβ-tracers have been developed. The most common PET tracers used in the diagnostics or differential diagnostics of AD are in Table 4. The first Aβ tracer was the ^{11}C-labeled Pittsburgh compound-B, **[^{11}C]PiB**, (N-methyl-[^{11}C]2-(4′-methylaminophenyl)-6-hydroxybenzothiazole), which can cross the blood–brain barrier and binds to Aβ with a high affinity. Neuropathological studies have shown that in vivo PiB uptake correlates well with post-mortem brain Aβ plaques. (Seo et al., 2016)

The **^{18}F-labeled tracers** were developed because of the longer half-time of ^{18}F than that of ^{11}C, enabling the wider utilization of amyloid-PET. The ^{18}F-labeled tracers (see Table 4) have high sensitivity and specificity. (Ricci et al., 2020) They have higher non-specific binding to white matter than [^{11}C]PiB, and in AD patients, PET imaging with ^{18}F-labeled tracers shows loss of the grey matter–white matter demarcation. (Rowe & Villemagne, 2011)

According to the Society of Nuclear Medicine and Molecular Imaging (SNMMI), Aβ PET imaging is considered appropriate if the patient has persistent or progressive unexplained MCI; the core clinical criteria for possible AD are satisfied, but an unclear clinical presentation exists (an atypical clinical course or etiologically mixed presentation) or the patient has progressive dementia at an untypically young age (usually ≤65 years). (Minoshima et al., 2016)

In clinical practice, Aβ PET scans are interpreted visually as “amyloid negative” or “amyloid positive”. Only a nonspecific tracer uptake is seen in the amyloid-

negative scans and little or no binding in the grey matter. (Minoshima et al., 2016) Most [^{11}C]PiB studies have quantified tracer binding using the cerebellar grey matter as a reference region, i.e. calculated the ratio of cortical binding to that of the cerebellum. The cerebellum is a suitable reference region because Aβ plaques in the cerebellum are usually far less dense than in other cortical areas. This ratio is typically described as an SUV ratio. The SUV ratio cut-off values that have been used for amyloid positivity of PiB PET have typically varied between 1.3 and 1.6. (Rowe & Villemagne, 2011)

Previous PET studies have shown that Aβ is found not only in patients with AD but in individuals with normal cognitive function as well. (Jansen et al., 2015; Roberts et al., 2018) The prevalence of cerebral Aβ is known to increase with age. Also, the *APOE ε4* genotype increases the risk for Aβ accumulation. According to a previous meta-analysis, among individuals with normal cognition, 10% of those aged 50 and 44% of those aged 90 were amyloid-positive (determined by PET or CSF studies). *APOE ε4* carriers had a two to three times higher prevalence of Aβ load than those without the APOE ε4 allele. (Jansen et al., 2015)

Although Aβ accumulation is common among asymptomatic individuals, amyloid-positive individuals have an increased risk of developing cognitive complaints. According to the population-based Mayo Clinic Study of Aging in Olmsted County, Minnesota, (n=1671), the risks for MCI and AD dementia were more than two-fold for amyloid-positive participants (determined by PiB-PET) compared to amyloid negative-participants. (Roberts et al., 2018)

Table 4. The most common PET tracers used in the diagnostics or differential diagnostics of Alzheimer's disease.

Tracer	Imaging target	Purpose
^{11}C-PIB	amyloid	AD vs no AD
^{18}F-Flutemetamol	amyloid	AD vs no AD
^{18}F-Florbetapir	amyloid	AD vs no AD
^{18}F-Florbetaben	amyloid	AD vs no AD
^{18}F-FDG	glucose metabolism	Dementia diagnostics and AD differential diagnostics

2.6.5 Tau imaging with PET

Besides accumulation of Aβ, AD is biologically characterized by intraneuronal neurofibrillary tangles consisting of tau protein. Neurofibrillary tangles first accumulate in the transentorhinal cortex, spread to the hippocampus, and finally to the neocortex. (Braak & Braak, 1997) Clinicopathological studies have shown that

the severity of cognitive impairment is associated with the amount of neocortical neurofibrillary tangles in AD patients. (Nelson et al., 2012) Recently, tracers for tau-PET imaging have been developed, enabling the evaluation of tau burden in vivo. The major limitation of the present tracers is the off-target binding, and new (second-generation) ligands are being developed. (Jack et al., 2018; Scheltens et al., 2021) Compared to amyloid-PET, tau-PET seems more strongly associated with cognitive performance and disease severity and might predict cognitive decline better. (Aschenbrenner et al., 2018; Brier et al., 2016, Pontecorvo et al., 2017)

The most widely studied tau tracer is [18F]flortaucipir, approved for clinical use by the US Food and Drug Administration in 2020. (Mattay et al., 2020) Tau-PET with flortaucipir seems able to discriminate AD dementia from other neurodegenerative diseases but the differentiation of MCI due to AD from other neurodegenerative disorders appears moderate. (Ossenkoppele et al., 2018) According to a previous study evaluating 217 participants including cognitively normal individuals, individuals with MCI, and patients with clinically defined possible or probable AD, high neocortical flortaucipir binding was associated with Aβ positivity (evaluated by florbetapir), and flortaucipir binding in the neocortex was significantly higher with more advanced clinical stages of AD continuum among Aβ positive participants. (Pontecorvo et al., 2017)

2.6.6 Measuring neuroinflammation with PET

PET is a possible way of measuring the current inflammatory state in the living human brain. (Edison & Brooks, 2018; Lagarde et al., 2018; Schain & Kreisl, 2017) The main target of the PET studies is the **translocator protein (TSPO)**, an 18 kDa protein structure (formerly known as the peripheral benzodiazepine receptor [PBR]), because it is thought to be overexpressed by reactive microglia during neuroinflammation. Previous TSPO PET studies have shown that TSPO-tracer uptake is altered in individuals with neurodegenerative, neuroinflammatory, and also psychiatric diseases compared to healthy controls. (Nutma et al., 2021)

TSPO, present on the outer mitochondrial membrane, is assumed to have numerous cellular functions that are essential to human health, including steroid hormone synthesis, cholesterol transport into mitochondria, and mitochondrial respiration. (Denora & Natile, 2017; Nutma et al., 2021) TSPO is minimally expressed in the normal brain. However, it is upregulated in neurodegenerative and neuroinflammatory diseases. In AD, overexpression of TSPO is seen within or surrounding Aβ depositions. (Cosenza-Nashat et al., 2009)

Traditionally, TSPO has been considered a marker of microglial activity. However, TSPO is also present in other brain cell types. In a recent neuropathological study, TSPO was expressed not only in microglia but especially

in astrocytes, endothelial cells, and vascular smooth muscle cells as well. Moreover, the cortical burden of TSPO did not correlate with the burden of reactive microglia or astrocytes, Aβ accumulation, or neurofibrillary tangles. (Gui et al., 2020)

The first PET tracer for TSPO imaging was [^{11}C]-(R)-PK11195. Limitations of this radioligand (limited brain entry, poor signal-to-noise ratio, plasma protein binding) have led to **"second-generation" TSPO traces** with lower non-specific binding (with higher signal-to-noise ratio) developing. These second-generation TSPO ligands seem to have better specific TSPO binding than [^{11}C]-(R)-PK11195. (Fujita et al., 2017) A study investigating monkey brains showed that specific TSPO binding was higher with [^{11}C]PBR28 than [^{11}C]-PK11195. (Kreisl et al., 2010) However, no head-to-head studies comparing [^{11}C]PBR28 and [^{11}C]-(R)-PK11195 PET in humans exist; thus, no convincing evidence is available that proves that one tracer would be more sensitive than another. (Edison & Brooks, 2018)

One challenge concerning most new tracers is the large interindividual variability in the binding affinity of the new tracers due to the rs6971 polymorphism of the TSPO binding site, resulting in the differential affinity of radioligands to TSPO. (Owen et al., 2012). Depending on the TSPO binding site, individuals are high-, low-, or mixed-affinity binders. In Europe, approximately 50% of the population are homozygous for the high-affinity binding site, 10% homozygous for the low-affinity binding site, and about 40% heterozygotes for the high- and low-affinity binding sites. (Kreisl, Jenko, et al., 2013)

Finding the most appropriate PET quantification method for different TSPO tracers has also been challenging. There is no optimal reference region for TSPO imaging since TSPO is expressed throughout the brain and blood vessels, and no brain region is devoid of specific TSPO binding sites. (Lagarde et al., 2018; Lyoo et al., 2015) Further, the function and behaviour of TSPO in normal and pathological states are not yet fully understood; thus, interpreting the TSPO PET results may be challenging. (Wimberley et al., 2021)

TSPO tracers used in previous PET studies to evaluate neuroinflammation in the AD continuum are listed in Table 5. Most of the earlier studies were performed with [^{11}C]-(R)-PK11195, reaping conflicting results, as recently reviewed by Lagarde et al. (Lagarde et al., 2018) A recent meta-analysis including 28 PET studies (n=755) suggested that individuals with AD dementia have a higher TSPO tracer uptake, especially within frontotemporal regions than healthy controls. Individuals with MCI had modestly higher TSPO levels than controls. (Bradburn et al., 2019). PET imaging studies with newer tracers ([^{11}C]PBR28, [^{18}F]DPA-714, and [^{11}C]DPA713) seem to have more consistent findings concerning tracer uptake in patients with AD than studies with [^{11}C]-(R)-PK11195. (Chauveau et al., 2021) However, there is variability in these results too.

Of the second-generation TSPO tracers [^{11}C]PBR28 is the most used to study AD dementia and its early stages. Kreisl *et al.* showed that [^{11}C]PBR28 uptake in cortical brain regions is higher among AD patients but not those with MCI compared to controls. (Kreisl, Lyoo, et al., 2013) The largest and most recent of the [^{11}C]PBR28 studies (n=130) by Pascoal et al. reported that [^{11}C]PBR28 binding was progressively higher from the cognitively unimpaired young to the cognitively unimpaired aged, as well as individuals with MCI and those with AD dementia. They also suggested that microglial activation would correlate with tau (measured with PET) hierarchically with each other following the Braak-like stage and that Aβ would potentiate the effects of microglial activation on tau spreading. (Pascoal et al., 2021) Fan *et al.* showed higher [^{11}C]PBR28 binding in amyloid-positive MCI subjects than healthy controls. (Fan et al., 2018) Similarly, Hamelin *et al*. found an increase in TSPO binding measured with [^{18}F]-DPA-714 in individuals with AD, especially at the prodromal stage, compared to controls. (Hamelin et al., 2016) In contrast, Dani et al. could not find group differences in [^{11}C]PBR28 binding between individuals with AD or MCI and healthy controls. (Dani et al., 2018)

Table 5. TSPO tracers that have been used to assess MCI- or AD-related neuroinflammation in previous PET studies.

First-generation TSPO tracers	Second-generation TSPO tracers
[^{11}C]PK11195 [^{11}C](R)-PK11195	[^{11}C]DAA1106 [^{11}C]Vinpocetine [^{11}C]PBR28 [^{18}F]FEDAA1106 [^{18}F]DPA-714 [^{18}F]FEPPA [^{18}F]FEMPA [^{11}C]DPA713

Aβ and neurofibrillary tangles have been suggested to serve as inflammatory stimuli to microglia. (Aktas et al., 2007). Studies that have evaluated the association between Aβ accumulation and neuroinflammation with PET are summarized in Table 6. Most, but not all, show an association between Aβ binding and TSPO binding. For example, Hamelin *et al.* found a correlation between [^{11}C]PIB and [^{18}F]DPA-714, and Dani et al. with [^{18}F]flutemetamol and [^{11}C]PBR28 binding. The association of the latter was stronger among individuals with MCI than AD patients. (Dani et al., 2018; Hamelin et al., 2016) The previous studies have been performed with different radioligands and study designs. Also, the quantification methods of the PET images have varied. These methodological issues might explain some of the variations in the results.

Table 6. PET studies that have evaluated the association between TSPO binding and amyloid accumulation.

Study	N	Design	TSPO tracer	Aβ tracer	Association between TSPO and Aβ binding
Edison et al., 2008	13 AD, 10 HC	C	[^{11}C](R)PK11195	[^{11}C]PIB	No regional correlation.
Wiley et al., 2009	6 MCI, 6 AD, 6 HC	C	[^{11}C](R)PK11195	[^{11}C]PIB	No regional correlation.
Okello et al., 2009	14 MCI, 15 AD, 10 HC	C	[^{11}C](R)PK11195	[^{11}C]PIB	No regional correlation.
Yokokura et al., 2011	11 AD, 10 HC	C	[^{11}C](R)PK11195	[^{11}C]PIB	A negative regional correlation in the posterior cingulate cortex.
Kreisl, Lyoo, et al., 2013	10 MCI, 19 AD, 13 HC	C	[^{11}C]PBR28	[^{11}C]PIB	A positive regional correlation in the inferior parietal lobule, superior temporal cortex, precuneus, hippocampus, and parahippocampal gyrus, but only after partial volume correction.
Fan, Okello, et al., 2015	8 AD, 14 HC	L	[^{11}C](R)PK11195	[^{11}C]PIB	A positive voxel-wise correlation at baseline in temporal, parietal, and occipital lobes among participants with AD.
Fan, Aman, et al., 2015	10 MCI, 10 AD, 10 HC	C	[^{11}C](R)PK11195	[^{11}C]PIB	Positive correlations in the frontal, temporal, parietal, and occipital cortices; parahippocampus; insula; and the thalamus among particpants with AD. Positive correlations were also found among those with MCI, but the number of voxels involved was much lower.
Kreisl et al., 2016	14 Aβ+, 11 Aβ-	L	[^{11}C]PBR28	[^{11}C]PIB	No regional correlation between change in PBR28 binding and change in PIB binding.
Hamelin et al., 2016	34 pd AD, 24 AD d, 26 HC	C/L	[^{18}F]DPA-714	[^{11}C]PIB	Positive correlation between DPA-714 and PIB global cortical binding.
Fan et al., 2017	8 MCI, 14 HC	L	[^{11}C](R)PK11195	[^{11}C]PIB	A positive voxel-wise correlation at baseline in the frontal, temporal, and occipital lobes among participants with MCI.
Parbo et al., 2017	42 MCI, 10 HC	C	[^{11}C](R)PK11195	[^{11}C]PIB	A positive regional correlation in the frontal, parietal and temporal cortices among those with MCI.

Study	N	Design	TSPO tracer	Aβ tracer	Association between TSPO and Aβ binding
Dani et al., 2018	16 MCI, 16 AD, 19 HC	C	[^{11}C]PBR28	[^{18}F]-Flutemetamol	Positive voxel-wise correlations throughout the cortex, especially in the parietal cortex in the AD group and the frontal and temporal cortex in the MCI group. Correlations were stronger among MCI than AD participants.
Hamelin et al., 2018	33 pd AD, 19 AD, 17 HC	C/L	[^{18}F]DPA-714	[^{11}C]PIB	Positive correlation between DPA-714 and PIB global cortical binding at baseline – as reported in (Hamelin et al., 2016).
Knezevic et al., 2018	11 MCI, 14 HC	C	[^{18}F]-FEPPA	[^{11}C]PIB	Positive correlation in the hippocampus in the MCI group.
Pascoal et al., 2021	28 MCI, 16 AD, 86 HC	C	[^{11}C]PBR28	[^{18}F] AZD4694	A positive correlation.

HC= healthy control, pd AD= prodromal AD, AD d= AD dementia, C=cross-sectional, L=longitudinal

3 Aims

I To evaluate whether midlife insulin resistance predicts cognitive performance and cerebrovascular lesions 15 years later and whether brain amyloid accumulation or cerebrovascular lesions are associated with cognitive functioning.

II To examine if early amyloid accumulation is associated with neuroinflammation and if metabolic risk factors (higher body mass index, insulin resistance, and systemic low-grade inflammation) are linked to neuroinflammation in elderly individuals without dementia. Additionally, to evaluate if CSF markers of neuroinflammation are associated with neuroinflammation measured with PET.

III To evaluate if glucose or insulin values measured at different time points of an oral glucose tolerance test and early insulin response to oral glucose load can predict cognitive decline ten years later in the Finnish population aged 45–74.

4 Materials and Methods

Studies I and II were based on a sample of 60 (Study I) or 54 (Study II) volunteers who all had attended the Finnish Health 2000 survey. **Studies III and IV** were based on a subpopulation of the Health 2000 Survey, and its follow-up, the Health 2011 Study. The Ethics Committee of the hospital district of Helsinki and Uusimaa approved the Health 2000 and Health 2011 surveys; the Ethics Committee of the Hospital District of Southwest Finland approved Studies I and II. Written informed consent was obtained from all participants before they attended the studies.

4.1 The Health 2000 and 2011 surveys

Figure 8 describes the basic design of the Health 2000 and 2011 surveys. The protocols and the methods of the surveys were described in detail in the reports of the studies. (Aromaa & Koskinen, 2004; Heistaro, Sami, 2008; Lundqvist & Mäki-Opas, 2016) Here, the studies will be summarized.

The Finnish National Institute for Health and Welfare conducted the Health 2000 survey to study Finland's major public health problems and evaluate the population's functional capacity. A random, nationally representative sample of the Finnish population was examined from 2000 to 2001. Altogether, 8028 individuals aged 30 years or over and living in mainland Finland were randomly selected with a stratified two-stage cluster sampling design. Altogether, 16 health care districts were sampled as clusters from each of the five university hospital regions (Helsinki, Turku, Tampere, Oulu, Kuopio) – 80 healthcare districts in total. The data was collected by 1) home-visit interviews and by 2) health examinations a few weeks after the interviews. If a participant could not participate in the health examination proper at a study site, 3) a less extensive health examination was performed at home. Those who dropped out of these stages of the study were contacted by phone and then by letter to receive basic information about their health. (Aromaa & Koskinen, 2004; Heistaro, Sami, 2008) (Figure 8).

Of the 8028, 87% (n=6986) were interviewed, 79% (n=6354) attended the health examination proper, and 5% (n=416) were examined at home. Hence, 84% (n=6770) of the invited participated in the health examination proper or the examination at home. The mean duration of the health interview at home was 90 minutes. The

participants also received a questionnaire to fill in and to bring to the health examination. The mean duration of the health examination was three hours and 15 minutes. The examination included symptom interview, clinical examination, measurements such as BMI and blood pressure, blood samples, oral examination, and functional capacity tests. (Aromaa & Koskinen, 2004)

A subsample of the leading Health 2000 study population was invited for more in-depth examinations from 2001 to 2002 to examine cardiovascular diseases and diabetes more thoroughly. From those who had participated in the health examination in 2000–2001, those aged 45–74 and who lived near one of the five university hospital cities in Finland (or near Joensuu) were invited to the supplemental investigation of the circulatory system (n=1867). Of the invited, 82% (n=1526) participated in the in-depth examinations. The participants were interviewed and examined, and fasting blood samples were drawn. Thereafter, OGTT was performed except for those with insulin-treated diabetes. Also, a carotid doppler ultrasound examination was performed. (Heistaro, Sami, 2008).

The Health 2011 follow-up survey was carried out from 2011 to 2012. The invitation to participate was sent to all individuals who had been invited to the original Health 2000 Survey and were still living, lived in Finland, and had not refused to participate. Comprehensive health examinations with questionnaires and interviews were performed. If one was unable or unwilling to attend the full health examination, she/he was invited to participate in a concise health examination at home and, if such was impossible, participate in a phone interview. (Lundqvist & Mäki-Opas, 2016).

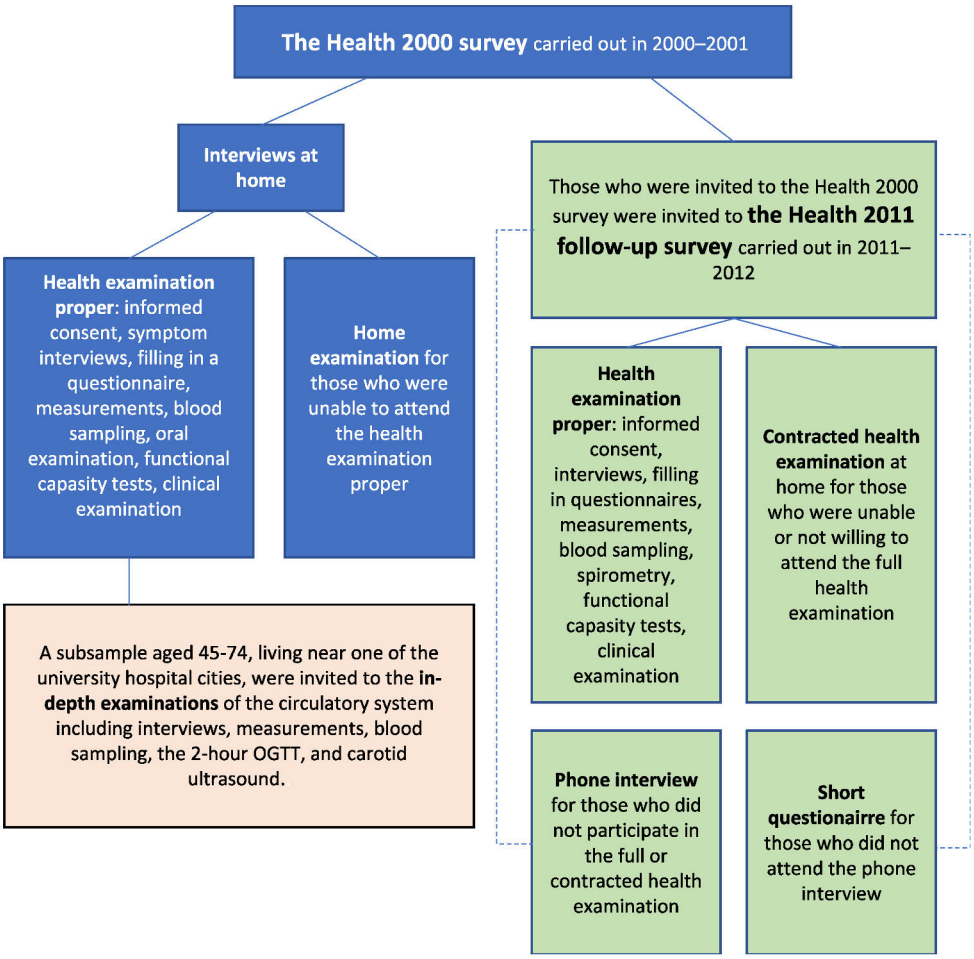

Figure 8. The study designs of the Health 2000 and Health 2011 surveys, based on (Aromaa & Koskinen, 2004; Heistaro, Sami, 2008; Lundqvist & Mäki-Opas, 2016).

4.2 Participants

4.2.1 Studies I and II

From those who had attended the health examination proper of the Health 2000 survey (n=6354), 60 volunteers were recruited to participate in a neuroimaging study (**Studies I and II**) at the Turku PET Centre from 2014 to 2016. The study aimed to investigate the associations among midlife insulin resistance, inflammation, and late-life brain Aβ accumulation, neuroinflammation, cerebrovascular lesions, and cognitive functioning. The sample was designed to consist of 30 individuals with and 30 without insulin resistance in the Health 2000 study. Insulin resistance was

determined using HOMA-IR, and both groups were designed to contain 15 *APOEε4* carriers.

The following criteria were used for recruitment: 1) birth year 1934–1949 (age at the time of neuroimaging being 65–80); 2) living site in the Hospital District of Southwest Finland or the Hospital District of Helsinki and Uusimaa; and 3) HOMA-IR (>2.17 for those with insulin resistance, i.e. the highest tertile of the Health 2000 study population) or (<1.25 for those with normal insulin sensitivity, i.e. the lowest tertile of the Health 2000 study population). Exclusion criteria were a fasting time of fewer than four hours in the Health 2000 study, insulin or unknown diabetes medication, missing HOMA-IR values, diagnosis of type 2 diabetes in 2000, and missing APOE genotyping or ε2/ε4 genotype. The National Institute of Health and Welfare sent a recruitment letter to 360 individuals, of which 98 were interested in attending the study. These prospective attendees were contacted by mail and interviewed by telephone. Further exclusion criteria were a history of a major stroke, previous dementia diagnosis, any other major neurological disorder, as well as a diagnosis of type 2 diabetes after the year 2000 for the insulin-sensitive group, and a contraindication for a PET or MRI scan. Information on an individual's insulin sensitivity/resistance in the Health 2000 survey and APOE genotype was obtained from the National Institution of Health and Welfare to finish with balanced study groups. By ending up with 15 insulin resistant individuals who were also *APOEε4* carriers, recruitment regarding this group was broadened to cover all of Finland, after which 15 volunteers, who met the criteria, were found.

The participants were genotyped for the rs6971 polymorphism of the TSPO gene before [^{11}C]PBR28 imaging to exclude low-affinity binders for PBR28. Among the 60 participants were five low-affinity binders excluded from the [^{11}C]PBR28 imaging. Also, one individual was excluded for being unable to finish the [^{11}C]PBR28 scanning. Thus, the **study population II** consisted of 54 individuals. The flow chart of the study populations is in Figure 9.

4.2.2 Studies III and IV

Studies III and IV were based on the Health 2000 survey and its in-depth studies, as well as the Health 2011 survey. Those who had attended all three studies were included in studies III and IV. Individuals who did not undergo an OGTT in the in-depth examinations due to insulin usage or some other reason, were excluded. Also, individuals with missing cognitive test results at baseline, i.e. in the Health 2000 survey or at follow-up, i.e. in the Health 2011 survey, were excluded. The study populations III and IV comprised 961 individuals (Figure 9).

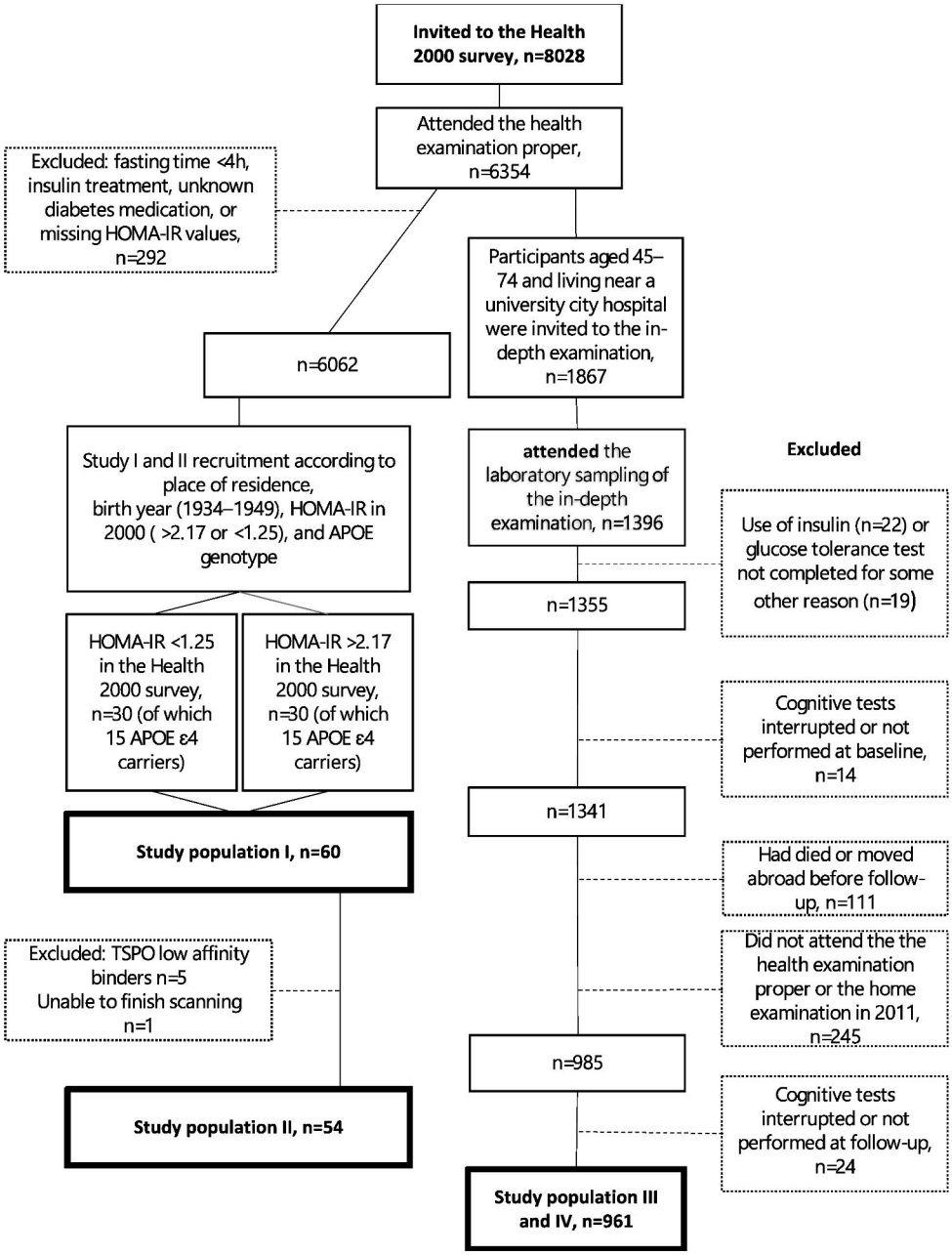

Figure 9. Flow chart of the study populations.

4.3 Laboratory examination and covariates

In the Health 2000 survey, participants were asked not to eat for at least four hours before the examination (**Studies I and II**). The duration of the fasting time before blood sampling was recorded. In the in-depth examinations of the Health 2000 study, fasting blood samples were drawn after an overnight fast of 10–12 hours (**Studies III and IV**). During the neuroimaging study in 2014–2016, blood samples were drawn after an overnight fast (minimum of ten hours) (**Study II**).

In the Health 2000 survey *APOE* genotyping was accomplished on participants who had given their written consent for DNA sampling. The genotyping was performed using the MassARRAY System (Sequenom, San Diego, CA, USA) with a modified protocol. (Jänis et al., 2004) Individuals with one or two ε4 alleles were considered *APOEε4*-carriers. (**Studies I, II, III, and IV**) HDL cholesterol values were analyzed by an HDL-C Plus test (Roche Diagnostics, Mannheim, Germany), triglycerides by a GPO PAP test (Olympus SystemReagent), glucose values by a hexokinase test (Olympus SystemReagent), and serum insulin by a microparticle enzyme immunoassay (Abbott Laboratories Dainabot, Tokyo, Japan) (**Studies I and II**). HbA1c (**Study IV**) was analyzed with an immunoturbidimetric method (Hemoglobin A1c assay, Abbott Laboratories), and high sensitivity C-reactive protein (hs-CRP) (**Study II**) was determined using an automated analyzer (Optima, Thermo Electron Oy, Vantaa, Finland) and an ultrasensitive immunoturbidimetric test (Ultrasensitive CRP, Orion Diagnostica, Espoo, Finland). (Heistaro, Sami, 2008)

In the in-depth examinations, serum total cholesterol and triglycerides were analyzed by the spectrophotometric enzymatic method and HDL by a direct method. Plasma glucose concentrations were determined by the glucose dehydrogenase method (Diagnostica Merck, Darmstadt, Germany) in an automated clinical chemistry analyzer (Thermo Clinical Labsystems, Konelab, Vantaa, Finland), and plasma insulin concentrations were measured with an RIA kit (Phadeseph insulin RIA; Pharmacia, Uppsala, Sweden). After the fasting samples had been drawn, an OGTT was performed: participants were given 300 ml of a glucose solution containing 75 g of glucose and water, and blood samples were drawn at 30 and 120 minutes after the glucose ingestion. (**Studies III and IV**)

During the neuroimaging study, hs-CRP (**Study II**) was determined by immunonephelometry with a BN ProSpec System (Siemens Healthcare GmbH, Marburg, Germany). Insulin was determined by ECLIA (electrochemiluminescence immunoassay) with a Cobas e602 immunochemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany) and glucose by enzymatic photometry with a Cobas c702 chemistry analyzer (Roche Diagnostics GmbH). (**Studies I and II**) The laboratory of Turku University Hospital (Tykslab) analyzed these blood samples. The participants' TSPO genotypes were analyzed in London, UK (Imperial Molecular Pathology Laboratory, Hammersmith Hospital). DNA was extracted from

peripheral blood by the Qiagen QIAamp DNA blood mini kit, and TSPO genotyping was accomplished with a TaqMan Allelic Discrimination assay. CSF samples were obtained from 11 individuals (who gave permission and did not have any contraindication for sampling). Approximately 10 ml of CSF was drawn, centrifuged within 30 minutes, and stored at -70°C. The samples were analyzed at the University of Gothenburg. CSF YKL-40 was measured using a YKL-40 ELISA kit (R&D Systems, Minneapolis, MN, USA). CSF Aβ40 and Aβ42 concentrations were determined with electrochemiluminescence technology and the MS6000 Human Abeta 3-Plex Ultra-Sensitive Kit (Meso Scale Discovery, Rockville, MD). CSF T-tau and P-tau were determined using commercially available INNOTEST sandwich ELISAs (Fujirebio Europe, Ghent, Belgium). CSF sTREM2 concentration was measured using an in-house ELISA. (Gisslén et al., 2019) (**Study II**)

In the Health 2000 survey, blood pressure was measured twice in the sitting position from the right arm using a standard mercury manometer (Mercuro 300; Speidel & Keller, Jungingen Germany) (**Study I**); however, in the in-depth examinations, blood pressure was measured three times with the oscillometric OMRON M4 blood pressure measuring device (Omron Matsusaka Co, Japan, OMRON Healthcare Europe B.V., Hoofddorp, The Netherlands) (**Studies III and IV**). The mean of the measurements was used in the analyses. BMI was determined in all studies (Health 2000 survey, its in-depth examinations, neuroimaging study, Health 2011 survey) (**Studies I, II, III, and IV**). Information on participant's level (classified as primary school, middle school/comprehensive school, high school, college/university) and duration of education, as well as medication and smoking status (defined as current smoking/no smoking), were obtained by interviews and questionnaires from the **Health 2000 survey** (**Studies I, III, and IV**). Depressive symptoms were evaluated with the Beck Depression Inventory (BDI). (**Studies III and IV**). (Heistaro, Sami, 2008)

HOMA-IR was calculated using the equation '(fasting insulin [μU/ml] times fasting glucose [mmol/l]) divided by 22.5' (**Studies I, II, and IV**). EIR to oral glucose load (**Study IV**) was defined as the ratio of the 30-min increment in insulin concentration (30-min insulin minus fasting insulin) to the 30-min increment in glucose concentration (30-min glucose minus fasting glucose). Hypertension was defined as systolic blood pressure ≥140 or diastolic ≥90 mmHg, or use of antihypertensive medication (**Studies I, III, and IV**), and hypercholesterolemia (**Studies III and IV**) as serum total cholesterol >6.5, or use of cholesterol-lowering treatment.

4.4 Cognitive tests

In the Health 2000 and 2011 surveys participants were tested for verbal fluency and encoding and retaining verbal material by selected tasks from the Finnish version

(Hänninen et al., 1999) of the CERAD test battery. (J. C. Morris et al., 1989) In the categorical verbal fluency test, a participant was asked to list as many animals as possible within a minute. In the word-list learning test, a participant was shown ten words. The participant was first asked to read them aloud, memorize them, and then recall as many of the words as possible in 90 seconds. In the Health 2000 survey, if a participant could recall all ten words in the first round, the result was 30 points (the range in the test score was 0–30 points). Otherwise, this procedure was repeated twice. In the Health 2011, all three rounds were accomplished in any case. In the word-list delayed recall test, a participant was asked to recall the ten words after a 5-minute delay. (Heistaro, Sami, 2008; Lundqvist & Mäki-Opas, 2016) The change in the cognitive test scores from baseline to follow-up was calculated as "cognitive test score in the Health 2011 survey minus cognitive test score in the Health 2000 survey". (**Studies III and IV**)

A thorough neuropsychological test battery was performed to the participants of the neuroimaging study by trained psychology students. The test battery included parts from the Finnish version of the Wechsler Memory Scale (WMS-R) and the Wechsler Adult Intelligence Scale (WAIS-R), Boston Naming Test (BNT), Trail Making Tests A and B (TMT-A and TMT-B), S-fluency, categorical fluency, and Stroop. Domain-specific Z-scores were calculated for executive functions, processing speed, language, and episodic memory. An experienced neuropsychologist was consulted on which tests to include in each domain. The executive function domain consisted of TMT-A and TMT-B (TMT-B minus TMT-A), Stroop (inhibition minus naming), digit span backwards, and S-fluency. The processing speed domain included TMT-A and digit symbol tests. The episodic memory domain consisted of WMS-R delayed logic memory and delayed verbal recall. The language domain included categorical fluency (animals), Boston naming test, and WAIS-R similarities. A Z-score for each test was first calculated by standardizing the raw test score to the study population's mean and standard deviation to combine the results from different cognitive tests (with different score ranges) into domain-specific scores. The domain-specific Z-scores were then determined by calculating the mean of the Z-scores of the tests included in the domain. (**Study I**)

4.5 Neuroimaging (Studies I and II)

Brain MRI and PET imaging with [^{11}C]PiB (**Studies I and II**) and [^{11}C]PBR28 (**Study II**) were carried out from 2014 to 2016 (the neuroimaging study). MRI imaging was performed using a 3T PET-MRI scanner (Philips Ingenuity TF PET-MR device, Philips Healthcare, the Netherlands) with sequences 3DT1, T2W, and fluid-attenuated inversion recovery (FLAIR). In **Study I,** cerebrovascular changes

were quantified from FLAIR images using an automatic image quantification tool. (J. Koikkalainen et al., 2016) Three measures used were the: 1) total volume of WMHs (normalized for age, sex, and total brain volume); 2) a computational counterpart for the Fazekas grading computed from the deep WMH volume using a regression-based model (J. R. Koikkalainen et al., 2019); and 3) total vascular burden measure (calculated as the weighted combination of deep WMH volume, the volume of cortical and lacunar infarcts). (J. Koikkalainen et al., 2016).

The dynamic 90-minute [^{11}C]PiB PET (**Studies I and II**) and 70-minute [^{11}C]PBR28 PET (**Study II**) scans were performed using a brain-dedicated high-resolution PET scanner, the ECAT HRRT (Siemens Medical Solutions, Knoxville, TN, USA). A thermoplastic mask (shaped individually) minimized head movement. The imaging procedure of the [^{11}C]PiB PET scanning has been described in detail. (Ekblad et al., 2018) [^{11}C]PiB was manufactured at the imaging site as described. (Snellman et al., 2017) [^{11}C]PiB (mean dose 489 MBq, SD 42) was injected intravenously before the scanning. Voxel-by-voxel [^{11}C]PiB SUVs were calculated using imaging data from 60 to 90 minutes after the tracer administration. 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. Six ROIs were formulated: parietal cortex, prefrontal cortex, anterior cingulum, posterior cingulum, precuneus, lateral temporal cortex, and cerebellar cortex. SUVRs were calculated by using the cerebellar cortex as a reference region. A composite cortical PiB score was calculated as the average PiB SUVR of all six ROIs. Based on previous studies on cognitively healthy elderly populations, the [^{11}C]PiB PET scan was considered Aβ positive when the [^{11}C]PiB composite SUVR score was >1.5. (Jack et al., 2008; Rowe et al., 2010)

Before the [^{11}C]PBR28 scanning (**Study II**) an anesthesiologist performed arterial catheterization for those without any contraindication (n=44). [^{11}C]PBR28 was injected as a rapid intravenous bolus (mean dose 496 MBq, SD 19). Arterial blood samples to measure radioactivity and metabolites were taken. Detailed information about the manufacture of the [^{11}C]PBR28, PET data acquisition, reconstruction, arterial blood sampling, and radiometabolite analysis have been described. (Tuisku et al., 2019) Automated ROI generation was conducted for [^{11}C]PBR28 data as it was for [^{11}C]PiB data (described above). SUVRs were calculated using imaging data from 30 to 70 min, and the cerebellum as a pseudo-reference region. V_T was calculated for those with arterial blood sampling (n=44) for additional analyses using Logan analysis in 30–70-minute interval. Distribution volume ratios (DVR) were calculated by dividing the V_T of the target ROI by that of the cerebellar cortex. A composite cortical [^{11}C]PBR28 score was calculated as a volume-weighted average [^{11}C]PBR28 SUVR over all six ROIs.

4.6 Statistical analyses

The normality of distribution for all variables was assessed visually before the analyses (**Studies I–IV**). Except for Study III, if the distribution was skewed to the right, a logarithmic transformation was used (provided the normal distribution was achieved with the transformation). Differences between groups (insulin-sensitive/-resistant [**Study I**]; amyloid-negative/-positive [**Study II**]; study population of the neuroimaging study/the Health 2000 study population [**Study II**]; study population III and IV/those aged 45–74 in the Health 2000 study who were not included in studies III and IV; participants with IFG/IGT [**Study IV**]) were assessed with a two-sample t-test or Wilcoxon Rank-Sum test for continuous variables and with Pearson's Chi-squared test or Cochran-Armitage Trend test (**Study I**) for categorical variables. In **Study IV,** p-values for differences in the descriptive data among the three glucose metabolism groups were assessed with Oneway Anova or a Chi Squared test. Multiple comparison p-values were calculated using Tukey's HSD or Bonferroni corrections. The associations between explanatory variables and the outcome variables were evaluated with simple linear regression; the adjusted analyses were performed with multivariable models (**Studies I, II, III, and IV**). Normality assumptions for the analyses were verified from the residuals. In **Study II,** nonparametric Spearman's correlation was also used (the association between the CSF proteins and PET results) because the parameters were not normally distributed.

In **Study I,** a maximum of three outliers per cognitive test were excluded before the z-score transformation to achieve normal distribution (three outliers in TMT B minus A; two outliers in TMT-A; two outliers in Boston naming test; and two outliers in WAIS-R similarities). A skewness from –1 to 1 was accepted for the distribution of the raw test scores. Additional analyses were performed to ensure that excluding the outliers did not change the results (Z-scores were calculated with the outliers included; the group comparisons were performed with outliers included using a nonparametric Wilcoxon Rank-Sum test). Effect sizes for the group comparisons (insulin-sensitive/-resistant) concerning cognitive performance and cerebrovascular changes on MRI were calculated using Cohen's d.

In Study I, previously reported protective/risk factors for cognitive impairment and cerebrovascular lesions were included as covariates in the adjusted models comparing the cognitive performance and presentation of cerebrovascular lesions between insulin-sensitive and insulin-resistant individuals. Model 1 was adjusted for education, Model 2 was adjusted more for hypertension and smoking, and Model 3 was further adjusted for BMI, triglycerides, and HDL cholesterol. Age, sex, or *APOE ε4* genotype were not added in the adjusted models of group comparisons because the groups were balanced regarding age, sex, and *APOE ε4* genotype. In the analyses evaluating the associations between cerebrovascular lesions or Aβ and the neurocognitive test scores, age, sex, education, and *APOE ε4* genotype were included

in the models. In **Study II**, the analyses evaluating the association between explanatory variables ([^{11}C]PiB SUVRs/BMI/HOMA-IR/hs-CRP) and the outcome variables [^{11}C]PBR28 SUVR composite score, [^{11}C]PBR28 SUVRs of the six cortical ROIs, and [^{11}C]PBR28 DVRs of the same ROIs were adjusted for age, sex, and TSPO genotype because, according to previous studies, they may affect [^{11}C]PBR28 binding. (Owen et al., 2012; Tuisku et al., 2019)

The number of participants in the neuroimaging studies was planned according to power calculations of the primary neuroimaging study (published previously), where Aβ accumulation ([^{11}C]PiB -PET) was the primary outcome. (Ekblad et al., 2018) The power calculations were based on test-retest analyses of [^{11}C]PiB -PET scans (Aalto et al., 2009), indicating that for a 90% power to obtain a statistically significant difference among the groups, five persons per group would be sufficient to detect a 15% difference in the frontal cortex [^{11}C]PiB binding.

In **Studies III and IV**, previously reported protective / risk factors for cognitive decline were included in the models. First, the analyses were adjusted for age, sex, and education duration. Then the analyses were adjusted further for *APOE ε4* genotype, type 2 diabetes, hypertension, hypercholesterolemia, BMI, BDI score, and smoking. The analyses concerning the change in cognitive tests after 11 years were adjusted further for baseline cognitive test scores.

Two-sided statistical significance was set at $p < 0.05$. The statistical analyses were performed with SAS JMP Pro 14 (SAS Institute, Cary; NC, USA). Voxel-by-voxel associations in **Studies I and II** were assessed with Statistical Parametric Mapping (SPM12; Wellcome Trust Centre for Neuroimaging, London, UK).

5 Results

5.1 Baseline characteristics (Studies I–IV)

Characteristics of the study populations of **Studies I–IV** at baseline (the Health 2000 survey) are summarized in Table 7. The study participants were on average 55 years old at baseline and 56% were women.

The participants of **Studies I and II** represented the original Health 2000 study population (n=6062) well. They did not differ from the Health 2000 study population concerning smoking, BMI, HbA1c, fasting glucose, systolic blood pressure, total cholesterol, LDL cholesterol, HDL cholesterol, or triglycerides values (all p-values calculated for the Study I population ≥0.07). Due to the recruitment protocol, participants in Studies I and II were older ($p<0.0001$) and more frequently *APOE ε4* carriers ($p=0.002$) than participants in the Health 2000 survey. (Data not shown.)

Individuals in **Studies III and IV** were younger (mean age at baseline 55.6 vs 57.8 years, $p<0.0001$), more educated (education in years: mean 11.6 vs. 10.0, $p<0.0001$), more frequently women (55.8% vs 51.4%, $p=0.02$), and less frequently smokers (9.0% vs. 24.1%, $p<0.0001$) than those 45–74-year-old individuals of the Health 2000 study who were not included in Studies III and IV. The individuals who did not participate in the follow-up examinations in 2011 were older (mean age at baseline 59.0 vs 55.6 years, $p<0.0001$), less educated (education in years, mean 10.3 vs 11.6, <0.0001), and more frequently smokers (29.7% vs 9.0%, $p<0.0001$) than those included in studies III and IV. (Data not shown.)

Table 7. Baseline characteristics of the study populations I-IV.

	Study I n=60	**Study II n=54**	**Studies III and IV n=961**
Baseline measurements			
age, mean (SD)	55.4 (3.3)	55.3 (3.2)	55.6 (7.4)
women, n/%	33/55	31/57	536/56
BMI (kg/m^2), mean (SD)	27.5 (4.0)	27.3 (4.4)	27.0 (4.4)
fasting glucose (mmol/l), median (Q1, Q3)	5.4 (5, 5.5)	5.4 (5.0, 5.5)	5.6 (5.2, 6.0)
fasting insulin (mU/l), median (Q1, Q3)	5.0 (4.0, 11.0)	6.5 (4.0, 11.3)	7.5 (5.5, 10.5)
HOMA-IR, median (Q1, Q3)	1.71 (0.89, 2.88)	1.71 (0.90, 2.77)	1.85 (1.31, 2.79)
HbA1c (%), mean (SD)	5.2 (0.3)	5.6 (0.3)	5.7
hypertension, n/%	31/52	27/50	518/54
total cholesterol (mmol/l), mean (SD)	6.2 (1.0)	6.1 (0.9)	5.6 (0.9)
HDL cholesterol (mmol/l), mean (SD)	1.4 (0.4)	1.4 (0.4)	1.6 (0.4)
triglycerides (mmol/l), mean (SD)	1.5 (0.8)	1.5 (0.8)	1.3 (0.7)
current smoking, n/%	9/15	3/6	86/9
years of education, mean (SD)	12.0 (4.1)	12.0 /4.0)	11.6 (3.8)
APOE ε4 genotype	30 (50)	27/50	304/33
BDI score	7.4 (7.4)	7.2 (6.0)	5
cognitive measures at baseline			
verbal fluency, mean (SD)	26 (6)	26 (6)	25 (7)
word-list learning, mean (SD)	21 (3)	21 (3)	21 (4)
word-list delayed recall, mean (SD)	7 (2)	7 (2)	7 (2)

The baseline measurements are otherwise from the main Health 2000 survey, but in Studies III and IV, the glucose, insulin, HOMA-IR, cholesterol, and BMI values are from the in-depth studies (from 2001 to 2002). BMI = body mass index, HOMA-IR = Homeostatic Model Assessment of Insulin Resistance, HbA1c = glycated hemoglobin, HDL = high-density lipoprotein, APOE ε4 = apolipoprotein E ε4 genotype, BDI = Beck Depression Inventory.

5.2 Insulin resistance, cognitive performance, and neuroimaging changes (Study I)

Descriptive data of the neuroimaging study (from 2014 to 2016) according to insulin sensitivity/resistance at baseline (in 2000) are shown in Table 8.

Table 8. The study population characteristics at follow-up (2014–2016), according to insulin sensitivity/resistance based on HOMA-IR values at baseline (in 2000).

	Insulin-sensitive, n=30	**Insulin-resistant, n=30**	**p**
age, mean (SD)	71.1 (3.7)	70.8 (2.8)	0.72
HOMA-IR, median (Q1, Q3)	1.73 (0.98, 2.45)	3.31 (2.11, 5.60)	<0.0001
total volume of WMHs, median (Q1, Q3)	3.7 (1.1, 5.6)	2.9 (1.5, 8.4)	0.88
Fazekas score, n/%			0.78
0	9/30	15/50	
1	16/53	6/20	
2	3/10	7/23	
3	2/7	2/7	
total vascular burden measure, median (Q1, Q3)	2.9 (0.6, 20.9)	4.3 (0.5, 21.3)	0.78
Aβ negative	20/67	12/40	0.04
Aβ positive	10/33	18/60	0.04

HOMA-IR = Homeostatic Model Assessment of Insulin Resistance, WMH = white matter hyperintensity, Aβ = beta-amyloid.
Insulin-sensitive: HOMA-IR in the lowest tertile of the Health 2000 study population (HOMA-IR<1.25); insulin-resistant: HOMA-IR in the highest tertile of the Health 2000 study population (HOMA-IR>2.17). Fazekas scores have been obtained by rounding the computed Fazekas score to the nearest whole number. PiB PET scan was considered Aβ negative when the PiB composite score was ≤1.5 and Aβ positive when the PiB composite score was >1.5. P-values for the differences between the IR groups were assessed with Student's t-test for continuous variables and with Pearson's Chi-Squared test for categorical variables except for the Fazekas score that was assessed with a Cochran–Armitage Trend Test. A logarithmic transformation is used for HOMA-IR, normalized total WMH volume, and the normalized total vascular burden measure to achieve normal distribution. Modified from Table 1 in Study I.

5.2.1 Group comparisons

Participants with insulin resistance in 2000 performed worse in executive functions and processing speed than participants with normal insulin sensitivity in 2000 (p-values after adjustments for the education level, hypertension, and smoking in 2000: for executive functions: p=0.04; for processing speed: p= 0.03). The differences between the groups were insignificant in episodic memory and language function. (Table 9 and Figure 1 in Study I) Using a nonparametric test and including the outliers in the (unadjusted) analyses, the difference between the groups was also significant in language function (p=0.02). (Data not shown).

Table 9. Domain-specific cognitive performance in 2014–2015, according to insulin sensitivity/resistance based on HOMA-IR values in 2000.

Z-score, mean (SD)	Insulin-sensitive	Insulin-resistant	P-value[a]	P-value[b]	P-value[c]	P-value[d]
Executive functions	0.30 (0.68)	-0.26 (0.68)	0.004	0.02	0.04	0.32
Processing speed	0.35 (0.65)	-0.32 (0.82)	0.001	0.007	0.03	0.03
Episodic memory	0.12 (1.0)	-0.13 (0.77)	0.28	0.99	0.98	0.53
Language	0.24 (0.77)	-0.10 (0.65)	0.09	0.30	0.18	0.93

Insulin-sensitive: HOMA-IR in the lowest tertile of the Health 2000 study population (HOMA-IR<1.25); insulin-resistant: HOMA-IR in the highest tertile of the Health 2000 study population (HOMA-IR>2.17). P-values for the differences between the insulin sensitive / resistant groups were assessed with student's t-test. [a]Unadjusted, [b]adjusted for the level of education, [c]further adjusted for hypertension and smoking in 2000, [d]further adjusted for BMI, HDL-cholesterol, and triglycerides. Modified from Table 2 in Study I.

No differences between the insulin-sensitive and -resistant groups were found in computer-based cerebrovascular lesions determined from MRI images: in computed Fazekas scores (unadjusted p=0.72), in normalized total WMH volume (unadjusted p=0.88), or normalized total vascular burden (unadjusted p=0.78) (Table 8). For more detailed results, see Table 2 in Study I.

5.2.2 Associations

A higher PiB composite score was associated with slower processing speed (after adjustments for age, education level, sex, and APOE ε4 genotype: slope -1.15, 95% CI -1.64 to -0.67, p<0.001). No associations were found between the PiB composite score and executive functions, episodic memory, or language function (all p-values ≥0.16). (Figure 10). In voxel-wise SPM analyses, a higher PiB SUVR was associated with slower processing speed in the frontal regions and parietal cortex (Study I, Figure 2). No association was found between cerebrovascular changes and cognitive Z-scores (executive functions, processing speed, episodic memory, and language function) (Study I, Table 3).

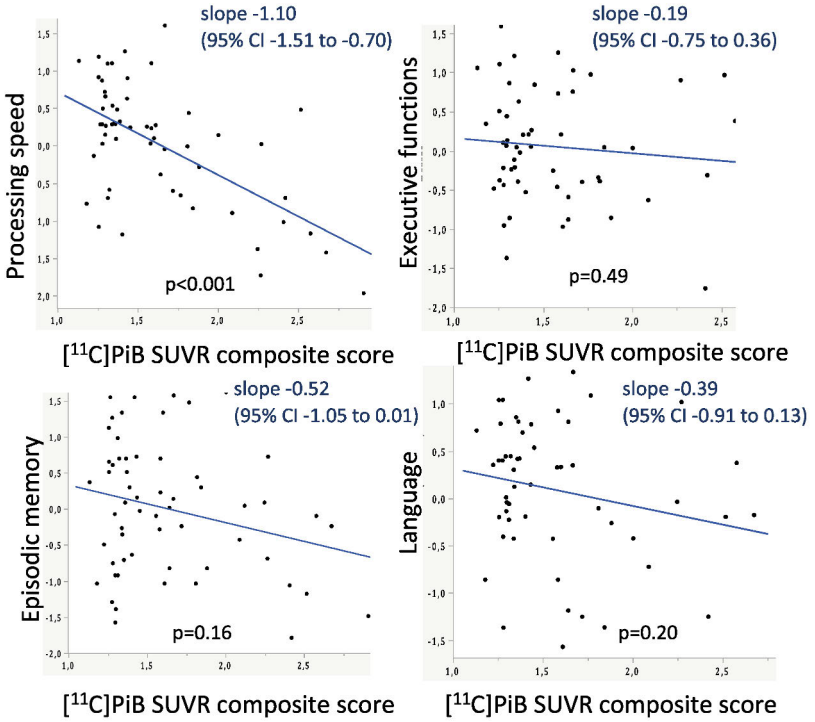

Figure 10. The (unadjusted) association between [^{11}C]PiB composite score and the cognitive Z-scores.

5.3 Beta-amyloid and neuroinflammation (Study II)

Characteristics of the study population at follow-up are in Table 10. Altogether, 54 individuals, of which 25 were mixed affinity binders and 29 were high-affinity binders for TSPO, were included in Study II. Arterial data, i.e. arterial blood samples measuring radioactivity and metabolites during imaging, were obtained from 44 and CSF sampling from 11 individuals. Of the participants, 25 (46%) were considered amyloid positive ([^{11}C]PiB composite SUVR score >1.5). No differences were found between amyloid-negative and -positive participants in age, sex, BMI, fasting glucose, hs-CRP, or total cholesterol (all p-values ≥0.59). Amyloid-positive participants were more often *APOE ε4* carriers (p<0.0001). (Data not shown). One individual (amyloid-positive, mixed affinity binder) had exceptionally high [^{11}C]PBR28 SUVR values in all ROIs ([^{11}C]PBR28 SUVR composite score 1.40). The same individual's BMI (38.8 kg/m2) and HOMA-IR value (21.4) were notably high among the study population. This outlier was excluded from all analyses.

Table 10. The study population characteristics of Study II at follow-up.

	All participants N=54	**Participants with CSF data N=11**
age, mean (SD)	70.0 (3.20)	68.1 (2.2)
women, n (%)	31 (57.4)	6 (54.5)
APOE ε4 genotype, n (%)	27 (50.0)	5 (45.5)
BMI (kg/m^2), mean (SD)	26.6 (4.0)	26.1 (3.5)
HbA1c (mmol/mol), median (Q1, Q3))	34 (33, 38)	34 (33, 34)
fasting glucose (mmol/l), median (Q1, Q3)	5.6 (5.3, 6.0)	5.6 (5.3, 5.9)
fasting insulin (mU/l), median (Q1, Q3)	9.5 (6.8, 14)	9 (4, 12)
HOMA-IR, median (Q1, Q3)	2.36 (1.52, 3.48)	2.32 (0.98, 3.15)
hs-CRP, median (Q1, Q3)	1.1 (0.6, 2.5)	0.8 (0.6, 2.6)
serum total cholesterol, mean (SD)	5.1 (0.96)	5.7 (0.73)
CERAD total score, median (Q1, Q3)	89 (83, 94)	94 (85, 95)
[^{11}C]PiB SUVR composite score, median (Q1, Q3)	1.43 (1.30, 1.82)	1.37 (1.30, 1.58)
Amyloid-positive, n (%)	25 (46)	3 (27.3)
TSPO binding genotype MAB, n (%)	25 (46.3)	5 (45.5)
TSPO binding genotype HAB, n (%)	29 (53.7)	6 (54.5)
[^{11}C]PBR28 SUVR composite score, median (Q1, Q3)	1.07 (1.04, 1.12)	1.05 (1.02, 1.12)
arterial data available, n (%)	44 (81.5)	10 (90.9)
CSF data		
Aβ40 (pg/ml), median (Q1, Q3)	N/A	7798 (5627–8355)
Aβ42 (pg/ml), median (Q1, Q3)	N/A	633.4 (475.5–1048.2)
Tau (pg/ml), median (Q1, Q3)	N/A	233.0 (202.3–323.3)
phosphorylated Tau (pg/ml), median (Q1, Q3)	N/A	40.8 (33.6–53.8)
YKL-40 (ng/ml), median (Q1, Q3)	N/A	133 (111–155)
sTREM2 (pg/ml), median (Q1, Q3)	N/A	3647 (3370–4502)

CSF = cerebrospinal fluid, APOE ε4 = apolipoprotein E ε4, HbA1c = glycated hemoglobin, HOMA-IR = Homeostatic Model Assessment of Insulin Resistance, hs-CRP = high sensitivity C-reactive protein, CERAD = Consortium to Establish a Registry for Alzheimer's Disease, SUVR = standardized uptake value ratio, TSPO = translocator protein 18 kDa, MAB = mixed affinity binder, HAB = high-affinity binder, Aβ = beta-amyloid, YKL-40 = chitinase 3-like protein 1, sTREM2 = soluble triggering receptor expressed on myeloid cells 2, Q1, Q3 = interquartile range. [^{11}C]-PIB-PET scan was considered amyloid-positive when [^{11}C]-PIB SUVR composite score was >1.5. Composite [^{11}C]-PBR28 and [^{11}C]-PIB SUVR scores were calculated as the average SUVR over all six ROIs.

5.3.1 [^{11}C]PiB and [^{11}C]PBR28

There were no associations between [^{11}C]PiB and [^{11}C]PBR28 SUVR composite scores (slope 0.02, p=0.30) or [^{11}C]PiB SUVRs and [^{11}C]PBR28 SUVRs in any of the ROIs (all p≥0.21) among the whole study population (amyloid-negative and -positive individuals). To evaluate if the association between [^{11}C]PiB and [^{11}C]PBR28 SUVR composite scores would be modulated by amyloid status (based on [^{11}C]PiB PET scans), the interaction '[^{11}C]PiB composite score × amyloid positivity' for predicting [^{11}C]PBR28 SUVR composite score was analyzed. The TSPO genotype was included in the model. The threshold for amyloid positivity was set at PiB composite score SUVR >1.5. The interaction for '[^{11}C]PiB composite score × amyloid positivity' was significant (p = 0.02); thus, the analyses were stratified according to amyloid status.

In the stratified analyses according to amyloid positivity (an individual was considered amyloid-negative when [^{11}C]PiB composite score was ≤1.5 and -positive when [^{11}C]PiB composite score was >1.5), a higher [^{11}C]PiB SUVR composite score was associated with a higher [^{11}C]PBR28 SUVR composite score among amyloid-negative participants, i.e. among those who had only small amounts of amyloid accumulation (slope 0.26, p=0.008). No association was found between [^{11}C]PiB and [^{11}C]PBR28 SUVR composite scores among amyloid-positive participants (slope −0.004, p=0.88). (Figure 11)

In the voxel-level analysis, [^{11}C]PiB uptake was associated with [^{11}C]PBR28 uptake among amyloid-negative participants, especially in the parietal cortex, and also in the frontal cortex, precuneus, and the posterior cingulum, reflecting cortical regions of early Aβ accumulation in AD (Figure 1 in Study II). A positive association was found in the false discovery rate (FDR) corrected voxel-level analysis in the entire study population. However, the association was explained by the amyloid-negative subgroup, as no association existed between [^{11}C]PiB and [^{11}C]PBR28 binding among the amyloid-positive participants.

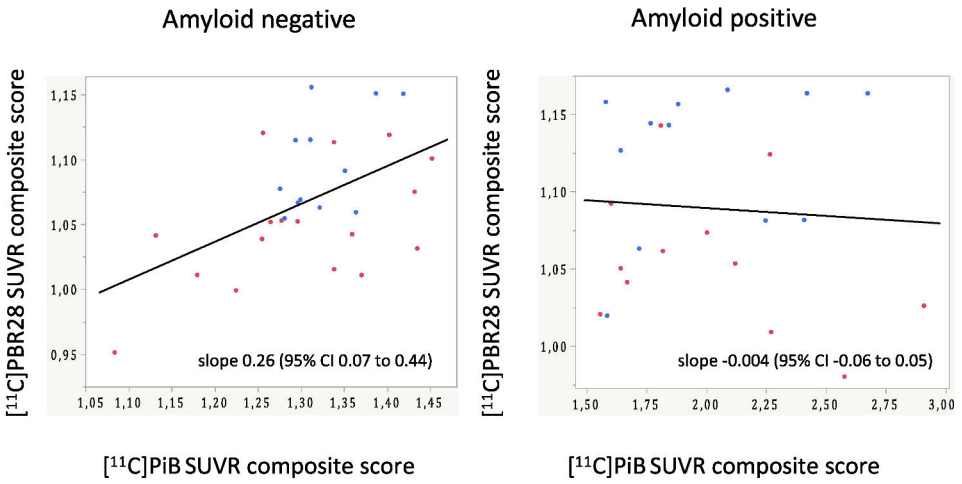

Figure 11. Association between [^{11}C]PiB and [^{11}C]PBR28 SUVR composite scores in the amyloid-negative ([^{11}C]-PIB SUVR composite score ≤1.5) and amyloid-positive ([^{11}C]-PIB SUVR composite score >1.5) participants. High-affinity binders are marked with red and mixed-affinity binders with blue dots. Age-, sex-, and translocator protein genotype -adjusted slopes (with 95% confidence intervals) for the association between [^{11}C]PiB and [^{11}C]PBR28 SUVR composite scores were assessed with multivariable linear models, Modified from Figure 1B in Study II.

5.3.2 CSF markers and PET imaging

Characteristics of the subpopulation with CSF sampling (n=11) are in Table 10. Proteins related to AD and inflammation were measured from the CSF samples to support the results from the PET scans. All participants who gave permission for CSF sampling had CSF T-tau and P-tau levels within the normal range. Only three of the participants who underwent CSF sampling were classified as amyloid positive according to the PiB PET imaging (the cut-off for amyloid positivity was set at > 1.5 PiB SUVR composite score). Associations between CSF biomarkers of inflammation and TSPO-PET and between CSF biomarkers of Aβ pathology and amyloid-PET were analyzed. The results are shown in Figure 12. Higher levels of inflammatory biomarkers measured from the CSF (sTREM2 and YKL-40) correlated with higher TSPO ligand binding in PET imaging ([^{11}C]PBR28 SUVR composite score). CSF biomarkers of Aβ pathology (lower Aβ42 and lower Aβ42/Aβ40 ratio) correlated with higher PiB binding in PET ([^{11}C]PiB SUVR composite score), a PET biomarker of amyloid pathology. (Figure 12)

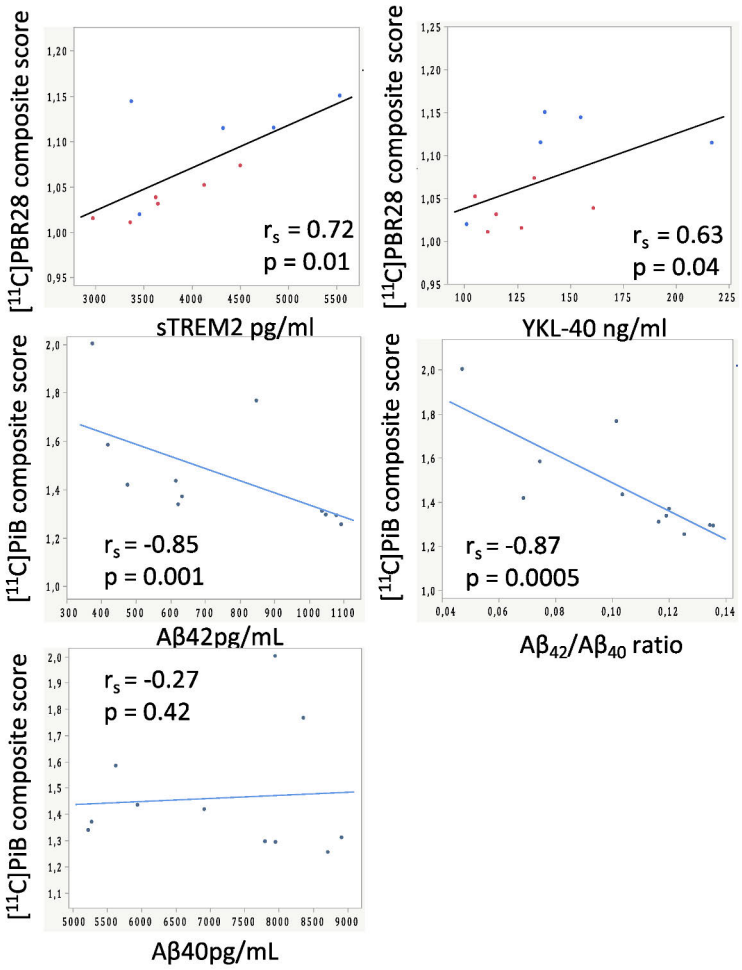

Figure 12. Spearman's correlation between CSF biomarkers of neuroinflammation and [^{11}C]PBR28 SUVR composite score and between CSF biomarkers of amyloid accumulation and [^{11}C]PiB SUVR composite score (n = 11). In the figures on top, high-affinity binders are marked with red and mixed affinity binders with blue dots. Modified from Figure 2 in Study II.

5.3.3 Metabolic risk factors and [^{11}C]PBR28

The association between metabolic risk factors measured at baseline 15 years before the PET scans and during the PET scans and [^{11}C]PBR28 SUVR were analyzed. A higher BMI at baseline was associated with a higher [^{11}C]PBR28 SUVR in the parietal cortex (uncorrected p=0.002) and precuneus (uncorrected p=0.03), but only the former survived Bonferroni correction (Bonferroni-corrected p=0.01). A positive association was found between BMI during PET scans and [^{11}C]PBR28 SUVR composite score (p=0.006). A higher BMI was associated with a higher [^{11}C]PBR28

SUVR in the parietal cortex (Bonferroni corrected p<0.0006) and the precuneus (Bonferroni-corrected p=0.002). No significant association was found in other ROIs (all uncorrected p≥0.10).

Neither baseline HOMA-IR nor hs-CRP was associated with [^{11}C]PBR28 SUVRs in any of the ROIs (all uncorrected p≥0.07). HOMA-IR and hs-CRP at the time of PET scans were unassociated with [^{11}C]PBR28 composite score (HOMA-IR p=0.21, hs-CRP p=0.29). However, a higher HOMA-IR and hs-CRP during PET scans were associated with a higher [^{11}C]PBR28 SUVR in the parietal cortex (HOMA-IR: uncorrected p=0.0009, Bonferroni-corrected p=0.005; hs-CRP: uncorrected p=0.01). A higher HOMA-IR was associated with a higher [^{11}C]PBR28 SUVR also in the precuneus (uncorrected p=0.04).

Additional analyses were performed with [^{11}C]PBR28 DVRs (based on the arterial data) instead of SUVRs (n=44). The results were almost like the results SUVRs obtained. A positive association was found between BMI during PET scans and [^{11}C]PBR28 DVR composite score (Bonferroni-corrected p=0.03). A higher BMI during PET scans was associated with a higher [^{11}C]PBR28 DVR in the parietal cortex (Bonferroni-corrected p < 0.0006) and in the precuneus (Bonferroni-corrected p=0.049). A positive association was also found between hs-CRP during PET scans and [^{11}C]PBR28 DVR in the parietal cortex (Bonferroni-corrected p=0.03).

5.4 OGTT and cognitive performance (Studies III and IV)

Altogether, 961 individuals were included in the analyses. For descriptive purposes only, the participants were categorized into three groups (normal glucose metabolism, IFG or IGT, and diabetes) according to WHO criteria for IFG, IGT, and diabetes (Table 1 in Study IV).

Compared to participants with normal glucose metabolism, those with type 2 diabetes or IFG/IGT were older (DM2: p=0.004, IFG/IGT: p=0.006), had higher systolic blood pressure (DM2: p<0.0001; IFG/IGT p<0.0001), and higher BMI (DM2: p<0.0001; IFG/IGT: p<0.0001). Participants with diabetes had also less education than those with normal glucose metabolism (p=0.006). Compared to individuals with IFG/IGT, those with type 2 diabetes had higher systolic blood pressure (p=0.01) and BMI (p=0.02). (For more detailed characteristics, see Table 1 in Study IV.)

The results of the OGTT at baseline and the cognitive test scores at baseline and follow-up are in Table 11; the results according to the glucose metabolism groups are in Table 1 in Study IV. Participants with IFG did not differ from participants with IGT in fasting or 30-min insulin levels (p≥0.90), EIR (p=0.59), HOMA-IR (p=0.19), or HbA1c (p=0.92) at baseline. (Data not shown.)

Table 11. The OGTT results at baseline and the cognitive test scores at baseline and follow-up.

	N=961
Baseline	
fasting glucose (mmol/l), median (Q1, Q3)	5.6 (5.2, 6.0)
30-min glucose (mmol/l) in OGGT, median (Q1, Q3)	8.6 (7.6, 9.7)
2h glucose (mmol/l) in OGTT, median (Q1, Q3)	6.3 (5.1, 7.7)
fasting insulin (mU/l), median (Q1, Q3)	7.5 (5.5, 10.5)
30-min insulin (mU/l) in OGTT, median (Q1, Q3)	40.3 (28.3, 58.4)
2h insulin (mU/l) in OGTT, median (Q1, Q3)	35.9 (23.0, 58.1)
early insulin response (mU/mmol), median (Q1, Q3)	11.4 (7.5, 18.7)
HOMA-IR, median (Q1, Q3)	1.8 (1.3, 2.8)
verbal fluency, mean (SD)	25.6 (6.8)
word-list learning, mean (SD)	21.6 (3.7)
word-list delayed recall, mean (SD)	7.3 (1.6)
Follow-up	
verbal fluency, mean (SD)	23.8 (7.1)
word-list learning, mean (SD)	20.5 (4.4)
word-list delayed recall, mean (SD)	6.9 (2.1)

OGTT = oral glucose tolerance test, HOMA-IR = Homeostatic Model Assessment of Insulin Resistance

5.4.1 2-hour glucose

The associations between the 2-hour glucose values of the OGTT at baseline (the in-depth examinations in 2001–2002) and the cognitive test results at follow-up (the Health 2011 survey) are in Table 12; the change in cognitive scores from 2000 to 2011 are in Table 13. Higher 2-hour glucose at baseline was associated with a worse score in the word-list delayed recall test after ten years (fully adjusted model slope -0.08; 95% CI -0.14 to -0.02; p=0.01) and a steeper decline from 2000–2001 to 2011 (fully adjusted model slope -0.07; 95% CI -0.13 to -0.02; p=0.007). Additional analyses were performed by adding fasting glucose into the fully adjusted model, after which associations remained significant.

Higher 2-hour glucose was also associated with worse performance (slope -0.14, 95% CI -0.23 to -0.06, p=0.0005) and steeper decline (slope -0.10, 95% CI -0.17 to -0.03, p=0.006) in the word-list learning test, but only in the model adjusted for age, sex, and education. No association was found between 2-hour glucose and verbal fluency (age, sex, and education adjusted slope -0.11, 95% CI -0.26 to 0.03, p=0.11).

Table 12. The associations between the 2-hour glucose values of the OGTT at baseline (in 2001–2002) and the cognitive test results at follow-up (in 2011).

	verbal fluency in 2011	word-list learning in 2011	word-list delayed recall in 2011
2-hour glucose[A], slope (95% CI)	-0.30 (-0.45 to -0.15)***	-0.29 (-0.38 to -0.19)***	-0.14 (-0.18 to -0.10)***
R^2_{adj}	0.01	0.04	0.04
2-hour glucose[B], slope (95% CI)	-0.11 (-0.26 to 0.03)	-0.14 (-0.23 to -0.06)***	-0.08 (-0.12 to -0.04)***
R^2_{adj}	0.16	0.29	0.22
2-hour glucose[C], slope (95% CI)	0.02 (-0.19 to 0.23)	-0.07 (-0.19 to 0.05)	-0.08 (-0.14 to -0.02)*
R^2_{adj}	0.16	0.29	0.22

[A]= unadjusted. [B]= model adjusted for age, sex, and education. [C]= model further adjusted for *APOEε4* genotype, type 2 diabetes, hypertension, hypercholesterolemia, BMI, Beck Depression Inventory score, and smoking. R^2_{adj} = adjusted coefficient of determination of the multivariable model. *p < 0.05, **p < 0.01, ***p < 0.001. Modified from Table 1 in Study III.

Table 13. The association between 2-hour glucose values at baseline (in 2001–2002) and the change in cognitive test scores from 2000 to 2011.

	change in verbal fluency 2000–2011	change in word-list learning 2000–2011	change in word-list delayed recall 2000–2011
2-hour glucose[A], slope (95% CI)	-0.05 (-0.18 to 0.08)	-0.11 (-0.19 to -0.03)**	-0.08 (-0.12 to -0.04)***
R^2_{adj}	0.00	0.01	0.02
2-hour glucose[B], slope (95% CI)	-0.05 (-0.17 to 0.07)	-0.10 (-0.17 to -0.03)**	-0.07 (-0.11 to -0.04)***
R^2_{adj}	0.23	0.21	0.20
2-hour glucose[C], slope (95% CI)	0.03 (-0.14 to 0.20)	-0.05 (-0.16 to 0.05)	-0.07 (-0.13 to -0.02)**
R^2_{adj}	0.25	0.22	0.21

The change in the cognitive test scores at follow-up was calculated by the following equation: "cognitive test score at follow-up (the year 2011) minus cognitive test score at baseline (the year 2000)". A negative change indicates a decline in cognitive performance. [A]= unadjusted. [B]= model adjusted for age, sex, education, and baseline cognitive test scores. [C]= model further adjusted for *APOEε4* genotype, type 2 diabetes, hypertension, hypercholesterolemia, BMI, Beck Depression Inventory score, and smoking. R^2_{adj} = adjusted coefficient of determination of the multivariable model. *p < 0.05, **p < 0.01, ***p < 0.001. Modified from Table 1 in Study III.

The possible modulating effects of type 2 diabetes, *APOE ε4* genotype, age, and sex on the association between 2-hour glucose and the cognitive test scores as well

as the association between 2-hour glucose and the change in the cognitive test scores (from 2000 to 2001) were studied by testing for interactions in the model adjusted for age, sex, and education. Only the interaction "2-hour glucose x age" was significant for predicting the change in word-list learning (p=0.04); thus, the analysis was stratified according to age group (<54 years [the median age] and ≥54 years). No other significant interactions were found (all p-values ≥0.06). In the stratified analysis, the association between 2-hour glucose and the change in word-list learning was significant among participants aged 54 and older but not among participants under 54 years (for age group ≥54 years: slope -0.15, 95% CI -0.24 to -0.05, p=0.003; for age group <54 years: slope -0.03, 95% CI -0.14 to 0.08, p=0.55).

5.4.2 Insulin and insulin-derived measures

The association between EIR and HOMA-IR at baseline and the cognitive test scores at follow-up are in Table 14. All the associations between insulin and insulin-derived measures from the OGTT at baseline (fasting insulin, 30-minute insulin, 2-hour insulin, EIR, and HOMA-IR) and cognitive test results at follow-up are demonstrated in Study IV, Table 2.

Higher insulin value measured 30 minutes after the glucose load was associated with weaker performance in verbal fluency in 2011 (fully adjusted model slope -1.06, 95% CI -1.94 to -0.17, p=0.02). Lower EIR was associated with worse performance in the word-list delayed recall test at follow-up (fully adjusted slope 0.21, 95% CI 0.02 to 0.40, p=0.03) (Table 14). After including HOMA-IR in the analyses, the association remained borderline significant (p=0.056). (Data not shown.) In the analyses adjusted only for age, sex, and education, higher fasting insulin and HOMA-IR were associated with weaker performance in verbal fluency (for fasting insulin: slope -1.41, 95% CI -2.29 to -0.53, p=0.002; for HOMA-IR: slope -1.25, 95% CI -2.00 to -0.50, p=0.001). The baseline 2-hour insulin was unassociated with any of the cognitive tests after follow-up.

The possible modulating effects of age, sex, *APOEε4* genotype, and type 2 diabetes on the associations between OGTT insulin values, EIR, and HOMA-IR and the cognitive test scores at follow-up were analyzed by testing for interactions (in the model adjusted for age, sex, and education). The interaction "2-hour insulin x *APOEε4"* was significant for predicting word-list recall in 2011 (p=0.01); thus, the analysis was stratified by APOE genotype. No other significant interactions were found (all p-values ≥ 0.06).

In the analyses stratified by *APOEε4* genotype (age, sex, education, type 2 diabetes, hypertension, hypercholesterolemia, BMI, BDI score, and smoking adjusted), no association was found between 2-hour insulin and verbal fluency in

2011 among *APOEε4* negative or positive participants (p-values ≥0.34). (Data not shown.)

Table 14. The association between baseline early insulin response and HOMA-IR (in 2001–2002) and the cognitive test scores at follow-up (in 2011).

	verbal fluency in 2011		**word-list learning in 2011**		**word-list delayed recall in 2011**	
	slope (95% CI)	r^2_{adj}	slope (95% CI)	r^2_{adj}	slope (95% CI)	r^2_{adj}
Age, sex, and education adjusted						
EIR	-0.36 (-0.94 to 0.22)	0.16	0.01 (-0.32 to 0.35)	0.28	0.24** (0.07 to 0.40)	0.22
HOMA-IR	-1.25** (-2.00 to -0.50)	0.17	-0.49* (-0.93 to -0.05)	0.29	-0.09 (-0.30 to 0.13)	0.21
Further adjusted for *APOEε4* genotype, type 2 diabetes, hypertension, hypercholesterolemia, BMI, Beck Depression Inventory score, and smoking						
EIR	-0.51 (-1.17 to 0.15)	0.17	-0.15 (-0.54 to 0.23)	0.30	0.21* (0.02 to 0.40)	0.23
HOMA-IR	-0.89 (-1.98 to 0.21)	0.17	0.36 (-0.28 to 0.99)	0.30	0.17 (-0.15 to 0.49)	0.23

EIR = early insulin response, HOMA-IR = Homeostatic Model Assessment of Insulin Resistance. A logarithmic transformation is used for EIR and HOMA-IR. r^2_{adj} = adjusted coefficient of determination of the multivariable model. *p < 0.05, **p < 0.01, ***p < 0.001. Modified from Table 2 in Study IV.

The association between EIR and HOMA-IR and the change in the cognitive tests are in Table 15. All the associations between insulin and insulin-derived measures from the OGTT at baseline (fasting insulin, 30-minute insulin, 2-hour insulin, EIR, and HOMA-IR) and the change in cognitive test scores during the follow-up are in Study IV, Table 3. Lower EIR in 2001–2002 predicted a greater decline in the word-list delayed recall test from baseline to follow-up (fully adjusted model slope 0.19, 95% CI 0.02 to 0.36, p=0.03). In the less adjusted analyses (age, sex, and education adjusted), higher fasting insulin and HOMA-IR predicted a steeper decline in verbal fluency (for fasting insulin: slope -0.76, 95% CI -1.48 to -0.03, p=0.04; for HOMA-IR: slope -0.67, 95% CI -1.29 to -0.04, p=0.04).

Table 15. The association between early insulin response and HOMA-IR at baseline (in 2001–2002) and the change in cognitive test scores from baseline to follow-up (2011).

	change in verbal fluency 2011–2000		**change in word-list learning 2011–2000**		**change in word-list delayed recall 2011–2000**	
	slope (95% CI)	r^2_{adj}	slope (95% CI)	r^2_{adj}	slope (95% CI)	r^2_{adj}
Age, sex, education, and baseline cognitive test scores adjusted						
EIR	-0.20 (-0.68 to 0.27)	0.23	-0.04 (-0.34 to 0.25)	0.20	0.21** (0.06 to 0.35)	0.19
HOMA-IR	-0.67* (-1.29 to -0.04)	0.23	-0.37 (-0.76 to 0.02)	0.20	0.005 (-0.22 to 0.23)	0.19
Further adjusted for *APOEε4* genotype, type 2 diabetes, hypertension, hypercholesterolemia, BMI, Beck depression inventory score, and smoking						
EIR	-0.28 (-0.82 to 0.27)	0.25	-0.19 (-0.54 to 0.15)	0.23	0.19* (0.016 to 0.36)	0.21
HOMA-IR	-0.54 (-1.45 to 0.36)	0.25	0.31 (-0.25 to 0.88)	0.22	0.13 (-0.16 to 0.42)	0.20

EIR = early insulin response, HOMA-IR = Homeostatic Model Assessment of Insulin Resistance. A logarithmic transformation is used for EIR and HOMA-IR. r^2_{adj} = adjusted coefficient of determination of the multivariable model. *p < 0.05, **p < 0.01, ***p < 0.001. Modified from Table 3 in Study IV.

6 Discussion

6.1 PET studies (Studies I and II)

6.1.1 Midlife insulin resistance as a predictor of cognitive performance and brain vascular changes

The main result of Study I was that individuals considered insulin-resistant at midlife performed poorer in cognitive tests measuring executive function and processing speed 15 years later than those considered insulin-sensitive at midlife. Contrary to the original hypothesis, midlife insulin resistance did not predict vascular brain changes after a follow-up of 15 years. Cerebrovascular changes were unassociated with cognitive performance. Instead, a higher brain Aβ load was associated with slower processing speed in the unadjusted analyses and the analyses adjusted for age, sex, education level, and *APOE ε4* genotype.

Finding an association between insulin resistance and poorer cognitive function (executive function and processing speed) aligns with previous extensive epidemiological studies. A few large cross-sectional studies have investigated the association between insulin resistance and cognitive function evaluated with a neuropsychological test battery, showing an association between increased insulin resistance and poorer cognitive performance. (Kuusisto et al., 1993; Laws et al., 2017; Maria Teixeira et al., 2020; Sanz et al., 2013; Schuur et al., 2010; Tan et al., 2011) Moreover, most have reported an inverse association between insulin resistance and measures of executive functions. (Laws et al., 2017; Schuur et al., 2010; Tan et al., 2011) Fewer studies have evaluated the association between insulin resistance and processing speed. Sanz et al. found an inverse association between insulin resistance and one of the study's two tests evaluating processing speed (n=1172, aged 35–64). (Sanz et al., 2013) In contrast, another smaller study (n=119, mean age 73 years) found no association between HOMA-IR and processing speed. Instead, they showed an association between higher insulin resistance and weaker performance in working memory and cognitive control – components of executive function. (Frazier et al., 2015)

Also, several longitudinal studies have revealed an association between insulin resistance and cognitive decline (Ekblad et al., 2017; Ennis et al., 2021; Hughes et

al., 2017; Neergaard et al., 2017; Young et al., 2006), but longitudinal studies with comprehensive neuropsychological testing are scarce. A study of 489 participants with coronary heart disease (mean age 58 years, follow-up 20 years) found that insulin resistance was associated with poorer overall cognitive performance, memory, and executive function (evaluated using computerized cognitive assessment) among participants without diabetes. (Lutski et al., 2017) A recent 7-year-follow-up on a subsample of the Finnish population-based Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) study (n=269, aged 65–79 years) found no associations between insulin resistance and cognitive functioning measured with a comprehensive battery of neuropsychological tests among the whole study population. Interestingly, however, after excluding incident dementia cases (n=19), higher HOMA-IR at baseline was related to worse performance in global cognition and psychomotor speed. (Hooshmand et al., 2019)

Unexpectedly, Study I found no association between midlife insulin resistance and late-life cerebrovascular changes. However, this result is supported by a previous longitudinal study (n=934) evaluating the risk of atherosclerosis among individuals aged 45 to 64 according to which HOMA-IR did not predict lacunar infarcts nor WMH progression over ten years. (Dearborn et al., 2015) A previous cross-sectional study (n=2326, mean age 56) of healthy adults suggested a positive correlation between insulin resistance and silent lacunar infarcts but not WMHs. (Lee et al., 2016) In contrast, in a previous population-based study of a relatively young study population (n=1597, mean age 40), insulin resistance was associated with WMHs cross-sectionally but only after adjustments for BMI and hypertension. (Weinstein et al., 2015) The relationship between insulin resistance and brain vascular changes remains inconclusive, although several studies have shown an association between MetS and cerebrovascular changes. (H. M. Kwon et al., 2006; Portet et al., 2012; Viscogliosi et al., 2015)

According to previous studies, the presence of cerebrovascular lesions is associated with weaker neurocognitive performance. WMHs, in particular, have been linked to an increased risk of future cognitive decline, especially in executive functions and processing speed. (Baune et al., 2009; Carey et al., 2008; Debette & Markus, 2010; Provenzano et al., 2013; Ylikoski et al., 1993) However, in Study I, no association between cerebrovascular lesions and cognitive performance was found even if individuals with insulin resistance performed poorer, specifically in the tests of executive functions and processing speed. Few participants in Study I had significant WMHs; only four were classified as Fazekas 3, scoring well in the neuropsychological tests. These somewhat atypical individuals probably affected the results. Nevertheless, previous studies support Study I's findings. A study of elderly individuals (n=80, aged 67-91 years) found that MetS was associated with lower performance in executive functions, but the association was not mediated by WMH

severity. (Viscogliosi et al., 2015) Interestingly, another study that investigated patients with hypertension (n=67, mean age 75) with Aβ-PET, MRI, and neuropsychological testing revealed similar results to Study I. Aβ but not cerebrovascular changes were associated with poorer cognitive performance. (Smith et al., 2018)

Study I found an association between a higher Aβ load and slower processing speed in the frontal regions and parietal cortex. According to neuropathological and PET studies, these are the brain regions of early Aβ accumulation. (Braak & Braak, 1997; Villemagne et al., 2017) Aβ accumulation in frontal regions could influence processing speed since processing speed is subserved by the prefrontal cortex. (Motes et al., 2011) However, episodic memory is typically the first domain that impairs in AD.

In large neuropathological studies, individuals with diabetes have shown increased cerebrovascular but not AD pathology. (Abner et al., 2016; Dos Santos Matioli et al., 2017) Cerebral macrovascular (e.g. atherosclerosis and infarction), microvascular (e.g. lacunar infarcts, WMHs, microbleeds), and neurodegenerative pathologies might cause additive brain damage and contribute to cognitive decline. Thus, cerebrovascular disease seems to play a role in determining the clinical expression of AD. (Attems & Jellinger, 2014; Iadecola & Gorelick, 2003) Brain hypoxia caused by cerebral atherosclerosis might increase the cleavage of Aβ from the APP. Further, cerebrovascular disease might impair Aβ clearance due to hypoperfusion in the brain resulting in increased cerebral Aβ accumulation. Aβ, in turn, could promote atherogenesis via vascular oxidative stress and endothelial dysfunction resulting in further vascular damage. (Gupta & Iadecola, 2015)

Vascular and metabolic diseases and brain vascular changes have been suggested to be "second hits" leading to the clinical manifestation of AD in the presence of AD neuropathology. The neuropathological Nun Study (n=102 women; 61 met the neuropathologic criteria for AD), for example, suggested that cerebrovascular disease promotes clinical symptoms of AD. Patients with AD pathology but without cerebrovascular disease tolerated more AD lesions in their brains before clinical symptoms emerged. (Snowdon et al., 1997) The theory about cerebrovascular and degenerative pathologies causing additive brain damage is also supported by studies showing that mixed dementia pathology (vascular and AD) accounts for a substantial number of dementia cases among elderly people. (Schneider et al., 2007; Toledo et al., 2013) However, neuropathological studies have also revealed that AD pathology and cerebrovascular changes can be found in the brains of persons without dementia. (Bennett et al., 2012; Crystal et al., 1988; Hyman et al., 2012; Price et al., 2009)

In Study I, participants with insulin resistance at baseline had more cerebral Aβ than insulin-sensitive individuals, but the groups did not differ in brain vascular changes. Furthermore, Aβ accumulation, but not cerebrovascular changes, was

associated with slower processing speed performance. Thus, Study I indicates that the increased risk for cognitive decline related to insulin resistance might be mediated by something other than merely vascular pathways, possibly Aβ accumulation. However, further research with larger study populations is needed.

6.1.2 Amyloid accumulation and neuroinflammation

The main finding of Study II was that neuroinflammation is associated with the early stages of Aβ accumulation in elderly individuals without dementia. Also, higher BMI, HOMA-IR, and hs-CRP were positively associated with neuroinflammation in brain regions where Aβ accumulation is first detected in AD. The results were supported by the finding that CSF sTREM2 and YKL-40 concentrations, regarded as markers of neuroinflammation, were positively associated with signs of neuroinflammation evaluated with PET (the [^{11}C]PBR28 SUVR composite score).

Previous TSPO-PET studies in elderly individuals without AD dementia but at risk for AD, are scarce. Several PET studies have investigated TSPO binding in patients with MCI or AD compared to healthy controls. (Bradburn et al., 2019). To date, no other studies than Study II have evaluated the association between midlife and late-life metabolic risk factors and late-life neuroinflammation and Aβ accumulation in individuals at risk for AD.

The exact role of neuroinflammation in the pathogenesis of AD (and other diseases causing dementia) is not yet fully understood. Still, neuroinflammation is presumably beneficial and detrimental, depending on the stage of the AD continuum. (Edison & Brooks, 2018) The initial microglial reactivity has been suggested to be protective, but neuroinflammation would become unfavourable as the disease progresses. (Hamelin et al., 2016; van Eldik et al., 2016). This theory is supported by a PET study with [^{11}C]PBR28, showing that there could be two microglial reactivity peaks in the pathogenesis of AD: an early protective peak and a later pro-inflammatory peak. (Fan et al., 2017) Accordingly, Hamelin et al. demonstrated that TSPO binding was higher in those whose condition declined slowly than in those whose fell fast, with no difference in Aβ load, suggesting that microglial activation could have a protective role. (Hamelin et al., 2016) However, in a longitudinal study, the same study group found that the subsequent increase in TSPO binding among AD patients was associated with disease worsening. (Hamelin et al., 2018)

Some previous PET studies have shown an association between Aβ and neuroinflammation (Table 6). In Study II, a positive association between Aβ accumulation and [^{11}C]PBR28 was found among amyloid-negative but not amyloid-positive participants, suggesting that neuroinflammation is associated with early stages of Aβ deposition. The finding is supported by previous TSPO-PET studies indicating that neuroinflammation occurs at the early stages of AD pathogenesis.

(Calsolaro & Edison, 2016; Edison & Brooks, 2018) Dani et al. found a positive association between Aβ accumulation (measured with [^{18}F]flutemetamol) and [^{11}C]PBR28 binding; interestingly, the association was stronger among individuals with MCI than patients with AD. (Dani et al., 2018) Hamelin et al. showed an increase of [^{18}F]DPA-714 binding in AD patients, especially in those at the prodromal stage, compared to healthy controls. They also found higher TSPO binding among amyloid-positive controls than amyloid-negative controls. (Hamelin et al., 2016) Contrary to this finding, no group difference in TSPO binding between amyloid-negative and -positive participants was discovered in Study II. However, the control groups of Hamelin et al. were smaller (amyloid-positive [n=6] and -negative [n=20]) than the Study II population. Also, the quantification was performed without arterial blood samples. (Hamelin et al., 2016)

In Study II, metabolic risk factors (higher BMI, insulin resistance, and hs-CRP) were associated positively with [^{11}C]PBR28 in brain regions where Aβ accumulation is first detected in AD. Epidemiological studies have showed that chronic low-grade inflammation (Pugazhenthi et al., 2017; Roberts et al., 2009; Singh-Manoux et al., 2014; Walker et al., 2019; Yaffe et al., 2003), obesity (Kivipelto et al., 2005) and insulin resistance (Ekblad et al., 2017; Kuusisto et al., 1997; Schrijvers et al., 2010; Whitmer et al., 2008) are associated with an increased risk for cognitive decline or AD. Study II indicates the link between metabolic risk factors and cognitive decline or AD could be mediated – at least partly – through neuroinflammation. However, in Study II, no longitudinal association was found between midlife insulin resistance or low-grade inflammation and neuroinflammation 15 years later, although midlife vascular and metabolic risk factors seem crucial in the development of later cognitive decline (Winblad et al., 2016) and midlife insulin resistance and obesity predict future Aβ accumulation. (Ekblad et al., 2018; Gottesman et al., 2017) The lack of longitudinal association might be explained by the dynamic nature of neuroinflammation, whereas cerebral Aβ accumulation is gradual, beginning to accumulate decades before the clinical symptoms of AD.

Few studies have analyzed the association between CSF markers of neuroinflammation and TSPO-PET imaging. A previous study evaluating neuroinflammation in treated HIV-positive individuals found no associations between CSF chemokines and [^{11}C]PBR28 binding. (Vera et al., 2016) In the Study II subsample (n=11), CSF sTREM2 and YKL-40 concentrations were positively associated with [^{11}C]PBR28 SUVR composite score. After Study II, the result was supported by Pascoal et al. with a larger sample size (n=75), finding. a correlation between higher [^{11}C]PBR28 SUVR and CSF sTREM2. (Pascoal et al., 2021)

6.1.3 Methodological considerations

No previous studies, to the best of my knowledge, have evaluated the effects of midlife insulin resistance on the ageing brain after a 15-year follow-up with a combination of MRI imaging, comprehensive neuropsychological testing, and Aβ imaging (Study I), or with a combination of Aβ imaging, TSPO-PET imaging, and analyzing CSF proteins related to AD and inflammation (Study II).

The size of Study I's population would have been modest to investigate merely the association between insulin resistance and cognitive function or insulin resistance and cerebrovascular changes. However, the possibility to combine different imaging modalities with comprehensive neuropsychological testing makes the study population of 60 valuable. Anyhow, potential group differences (between the insulin-sensitive /-resistant individuals) in the cerebrovascular lesions may have been undetected because of a small and relatively healthy study population. Another limitation is the lack of data from imaging modalities that may detect more subtle forms of cerebrovascular changes (such as diffusion tensor imaging). Furthermore, microbleeds were unconsidered in the normalized total vascular burden measure, which can be seen as a limitation. Individuals with a previous diagnosis of major stroke were excluded at recruitment, which might have affected the results concerning group differences. However, the study aimed to evaluate the association between midlife insulin resistance and late-life cognitive functioning and brain imaging changes in individuals without dementia; major stroke can be considered a confounding factor possibly affecting cognitive performance.

In Study I, neuropsychological testing was performed only at follow-up, which can be considered a limitation. However, performing such time-consuming cognitive testing would be challenging in large epidemiological studies. In Study I, outliers of the raw cognitive test were excluded to achieve normal distribution before formulating the Z-scores. All the outliers excluded were individuals with baseline insulin resistance and had worse test scores than the average study population. Thus, excluding the outliers possibly attenuated the differences in cognitive function between the insulin-sensitive/-resistant groups. However, additional analyses with outliers were performed, and the results remained similar.

At baseline, the fasting time before blood sampling varied, which could have affected the HOMA-IR values. Nonetheless, the groups (insulin-sensitive/-resistant) did not differ regarding fasting time ($p=0.24$). There was a significant difference in the HOMA-IR values between the groups (insulin-sensitive/-resistant) at follow-up indicating the groups differed in insulin sensitivity throughout the follow-up. Estimates of insulin resistance other than HOMA-IR could have been more accurate. However, the euglycemic -hyperinsulinemic clamp, for example, is unsuitable for large epidemiological studies due to its laboriousness. Another limitation is that the association between waist circumference, which can be regarded as a useful

screening tool for MetS (K.G.M.M. Alberti et al., 2009), and cognitive performance or neuroimaging changes were unevaluated in these studies.

Quantifying TSPO-PET is challenging (Study II). There is vast interindividual variability in the binding affinity of [^{11}C]PBR28 because of the rs6971 polymorphism. (Owen et al., 2012) Furthermore, no proper reference region exists in the brain for [^{11}C]PBR28 imaging since TSPO is expressed ubiquitously in the brain and blood vessels. (Lagarde et al., 2018; Lyoo et al., 2015) Traditionally, arterial blood sampling is needed to quantify [^{11}C]PBR28 PET uptake. In Study II, arterial blood sampling was not possible for every participant which can be seen as a limitation. In Study II, the SUVR method was used (n=54) with the cerebellum as a pseudo-reference region, a method validated by Lyoo et al in [^{11}C]PBR28-PET imaging of AD patients. (Lyoo et al., 2015) The method is based on the findings that the cerebellar cortex usually lacks AD-related pathological changes, including inflammation. (Braak & Braak, 1991; Mattiace et al., 1990; Wood, 2003) According to Lyoo et al the SUVR method may be even more sensitive than the traditional method. (Lyoo et al., 2015) In Study II, additional analyses were performed with the DVR method using the arterial data (n=44). The results were similar regardless of the method, strengthening the results.

Also, interpreting TSPO-PET results may be demanding since tracer binding can be influenced not only by microglia but other cell types as well. (Gui et al., 2020) Furthermore, TSPO-PET imaging cannot differentiate between the different phenotypes of microglia.

The strengths of studies I and II were the combination of different imaging modalities, a study population of elderly individuals without dementia, and the long follow-up time (15 years) regarding the metabolic risk factors. Another benefit of Study I was that cognitive function was assessed with comprehensive neuropsychological testing. Advantages of Study II were also the arterial blood sampling during [^{11}C]PBR28 PET scanning from most of the study participants and using CSF results to support the results from the PET imaging, even though CSF samples were obtained from 11 participants only.

6.2 Epidemiological studies (Studies III and IV)

6.2.1 OGTT as a predictor of cognitive performance

The main results of Studies III and IV were that higher 2-hour glucose and a lower EIR in the OGTT predicted a decline in episodic memory after 10 years. Higher 2-hour glucose (reflecting impaired glucose tolerance) and a lower EIR (an indicator of beta-cell function) were associated with poorer performance in word-list delayed

recall at follow-up and a greater decline from the baseline. Also, higher 30-min insulin in the OGTT predicted weaker performance in verbal fluency at follow-up.

These results align with previous studies showing an association between higher 2-hour glucose (or IGT) or a lower EIR and poorer cognitive performance (summarized in Tables 2 and 3). Most of the studies have been cross-sectional and only a few have measured glucose and insulin values at multiple time points during OGTT. Furthermore, most of the studies have categorized participants into groups of normal glucose tolerance, IFG, IGT, or diabetes; they have not evaluated the association between 2-hour glucose as a continuous variable and cognitive decline.

Several cross-sectional studies have suggested an association between higher OGTT 2-hour glucose and poorer cognitive performance. (Dybjer et al., 2018; Lamport et al., 2009; Rolandsson et al., 2008; Vanhanen et al., 1997) However, the large longitudinal studies investigating the association between OGTT and cognitive decline have conflicts (Table 3). (Kanaya et al., 2004; Kumari & Marmot, 2005; Lamport et al., 2009; Rönnemaa et al., 2009; Vanhanen et al., 1998) Study III's results agree with Vanhanen et al., who showed that persistent IGT increased the risk of cognitive decline after a follow-up of 3.5 years (n=586, mean age 73). (Vanhanen et al., 1998) Another longitudinal study (n=999, age 42–89, follow-up four years) found that IGT was associated with poorer verbal fluency but only when the cut-off point for cognitive decline was broadened (when the 25 percentile cut-off point was used). (Kanaya et al., 2004) In a study of elderly Swedish men (n=1125, follow-up 12 years) a 2-hour glucose value in the OGTT was associated with an increased risk for cognitive decline and dementia. (Rönnemaa et al., 2009) Contrary to these and Study III's results, in the Whitehall II cohort study (n=5635), participants with midlife prediabetes – classified by fasting and 2-hour glucose values – had similar rates of cognitive decline after ten years of follow-up compared to those with normoglycemia. (Tuligenga et al., 2014) Similarly, Kumari & Marmot (n= 5647, mean age of 56 at follow-up) found no association between IGT and cognitive decline in a younger study population (the Whitehall II cohort) than Study III. Instead, they showed an association between diabetes and an increased risk of cognitive decline (Kumari & Marmot, 2005).

The association between lower EIR and cognitive decline is supported by Rönnemaa et al. (n=1125 men, follow-up 12 years), who found that a lower EIR was associated with an increased risk of AD. (Rönnemaa et al., 2009) Accordingly, the same study group showed that impaired insulin secretion at midlife, assessed with the IVGTT, was associated with an increased risk for cognitive decline, AD, and any dementia (n=1792 men, follow-up 32 years). (Rönnemaa et al., 2008)

An elevated 2-hour glucose level in the OGTT reflects postprandial hyperglycemia. However, this level does not distinguish between the underlying pathophysiologic disturbances: insulin resistance or impaired pancreatic β-cell

function. EIR can be regarded as indicative of β-cell function, while hyperinsulinemia is closely related to insulin resistance. In Study IV, a higher insulin value at 30 minutes predicted weaker performance in verbal fluency, and lower EIR predicted weaker performance in episodic memory after ten years. In the less-adjusted analyses (age, sex, and education) fasting insulin and HOMA-IR predicted poorer performance and a steeper decline in verbal fluency. Thus, impaired early insulin secretion, insulin resistance, and hyperinsulinemia all seem associated with cognitive decline. The different disease mechanisms underlying type 2 diabetes and the preceding stages might contribute to the different associations between insulin sensitivity and secretion and different endpoints.

EIR and 2-hour glucose were associated with the CERAD word-list delayed recall test – a relatively crude test of episodic memory. A decline in episodic memory is an early symptom of AD. Findings from Studies III and IV strengthen the previous studies suggesting an association between midlife metabolic risk factors and late-life cognitive decline (Winblad et al., 2016) since the mean age of the study population III and IV was 56 years at baseline. The exact mechanisms behind the relationship between midlife metabolic risk factors and late-life cognitive decline and dementia are unclear. However, the pathological process of AD is slow, and Aβ is estimated to accumulate years before clinical symptoms appear. (Jack et al., 2010) A previous neuropathological study showed that higher fasting insulin, HOMA-IR, and 2-hour glucose in OGTT measured on average 12.5 years before death, were associated with an increased Aβ load. (Matsuzaki et al., 2010) Conversely, another study found no association among HOMA-IR, diabetes, or IGT and neither cerebral Aβ load detected by PET imaging nor postmortem AD pathology. (Thambisetty et al., 2013)

6.2.2 Methodological considerations

Most of the previous longitudinal studies on the association between OGTT and cognitive decline were performed with a study population much smaller than Study population III and IV, the follow-up time has been shorter, or the sample has included only one sex. The novelty and the main strengths of Studies III and IV were the large study sample (n=961) including both sexes, the inclusion of middle-aged individuals (mean age 56 years at baseline), long follow-up time (ten years), the handling of the variables of the OGTT as continuous variables, and the possibility to adjust the analyses for known risk factors of cognitive decline, including *APOEε4* genotype. A further benefit was that cognitive function was evaluated both at baseline and follow-up, and the time between the cognitive tests in 2000–2001 and 2011–2012 was almost constant among the study participants (the mean time between the tests was 11.02 years, SD 0.13, 95% CI 11.01-11.03). A limitation is that comprehensive neuropsychological test batteries were unused, which would have been more

sensitive to detect subtle cognitive decline than categorical verbal fluency, word-list learning, and word-list delayed recall tests. However, comprehensive neuropsychological testing is time-consuming and often cannot be performed in extensive health examination surveys. Another limitation is that the word-list learning test was performed slightly differently in 2000 and in 2011, which, in theory, could have affected the results. Nevertheless, only one individual in the study population learned all the ten words in the first round; thus, no effect was detected.

A limitation is that OGTT was performed at baseline only. Further, insulin and glucose were measured at only two time-points (30 min and 120 min) after the glucose load; thus, the area under the curve, for example, could not been calculated. Conversely, measuring glucose and insulin not only at 120 minutes but at 30 can be regarded as advantageous. Brain imaging could have given more information about the pathophysiological mechanisms behind the association between OGTT and cognitive decline. However, brain imaging was not performed in this extensive health examination survey. In Study IV, multiple tests on the associations among different measurements of OGTT and three cognitive tests were performed without corrections for multiple comparisons, which could result in false positive associations. However, the analyses were based on a solid theoretical background.

Because of the observational study design, causality cannot be interpreted from the results. Furthermore, the predictive value of 2-hour glucose, 30-min insulin, or EIR alone for explaining cognitive test scores at follow up is fairly modest compared to the variation explained by age, sex, and education level. However, on a population level, detecting an association between modifiable midlife risk factors and cognitive decline is important as it could help plan and target interventions to prevent cognitive decline later in life.

6.3 Clinical relevance and future considerations

Cognitive decline and diseases leading to dementia are presumably caused by the interplay between genetic factors and biological processes. Results of this thesis further support the evidence indicating that midlife metabolic and vascular risk factors are associated with future cognitive decline. (Winblad et al., 2016) Several studies show that a substantial number of dementia-causing diseases could be delayed or potentially prevented by managing the modifiable risk factors of cognitive decline. (Ngandu et al., 2015; Norton et al., 2014) According to the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER), a two-year multidomain intervention (including diet, exercise, cognitive training, vascular risk monitoring) seemed to maintain or even improve cognitive functioning among people aged 60–77 with increased risk for cognitive decline. (Ngandu et al., 2015)

Prediabetes and insulin resistance have been suggested as particularly critical phases in the pathogenesis of dementia. Further, the influence of insulin resistance on cognitive decline could be mediated by the direct and indirect effects of insulin signalling on vascular tone and neuronal health. (Hughes & Craft, 2016; Provenzano et al., 2013) In this thesis, higher levels of insulin resistance, higher 2-hour glucose and a lower EIR in the OGTT – indicators of a prediabetic state or impaired insulin secretion – were all associated with an increased risk for cognitive decline. The results further suggest that the association could be mediated by increased risk for Aβ accumulation and neuroinflammation. The findings highlight the importance of targeting interventions, including health advice and lifestyle guidance, to people at risk cognitive decline to postpone or even prevent cognitive impairment. Measuring insulin sensitivity with HOMA-IR or other methods is uncommon in the clinical setting, limiting the use of measures of insulin resistance to predict cognitive decline. In contrast, OGTT is readily available and widely used to diagnose IGT and diabetes. Thus, OGTT might help detect individuals at increased risk for cognitive decline, even in the prediabetic stage.

Diabetes medications such as metformin, GLP-1 receptor agonists, dipeptidyl peptidase-4 (DPP-4) inhibitors, as well as intranasal insulin, have been investigated as potential drugs for AD. (Kellar & Craft, 2020) A small randomized controlled study suggested that liraglutide might hinder cognitive decline in patients with diabetes. (Vadini et al., 2020). A recent study showed that among patients with diabetes and AD-related cognitive impairment, those treated with DPP-4 inhibitors had a lower global Aβ burden than those without DPP-4 inhibitors. (Jeong et al., 2021) Also, patients with DPP-4 inhibitors showed slower cognitive decline longitudinally. (Jeong et al., 2021) Considering the observed association between insulin resistance and cognitive decline, treatments improving insulin sensitivity might be beneficial in postponing cognitive decline in the future.

However, larger longitudinal studies combining assessments of metabolic risk factors (including OGTT), comprehensive neuropsychological testing, CSF-sampling, and brain imaging with different modalities (including Aβ- and tau-PET and imaging of neuroinflammation), are needed to understand the complex relationship among metabolic risk factors, cognitive decline, neuroinflammation, and AD pathology. Future studies evaluating the association between glucose effectiveness and the risk for cognitive decline could be of interest. Further studies are also needed to solve the time course and relevance of neuroinflammation in the pathogenesis of diseases leading to dementia, which could provide new targets to develop novel therapies. Also, interventions and therapies targeted at insulin resistance, IGT, and impaired insulin secretion might be useful to prevent cognitive decline in the future.

7 Conclusions

The main findings of this thesis can be summarized as follows:

1. Midlife insulin resistance predicted poorer cognitive performance in tests of executive functions and processing speed but not episodic memory or language function after 15 years. Midlife insulin resistance was unassociated with cerebrovascular lesions at follow-up. Brain amyloid accumulation, but not cerebrovascular lesions, was associated with slower processing speed.
2. Early stages of amyloid accumulation were associated with neuroinflammation measured with [^{11}C]PBR28 PET in elderly individuals without dementia. Amyloid accumulation, measured with [^{11}C]PiB PET, was associated with [^{11}C]PBR28 uptake among those who had only small amounts of [^{11}C]PiB binding and thus considered amyloid-negative but not among amyloid-positive participants. Higher BMI and higher levels of insulin resistance and systemic low-grade inflammation were associated with higher levels of neuroinflammation in brain regions where amyloid accumulation is first detected in AD. Higher CSF soluble TREM2 and YKL-40 concentrations, two previously established markers of neuroinflammation, were associated with a higher [^{11}C]PBR28 composite score.
3. In a subpopulation (aged 45–74) of a Finnish population-based health examination survey, a higher 2-hour glucose value in the OGTT and a lower early insulin response to glucose load predicted worse performance and greater decline in the word-list delayed recall test – a test of episodic memory – after ten years. Higher 30-minute insulin in the OGTT predicted weaker performance in the verbal fluency test ten years later but not a decline from the baseline.

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References

Aalto, S., Scheinin, N. M., Kemppainen, N. M., Någren, K., Kailajärvi, M., Leinonen, M., Scheinin, M., & Rinne, J. O. (2009). Reproducibility of automated simplified voxel-based analysis of PET amyloid ligand [11C]PIB uptake using 30-min scanning data. *Eur J Nucl Med Mol Imaging*, 36(10):1651–1660. https://doi.org/10.1007/S00259-009-1174-1

Abdul-Ghani, M. A. (2006). Contributions of Beta-Cell Dysfunction and Insulin Resistance to the Pathogenesis of Impaired Glucose Tolerance and Impaired Fasting Glucose. *Diabetes Care*, *29*(5): 1130–1139. https://doi.org/10.2337/diacare.2951130

Abdul-Ghani, M. A., Jenkinson, C. P., Richardson, D. K., Tripathy, D., & DeFronzo, R. A. (2006). Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: Results from the veterans administration genetic epidemiology study. *Diabetes*, *55*(5):1430–1435. https://doi.org/10.2337/db05-1200

Abner, E. L., Nelson, P. T., Kryscio, R. J., Schmitt, F. A., Fardo, D. W., Woltjer, R. L., Cairns, N. J., Yu, L., Dodge, H. H., Xiong, C., Masaki, K., Tyas, S. L., Bennett, D. A., Schneider, J. A., & Arvanitakis, Z. (2016). Diabetes is associated with cerebrovascular but not Alzheimer's disease neuropathology. *Alzheimers Dement*, *12*(8):882–889. https://doi.org/10.1016/j.jalz.2015.12.006

Ahlqvist, E., Storm, P., Käräjämäki, A., Martinell, M., Dorkhan, M., Carlsson, A., Vikman, P., Prasad, R. B., Aly, D. M., Almgren, P., Wessman, Y., Shaat, N., Spégel, P., Mulder, H., Lindholm, E., Melander, O., Hansson, O., Malmqvist, U., Lernmark, Å., … Groop, L. (2018). Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *Lancet Diabetes Endocrinol,* 6(5), 361–369. https://doi.org/10.1016/S2213-8587(18)30051-2

Ahrén, B., & Pacini, G. (2021). Glucose effectiveness: Lessons from studies on insulin-independent glucose clearance in mice. J Diabetes Investig, 12(5), 675–685. https://doi.org/10.1111/JDI.13446

Aktas, O., Ullrich, O., Infante-Duarte, C., Nitsch, R., & Zipp, F. (2007). Neuronal Damage in Brain Inflammation. *Arch Neurol*, *64*(2):185. https://doi.org/10.1001/archneur.64.2.185

Albert, M. S., DeKosky, S. T., Dickson, D., Dubois, B., Feldman, H. H., Fox, N. C., Gamst, A., Holtzman, D. M., Jagust, W. J., Petersen, R. C., Snyder, P. J., Carrillo, M. C., Thies, B., & Phelps, C. H. (2011). The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*, *7*(3):270–279. https://doi.org/10.1016/j.jalz.2011.03.008

Alberti, K. G. M. M., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., Fruchart, J. C., James, W. P. T., Loria, C. M., & Smith, S. C. (2009). Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation, 120(16), 1640–1645. https://doi.org/10.1161/CIRCULATIONAHA.109.192644

Alberti, K., & Zimmet, P. (1998). Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications. Part 1: Diagnosis and Classification of Diabetes Mellitus Provisional Report of a

WHO Consultation. *Diabet Med, 15*(7). https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S

Alford, F. P., Henriksen, J. E., Rantzau, C., & Beck-Nielsen, H. (2018). Glucose effectiveness is a critical pathogenic factor leading to glucose intolerance and type 2 diabetes: An ignored hypothesis. Diabetes Metab Res Rev, 34(4). https://doi.org/10.1002/DMRR.2989

American Diabetes Association. (2018). Classification and diagnosis of diabetes: Standards of medical care in Diabetes 2018. *Diabetes Care*, *41*(Suppl 1), S13–S27. https://doi.org/10.2337/dc18-S002

Arnold, S. E., Arvanitakis, Z., Macauley-Rambach, S. L., Koenig, A. M., Wang, H. Y., Ahima, R. S., Craft, S., Gandy, S., Buettner, C., Stoeckel, L. E., Holtzman, D. M., & Nathan, D. M. (2018). Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. *Nat Rev. Neurol*, *14*(3):168–181. https://doi.org/10.1038/NRNEUROL.2017.185

Arnold, S. E., Lucki, I., Brookshire, B. R., Carlson, G. C., Browne, C. A., Kazi, H., Bang, S., Choi, B. R., Chen, Y., McMullen, M. F., & Kim, S. F. (2014). High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice. *Neurobiol Dis*, *67*:79–87. https://doi.org/10.1016/j.nbd.2014.03.011

Aromaa, A., & Koskinen, S. (2004). Health and functional capacity in Finland : Baseline results of the Health 2000 health examination survey. Publications of the National Public Health Institute, Helsinki. Available from http://www.julkari.fi/handle/10024/78534

Aschenbrenner, A. J., Gordon, B. A., Benzinger, T. L. S., Morris, J. C., & Hassenstab, J. J. (2018). Influence of tau PET, amyloid PET, and hippocampal volume on cognition in Alzheimer disease. *Neurology*, 91(9):e859–e866. https://doi.org/10.1212/WNL.0000000000006075

Atkinson, A. J., Colburn, W. A., DeGruttola, V. G., DeMets, D. L., Downing, G. J., Hoth, D. F., Oates, J. A., Peck, C. C., Schooley, R. T., Spilker, B. A., Woodcock, J., & Zeger, S. L. (2001). Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther*, *69*(3):89–95. https://doi.org/10.1067/MCP.2001.113989

Attems, J., & Jellinger, K. A. (2014). The overlap between vascular disease and Alzheimer's disease - lessons from pathology. *BMC Med*, *12*(1). https://doi.org/10.1186/s12916-014-0206-2

Baker, L. D., Cross, D. J., Minoshima, S., Belongia, D., Stennis Watson, G., & Craft, S. (2011). Insulin resistance and alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. *Arch Neurol*, *68*(1):51–57. https://doi.org/10.1001/archneurol.2010.225

Banks, W. A., Owen, J. B., & Erickson, M. A. (2012). Insulin in the brain: There and back again. *Pharmacol Ther,* 136(1), 82–93. https://doi.org/10.1016/j.pharmthera.2012.07.006

Baune, B. T., Roesler, A., Knecht, S., & Berger, K. (2009). Single and combined effects of cerebral white matter lesions and lacunar infarctions on cognitive function in an elderly population. *J Gerontol. A Biol Sci Med Sci*, *64*(1):118–124. https://doi.org/10.1093/GERONA/GLN004

Bellenguez, C., Grenier-Boley, B., & Lambert, J. C. (2020). Genetics of Alzheimer's disease: where we are, and where we are going. C*urr Opin Neurobiol,* 61:40–48. https://doi.org/10.1016/j.conb.2019.11.024

Bennett, D. A., Arnold, S. E., Valenzuela, M. J., Brayne, C., & Schneider, J. A. (2014). Cognitive and social lifestyle: Links with neuropathology and cognition in late life. *Acta Neuropathol*, 127(1):137–150. https://doi.org/10.1007/s00401-013-1226-2

Bennett, D. A., Schneider, J. A., Tang, Y., Arnold, S. E., & Wilson, R. S. (2006). The effect of social networks on the relation between Alzheimer's disease pathology and level of cognitive function in old people: a longitudinal cohort study. *Lancet Neurol*, *5*(5):406–412. https://doi.org/10.1016/S1474-4422(06)70417-3

Bennett, D. A., Wilson, R. S., Boyle, P. A., Buchman, A. S., & Schneider, J. A. (2012). Relation of neuropathology to cognition in persons without cognitive impairment. *Ann Neurol 72*(4):599–609. https://doi.org/10.1002/ana.23654

Bergman, R. N., Phillips, L. S., & Cobelli, C. (1981). Physiologic evaluation of factors controlling glucose tolerance in man. Measurement of insulin sensitivity and β-cell glucose sensitivity from

the response to intravenous glucose. *J Clin Invest*, *68*(6):1456–1467. https://doi.org/10.1172/JCI110398

Bergman, R. N., Piccinini, F., Kabir, M., Kolka, C. M., & Ader, M. (2019). Hypothesis: Role of Reduced Hepatic Insulin Clearance in the Pathogenesis of Type 2 Diabetes. *Diabetes*, 68(9), 1709–1716. https://doi.org/10.2337/DB19-0098

Bettens, K., Sleegers, K., & van Broeckhoven, C. (2013). Genetic insights in Alzheimer's disease. *Lancet Neurol,* 12,(1):92–104. https://doi.org/10.1016/S1474-4422(12)70259-4

Biessels, G. J., Nobili, F., Teunissen, C. E., Simó, R., & Scheltens, P. (2020). Understanding multifactorial brain changes in type 2 diabetes: a biomarker perspective. *Lancet Neurol,* 19(8):699–710. https://doi.org/10.1016/S1474-4422(20)30139-3

Biessels, G. J., Strachan, M. W. J., Visseren, F. L. J., Kappelle, L. J., & Whitmer, R. A. (2014). Dementia and cognitive decline in type 2 diabetes and prediabetic stages: Towards targeted interventions. *Lancet Diabetes Endocrinol,* 2(3):246-2555. https://doi.org/10.1016/S2213-8587(13)70088-3

Blennow, K., Hampel, H., Weiner, M., & Zetterberg, H. (2010). Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol,* 6,(3):131–144. https://doi.org/10.1038/nrneurol.2010.4

Blennow, K., & Zetterberg, H. (2018). Biomarkers for Alzheimer's disease: current status and prospects for the future. *J Intern Med*, 284,(6):643–663. Blackwell Publishing Ltd. https://doi.org/10.1111/joim.12816

Braak, H., & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathologica*, *82*(4), 239–259. https://doi.org/10.1007/BF00308809

Braak, H., & Braak, E. (1997). Frequency of Stages of Alzheimer-Related Lesions in Different Age Categories. *Neurobiol Aging*, *18*(4):351–357. https://doi.org/10.1016/S0197-4580(97)00056-0

Bradburn, S., Murgatroyd, C., & Ray, N. (2019). Neuroinflammation in mild cognitive impairment and Alzheimer's disease: A meta-analysis. *Ageing Res Rev*, *50*:1–8. https://doi.org/10.1016/j.arr.2019.01.002

Brendle, C., Kupferschläger, J., Nikolaou, K., la Fougère, C., Gatidis, S., & Pfannenberg, C. (2015). Is the standard uptake value (SUV) appropriate for quantification in clinical PET imaging? – Variability induced by different SUV measurements and varying reconstruction methods. *Eur J Radiol*, *84*(1):158–162. https://doi.org/10.1016/J.EJRAD.2014.10.018

Brier, M. R., Gordon, B., Friedrichsen, K., McCarthy, J., Stern, A., Christensen, J., Owen, C., Aldea, P., Su, Y., Hassenstab, J., Cairns, N. J., Holtzman, D. M., Fagan, A. M., Morris, J. C., Benzinger, T. L. S., & Ances, B. M. (2016). Tau and Aβ imaging, CSF measures, and cognition in Alzheimer's disease. *Sci Transl Med*, 8(338). https://doi.org/10.1126/SCITRANSLMED.AAF2362

Brosseron, F., Krauthausen, M., Kummer, M., & Heneka, M. T. (2014). Body Fluid Cytokine Levels in Mild Cognitive Impairment and Alzheimer's Disease: a Comparative Overview. *Mol Neurobiol*, *50*(2):534–544. https://doi.org/10.1007/s12035-014-8657-1

Cai, Z., Hussain, M. D., & Yan, L. J. (2014). Microglia, neuroinflammation, and beta-amyloid protein in Alzheimer's disease. *Int J Neurosci*, *124*(5):307–321. https://doi.org/10.3109/00207454.2013.833510

Calsolaro, V., & Edison, P. (2016). Neuroinflammation in Alzheimer's disease: Current evidence and future directions. *Alzheimers Dement*, *12*(6):719–732. https://doi.org/10.1016/j.jalz.2016.02.010

Cantó, E., Tintoré, M., Villar, L. M., Costa, C., Nurtdinov, R., Álvarez-Cermeño, J. C., Arrambide, G., Reverter, F., Deisenhammer, F., Hegen, H., Khademi, M., Olsson, T., Tumani, H., Rodríguez-Martín, E., Piehl, F., Bartos, A., Zimova, D., Kotoucova, J., Kuhle, J., … Comabella, M. (2015). Chitinase 3-like 1: prognostic biomarker in clinically isolated syndromes. *Brain*, *138*(4):918–931. https://doi.org/10.1093/brain/awv017

Carey, C. L., Kramer, J. H., Josephson, S. A., Mungas, D., Reed, B. R., Schuff, N., Weiner, M. W., & Chui, H. C. (2008). Subcortical lacunes are associated with executive dysfunction in cognitively normal elderly. *Stroke*, *39*(2):397–402. https://doi.org/10.1161/STROKEAHA.107.491795

Castellani, R. J., Rolston, R. K., & Smith, M. A. (2010). Alzheimer disease. *Dis Mon* 56(9):484–546. https://doi.org/10.1016/j.disamonth.2010.06.001

Chatterjee, S., Khunti, K., & Davies, M. J. (2017). Type 2 diabetes. *Lancet*, 389(10085):2239–2251. https://doi.org/10.1016/S0140-6736(17)30058-2

Chauveau, F., Becker, G., & Boutin, H. (2021). Have (R)-[11 C]PK11195 challengers fulfilled the promise? A scoping review of clinical TSPO PET studies. *Eur J Nucl Med Mol Imaging*, *49*(1):201–220. https://doi.org/10.1007/S00259-021-05425-W

Cheng, G., Huang, C., Deng, H., & Wang, H. (2012). Diabetes as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies. *Intern Med J*, *42*(5):484–491. https://doi.org/10.1111/j.1445-5994.2012.02758.x

Cholerton, B., Baker, L. D., & Craft, S. (2013). Insulin, cognition, and dementia. *Eur J Pharmacol,* 719(1–3):170–179. https://doi.org/10.1016/j.ejphar.2013.08.008

Christman, A. L., Matsushita, K., Gottesman, R. F., Mosley, T., Alonso, A., Coresh, J., Hill-Briggs, F., Sharrett, A. R., & Selvin, E. (2011). Glycated haemoglobin and cognitive decline: The Atherosclerosis Risk in Communities (ARIC) study. *Diabetologia*, *54*(7):1645–1652. https://doi.org/10.1007/s00125-011-2095-7

Cohen, R. A., Marsiske, M. M., & Smith, G. E. (2019). Neuropsychology of aging. *Handb Clin Neurol,* 167:149–180 https://doi.org/10.1016/B978-0-12-804766-8.00010-8

Convit, A., Wolf, O. T., Tarshish, C., & de Leon, M. J. (2003). Reduced glucose tolerance is associated with poor memory performance and hippocampal atrophy among normal elderly. *Proc Natl Acad Sci USA, 100*(4):2019–2022. https://doi.org/10.1073/pnas.0336073100

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L., & Pericak-Vance, M. A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*, *261*(5123):921–923. https://doi.org/10.1126/science.8346443

Cosenza-Nashat, M., Zhao, M. L., Suh, H. S., Morgan, J., Natividad, R., Morgello, S., & Lee, S. C. (2009). Expression of the translocator protein of 18 kDa by microglia, macrophages and astrocytes based on immunohistochemical localization in abnormal human brain. *Neuropathol Appl Neurobiol*, *35*(3):306–328. https://doi.org/10.1111/j.1365-2990.2008.01006.x

Crane, P. K., Walker, R., Hubbard, R. A., Li, G., Nathan, D. M., Zheng, H., Haneuse, S., Craft, S., Montine, T. J., Kahn, S. E., McCormick, W., McCurry, S. M., Bowen, J. D., & Larson, E. B. (2013). Glucose levels and risk of dementia. *N Engl J Med*, *369*(6):540–548. https://doi.org/10.1056/NEJMoa1215740

Crystal, H., Dickson, D., Fuld, P., Masur, D., Scott, R., Mehler, M., Masdeu, J., Kawas, C., Aronson, M., & Wolfson, L. (1988). Clinico-pathologic studies in dementia: Nondemented subjects with pathologically confirmed alzheimer's disease. *Neurology*, *38*(11):1682–1687. https://doi.org/10.1212/wnl.38.11.1682

Czirr, E., & Wyss-Coray, T. (2012). The immunology of neurodegeneration. *J Clin Invest*, *122*(4):1156–1163. https://doi.org/10.1172/JCI58656

Dá Mesquita, S., Ferreira, A. C., Sousa, J. C., Correia-Neves, M., Sousa, N., & Marques, F. (2016). Insights on the pathophysiology of Alzheimer's disease: The crosstalk between amyloid pathology, neuroinflammation and the peripheral immune system. *Neurosci Biobehav Rev*, *68*:547–562. https://doi.org/10.1016/j.neubiorev.2016.06.014

Danat, I. M., Clifford, A., Partridge, M., Zhou, W., Bakre, A. T., Chen, A., McFeeters, D., Smith, T., Wan, Y., Copeland, J., Anstey, K. J., & Chen, R. (2019). Impacts of Overweight and Obesity in Older Age on the Risk of Dementia: A Systematic Literature Review and a Meta-Analysis. *J Alzheimers Dis,* 70(s1):S87–S99. https://doi.org/10.3233/JAD-180763

Dani, M., Wood, M., Mizoguchi, R., Fan, Z., Walker, Z., Morgan, R., Hinz, R., Biju, M., Kuruvilla, T., Brooks, D. J., & Edison, P. (2018). Microglial activation correlates in vivo with both tau and amyloid in Alzheimer's disease. *Brain*, *141*(9):2740–2754. https://doi.org/10.1093/brain/awy188

Dave, A., Hansen, N., Downey, R., & Johnson, C. (2020). FDG-PET Imaging of Dementia and Neurodegenerative Disease. *Semin Ultrasound CT MR*, *41*(6):562–571. https://doi.org/10.1053/J.SULT.2020.08.010

de Felice, F. G., & Ferreira, S. T. (2014). Inflammation, Defective Insulin Signaling, and Mitochondrial Dysfunction as Common Molecular Denominators Connecting Type 2 Diabetes to Alzheimer Disease. *Diabetes*, *63*(7):2262–2272. https://doi.org/10.2337/db13-1954

Dearborn, J. L., Schneider, A. L. C., Sharrett, A. R., Mosley, T. H., Bezerra, D. C., Knopman, D. S., Selvin, E., Jack, C. R., Coker, L. H., Alonso, A., Wagenknecht, L. E., Windham, B. G., & Gottesman, R. F. (2015). Obesity, Insulin Resistance, and Incident Small Vessel Disease on Magnetic Resonance Imaging: Atherosclerosis Risk in Communities Study. *Stroke*, *46*(11):3131–3136. https://doi.org/10.1161/STROKEAHA.115.010060

Debette, S., & Markus, H. S. (2010). The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ*, *341*:c3666. https://doi.org/10.1136/BMJ.C3666

DeFronzo, R. A., Tobin, J. D., & Andres, R. (1979). Glucose clamp technique: A method for quantifying insulin secretion and resistance. *Am J Physiol*, 237(3):E214-223. https://doi.org/10.1152/ajpendo.1979.237.3.e214

Denora, N., & Natile, G. (2017). An updated view of translocator protein (TSPO). *Int J Mol Sci,* 18(12):2640. https://doi.org/10.3390/ijms18122640

Diniz, B. S., Butters, M. A., Albert, S. M., Dew, M. A., & Reynolds, C. F. (2013). Late-life depression and risk of vascular dementia and Alzheimer's disease: Systematic review and meta-analysis of community-based cohort studies. *Br J Psychiatry*, 202(5):329–335. https://doi.org/10.1192/bjp.bp.112.118307

Dos Santos Matioli, M. N. P., Suemoto, C. K., Rodriguez, R. D., Farias, D. S., Da Silva, M. M., Leite, R. E. P., Ferretti-Rebustini, R. E. L., Farfel, J. M., Pasqualucci, C. A., Jacob Filho, W., Arvanitakis, Z., Naslavsky, M. S., Zatz, M., Grinberg, L. T., & Nitrini, R. (2017). Diabetes is Not Associated with Alzheimer's Disease Neuropathology. *J Alzheimer's Dis*, *60*(3):1035–1043. https://doi.org/10.3233/JAD-170179

Dubois, B., Feldman, H. H., Jacova, C., DeKosky, S. T., Barberger-Gateau, P., Cummings, J., Delacourte, A., Galasko, D., Gauthier, S., Jicha, G., Meguro, K., O'Brien, J., Pasquier, F., Robert, P., Rossor, M., Salloway, S., Stern, Y., Visser, P. J., & Scheltens, P. (2007). Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol,* 6(8):734–746). https://doi.org/10.1016/S1474-4422(07)70178-3

Dubois, B., Feldman, H. H., Jacova, C., Hampel, H., Molinuevo, J. L., Blennow, K., Dekosky, S. T., Gauthier, S., Selkoe, D., Bateman, R., Cappa, S., Crutch, S., Engelborghs, S., Frisoni, G. B., Fox, N. C., Galasko, D., Habert, M. O., Jicha, G. A., Nordberg, A., … Cummings, J. L. (2014). Advancing research diagnostic criteria for Alzheimer's disease: The IWG-2 criteria. *Lancet Neurol, 13(6):*614–629). https://doi.org/10.1016/S1474-4422(14)70090-0

Dybjer, E., Nilsson, P. M., Engström, G., Helmer, C., & Nägga, K. (2018). Pre-diabetes and diabetes are independently associated with adverse cognitive test results: A cross-sectional, population-based study. *BMC Endocr Disord*, *18*(1):91. https://doi.org/10.1186/s12902-018-0318-3

Edison, P., Archer, H. A., Gerhard, A., Hinz, R., Pavese, N., Turkheimer, F. E., Hammers, A., Tai, Y. F., Fox, N., Kennedy, A., Rossor, M., & Brooks, D. J. (2008). Microglia, amyloid, and cognition in Alzheimer's disease: An [11C](R)PK11195-PET and [11C]PIB-PET study. *Neurobiology of Disease*, *32*(3), 412–419. https://doi.org/10.1016/J.NBD.2008.08.001

Edison, P., & Brooks, D. J. (2018). Role of Neuroinflammation in the Trajectory of Alzheimer's Disease and in vivo Quantification Using PET. *J Alzheimer's Dis, 64*(s1):S339–S351. https://doi.org/10.3233/JAD-179929

Ekblad, L. L., Johansson, J., Helin, S., Viitanen, M., Laine, H., Puukka, P., Jula, A., & Rinne, J. O. (2018). Midlife insulin resistance, APOE genotype, and late-life brain amyloid accumulation. *Neurology*, *90*(13):e1150–e1157. https://doi.org/10.1212/WNL.0000000000005214

Ekblad, L. L., Rinne, J. O., Puukka, P. J., Laine, H. K., Ahtiluoto, S. E., Sulkava, R. O., Viitanen, M. H., & Jula, A. M. (2015). Insulin resistance is associated with poorer verbal fluency performance in women. *Diabetologia*, *58*(11):2545–2553. https://doi.org/10.1007/S00125-015-3715-4

Ekblad, L. L., Rinne, J. O., Puukka, P., Laine, H., Ahtiluoto, S., Sulkava, R., Viitanen, M., & Jula, A. (2017). Insulin Resistance Predicts Cognitive Decline: An 11-Year Follow-up of a Nationally Representative Adult Population Sample. *Diabetes Care*, *40*(6):751–758. https://doi.org/10.2337/dc16-2001

Ennis, G. E., Koscik, R. L., Ma, Y., Jonaitis, E. M., van Hulle, C. A., Betthauser, T. J., Randall, A. M., Chin, N., Engelman, C. D., Anderson, R., Suridjan, I., Kollmorgen, G., Christian, B. T., Carlsson, C. M., Asthana, S., Johnson, S. C., Zetterberg, H., Blennow, K., & Bendlin, B. B. (2021). Insulin resistance is related to cognitive decline but not change in CSF biomarkers of Alzheimer's disease in non-demented adults. *Alzheimers Dement: 13*(1):e12220. https://doi.org/10.1002/dad2.12220

Enoki, S., Mitsukawa, T., Takemura, J., Nakazato, M., Aburaya, J., Toshimori, H., & Matsukara, S. (1992). Plasma islet amyloid polypeptide levels in obesity, impaired glucose tolerance and non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract*, *15*(1):97–102. https://doi.org/10.1016/0168-8227(92)90074-2

Esser, N., Legrand-Poels, S., Piette, J., Scheen, A. J., & Paquot, N. (2014). Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res Clinl Pract*, *105*(2):141–150. https://doi.org/10.1016/j.diabres.2014.04.006

Fadel, J. R., & Reagan, L. P. (2016). Stop signs in hippocampal insulin signaling: The role of insulin resistance in structural, functional and behavioral deficits. *Curr Opin Behav Sci, 9:*47–54. https://doi.org/10.1016/j.cobeha.2015.12.004

Fan, Z., Aman, Y., Ahmed, I., Chetelat, G., Landeau, B., Ray Chaudhuri, K., Brooks, D. J., & Edison, P. (2015). Influence of microglial activation on neuronal function in Alzheimer's and Parkinson's disease dementia. *Alzheimers Dement ,11*(6):608-621.e7. https://doi.org/10.1016/J.JALZ.2014.06.016

Fan, Z., Brooks, D. J., Okello, A., & Edison, P. (2017). An early and late peak in microglial activation in Alzheimer's disease trajectory. *Brain, 140*(3):792–803. https://doi.org/10.1093/brain/aww349

Fan, Z., Dani, M., Femminella, G. D., Wood, M., Calsolaro, V., Veronese, M., Turkheimer, F., Gentleman, S., Brooks, D. J., Hinz, R., & Edison, P. (2018). Parametric mapping using spectral analysis for 11C-PBR28 PET reveals neuroinflammation in mild cognitive impairment subjects. *Eur J Nucl Med, 45*(8):1432–1441. https://doi.org/10.1007/s00259-018-3984-5

Fan, Z., Okello, A. A., Brooks, D. J., & Edison, P. (2015). Longitudinal influence of microglial activation and amyloid on neuronal function in Alzheimer's disease. *Brain, 138*(Pt 12):3685–3698. https://doi.org/10.1093/BRAIN/AWV288

Farris, W., Mansourian, S., Chang, Y., Lindsley, L., Eckman, E. A., Frosch, M. P., Eckman, C. B., Tanzi, R. E., Selkoe, D. J., & Guénette, S. (2003). Insulin-degrading enzyme regulates the levels of insulin, amyloid β-protein, and the β-amyloid precursor protein intracellular domain in vivo. *Proc Natl Acad Sci USA 100*(7):4162–4167. https://doi.org/10.1073/pnas.0230450100

Fazekas, F., Chawluk, J. B., Alavi, A., Hurtig, H. I., & Zimmerman, R. A. (1987). MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol*, *149*(2):351–356. https://doi.org/10.2214/ajr.149.2.351

Ferrannini, E., Gastaldelli, A., & Iozzo, P. (2011). Pathophysiology of Prediabetes. *Med Clin North Am,* 95(2): 327–339). Med Clin North Am. https://doi.org/10.1016/j.mcna.2010.11.005

Ferrannini, E., & Mari, A. (1998). How to measure insulin sensitivity. *J Hypertens*, *16*(7):895–906. https://doi.org/10.1097/00004872-199816070-00001

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*, *12*(3):189–198. https://doi.org/10.1016/0022-3956(75)90026-6

Frazier, D. T., Bettcher, B. M., Dutt, S., Patel, N., Mungas, D., Miller, J., Green, R., & Kramer, J. H. (2015). Relationship between Insulin-Resistance Processing Speed and Specific Executive

Function Profiles in Neurologically Intact Older Adults. *J Int Neuropsychol Soc, 21*(8):622–628. https://doi.org/10.1017/S1355617715000624

Freeman, A. M., & Pennings, N. (2022). Insulin Resistance. StatPearls Publishing. https://pubmed-ncbi-nlm-nih-gov.ezproxy.utu.fi/29939616/

Fujita, M., Kobayashi, M., Ikawa, M., Gunn, R. N., Rabiner, E. A., Owen, D. R., Zoghbi, S. S., Haskali, M. B., Telu, S., Pike, V. W., & Innis, R. B. (2017). Comparison of four 11C-labeled PET ligands to quantify translocator protein 18 kDa (TSPO) in human brain: (R)-PK11195, PBR28, DPA-713, and ER176—based on recent publications that measured specific-to-non-displaceable ratios. *EJNMMI Res 7*(1):84. https://doi.org/10.1186/s13550-017-0334-8

Gastaldelli, A., Ferrannini, E., Miyazaki, Y., Matsuda, M., & DeFronzo, R. A. (2004). Beta-cell dysfunction and glucose intolerance: Results from the San Antonio metabolism (SAM) study. *Diabetologia*, *47*(1):31–39. https://doi.org/10.1007/s00125-003-1263-9

Gisslén, M., Heslegrave, A., Veleva, E., Yilmaz, A., Andersson, L. M., Hagberg, L., Spudich, S., Fuchs, D., Price, R. W., & Zetterberg, H. (2019). CSF concentrations of soluble TREM2 as a marker of microglial activation in HIV-1 infection. *Neurol: Neuroimmunol NeuroInflamm*, *6*(1):e512. https://doi.org/10.1212/NXI.0000000000000512

Goldstein, B. J. (2002). Insulin resistance as the core defect in type 2 diabetes mellitus. *Am J Cardiol*, *90*(5A):3–10. https://doi.org/10.1016/S0002-9149(02)02553-5

Gottesman, R. F., Schneider, A. L. C., Zhou, Y., Coresh, J., Green, E., Gupta, N., Knopman, D. S., Mintz, A., Rahmim, A., Sharrett, A. R., Wagenknecht, L. E., Wong, D. F., & Mosley, T. H. (2017). Association between midlife vascular risk factors and estimated brain amyloid deposition. *JAMA,317*(14):1443–1450. https://doi.org/10.1001/jama.2017.3090

Guerreiro, R., Wojtas, A., Bras, J., Carrasquillo, M., Rogaeva, E., Majounie, E., Cruchaga, C., Sassi, C., Kauwe, J. S. K., Younkin, S., Hazrati, L., Collinge, J., Pocock, J., Lashley, T., Williams, J., Lambert, J.-C., Amouyel, P., Goate, A., Rademakers, R., … Hardy, J. (2013). TREM2 Variants in Alzheimer's Disease . *N Engl J Med*, *368*(2):117–127. https://doi.org/10.1056/nejmoa1211851

Gui, Y., Marks, J. D., Das, S., Hyman, B. T., & Serrano-Pozo, A. (2020). Characterization of the 18 kDa translocator protein (TSPO) expression in post-mortem normal and Alzheimer's disease brains. *Brain Pathol, 30*(1):151–164. https://doi.org/10.1111/BPA.12763

Gupta, A., & Iadecola, C. (2015). Impaired Aß clearance: A potential link between atherosclerosis and Alzheimer's disease. *Front Aging Neurosci*, *7*:115. https://doi.org/10.3389/fnagi.2015.00115

Hamelin, L., Lagarde, J., Dorothée, G., Leroy, C., Labit, M., Comley, R. A., de Souza, L. C., Corne, H., Dauphinot, L., Bertoux, M., Dubois, B., Gervais, P., Colliot, O., Potier, M. C., Bottlaender, M., Sarazin, M., & Clinical IMABio3 team. (2016). Early and protective microglial activation in Alzheimer's disease: a prospective study using 18F-DPA-714 PET imaging. *Brain, 139*(Pt 4):1252–1264. https://doi.org/10.1093/brain/aww017

Hamelin, L., Lagarde, J., Dorothée, G., Potier, M. C., Corlier, F., Kuhnast, B., Caillé, F., Dubois, B., Fillon, L., Chupin, M., Bottlaender, M., & Sarazin, M. (2018). Distinct dynamic profiles of microglial activation are associated with progression of Alzheimer's disease. *Brain*, *141*(6):1855–1870. https://doi.org/10.1093/brain/awy079

Hane, F. T., Robinson, M., Lee, B. Y., Bai, O., Leonenko, Z., & Albert, M. S. (2017). Recent Progress in Alzheimer's Disease Research, Part 3: Diagnosis and Treatment. *J Alzheimer's Dis,* 57(3):645–665). IOS Press. https://doi.org/10.3233/JAD-160907

Hänninen, T., Pulliainen, V., Salo, J., Hokkanen, L., Erkinjuntti, T., Koivisto, K., Viramo, P., Soininen, H., & Asiantuntijaryhmä, S. M. (1999). Kognitiiviset testit muistihäiriöiden ja alkavan dementian varhaisdiagnostiikassa: CERAD-tehtäväsarja. [Cognitive tests in diagnosing memory disorders and early dementia: CERAD-nb]. *Suom Laakaril*, 54(15):1967–1975.

Harvey, P. D. (2019). Domains of cognition and their assessment. *Dialogues Clin Neurosci,21*(3):227–237. https://doi.org/10.31887/DCNS.2019.21.3/pharvey

Havrankova, J., Schmechel, D., Roth, J., & Brownstein, M. (1978). Identification of insulin in rat brain. *Proc Natl Acad Sci USA*, *75*(11):5737–5741. https://doi.org/10.1073/pnas.75.11.5737

Heistaro, Sami, editor. (2008). *Methodology report: Health 2000 survey*. Publications of the National Public Health Institute, Helsinki. http://www.julkari.fi/handle/10024/78185

Helkala, E.-L., Lakka, T., Vanhanen, M., Tuomainen, T.-P., Ehnholm, C., Kaplan, G. A., & Salonen, J. T. (2001). Associations between apolipoprotein E phenotype, glucose metabolism and cognitive function in men. An explorative study in a population sample. *Diabet Med*, *18*(12):991–997. https://doi.org/10.1046/j.0742-3071.2001.00588.x

Heneka, M. T., Carson, M. J., el Khoury, J., Landreth, G. E., Brosseron, F., Feinstein, D. L., Jacobs, A. H., Wyss-Coray, T., Vitorica, J., Ransohoff, R. M., Herrup, K., Frautschy, S. A., Finsen, B., Brown, G. C., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz, E., Halle, A., … Kummer, M. P. (2015). Neuroinflammation in Alzheimer's disease. *Lancet. Neurol*, *14*(4):388–405. https://doi.org/10.1016/S1474-4422(15)70016-5

Heni, M., Kullmann, S., Preissl, H., Fritsche, A., & Häring, H. U. (2015). Impaired insulin action in the human brain: causes and metabolic consequences. *Nat Rev. Endocrinol*, *11*(12):701–711. https://doi.org/10.1038/NRENDO.2015.173

Heni, M., Schöpfer, P., Peter, A., Sartorius, T., Fritsche, A., Synofzik, M., Häring, H. U., Maetzler, W., & Hennige, A. M. (2014). Evidence for altered transport of insulin across the blood-brain barrier in insulin-resistant humans. *Acta Diabetol*, *51*(4):679–681. https://doi.org/10.1007/s00592-013-0546-y

Hiltunen, L., Keinänen-Kiukaanniemi, S., & Läärä, E. (2001). Glucose tolerance and cognitive impairment in an elderly population. *Public Health*, *115*(3):197-200. https://doi.org/10.1038/SJ/PH/1900758

Hooshmand, B., Rusanen, M., Ngandu, T., Leiviskä, J., Sindi, S., von Arnim, C. A. F., Falkai, P., Soininen, H., Tuomilehto, J., & Kivipelto, M. (2019). Serum Insulin and Cognitive Performance in Older Adults: A Longitudinal Study. *Am J Med*, *132*(3):367–373. https://doi.org/10.1016/J.AMJMED.2018.11.013

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature*, *444*(7121):860–867. https://doi.org/10.1038/nature05485

Hughes, T. M., & Craft, S. (2016). The role of insulin in the vascular contributions to age-related dementia. *Biochim Biophys Acta*, *1862*(5):983–991. https://doi.org/10.1016/J.BBADIS.2015.11.013

Hughes, T. M., Craft, S., Baker, L. D., Espeland, M. A., Rapp, S. R., Sink, K. M., Bertoni, A. G., Burke, G. L., Gottesman, R. F., Michos, E. D., Luchsinger, J. A., Fitzpatrick, A. L., & Hayden, K. M. (2017). Changes in metabolic risk factors over 10 years and their associations with late-life cognitive performance: The Multi-Ethnic Study of Atherosclerosis. *Alzheimers Dement, 8*:18–25. https://doi.org/10.1016/j.dadm.2017.03.003

Hyman, B. T., Phelps, C. H., Beach, T. G., Bigio, E. H., Cairns, N. J., Carrillo, M. C., Dickson, D. W., Duyckaerts, C., Frosch, M. P., Masliah, E., Mirra, S. S., Nelson, P. T., Schneider, J. A., Thal, D. R., Thies, B., Trojanowski, J. Q., Vinters, H. V., & Montine, T. J. (2012). National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimers Dement*, *8*(1):1–13. https://doi.org/10.1016/j.jalz.2011.10.007

Iadecola, C., & Gorelick, P. B. (2003). Converging pathogenic mechanisms in vascular and neurodegenerative dementia. *Stroke*, 34(2):335–337. https://doi.org/10.1161/01.STR.0000054050.51530.76

Jack, C. R., Albert, M. S., Knopman, D. S., McKhann, G. M., Sperling, R. A., Carrillo, M. C., Thies, B., & Phelps, C. H. (2011). Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*, *7*(3):257–262. https://doi.org/10.1016/j.jalz.2011.03.004

Jack, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., Holtzman, D. M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J. L., Montine, T., Phelps, C., Rankin, K. P., Rowe, C. C., Scheltens, P., Siemers, E., Snyder, H. M., … Silverberg, N. (2018). NIA-AA

Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement,* 14(4):535–562. https://doi.org/10.1016/j.jalz.2018.02.018

Jack, C. R., Knopman, D. S., Jagust, W. J., Shaw, L. M., Aisen, P. S., Weiner, M. W., Petersen, R. C., & Trojanowski, J. Q. (2010). Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol*, 9(1):119–128. https://doi.org/10.1016/S1474-4422(09)70299-6

Jack, C. R., Lowe, V. J., Senjem, M. L., Weigand, S. D., Kemp, B. J., Shiung, M. M., Knopman, D. S., Boeve, B. F., Klunk, W. E., Mathis, C. A., & Petersen, R. C. (2008). 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. *Brain*, *131*(3):665–680. https://doi.org/10.1093/brain/awm336

Jackson, K., Barisone, G. A., Diaz, E., Jin, L. W., DeCarli, C., & Despa, F. (2013). Amylin deposition in the brain: A second amyloid in Alzheimer disease? *Ann Neurol*, *74*(4):517–526. https://doi.org/10.1002/ana.23956

James, B. D., Wilson, R. S., Boyle, P. A., Trojanowski, J. Q., Bennett, D. A., & Schneider, J. A. (2016). TDP-43 stage, mixed pathologies, and clinical Alzheimer's-type dementia. *Brain*, *139*(11):2983–2993. https://doi.org/10.1093/brain/aww224

Janatuinen, T., & Kemppainen, J. (2020). PET-kuvantamisen menetelmät yleistajuisesti. *Duodecim*, *136(9)*:1062–1067.

Janelidze, S., Mattsson, N., Stomrud, E., Lindberg, O., Palmqvist, S., Zetterberg, H., Blennow, K., & Hansson, O. (2018). CSF biomarkers of neuroinflammation and cerebrovascular dysfunction in early Alzheimer disease. *Neurology*, *91*(9):e867–e877. https://doi.org/10.1212/WNL.0000000000006082

Jänis, M. T., Siggins, S., Tahvanainen, E., Vikstedt, R., Silander, K., Metso, J., Aromaa, A., Taskinen, M. R., Olkkonen, V. M., Jauhiainen, M., & Ehnholm, C. (2004). Active and low-active forms of serum phospholipid transfer protein in a normal Finnish population sample. *J Lipid Res*, *45*(12):2303–2309. https://doi.org/10.1194/jlr.M400250-JLR200

Jansen, W. J., Ossenkoppele, R., Knol, D. L., Tijms, B. M., Scheltens, P., Verhey, F. R. J., Visser, P. J., Aalten, P., Aarsland, D., Alcolea, D., Alexander, M., Almdahl, I. S., Arnold, S. E., Baldeiras, I., Barthel, H., van Berckel, B. N. M., Bibeau, K., Blennow, K., Brooks, D. J., … Zetterberg, H. (2015). Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. *JAMA*, *313*(19):1924–1938. https://doi.org/10.1001/JAMA.2015.4668

Jaturapatporn, D., Isaac, M. G. E. K. N., McCleery, J., & Tabet, N. (2012). Aspirin, steroidal and non-steroidal anti-inflammatory drugs for the treatment of Alzheimer's disease. *Cochrane Database Syst Rev, 15*(2), CD006378. https://doi.org/10.1002/14651858.CD006378.pub2

Jeong, S. H., Kim, H. R., Kim, J., Kim, H., Hong, N., Jung, J. H., Baik, K., Cho, H., Lyoo, C. H., Ye, B. S., Sohn, Y. H., Seong, J.-K., & Lee, P. H. (2021). Association of Dipeptidyl Peptidase-4 Inhibitor Use and Amyloid Burden in Diabetic Patients With AD-Related Cognitive Impairment. *Neurology*, *97*(11):e1110–e1122. https://doi.org/10.1212/wnl.0000000000012534

Jiang, T., Yu, J. T., Zhu, X. C., & Tan, L. (2013). TREM2 in Alzheimer's disease. *Mol Neurobiol,* 48(1):180–185. https://doi.org/10.1007/s12035-013-8424-8

Jonsson, T., Stefansson, H., Steinberg, S., Jonsdottir, I., Jonsson, P. V., Snaedal, J., Bjornsson, S., Huttenlocher, J., Levey, A. I., Lah, J. J., Rujescu, D., Hampel, H., Giegling, I., Andreassen, O. A., Engedal, K., Ulstein, I., Djurovic, S., Ibrahim-Verbaas, C., Hofman, A., … Stefansson, K. (2013). Variant of TREM2 Associated with the Risk of Alzheimer's Disease . *N Engl J Med*, *368*(2):107–116. https://doi.org/10.1056/nejmoa1211103

Jula, A., Marniemi, J., Huupponen, R., Virtanen, A., Rastas, M., & Rönnemaa, T. (2002). Effects of diet and simvastatin on serum lipids, insulin, and antioxidants in hypercholesterolemic men: a randomized controlled trial. JAMA, 287(5):598–605. https://doi.org/10.1001/JAMA.287.5.598

Kanat, M., Mari, A., Norton, L., Winnier, D., DeFronzo, R. A., Jenkinson, C., & Abdul-Ghani, M. A. (2012). Distinct β-cell defects in impaired fasting glucose and impaired glucose tolerance. *Diabetes*, *61*(2):447–453. https://doi.org/10.2337/db11-0995

Kanaya, A. M., Barrett-Connor, E., Gildengorin, G., & Yaffe, K. (2004). Change in cognitive function by glucose tolerance status in older adults: A 4-year prospective study of the Rancho Bernardo Study cohort. *Arch Intern Med*, *164*(12):1327–1333. https://doi.org/10.1001/archinte.164.12.1327

Katz, A., Nambi, S. S., Mather, K., Baron, A. D., Follmann, D. A., Sullivan, G., & Quon, M. J. (2000). Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab*, *85*(7):2402–2410. https://doi.org/10.1210/jcem.85.7.6661

Kellar, D., & Craft, S. (2020). Brain insulin resistance in Alzheimer's disease and related disorders: mechanisms and therapeutic approaches. *Lancet Neurol,* 19(9):758–766. https://doi.org/10.1016/S1474-4422(20)30231-3

Kivipelto, M., Ngandu, T., Fratiglioni, L., Viitanen, M., Kåreholt, I., Winblad, B., Helkala, E. L., Tuomilehto, J., Soininen, H., & Nissinen, A. (2005). Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Arch Neurol*, *62*(10):1556–1560. https://doi.org/10.1001/archneur.62.10.1556

Kivipelto, M., Ngandu, T., Laatikainen, T., Winblad, B., Soininen, H., & Tuomilehto, J. (2006). Risk score for the prediction of dementia risk in 20 years among middle aged people: a longitudinal, population-based study. *Lancet Neurol*, *5*(9):735–741. https://doi.org/10.1016/S1474-4422(06)70537-3

Knezevic, D., Verhoeff, N. P. L., Hafizi, S., Strafella, A. P., Graff-Guerrero, A., Rajji, T., Pollock, B. G., Houle, S., Rusjan, P. M., & Mizrahi, R. (2018). Imaging microglial activation and amyloid burden in amnestic mild cognitive impairment. *J Cereb Blood Flow Metab, 38*(11):1885–1895. https://doi.org/10.1177/0271678X17741395

Koikkalainen, J. R., Rhodius-Meester, H. F. M., Frederiksen, K. S., Bruun, M., Hasselbalch, S. G., Baroni, M., Mecocci, P., Vanninen, R., Remes, A., Soininen, H., van Gils, M., van der Flier, W. M., Scheltens, P., Barkhof, F., Erkinjuntti, T., & Lötjönen, J. M. P. (2019). Automatically computed rating scales from MRI for patients with cognitive disorders. *Eur Radiology*, *29*(9):4937–4947. https://doi.org/10.1007/S00330-019-06067-1

Koikkalainen, J., Rhodius-Meester, H., Tolonen, A., Barkhof, F., Tijms, B., Lemstra, A. W., Tong, T., Guerrero, R., Schuh, A., Ledig, C., Rueckert, D., Soininen, H., Remes, A. M., Waldemar, G., Hasselbalch, S., Mecocci, P., van der Flier, W., & Lötjönen, J. (2016). Differential diagnosis of neurodegenerative diseases using structural MRI data. *NeuroImage: Clin*, *11*:435–449. https://doi.org/10.1016/j.nicl.2016.02.019

Kokkonen, A., Kaasinen, V., Jutila, L., Tuominen, L., Rinne, J. O., & Joutsa, J. (2020). PET aivosairauksissa. *Duodecim*, *136*(9):1110–1117.

Kolb, H., Kempf, K., Röhling, M., & Martin, S. (2020). Insulin: Too much of a good thing is bad. *BMC Med,* 18(1):224. https://doi.org/10.1186/s12916-020-01688-6

Kothari, V., Luo, Y., Tornabene, T., O'Neill, A. M., Greene, M. W., Geetha, T., & Babu, J. R. (2017). High fat diet induces brain insulin resistance and cognitive impairment in mice. *Biochim Biophys Acta Mol Basis Dis*, *1863*(2):499–508. https://doi.org/10.1016/j.bbadis.2016.10.006

Kreisl, W. C., Fujita, M., Fujimura, Y., Kimura, N., Jenko, K. J., Kannan, P., Hong, J., Morse, C. L., Zoghbi, S. S., Gladding, R. L., Jacobson, S., Oh, U., Pike, V. W., & Innis, R. B. (2010). Comparison of [(11)C]-(R)-PK 11195 and [(11)C]PBR28, two radioligands for translocator protein (18 kDa) in human and monkey: Implications for positron emission tomographic imaging of this inflammation biomarker. *NeuroImage*, *49*(4), 2924–2932. https://doi.org/10.1016/J.NEUROIMAGE.2009.11.056

Kreisl, W. C., Jenko, K. J., Hines, C. S., Hyoung Lyoo, C., Corona, W., Morse, C. L., Zoghbi, S. S., Hyde, T., Kleinman, J. E., Pike, V. W., McMahon, F. J., & Innis, R. B. (2013). A genetic polymorphism for translocator protein 18 kDa affects both in vitro and in vivo radioligand binding in human brain to this putative biomarker of neuroinflammation. *Journal of Cerebral Blood Flow and Metabolism*. https://doi.org/10.1038/jcbfm.2012.131

Kreisl, W. C., Lyoo, C. H., Liow, J. S., Wei, M., Snow, J., Page, E., Jenko, K. J., Morse, C. L., Zoghbi, S. S., Pike, V. W., Turner, R. S., & Innis, R. B. (2016). 11C-PBR28 binding to translocator protein increases with progression of Alzheimer's disease. *Neurobiol Aging*, *44*:53–61. https://doi.org/10.1016/j.neurobiolaging.2016.04.011

Kreisl, W. C., Lyoo, C. H., McGwier, M., Snow, J., Jenko, K. J., Kimura, N., Corona, W., Morse, C. L., Zoghbi, S. S., Pike, V. W., McMahon, F. J., Turner, R. S., & Innis, R. B. (2013). In vivo radioligand binding to translocator protein correlates with severity of Alzheimer's disease. *Brain*, *136*:2228–2238. https://doi.org/10.1093/brain/awt145

Kullmann, S., Heni, M., Hallschmid, M., Fritsche, A., Preissl, H., & Häring, H. U. (2016). Brain insulin resistance at the crossroads of metabolic and cognitive disorders in humans. *Physiol Rev*, *96*(4):1169–1209. https://doi.org/10.1152/physrev.00032.2015

Kullmann, S., Kleinridders, A., Small, D. M., Fritsche, A., Häring, H. U., Preissl, H., & Heni, M. (2020). Central nervous pathways of insulin action in the control of metabolism and food intake. *Lancet Diabetes Endocrinol,* 8(6):524–534. https://doi.org/10.1016/S2213-8587(20)30113-3

Kumari, M., & Marmot, M. (2005). Diabetes and cognitive function in a middle-aged cohort: Findings from the Whitehall II study. *Neurology*, *65*(10):1597–1603. https://doi.org/10.1212/01.wnl.0000184521.80820.e4

Kuusisto, J., Koivisto, K., Mykkänen, L., Helkala, E. L., Vanhanen, M., Hänninen, T., Kervinen, K., Kesäniemi, Y. A., Riekkinen, P. J., & Laakso, M. (1997). Association between features of the insulin resistance syndrome and Alzheimer's disease independently of apolipoprotein E4 phenotype: Cross sectional population based study. *BMJ*, *315*(7115):1045–1049. https://doi.org/10.1136/bmj.315.7115.1045

Kuusisto, J., Koivisto, K., Mykkänen, L., Helkala, E. L., Vanhanen, M., Hänninen, T., Pyörälä, K., Riekkinen, P., & Laakso, M. (1993). Essential hypertension and cognitive function: The role of hyperinsulinemia. *Hypertension*, *22*(5):771–779. https://doi.org/10.1161/01.HYP.22.5.771

Kwon, H. M., Beom, J. K., Lee, S. H., Seung, H. C., Oh, B. H., & Yoon, B. W. (2006). Metabolic syndrome as an independent risk factor of silent brain infarction in healthy people. *Stroke*, *37*(2):466–470. https://doi.org/10.1161/01.STR.0000199081.17935.81

Kwon, H. S., & Koh, S. H. (2020). Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Transl Neurodegener*, *9*(1):42. https://doi.org/10.1186/S40035-020-00221-2

Lagarde, J., Sarazin, M., & Bottlaender, M. (2018). In vivo PET imaging of neuroinflammation in Alzheimer's disease. *J Neural Transm*, *125*(5):847–867. https://doi.org/10.1007/s00702-017-1731-x

Lameka, K., Farwell, M. D., & Ichise, M. (2016). Positron Emission Tomography. *Handb Clin Neurol*, *135*:209–227. https://doi.org/10.1016/B978-0-444-53485-9.00011-8

Lamport, D. J., Lawton, C. L., Mansfield, M. W., & Dye, L. (2009). Impairments in glucose tolerance can have a negative impact on cognitive function: A systematic research review. *Neurosci Biobehav Rev,* 33(3):394–413. https://doi.org/10.1016/j.neubiorev.2008.10.008

Laws, S. M., Gaskin, S., Woodfield, A., Srikanth, V., Bruce, D., Fraser, P. E., Porter, T., Newsholme, P., Wijesekara, N., Burnham, S., Doré, V., Li, Q. X., Maruff, P., Masters, C. L., Rainey-Smith, S., Rowe, C. C., Salvado, O., Villemagne, V. L., Martins, R. N., & Verdile, G. (2017). Insulin resistance is associated with reductions in specific cognitive domains and increases in CSF tau in cognitively normal adults. *Sci Rep*, *7*(1):9766. https://doi.org/10.1038/s41598-017-09577-4

Leandrou, S., Petroudi, S., Kyriacou, P. A., Reyes-Aldasoro, C. C., & Pattichis, C. S. (2018). Quantitative MRI Brain Studies in Mild Cognitive Impairment and Alzheimer's Disease: A Methodological Review. *IEEE Rev in Biomed Eng*, *11*:97–111. https://doi.org/10.1109/RBME.2018.2796598

Lee, J. E., Shin, D. W., Yun, J. M., Kim, S. H., Nam, Y. S., Cho, B., Lim, J. S., Jeong, H. Y., Kwon, H. M., & Park, J. H. (2016). Insulin Resistance Is a Risk Factor for Silent Lacunar Infarction. *Stroke*, *47*(12):2938–2944. https://doi.org/10.1161/STROKEAHA.116.014097

Leng, F., & Edison, P. (2021). Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? *Nat Rev. Neurol*, 17(3):157–172. https://doi.org/10.1038/S41582-020-00435-Y

Lillioja, S., Mott, D. M., Spraul, M., Ferraro, R., Foley, J. E., Ravussin, E., Knowler, W. C., Bennett, P. H., & Bogardus, C. (1993). Insulin Resistance and Insulin Secretory Dysfunction as Precursors of Non-Insulin-Dependent Diabetes Mellitus: Prospective Studies of Pima Indians. *N Engl J Med*, *329*(27):1988–1992. https://doi.org/10.1056/nejm199312303292703

Lingvay, I., Sumithran, P., Cohen, R. v., & le Roux, C. W. (2022). Obesity management as a primary treatment goal for type 2 diabetes: time to reframe the conversation. Lancet, 399(10322):394–405. https://doi.org/10.1016/S0140-6736(21)01919-X

Liu, Z., Patil, I. Y., Jiang, T., Sancheti, H., Walsh, J. P., Stiles, B. L., Yin, F., & Cadenas, E. (2015). High-fat diet induces hepatic insulin resistance and impairment of synaptic plasticity. *PLoS ONE*, *10*(5). https://doi.org/10.1371/journal.pone.0128274

Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., Brayne, C., Burns, A., Cohen-Mansfield, J., Cooper, C., Costafreda, S. G., Dias, A., Fox, N., Gitlin, L. N., Howard, R., Kales, H. C., Kivimäki, M., Larson, E. B., Ogunniyi, A., … Mukadam, N. (2020). Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet,* 396(10248):413–446. https://doi.org/10.1016/S0140-6736(20)30367-6

Luchsinger, J. A., Tang, M. X., Shea, S., & Mayeux, R. (2004). Hyperinsulinemia and risk of Alzheimer disease. *Neurology*, *63*(7):1187–1192. https://doi.org/10.1212/01.WNL.0000140292.04932.87

Lundqvist, A., & Mäki-Opas, T. (2016). *Health 2011 Survey - Methods. Publications of the Finnish National Institute for Health and Welfare. Helsinki.* THL. http://urn.fi/URN:ISBN:978-952-302-669-8

Lutski, M., Weinstein, G., Goldbourt, U., & Tanne, D. (2017). Insulin Resistance and Future Cognitive Performance and Cognitive Decline in Elderly Patients with Cardiovascular Disease. *J Alzheimer's Dis, 57*(2):633–643. https://doi.org/10.3233/JAD-161016

Lyoo, C. H., Ikawa, M., Liow, J. S., Zoghbi, S. S., Morse, C. L., Pike, V. W., Fujita, M., Innis, R. B., & Kreisl, W. C. (2015). Cerebellum can serve as a pseudo-reference region in Alzheimer disease to detect neuroinflammation measured with PET radioligand binding to translocator protein. *J Nucl Med*, *56*(5):701–706. https://doi.org/10.2967/jnumed.114.146027

Maria Teixeira, M., Passos, V., Barreto, S., Inês Schmidt, M., Duncan, B., Beleigoli, A., de Jesus Fonseca, M., Vidigal, P., Figueiredo, R., Colosimo, E., Araújo, L., & de Fátima Haueisen S. Diniz, M. (2020). Markers of adiposity, insulin resistance, prediabetes and cognitive function at baseline of the Brazilian Longitudinal Study of Adult Health (ELSA - Brasil). *Diabetes Res Clin Pract*, 170:108499. https://doi.org/10.1016/J.DIABRES.2020.108499

Marseglia, A., Fratiglioni, L., Kalpouzos, G., Wang, R., Bäckman, L., & Xu, W. (2019). Prediabetes and diabetes accelerate cognitive decline and predict microvascular lesions: A population-based cohort study. *Alzheimers Dement*, *15*(1):25–33. https://doi.org/10.1016/j.jalz.2018.06.3060

Masdeu, J. C. (2020). Neuroimaging of Diseases Causing Dementia. *Neurol Clin,* 38(1): 65–94. https://doi.org/10.1016/j.ncl.2019.08.003

Masur, D., Sliwinski, M., Lipton, R. B., Blau, A. D., & Crystal, H. A. (1994). Neuropsychological prediction of dementia and the absence of dementia in healthy elderly persons. *Neurology*, *44*(8):1427–1432. https://doi.org/10.1212/wnl.44.8.1427

Mather, K. J., Hunt, A. E., Steinberg, H. O., Paradisi, G., Hook, G., Katz, A., Quon, M. J., & Baron, A. D. (2001). Repeatability characteristics of simple indices of insulin resistance: Implications for research applications. *J Clin Endocrinol Metab*, *86*(11):5457–5464. https://doi.org/10.1210/jcem.86.11.7880

Matsuda, M., & DeFronzo, R. A. (1999). Insulin sensitivity indices obtained from oral glucose tolerance testing: Comparison with the euglycemic insulin clamp. *Diabetes Care*, *22*(9):1462–1470. https://doi.org/10.2337/diacare.22.9.1462

Matsuzaki, T., Sasaki, K., Tanizaki, Y., Hata, J., Fujimi, K., Matsui, Y., Sekita, A., Suzuki, S. O., Kanba, S., Kiyohara, Y., & Iwaki, T. (2010). Insulin resistance is associated with the pathology of Alzheimer disease: The Hisayama study. *Neurology*, *75*(9):764–770. https://doi.org/10.1212/WNL.0b013e3181eee25f

Mattay, V. S., Fotenos, A. F., Ganley, C. J., & Marzella, L. (2020). Brain Tau Imaging: Food and Drug Administration Approval of 18 F-Flortaucipir Injection. *J Nucl Med*, 61(10):1411–1412. https://doi.org/10.2967/JNUMED.120.252254

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, *28*(7):412–419. https://doi.org/10.1007/bf00280883

Mattiace, L. A., Davies, P., Yen, S. H., & Dickson, D. W. (1990). Microglia in cerebellar plaques in Alzheimer's disease. *Acta Neuropathol*, *80*(5):493–498. https://doi.org/10.1007/bf00294609

McGeer, P. L., & McGeer, E. G. (2013). The amyloid cascade-inflammatory hypothesis of Alzheimer disease: Implications for therapy. *Acta Neuropathol*, *126*(4):479–497. https://doi.org/10.1007/s00401-013-1177-7

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack, C. R., Kawas, C. H., Klunk, W. E., Koroshetz, W. J., Manly, J. J., Mayeux, R., Mohs, R. C., Morris, J. C., Rossor, M. N., Scheltens, P., Carrillo, M. C., Thies, B., Weintraub, S., & Phelps, C. H. (2011). The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*, *7*(3):263–269. https://doi.org/10.1016/j.jalz.2011.03.005

Mehlig, K., Lapidus, L., Thelle, D. S., Waern, M., Zetterberg, H., Björkelund, C., Skoog, I., & Lissner, L. (2018). Low fasting serum insulin and dementia in nondiabetic women followed for 34 years. *Neurology*, *91*(5):e427–e435. https://doi.org/10.1212/WNL.0000000000005911

Meng, X., & D'Arcy, C. (2012). Education and dementia in the context of the cognitive reserve hypothesis: A systematic review with meta-analyses and qualitative analyses. *PLoS ONE*, *7*(6):e38268. https://doi.org/10.1371/journal.pone.0038268

Menke, A., Casagrande, S., Geiss, L., & Cowie, C. C. (2015). Prevalence of and Trends in Diabetes Among Adults in the United States, 1988-2012. JAMA, 314(10):1021–1029. https://doi.org/10.1001/JAMA.2015.10029

Messier, C., Tsiakas, M., Gagnon, M., Desrochers, A., & Awad, N. (2003). Effect of age and glucoregulation on cognitive performance. *Neurobiol Aging*, *24*(7):985–1003. https://doi.org/10.1016/S0197-4580(03)00004-6

Minoshima, S., Drzezga, A. E., Barthel, H., Bohnen, N., Djekidel, M., Lewis, D. H., Mathis, C. A., McConathy, J., Nordberg, A., Sabri, O., Seibyl, J. P., Stokes, M. K., & van Laere, K. (2016). SNMMI Procedure Standard/EANM Practice Guideline for Amyloid PET Imaging of the Brain 1.0. *J Nucl Med, 57*(8):1316–1322. https://doi.org/10.2967/JNUMED.116.174615

Minoshima, S., Giordani, B., Berent, S., Frey, K. A., Foster, N. L., & Kuhl, D. E. (1997). Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. *Annals of Neurology*, *42*(1), 85–94. https://doi.org/10.1002/ana.410420114

Mishra, S., Blazey, T. M., Holtzman, D. M., Cruchaga, C., Su, Y., Morris, J. C., Benzinger, T. L. S., & Gordon, B. A. (2018). Longitudinal brain imaging in preclinical Alzheimer disease: Impact of APOE ϵ4 genotype. *Brain*, *141*(6):1828–1839. https://doi.org/10.1093/brain/awy103

Mistur, R., Mosconi, L., de Santi, S., Guzman, M., Li, Y., Tsui, W., & de Leon, M. J. (2009). Current challenges for the early detection of alzheimer's disease: Brain imaging and CSF studies. *J Clin Neurol,* 5(4):153–166). https://doi.org/10.3988/jcn.2009.5.4.153

Morris, D. H., Khunti, K., Achana, F., Srinivasan, B., Gray, L. J., Davies, M. J., & Webb, D. (2013). Progression rates from HbA1c 6.0-6.4% and other prediabetes definitions to type 2 diabetes: a meta-analysis. *Diabetologia*, *56*(7):1489–1493. https://doi.org/10.1007/S00125-013-2902-4

Morris, J. C. (2005). Early-stage and preclinical Alzheimer disease. *Alzheimer Dis Assoc Disord*, *19*(3):163–165. https://doi.org/10.1097/01.wad.0000184005.22611.cc

Morris, J. C., Heyman, A., Mohs, R. C., Hughes, J. P., van Belle, G., Fillenbaum, G., Mellits, E. D., & Clark, C. (1989). The consortium to establish a registry for alzheimer's disease (CERAD). Part I. Clinical and neuropsychological assessment of alzheimer's disease. *Neurology*, *39*(9):1159–1165. https://doi.org/10.1212/wnl.39.9.1159

Motes, A. A., Biswal, B. B., & Rypma, B. (2011). Age-Dependent Relationships between Prefrontal Cortex Activation and Processing Efficiency. *Cogn Neurosci*, *2*(1):1–10. https://doi.org/10.1080/17588928.2010.512974

Mullins, R. J., Diehl, T. C., Chia, C. W., & Kapogiannis, D. (2017). Insulin Resistance as a Link between Amyloid-Beta and Tau Pathologies in Alzheimer's Disease. *Front Aging Neurosci*, 9:118. https://doi.org/10.3389/FNAGI.2017.00118

Muniyappa, R., Madan, R., & Varghese, R. T. (2021). Endotext: Assessing Insulin Sensitivity and Resistance in Humans. *Endotext*. MDText.com, Inc. https://pubmed-ncbi-nlm-nih-gov.ezproxy.utu.fi/25905189/

Muszyński, P., Groblewska, M., Kulczyńska-Przybik, A., Kułakowska, A., & Mroczko, B. (2017). YKL-40 as a Potential Biomarker and a Possible Target in Therapeutic Strategies of Alzheimer's Disease. *Curr Neuropharmacol*, *15*(6):906–917. https://doi.org/10.2174/1570159X15666170208124324

Nasreddine, Z. S., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., Cummings, J. L., & Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: A brief screening tool for mild cognitive impairment. *J Am Geriatr Soc*, *53*(4):695–699. https://doi.org/10.1111/j.1532-5415.2005.53221.x

Neergaard, J. S., Dragsbæk, K., Christiansen, C., Nielsen, H. B., Brix, S., Karsdal, M. A., & Henriksen, K. (2017). Metabolic Syndrome, Insulin Resistance, and Cognitive Dysfunction: Does Your Metabolic Profile Affect Your Brain? *Diabetes*, *66*(7):1957–1963. https://doi.org/10.2337/DB16-1444

Nelson, P. T., Alafuzoff, I., Bigio, E. H., Bouras, C., Braak, H., Cairns, N. J., Castellani, R. J., Crain, B. J., Davies, P., Tredici, K. del, Duyckaerts, C., Frosch, M. P., Haroutunian, V., Hof, P. R., Hulette, C. M., Hyman, B. T., Iwatsubo, T., Jellinger, K. A., Jicha, G. A., … Beach, T. G. (2012). Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. J Neuropathol Exp Neurol, 71(5), 362–381. https://doi.org/10.1097/NEN.0B013E31825018F7

Neumann, H., & Daly, M. J. (2013). Variant TREM2 as Risk Factor for Alzheimer's Disease . *N Engl J Med*, *368*(2):182–184. https://doi.org/10.1056/nejme1213157

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E. C., Biryukov, S., Abbafati, C., Abera, S. F., Abraham, J. P., Abu-Rmeileh, N. M. E., Achoki, T., Albuhairan, F. S., Alemu, Z. A., Alfonso, R., Ali, M. K., Ali, R., Guzman, N. A., … Gakidou, E. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 384(9945):766–781. https://doi.org/10.1016/S0140-6736(14)60460-8

Ngandu, T., Lehtisalo, J., Solomon, A., Levälahti, E., Ahtiluoto, S., Antikainen, R., Bäckman, L., Hänninen, T., Jula, A., Laatikainen, T., Lindström, J., Mangialasche, F., Paajanen, T., Pajala, S., Peltonen, M., Rauramaa, R., Stigsdotter-Neely, A., Strandberg, T., Tuomilehto, J., … Kivipelto, M. (2015). A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): A randomised controlled trial. *Lancet*, *385*(9984):2255–2263. https://doi.org/10.1016/S0140-6736(15)60461-5

Nordengen, K., Kirsebom, B. E., Henjum, K., Selnes, P., Gísladóttir, B., Wettergreen, M., Torsetnes, S. B., Grøntvedt, G. R., Waterloo, K. K., Aarsland, D., Nilsson, L. N. G., & Fladby, T. (2019).

Glial activation and inflammation along the Alzheimer's disease continuum. *J Neuroinflammation*, *16*(1):46. https://doi.org/10.1186/s12974-019-1399-2

Norton, S., Matthews, F. E., Barnes, D. E., Yaffe, K., & Brayne, C. (2014). Potential for primary prevention of Alzheimer's disease: An analysis of population-based data. *Lancet Neurol*, *13*(8):788–794. https://doi.org/10.1016/S1474-4422(14)70136-X

Nutma, E., Ceyzériat, K., Amor, S., Tsartsalis, S., Millet, P., Owen, D. R., Papadopoulos, V., & Tournier, B. B. (2021). Cellular sources of TSPO expression in healthy and diseased brain. *Eur J Nucl Med Mol Imaging*, *49*(1):146–163. https://doi.org/10.1007/S00259-020-05166-2

O'Brien, J. T., & Thomas, A. (2015). Vascular dementia. *Lancet*, 386(10004):1698–1706. https://doi.org/10.1016/S0140-6736(15)00463-8

Oikonen, V. (n.d.). *PET data analysis and modelling*. Retrieved January 6, 2022, from http://www.turkupetcentre.net

Okello, A., Edison, P., Archer, H. A., Turkheimer, F. E., Kennedy, J., Bullock, R., Walker, Z., Kennedy, A., Fox, N., Rossor, M., & Brooks, D. J. (2009). Microglial activation and amyloid deposition in mild cognitive impairment: a PET study. *Neurology*, *72*(1):56–62. https://doi.org/10.1212/01.WNL.0000338622.27876.0D

Oksanen, M., Lehtonen, S., Jaronen, M., Goldsteins, G., Hämäläinen, R. H., & Koistinaho, J. (2019). Astrocyte alterations in neurodegenerative pathologies and their modeling in human induced pluripotent stem cell platforms. *Cell Mol Life Sci,* *76*(14):2739–2760. https://doi.org/10.1007/S00018-019-03111-7

Olsson, B., Lautner, R., Andreasson, U., Öhrfelt, A., Portelius, E., Bjerke, M., Hölttä, M., Rosén, C., Olsson, C., Strobel, G., Wu, E., Dakin, K., Petzold, M., Blennow, K., & Zetterberg, H. (2016). CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet. Neurol*, *15*(7):673–684. https://doi.org/10.1016/S1474-4422(16)00070-3

Ossenkoppele, R., Rabinovici, G. D., Smith, R., Cho, H., Scholl, M., Strandberg, O., Palmqvist, S., Mattsson, N., Janelidze, S., Santillo, A., Ohlsson, T., Jogi, J., Tsai, R., la Joie, R., Kramer, J., Boxer, A. L., Gorno-Tempini, M. L., Miller, B. L., Choi, J. Y., … Hansson, O. (2018). Discriminative Accuracy of [18F]flortaucipir Positron Emission Tomography for Alzheimer Disease vs Other Neurodegenerative Disorders. *JAMA*, 320(11):1151–1162. https://doi.org/10.1001/JAMA.2018.12917

Owen, D. R., Yeo, A. J., Gunn, R. N., Song, K., Wadsworth, G., Lewis, A., Rhodes, C., Pulford, D. J., Bennacef, I., Parker, C. A., Stjean, P. L., Cardon, L. R., Mooser, V. E., Matthews, P. M., Rabiner, E. A., & Rubio, J. P. (2012). An 18-kDa Translocator Protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. *J Cereb Blood Flow Metab*, *32*(1):1–5. https://doi.org/10.1038/jcbfm.2011.147

Pahkala, K., Laitinen, T. T., Niinikoski, H., Kartiosuo, N., Rovio, S. P., Lagström, H., Loo, B. M., Salo, P., Jokinen, E., Magnussen, C. G., Juonala, M., Simell, O., Jula, A., Rönnemaa, T., Viikari, J., & Raitakari, O. T. (2020). Effects of 20-year infancy-onset dietary counselling on cardiometabolic risk factors in the Special Turku Coronary Risk Factor Intervention Project (STRIP): 6-year post-intervention follow-up. *Lancet. Child Adolesc Health*, *4*(5):359–369. https://doi.org/10.1016/S2352-4642(20)30059-6

Paloneva, J., Manninen, T., Christman, G., Hovanes, K., Mandelin, J., Adolfsson, R., Bianchin, M., Bird, T., Miranda, R., Salmaggi, A., Tranebjærg, L., Konttinen, Y., & Peltonen, L. (2002). Mutations in two genes encoding different subunits of a receptor signaling complex result in an identical disease phenotype. *Am J Hum Genet*, *71*(3):656–662. https://doi.org/10.1086/342259

Parbo, P., Ismail, R., Hansen, K. v., Amidi, A., Mårup, F. H., Gottrup, H., Brændgaard, H., Eriksson, B. O., Eskildsen, S. F., Lund, T. E., Tietze, A., Edison, P., Pavese, N., Stokholm, M. G., Borghammer, P., Hinz, R., Aanerud, J., & Brooks, D. J. (2017). Brain inflammation accompanies amyloid in the majority of mild cognitive impairment cases due to Alzheimer's disease. *Brain*, *140*(7):2002–2011. https://doi.org/10.1093/BRAIN/AWX120

Pascoal, T. A., Benedet, A. L., Ashton, N. J., Kang, M. S., Therriault, J., Chamoun, M., Savard, M., Lussier, F. Z., Tissot, C., Karikari, T. K., Ottoy, J., Mathotaarachchi, S., Stevenson, J., Massarweh, G., Schöll, M., de Leon, M. J., Soucy, J. P., Edison, P., Blennow, K., … Rosa-Neto, P. (2021). Microglial activation and tau propagate jointly across Braak stages. *Nat Med*, *27*(9):1592–1599. https://doi.org/10.1038/S41591-021-01456-W

Peila, R., Rodriguez, B. L., White, L. R., & Launer, L. J. (2004). Fasting insulin and incident dementia in an elderly population of Japanese-American men. *Neurology*, *63*(2):228–233. https://doi.org/10.1212/01.WNL.0000129989.28404.9B

Pekkala, T., Hall, A., Mangialasche, F., Kemppainen, N., Mecocci, P., Ngandu, T., Rinne, J. O., Soininen, H., Tuomilehto, J., Kivipelto, M., Solomon, A., & Ashford, J. W. (2020). Association of Peripheral Insulin Resistance and Other Markers of Type 2 Diabetes Mellitus with Brain Amyloid Deposition in Healthy Individuals at Risk of Dementia. *J Alzheimer's Dis, 76*(4):1243–1248. https://doi.org/10.3233/JAD-200145

Perreault, L. (2019). *Endotext: Prediabetes*. MDText.com, Inc. https://pubmed-ncbi-nlm-nih-gov.ezproxy.utu.fi/30860692/

Petersen, R. C., Lopez, O., Armstrong, M. J., Getchius, T. S. D., Ganguli, M., Gloss, D., Gronseth, G. S., Marson, D., Pringsheim, T., Day, G. S., Sager, M., Stevens, J., & Rae-Grant, A. (2018). Practice guideline update summary: Mild cognitive impairment report of theguideline development, dissemination, and implementation. *Neurology*, *90*(3):126–135. https://doi.org/10.1212/WNL.0000000000004826

Petersen, R. C., Smith, G. E., Waring, S. C., Ivnik, R. J., Kokmen, E., & Tangelos, E. G. (1997). Aging, memory, and mild cognitive impairment. *Int Psychogeriatr*, *9*(SUPPL.1):65–69. https://doi.org/10.1017/s1041610297004717

Petersen, R. C., Smith, G. E., Waring, S. C., Ivnik, R. J., Tangalos, E. G., & Kokmen, E. (1999). Mild cognitive impairment: Clinical characterization and outcome. *Arch Neurol*, *56*(3):303–308. https://doi.org/10.1001/archneur.56.3.303

Phillips, D. I. W., Clark, P. M., Hales, C. N., & Osmond, C. (1994). Understanding Oral Glucose Tolerance: Comparison of Glucose or Insulin Measurements During the Oral Glucose Tolerance Test with Specific Measurements of Insulin Resistance and Insulin Secretion. *Diabet Med*, *11*(3):286–292. https://doi.org/10.1111/j.1464-5491.1994.tb00273.x

Pontecorvo, M. J., Devous, M. D., Navitsky, M., Lu, M., Salloway, S., Schaerf, F. W., Jennings, D., Arora, A. K., McGeehan, A., Lim, N. C., Xiong, H., Joshi, A. D., Siderowf, A., & Mintun, M. A. (2017). Relationships between flortaucipir PET tau binding and amyloid burden, clinical diagnosis, age and cognition. *Brain*, 140(3):748–763. https://doi.org/10.1093/BRAIN/AWW334

Portet, F., Brickman, A. M., Stern, Y., Scarmeas, N., Muraskin, J., Provenzano, F. A., Berr, C., Bonafé, A., Artero, S., Ritchie, K., & Akbaraly, T. N. (2012). Metabolic syndrome and localization of white matter hyperintensities in the elderly population. *Alzheimers Dement*, *8*(SUPPL.5):S88-95.e1. https://doi.org/10.1016/j.jalz.2011.11.007

Price, J. L., McKeel, D. W., Buckles, V. D., Roe, C. M., Xiong, C., Grundman, M., Hansen, L. A., Petersen, R. C., Parisi, J. E., Dickson, D. W., Smith, C. D., Davis, D. G., Schmitt, F. A., Markesbery, W. R., Kaye, J., Kurlan, R., Hulette, C., Kurland, B. F., Higdon, R., … Morris, J. C. (2009). Neuropathology of nondemented aging: Presumptive evidence for preclinical Alzheimer disease. *Neurobiolol Aging*, *30*(7):1026–1036. https://doi.org/10.1016/j.neurobiolaging.2009.04.002

Provenzano, F. A., Muraskin, J., Tosto, G., Narkhede, A., Wasserman, B. T., Griffith, E. Y., Guzman, V. A., Meier, I. B., Zimmerman, M. E., & Brickman, A. M. (2013). White matter hyperintensities and cerebral amyloidosis: necessary and sufficient for clinical expression of Alzheimer disease? *JAMA Neurol*, *70*(4):455–461. https://doi.org/10.1001/JAMANEUROL.2013.1321

Pugazhenthi, S., Qin, L., & Reddy, P. H. (2017). Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. *Biochim Biophys Acta Mol Basis Dis*, *1863*(5):1037–1045. https://doi.org/10.1016/j.bbadis.2016.04.017

Qiu, W. Q., & Folstein, M. F. (2006). Insulin, insulin-degrading enzyme and amyloid-β peptide in Alzheimer's disease: Review and hypothesis. *Neurobiol Aging*, 27,(2):190–198. https://doi.org/10.1016/j.neurobiolaging.2005.01.004

Rawlings, A. M., Sharrett, A. R., Schneider, A. L. C., Coresh, J., Albert, M., Couper, D., Griswold, M., Gottesman, R. F., Wagenknecht, L. E., Windham, B. G., & Selvin, E. (2014). Diabetes in midlife and cognitive change over 20 years: A cohort study. *Ann Intern Med*, *161*(11):785–793. https://doi.org/10.7326/M14-0737

Reaven, G. (1988). Banting Lecture 1988. Role of insulin resistance in human disease. *Diabetes,* 37(12):1595-1607.

Reaven, G. (2004). The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. *Endocrinol Metab Clin North Am,* 33(2):283–303). https://doi.org/10.1016/j.ecl.2004.03.002

Reger, M. A., Watson, G. S., Green, P. S., Wilkinson, C. W., Baker, L. D., Cholerton, B., Fishel, M. A., Plymate, S. R., Breitner, J. C. S., DeGroodt, W., Mehta, P., & Craft, S. (2008). Intranasal insulin improves cognition and modulates beta-amyloid in early AD. *Neurology*, *70*(6):440–448. https://doi.org/10.1212/01.WNL.0000265401.62434.36

Reiman, E. M., Arboleda-Velasquez, J. F., Quiroz, Y. T., Huentelman, M. J., Beach, T. G., Caselli, R. J., Chen, Y., Su, Y., Myers, A. J., Hardy, J., Paul Vonsattel, J., Younkin, S. G., Bennett, D. A., De Jager, P. L., Larson, E. B., Crane, P. K., Keene, C. D., Kamboh, M. I., Kofler, J. K., … Zhao, Y. (2020). Exceptionally low likelihood of Alzheimer's dementia in APOE2 homozygotes from a 5,000-person neuropathological study. *Nat Commun*, *11*(1):667. https://doi.org/10.1038/s41467-019-14279-8

Ribe, E. M., & Lovestone, S. (2016). Insulin signalling in Alzheimer′s disease and diabetes: from epidemiology to molecular links. *J Intern Med,* 280(5):430–442. https://doi.org/10.1111/joim.12534

Ricci, M., Cimini, A., Chiaravalloti, A., Filippi, L., & Schillaci, O. (2020). Positron Emission Tomography (PET) and Neuroimaging in the Personalized Approach to Neurodegenerative Causes of Dementia. *Int J Mol Sci*, *21*(20):1–18. https://doi.org/10.3390/IJMS21207481

Roberts, R. O., Aakre, J. A., Kremers, W. K., Vassilaki, M., Knopman, D. S., Mielke, M. M., Alhurani, R., Geda, Y. E., Machulda, M. M., Coloma, P., Schauble, B., Lowe, V. J., Jack, C. R., & Petersen, R. C. (2018). Prevalence and Outcomes of Amyloid Positivity Among Persons Without Dementia in a Longitudinal, Population-Based Setting. *JAMA Neurol 75*(8):970–979. https://doi.org/10.1001/JAMANEUROL.2018.0629

Roberts, R. O., Geda, Y. E., Knopman, D. S., Boeve, B. F., Christianson, T. J. H., Pankratz, V. S., Kullo, I. J., Tangalos, E. G., Ivnik, R. J., & Petersen, R. C. (2009). Association of C-reactive protein with mild cognitive impairment. *Alzheimers Dement*, *5*(5):398–405. https://doi.org/10.1016/j.jalz.2009.01.025

Rolandsson, O., Backeström, A., Eriksson, S., Hallmans, G., & Nilsson, L. G. (2008). Increased glucose levels are associated with episodic memory in nondiabetic women. *Diabetes*, *57*(2):440–443. https://doi.org/10.2337/db07-1215

Rönnemaa, E., Zethelius, B., Sundelöf, J., Sundström, J., Degerman-Gunnarsson, M., Berne, C., Lannfelt, L., & Kilander, L. (2008). Impaired insulin secretion increases the risk of Alzheimer disease. *Neurology*, *71*(14):1065–1071. https://doi.org/10.1212/01.wnl.0000310646.32212.3a

Rönnemaa, E., Zethelius, B., Sundelöf, J., Sundström, J., Degerman-Gunnarsson, M., Lannfelt, L., Berne, C., & Kilander, L. (2009). Glucose metabolism and the risk of Alzheimer's disease and dementia: A population-based 12 year follow-up study in 71-year-old men. *Diabetologia*, *52*(8):1504–1510. https://doi.org/10.1007/s00125-009-1393-9

Rowe, C. C., Ellis, K. A., Rimajova, M., Bourgeat, P., Pike, K. E., Jones, G., Fripp, J., Tochon-Danguy, H., Morandeau, L., O'Keefe, G., Price, R., Raniga, P., Robins, P., Acosta, O., Lenzo, N., Szoeke, C., Salvado, O., Head, R., Martins, R., … Villemagne, V. L. (2010). Amyloid imaging results from

the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. *Neurobiol Aging*, *31*(8):1275–1283. https://doi.org/10.1016/j.neurobiolaging.2010.04.007

Rowe, C. C., & Villemagne, V. L. (2011). Brain amyloid imaging. *J Nucl Med, 52*(11):1733–1740. https://doi.org/10.2967/jnumed.110.076315

Sacuiu, S. F. (2016). Dementias. *Handb Clin Neurol,* 138:123–151. https://doi.org/10.1016/B978-0-12-802973-2.00008-2

Saltiel, A. R., & Olefsky, J. M. (2017). Inflammatory mechanisms linking obesity and metabolic disease. *J Clin Invest*, *127*(1):1–4. https://doi.org/10.1172/JCI92035

Sanz, C. M., Ruidavets, J. B., Bongard, V., Marquié, J. C., Hanaire, H., Ferrières, J., & Andrieu, S. (2013). Relationship between markers of insulin resistance, markers of adiposity, HbA1c, and cognitive functions in a middle-aged population-based sample: the MONA LISA study. *Diabetes Care*, *36*(6):1512–1521. https://doi.org/10.2337/DC12-1017

Schain, M., & Kreisl, W. C. (2017). Neuroinflammation in Neurodegenerative Disorders—a Review. *Curr Neurol Neurosci Rep*, *17*(3):25. https://doi.org/10.1007/s11910-017-0733-2

Scheltens, P., De Strooper, B., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C. E., Cummings, J., & van der Flier, W. M. (2021). Alzheimer's disease. *Lancet*, *397*(10284):1577–1590. https://doi.org/10.1016/S0140-6736(20)32205-4

Scheltens, P., Fox, N., Barkhof, F., & De Carli, C. (2002). Structural magnetic resonance imaging in the practical assessment of dementia: Beyond exclusion. *Lancet Neurol,* 1(1):13–21. https://doi.org/10.1016/S1474-4422(02)00002-9

Scheltens, P., Kuiper, M., Ch Wolters, E., Barkhof, F., Valk, J., Weinsten, H. C., Leys, D., Vermersch, P., Huglo, D., & Steinling, M. (1992). Atrophy of medial temporal lobes on MRI in "probable" Alzheimer's disease and normal ageing: diagnostic value and neuropsychological correlates. *J Neurol Neurosurg Psychiatry*, *55*(10):967–972. https://doi.org/10.1136/jnnp.55.10.967

Schneider, J. A., Arvanitakis, Z., Bang, W., & Bennett, D. A. (2007). Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology*, *69*(24):2197–2204. https://doi.org/10.1212/01.wnl.0000271090.28148.24

Schrijvers, E. M. C., Witteman, J. C. M., Sijbrands, E. J. G., Hofman, A., Koudstaal, P. J., & Breteler, M. M. B. (2010). Insulin metabolism and the risk of Alzheimer disease: the Rotterdam Study. *Neurology*, *75*(22):1982–1987. https://doi.org/10.1212/WNL.0b013e3181ffe4f6

Schuur, M., Henneman, P., van Swieten, J. C., Zillikens, M. C., de Koning, I., Janssens, A. C. J. W., Witteman, J. C. M., Aulchenko, Y. S., Frants, R. R., Oostra, B. A., van Dijk, K. W., & van Duijn, C. M. (2010). Insulin-resistance and metabolic syndrome are related to executive function in women in a large family-based study. *Eur J Epidemiol*, *25*(8):561–568. https://doi.org/10.1007/S10654-010-9476-Y

Schwartz, M. W., Figlewicz, D. F., Kahn, S. E., Baskin, D. G., Greenwood, M. R. C., & Porte, D. (1990). Insulin binding to brain capillaries is reduced in genetically obese, hyperinsulinemic Zucker rats. *Peptides*, *11*(3):467–472. https://doi.org/10.1016/0196-9781(90)90044-6

Seo, S. W., Ayakta, N., Grinberg, L. T., Villeneuve, S., Lehmann, M., Reed, B., DeCarli, C., Miller, B. L., Rosen, H. J., Boxer, A. L., O'Neil, J. P., Jin, L. W., Seeley, W. W., Jagust, W. J., & Rabinovici, G. D. (2016). Regional correlations between [11 C]PIB PET and post-mortem burden of amyloid-beta pathology in a diverse neuropathological cohort. *NeuroImage. Clin*, *13*:130–137. https://doi.org/10.1016/J.NICL.2016.11.008

Serrano-Pozo, A., Das, S., & Hyman, B. T. (2021). APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches. *Lancet Neurol,* 20(1):68–80. https://doi.org/10.1016/S1474-4422(20)30412-9

Shanik, M. H., Xu, Y., Skrha, J., Dankner, R., Zick, Y., & Roth, J. (2008). Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse? *Diabetes care,* 31(Suppl 2):S262-268. https://doi.org/10.2337/dc08-s264

Shen, X. N., Niu, L. D., Wang, Y. J., Cao, X. P., Liu, Q., Tan, L., Zhang, C., & Yu, J. T. (2019). Inflammatory markers in Alzheimer's disease and mild cognitive impairment: A meta-analysis and

systematic review of 170 studies. *J Neurol Neurosurg Psychiatry*, *90*(5):590–598. https://doi.org/10.1136/jnnp-2018-319148

Sims, R., Hill, M., & Williams, J. (2020). The multiplex model of the genetics of Alzheimer's disease. In *Nat Neurosci,* 23(3):311–322. https://doi.org/10.1038/s41593-020-0599-5

Singh-Manoux, A., Dugravot, A., Brunner, E., Kumari, M., Shipley, M., Elbaz, A., & Kivimaki, M. (2014). Interleukin-6 and C-reactive protein as predictors of cognitive decline in late midlife. *Neurology*, *83*(6):486–493. https://doi.org/10.1212/WNL.0000000000000665

Singh-Manoux, A., Dugravot, A., Shipley, M., Brunner, E. J., Elbaz, A., Sabia, S., & Kivimaki, M. (2018). Obesity trajectories and risk of dementia: 28 years of follow-up in the Whitehall II Study. *Alzheimers Dement*, *14*(2):178–186. https://doi.org/10.1016/j.jalz.2017.06.2637

Slade, K., Plack, C. J., & Nuttall, H. E. (2020). The Effects of Age-Related Hearing Loss on the Brain and Cognitive Function. *Trends Neurosci,* 43(10):810–821. https://doi.org/10.1016/J.TINS.2020.07.005

Smith, E. E., Muzikansky, A., McCreary, C. R., Batool, S., Viswanathan, A., Dickerson, B. C., Johnson, K., Greenberg, S. M., & Blacker, D. (2018). Impaired memory is more closely associated with brain beta-amyloid than leukoaraiosis in hypertensive patients with cognitive symptoms. *PloS One*, *13*(1):e0191345. https://doi.org/10.1371/JOURNAL.PONE.0191345

Snellman, A., Rokka, J., López-Picón, F. R., Helin, S., Re, F., Löyttyniemi, E., Pihlaja, R., Forloni, G., Salmona, M., Masserini, M., Solin, O., Rinne, J. O., & Haaparanta-Solin, M. (2017). Applicability of [11C]PIB micro-PET imaging for in vivo follow-up of anti-amyloid treatment effects in APP23 mouse model. *Neurobiol Aging*, *57*:84–94. https://doi.org/10.1016/j.neurobiolaging.2017.05.008

Snowdon, D. A., Greiner, L. H., Mortimer, J. A., Riley, K. P., Greiner, P. A., & Markesbery, W. R. (1997). Brain infarction and the clinical expression of Alzheimer disease: The Nun Study. *JAMA*, *277*(10):813–817. https://doi.org/10.1001/jama.277.10.813

Solano, D. C., Sironi, M., Bonfini, C., Solerte, S. B., Govoni, S., & Racchi, M. (2000). Insulin regulates soluble amyloid precursor protein release via phosphatidyl inositol 3 kinase-dependent pathway. *FASEB J*, *14*(7):1015–1022. https://doi.org/10.1096/fasebj.14.7.1015

Solis-Herrera, C., Triplitt, C., Reasner, C., DeFronzo, R. A., & Cersosimo, E. (2018). *Endotext: Classification of Diabetes Mellitus.* MDText.com. Inc. https://pubmed-ncbi-nlm-nih-gov.ezproxy.utu.fi/25905343/

Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., Iwatsubo, T., Jack, C. R., Kaye, J., Montine, T. J., Park, D. C., Reiman, E. M., Rowe, C. C., Siemers, E., Stern, Y., Yaffe, K., Carrillo, M. C., Thies, B., Morrison-Bogorad, M., … Phelps, C. H. (2011). Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*, *7*(3):280–292. https://doi.org/10.1016/j.jalz.2011.03.003

Srikanth, V., Sinclair, A. J., Hill-Briggs, F., Moran, C., & Biessels, G. J. (2020). Type 2 diabetes and cognitive dysfunction—towards effective management of both comorbidities. *Lancet Diabetes Endocrinol,* 8(6):535–545. https://doi.org/10.1016/S2213-8587(20)30118-2

Stern, Y. (2012). Cognitive reserve in ageing and Alzheimer's disease. *Lancet Neurol,* 11(11):1006–1012. https://doi.org/10.1016/S1474-4422(12)70191-6

Stolk, R. P., Breteler, M. M. B., Ott, A., Pols, H. A. P., Lamberts, S. W. J., Grobbee, D. E., & Hofman, A. (1997). Insulin and cognitive function in an elderly population the rotterdam study. *Diabetes Care*, *20*(5):792–795. https://doi.org/10.2337/diacare.20.5.792

Suárez-Calvet, M., Kleinberger, G., Araque Caballero, M. Á., Brendel, M., Rominger, A., Alcolea, D., Fortea, J., Lleó, A., Blesa, R., Gispert, J. D., Sánchez-Valle, R., Antonell, A., Rami, L., Molinuevo, J. L., Brosseron, F., Traschütz, A., Heneka, M. T., Struyfs, H., Engelborghs, S., … Haass, C. (2016). sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. *EMBO Mol Med*, *8*(5):466–476. https://doi.org/10.15252/emmm.201506123

Syed IA. (2011). Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus. World Health Organization, 61(4), 383. https://www-ncbi-nlm-nih-gov.ezproxy.utu.fi/books/NBK304267/

Tabák, A. G., Herder, C., Rathmann, W., Brunner, E. J., & Kivimäki, M. (2012). Prediabetes: A high-risk state for diabetes development. *Lancet*, 379(9833):2279–2290. https://doi.org/10.1016/S0140-6736(12)60283-9

Tabák, A. G., Jokela, M., Akbaraly, T. N., Brunner, E. J., Kivimäki, M., & Witte, D. R. (2009). Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. *Lancet*, *373*(9682):2215–2221. https://doi.org/10.1016/S0140-6736(09)60619-X

Talbot, K., Wang, H. Y., Kazi, H., Han, L. Y., Bakshi, K. P., Stucky, A., Fuino, R. L., Kawaguchi, K. R., Samoyedny, A. J., Wilson, R. S., Arvanitakis, Z., Schneider, J. A., Wolf, B. A., Bennett, D. A., Trojanowski, J. Q., & Arnold, S. E. (2012). Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J Clin Invest*, *122*(4):1316–1338. https://doi.org/10.1172/JCI59903

Tan, Z. S., Beiser, A. S., Fox, C. S., Au, R., Himali, J. J., Debette, S., Decarli, C., Vasan, R. S., Wolf, P. A., & Seshadri, S. (2011). Association of metabolic dysregulation with volumetric brain magnetic resonance imaging and cognitive markers of subclinical brain aging in middle-aged adults: The Framingham offspring study. *Diabetes Care*, *34*(8):1766–1770. https://doi.org/10.2337/dc11-0308

Tan, Z. S., Beiser, A. S., Vasan, R. S., Roubenoff, R., Dinarello, C. A., Harris, T. B., Benjamin, E. J., Au, R., Kiel, D. P., Wolf, P. A., & Seshadri, S. (2007). Inflammatory markers and the risk of Alzheimer disease: the Framingham Study. *Neurology*, *68*(22):1902–1908. https://doi.org/10.1212/01.WNL.0000263217.36439.DA

Tao, Q., Ang, T. F. A., DeCarli, C., Auerbach, S. H., Devine, S., Stein, T. D., Zhang, X., Massaro, J., Au, R., & Qiu, W. Q. (2018). Association of Chronic Low-grade Inflammation With Risk of Alzheimer Disease in ApoE4 Carriers. *JAMA Netw Open*, *1*(6):e183597. https://doi.org/10.1001/JAMANETWORKOPEN.2018.3597

Taylor, R. (2021). Type 2 diabetes and remission: practical management guided by pathophysiology. J Intern Med, 289(6), 754–770. https://doi.org/10.1111/JOIM.13214

Thambisetty, M., Beason-Held, L. L., An, Y., Kraut, M., Metter, J., Egan, J., Ferrucci, L., O'Brien, R., & Resnick, S. M. (2013). Impaired glucose tolerance in midlife and longitudinal changes in brain function during aging. *Neurobiol Aging*, *34*(10):2271–2276. https://doi.org/10.1016/j.neurobiolaging.2013.03.025

Thambisetty, M., Metter, E. J., Yang, A., Dolan, H., Marano, C., Zonderman, A. B., Troncoso, J. C., Zhou, Y., Wong, D. F., Ferrucci, L., Egan, J., Resnick, S. M., & O'Brien, R. J. (2013). Glucose intolerance, insulin resistance, and pathological features of Alzheimer disease in the Baltimore longitudinal study of aging. *JAMA Neurol* *70*(9):1167–1172. https://doi.org/10.1001/jamaneurol.2013.284

Toledo, J. B., Arnold, S. E., Raible, K., Brettschneider, J., Xie, S. X., Grossman, M., Monsell, S. E., Kukull, W. A., & Trojanowski, J. Q. (2013). Contribution of cerebrovascular disease in autopsy confirmed neurodegenerative disease cases in the National Alzheimer's Coordinating Centre. *Brain*, *136*(9):2697–2706. https://doi.org/10.1093/brain/awt188

Trouwborst, I., Bowser, S. M., Goossens, G. H., & Blaak, E. E. (2018). Ectopic Fat Accumulation in Distinct Insulin Resistant Phenotypes; Targets for Personalized Nutritional Interventions. Frontiers in Nutrition, 5, 77. https://doi.org/10.3389/FNUT.2018.00077

Tuisku, J., Plavén-Sigray, P., Gaiser, E. C., Airas, L., Al-Abdulrasul, H., Brück, A., Carson, R. E., Chen, M.-K., Cosgrove, K. P., Ekblad, L., Esterlis, I., Farde, L., Forsberg, A., Halldin, C., Helin, S., Kosek, E., Lekander, M., Lindgren, N., Marjamäki, P., … Cervenka, S. (2019). Effects of age, BMI and sex on the glial cell marker TSPO — a multicentre [11C]PBR28 HRRT PET study. *Eur J Nucl Med Mol Imaging*, *46*(11):2329–2338. https://doi.org/10.1007/s00259-019-04403-7

Tuligenga, R. H., Dugravot, A., Tabák, A. G., Elbaz, A., Brunner, E. J., Kivimäki, M., & Singh-Manoux, A. (2014). Midlife type 2 diabetes and poor glycaemic control as risk factors for cognitive decline in early old age: A post-hoc analysis of the Whitehall II cohort study. *Lancet Diabetes Endocrinol*, *2*(3):228–235. https://doi.org/10.1016/S2213-8587(13)70192-X

Vadini, F., Simeone, P. G., Boccatonda, A., Guagnano, M. T., Liani, R., Tripaldi, R., Di Castelnuovo, A., Cipollone, F., Consoli, A., & Santilli, F. (2020). Liraglutide improves memory in obese patients with prediabetes or early type 2 diabetes: a randomized, controlled study. *Int J Obes*, *44*(6):1254–1263.

van der Veldt, A. A. M., Smit, E. F., & Lammertsma, A. A. (2013). Positron Emission Tomography as a Method for Measuring Drug Delivery to Tumors in vivo: The Example of [(11)C]docetaxel. *Front Oncol*, 3:208. https://doi.org/10.3389/FONC.2013.00208

van Eldik, L. J., Carrillo, M. C., Cole, P. E., Feuerbach, D., Greenberg, B. D., Hendrix, J. A., Kennedy, M., Kozauer, N., Margolin, R. A., Molinuevo, J. L., Mueller, R., Ransohoff, R. M., Wilcock, D. M., Bain, L., & Bales, K. (2016). The roles of inflammation and immune mechanisms in Alzheimer's disease. *Alzheimers Dement*, *2*(2):99–109. https://doi.org/10.1016/j.trci.2016.05.001

Vanhanen, M., Koivisto, K., Karjalainen, L., Helkala, E. L., Laakso, M., Soininen, H., & Riekkinen, P. (1997). Risk for non-insulin-dependent diabetes in the normoglycaemic elderly is associated with impaired cognitive function. *NeuroReport*, *8*(6):1527–1530. https://doi.org/10.1097/00001756-199704140-00041

Vanhanen, M., Koivisto, K., Kuusisto, J., Mykkänen, L., Helkala, E. L., Hänninen, T., Riekkinen, P., Soininen, H., & Laakso, M. (1998). Cognitive function in an elderly population with persistent impaired glucose tolerance. *Diabetes Care*, *21*(3):398–402. https://doi.org/10.2337/diacare.21.3.398

Vanninen, R., Mäntylä, R., Salonen, O., Valanne, L., Rinne, J., & Erkinjuntti, T. (2011). Brain imaging of patients with memory disorders. *Duodecim*, *127*(24):2615–2626.

Vera, J. H., Guo, Q., Cole, J. H., Boasso, A., Greathead, L., Kelleher, P., Rabiner, E. A., Kalk, N., Bishop, C., Gunn, R. N., Matthews, P. M., & Winston, A. (2016). Neuroinflammation in treated HIV-positive individuals: A TSPO PET study. *Neurology*, *86*(15):1425–1432. https://doi.org/10.1212/WNL.0000000000002485

Vessby, B., Uusitupa, M., Hermansen, K., Riccardi, G., Rivellese, A. A., Tapsell, L. C., Nälsén, C., Berglund, L., Louheranta, A., Rasmussen, B. M., Calvert, G. D., Maffetone, A., Pedersen, E., Gustafsson, I. B., & Storlien, L. H. (2001). Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia*, *44*(3):312–319. https://doi.org/10.1007/S001250051620

Villemagne, V. L., Doré, V., Bourgeat, P., Burnham, S. C., Laws, S., Salvado, O., Masters, C. L., & Rowe, C. C. (2017). Aβ-amyloid and Tau Imaging in Dementia. *Semin Nucl Med*, *47*(1):75–88. https://doi.org/10.1053/J.SEMNUCLMED.2016.09.006

Viscogliosi, G., Chiriac, I. M., Andreozzi, P., & Ettorre, E. (2015). Executive dysfunction assessed by Clock-Drawing Test in older non-demented subjects with metabolic syndrome is not mediated by white matter lesions. *Psychiatry Clin Neurosci*, *69*(10):620–629. https://doi.org/10.1111/PCN.12296

Vlad, S. C., Miller, D. R., Kowall, N. W., & Felson, D. T. (2008). Protective effects of NSAIDs on the development of Alzheimer disease. *Neurology*, *70*(19):1672–1677. https://doi.org/10.1212/01.wnl.0000311269.57716.63

Walker, K. A., Gottesman, R. F., Wu, A., Knopman, D. S., Gross, A. L., Mosley, T. H., Selvin, E., & Windham, B. G. (2019). Systemic inflammation during midlife and cognitive change over 20 years: The ARIC Study. *Neurology*, *92*(11):e1256–e1267. https://doi.org/10.1212/WNL.0000000000007094

Wallace, T. M., Levy, J. C., & Matthews, D. R. (2004). Use and abuse of HOMA modeling. *Diabetes Care*, 27(6):1487–1495. https://doi.org/10.2337/diacare.27.6.1487

Wallace, T. M., Matthews, D. R. (2002). The assessment of insulin resistance in man. Diabet Med, 19(7):527–534. https://doi.org/ 10.1046/j.1464-5491.2002.00745.x

Wang, J., Tan, L., Wang, H. F., Tan, C. C., Meng, X. F., Wang, C., Tang, S. W., & Yu, J. T. (2015). Anti-inflammatory drugs and risk of Alzheimer's Disease: An updated systematic review and meta-analysis. *J Alzheimer's Dis,* 44(2):385–396. https://doi.org/10.3233/JAD-141506

Wattjes, M. P., Henneman, W. J. P., Van Der Flier, W. M., De Vries, O., Träber, F., Geurts, J. J. G., Scheltens, P., Vrenken, H., & Barkhof, F. (2009). Diagnostic imaging of patients in a memory clinic: Comparison of MR imaging and 64-detector row CT. *Radiology*, *253*(1):174–183. https://doi.org/10.1148/radiol.2531082262

Weinstein, G., Maillard, P., Himali, J. J., Beiser, A. S., Au, R., Wolf, P. A., Seshadri, S., & Decarli, C. (2015). Glucose indices are associated with cognitive and structural brain measures in young adults. *Neurology*, *84*(23):2329–2337. https://doi.org/10.1212/WNL.0000000000001655

Whitmer, R. A., Gustafson, D. R., Barrett-Connor, E., Haan, M. N., Gunderson, E. P., & Yaffe, K. (2008). Central obesity and increased risk of dementia more than three decades later. *Neurology*, *71*(14):1057–1064. https://doi.org/10.1212/01.wnl.0000306313.89165.ef

Wiley, C. A., Lopresti, B. J., Venneti, S., Price, J., Klunk, W. E., DeKosky, S. T., & Mathis, C. A. (2009). Carbon 11-labeled Pittsburgh Compound B and carbon 11-labeled (R)-PK11195 positron emission tomographic imaging in Alzheimer disease. *Arch Neurol*, *66*(1):60–67. https://doi.org/10.1001/ARCHNEUROL.2008.511

Willette, A. A., Bendlin, B. B., Starks, E. J., Birdsill, A. C., Johnson, S. C., Christian, B. T., Okonkwo, O. C., la Rue, A., Hermann, B. P., Koscik, R. L., Jonaitis, E. M., Sager, M. A., & Asthana, S. (2015). Association of Insulin Resistance With Cerebral Glucose Uptake in Late Middle-Aged Adults at Risk for Alzheimer Disease. *JAMA Neurol*, *72*(9):1013–1020. https://doi.org/10.1001/JAMANEUROL.2015.0613

Willette, A. A., Johnson, S. C., Birdsill, A. C., Sager, M. A., Christian, B., Baker, L. D., Craft, S., Oh, J., Statz, E., Hermann, B. P., Jonaitis, E. M., Koscik, R. L., La Rue, A., Asthana, S., & Bendlin, B. B. (2015). Insulin resistance predicts brain amyloid deposition in late middle-aged adults. *Alzheimers Dement*, *11*(5):504-510.e1. https://doi.org/10.1016/j.jalz.2014.03.011

Willette, A. A., Xu, G., Johnson, S. C., Birdsill, A. C., Jonaitis, E. M., Sager, M. A., Hermann, B. P., Rue, A. la, Asthana, S., & Bendlin, B. B. (2013). Insulin resistance, brain atrophy, and cognitive performance in late middle-aged adults. *Diabetes Care,* 36(2):443-449. https://doi.org/10.2337/dc12-0922

Willmann, C., Brockmann, K., Wagner, R., Kullmann, S., Preissl, H., Schnauder, G., Maetzler, W., Gasser, T., Berg, D., Eschweiler, G. W., Metzger, F., Fallgatter, A. J., Häring, H.-U., Fritsche, A., & Heni, M. (2020). Insulin sensitivity predicts cognitive decline in individuals with prediabetes. *BMJ Open Diabetes Res Care*, *8*(2):e001741. https://doi.org/10.1136/bmjdrc-2020-001741

Wimberley, C., Buvat, I., & Boutin, H. (2021). Imaging translocator protein expression with positron emission tomography. *Eur J Nucl Med Mol Imaging*, *49*(1):74–76. https://doi.org/10.1007/S00259-021-05601-Y

Winblad, B., Amouyel, P., Andrieu, S., Ballard, C., Brayne, C., Brodaty, H., Cedazo-Minguez, A., Dubois, B., Edvardsson, D., Feldman, H., Fratiglioni, L., Frisoni, G. B., Gauthier, S., Georges, J., Graff, C., Iqbal, K., Jessen, F., Johansson, G., Jönsson, L., … Zetterberg, H. (2016). Defeating Alzheimer's disease and other dementias: A priority for European science and society. *Lancet Neurol,* 15(15):455–532. https://doi.org/10.1016/S1474-4422(16)00062-4

Winblad, B., Palmer, K., Kivipelto, M., Jelic, V., Fratiglioni, L., Wahlund, L. O., Nordberg, A., Bäckman, L., Albert, M., Almkvist, O., Arai, H., Basun, H., Blennow, K., De Leon, M., Decarli, C., Erkinjuntti, T., Giacobini, E., Graff, C., Hardy, J., … Petersen, R. C. (2004). Mild cognitive impairment - Beyond controversies, towards a consensus: Report of the International Working Group on Mild Cognitive Impairment. *J Int Med*, *256*(3):240–246. https://doi.org/10.1111/j.1365-2796.2004.01380.x

Wood, P. L. (2003). *The Cerebellum in AD.* Neuroinflammation. Humana Press, Totowa, NJ; 2003

Wright, R. S., Levy, S. A. T., Katzel, L. I., Rosenberger, W. F., Manukyan, Z., Whitfield, K. E., & Waldstein, S. R. (2015). Fasting glucose and glucose tolerance as potential predictors of neurocognitive function among nondiabetic older adults. *J Clin Exp Neuropsychol*, *37*(1):49–60. https://doi.org/10.1080/13803395.2014.985189

Yaffe, K., Kanaya, A., Lindquist, K., Simonsick, E. M., Harris, T., Shorr, R. I., Tylavsky, F. A., & Newman, A. B. (2004). The metabolic syndrome, inflammation, and risk of cognitive decline. *JAMA*, *292*(18):2237–2242. https://doi.org/10.1001/jama.292.18.2237

Yaffe, K., Lindquist, K., Penninx, Simonsick, E. M., Pahor, M., Kritchevsky, S., Launer, L., Kuller, L., Rubin, S., & Harris, T. (2003). Inflammatory markers and cognition in well-functioning African-American and white elders. *Neurology*, *61*(1):76–80. https://doi.org/10.1212/01.WNL.0000073620.42047.D7

Ylikoski, R., Ylikoski, A., Erkinjuntti, T., Sulkava, R., Raininko, R., & Tilvis, R. (1993). White matter changes in healthy elderly persons correlate with attention and speed of mental processing. *Arch Neurol*, *50*(8):818–824. https://doi.org/10.1001/ARCHNEUR.1993.00540080029009

Yokokura, M., Mori, N., Yagi, S., Yoshikawa, E., Kikuchi, M., Yoshihara, Y., Wakuda, T., Sugihara, G., Takebayashi, K., Suda, S., Iwata, Y., Ueki, T., Tsuchiya, K. J., Suzuki, K., Nakamura, K., & Ouchi, Y. (2011). In vivo changes in microglial activation and amyloid deposits in brain regions with hypometabolism in Alzheimer's disease. *Eur J Nucl Med Mol Imaging*, *38*(2):343–351. https://doi.org/10.1007/S00259-010-1612-0

Young, S. E., Mainous, A. G., & Carnemolla, M. (2006). Hyperinsulinemia and cognitive decline in a middle-aged cohort. *Diabetes Care*, *29*(12):2688–2693. https://doi.org/10.2337/dc06-0915

Zethelius, B., Hales, C. N., Lithell, H. O., & Berne, C. (2004). Insulin resistance, impaired early insulin response, and insulin propeptides as predictors of the development of type 2 diabetes: A population-based, 7-year follow-up study in 70-year-old men. *Diabetes Care*, *27*(6):1433–1438. https://doi.org/10.2337/diacare.27.6.1433

Zhang, J., Chen, C., Hua, S., Liao, H., Wang, M., Xiong, Y., & Cao, F. (2017). An updated meta-analysis of cohort studies: Diabetes and risk of Alzheimer's disease. *Diabetes Res Clin Pract,* 124:41–47. https://doi.org/10.1016/j.diabres.2016.10.024

Zheng, F., Yan, L., Yang, Z., Zhong, B., & Xie, W. (2018). HbA1c, diabetes and cognitive decline: the English Longitudinal Study of Ageing. *Diabetologia*, *61*(4):839–848. https://doi.org/10.1007/s00125-017-4541-7

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