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Diabetes, Cognitive Ability and Dementia - Epidemiological, Metabolic and Genetic Aspects

Dybjær, Elin

2022

Document Version:

Publisher's PDF, also known as Version of record

[Link to publication](#)

Citation for published version (APA):

Dybjær, E. (2022). *Diabetes, Cognitive Ability and Dementia - Epidemiological, Metabolic and Genetic Aspects*. [Doctoral Thesis (compilation), Department of Clinical Sciences, Malmö]. Lund University, Faculty of Medicine.

Total number of authors:

1

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Diabetes, Cognitive Ability and Dementia

Epidemiological, Metabolic and Genetic Aspects

ELIN DYBJER

DEPARTMENT OF CLINICAL SCIENCES MALMÖ | LUND UNIVERSITY



Diabetes, Cognitive Ability and Dementia

Diabetes, Cognitive Ability and Dementia

Epidemiological, Metabolic and Genetic Aspects

Elin Dybjer



LUND
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DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 16th of December at 13.00 in Agardhsalen/the CRC Aula, Clinical Research Center, Jan Waldenströms gata 35

Faculty opponent

Associate professor Alina Solomon, Karolinska Institutet

Organization LUND UNIVERSITY Department of Clinical Sciences Malmö Internal Medicine Research Group	Document name DOCTORAL DISSERTATION	
	Date of issue November 3 rd 2022	
	Author: Elin Dybjer	
Sponsoring organization		
Title and subtitle Diabetes, Cognitive Ability and Dementia – Epidemiological, Metabolic and Genetic Aspects		
Abstract Diabetes is associated with mild negative effects on cognitive ability during the whole life course and with a two-fold risk of dementia in old age. The proportion of older people in the population and the prevalence of both diabetes and dementia are increasing worldwide. It is therefore important to further the understanding of the association between the two diseases, with a view to implementing health promotion and preventive strategies in the future. The aim of this thesis is to investigate the impact of impaired glucose metabolism and diabetes on cognitive performance and risk of dementia in a population-based setting. The specific aims of the four studies are to investigate associations between (1) pre-diabetes and diabetes and cognitive test results, (2) physiological levels of glucometabolic biomarkers and cognitive test results, (3) type 1 diabetes, cognitive ability and long-term morbidity and mortality in a historical cohort and (4) genetic risk markers of type 2 diabetes and dementia outcomes. Studies I, II and IV are based on the population-based Malmö Diet and Cancer Study (MDCS) with 30 446 participants at baseline 1991-96 and 3 734 participants at follow-up 2007–12 (the cardiovascular cohort, MDCS-CV). Data from questionnaires, physical health examinations, blood samples, genome wide association study (GWAS) screening and national health registers were obtained. Dementia diagnoses were validated by physicians. Study III is based on the Southern Sweden Diabetes in Conscripts Study (SSDCS) consisting of 120 men with type 1 diabetes and 469 control men that were examined at baseline during 1953–59 and followed up until 2018 in national registers. Statistical methods in the thesis include multiple regression and Cox regression analyses for epidemiological associations and 2-sample Mendelian randomization (MR) analyses for causal associations. Study I. In the MDCS-CV pre-diabetes, diabetes, fasting glucose and 2-h glucose (post oral glucose tolerance testing), were cross-sectionally associated with lower Mini-Mental State Examination (MMSE) results (a test of global cognition used to screen for dementia), as well as with worse results in A Quick Test of cognitive speed (AQT) Study II. In the MDCS-CV positive associations were found between insulin sensitivity, plasma glucagon and incretin levels on the one hand and cognitive test results on the other. Moreover, negative associations were found between insulin resistance and advanced glycation end products (AGEs) on the one hand and cognitive test results on the other. Study III. In the SSDCS based on material ranging back to 1953, men with type 1 diabetes had dramatically higher incidence rates of mortality and cardiovascular events compared to men without type 1 diabetes in a control group. Higher cognitive ability at baseline (at 18 years of age) was associated with lower mortality in the control group, but not in the group with type 1 diabetes. Study IV. In the MDCS a polygenic risk score (PRS) for type 2 diabetes was associated with all-cause dementia, mixed dementia and vascular dementia (VaD). Associations were stronger for non-carriers of the risk gene APOE-ε4 for Alzheimer's disease (AD). However, two-sample-Mendelian Randomization (MR) analyses could not confirm a causal link between type 2 diabetes and dementia. In conclusion, we identified genetic risk markers associated with type 2 diabetes that were associated with vascular dementia (VaD) in a population-based cohort, but no causal association between diabetes and dementia was established. Pre-diabetes as well as diabetes were associated with adverse cognitive test results. Glucose levels, incretin levels, insulin resistance, glucagon and AGEs may be important biomarkers for the association between diabetes and cognition. Cognitive ability in early adulthood may predict risk of mortality in the general population. However, we could not identify this phenomenon in men with type 1 diabetes in a historic cohort where strong effects of cardiovascular mortality were present.		
Key words: cognitive ability, dementia, diabetes, epidemiology, polygenic risk score, Mendelian randomization		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language English
ISSN and key title: 1652-8220 Lund University, Faculty of Medicine Doctoral Dissertation Series		ISBN 978-91-8021-327-1
Recipient's notes	Number of pages 102	Price
	Security classification	

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Date 2022-11-04

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Epidemiological, Metabolic and Genetic Aspects

Elin Dybjer



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Study 4 © The Authors (Manuscript unpublished).

Faculty of Medicine

Department of Clinical Sciences Malmö

Lund University, Doctoral Dissertation Series 2022:165

ISSN 1652-8220

ISBN 978-91-8021-327-1

Printed in Sweden by Media-Tryck, Lund University

Lund 2022



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MADE IN SWEDEN 

To my family

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Preface

I once asked my grandmother Hester what it is like to be old, and she told me that you feel exactly the same on the inside as you did when you were young, except that you have to resist the impulse of jumping over a fence or doing other strenuous things. She was an energetic and sociable old lady, was in charge of a book group, and rode an adult-sized tricycle around town at the age of 92. When I flew over to Cambridge to visit her once, I told her I had started working on a PhD on diabetes and dementia. She said ‘Oh, I have dementia, you know’, with a certain tongue-in-cheek attitude, which was characteristic of her sense of humour. And sadly, the next day, she had forgotten what I had told her.

My grandmother was very old when she started showing signs of dementia, and she lived a happy life for many years before that. I have other relatives on my dad’s side in Sweden, several in the same family, who have suffered from Alzheimer’s disease earlier than this. My encounters with family members with dementia, and also my experiences from working in care homes during my student years have led me to be interested in the subject of what causes dementia and whether anything can be done to prevent it.

During medical school in Lund and Malmö I met Professor Peter M Nilsson, who guided me into the world of research. He has always been good at transferring his enthusiasm for research to his students and is a well-known name in many different research fields. My friend and course mate Jenny Linvik and I went to Kenya in 2012 and carried out a study with Peter’s help on intrauterine growth restriction in an area recently affected by a conflict. We travelled around escorted on motorbikes on red dusty roads. This was my first experience of research. When Peter later suggested I should embark on a PhD on the topic of diabetes and cognitive outcomes and dementia, I was happy to continue working with him, and with a topic that felt meaningful to me.

Peter has now partly retired and Gunnar Engström is my main supervisor. Thanks to them and to my co-supervisor Linda B Hassing, I have been able to pursue this PhD. Their enthusiasm, and my interest in the subject of diabetes related to cognitive ability, has inspired me to continue to do research in the future. I also hope that this growing research field will eventually provide more opportunities to improve the life circumstances of those suffering from diabetes and cognitive impairment, and my feeling is that there is good hope of this.

Abstract

Background

Diabetes is associated with mild negative effects on cognitive ability during the whole life course and with a two-fold risk of dementia in old age. The proportion of older people in the population and the prevalence of both diabetes and dementia are increasing worldwide. It is therefore important to further the understanding of the association between the two diseases, with a view to implementing health promotion and preventive strategies in the future.

Aims

The aim of this thesis is to investigate the impact of impaired glucose metabolism and diabetes on cognitive performance and risk of dementia in a population-based setting. The specific aims of the four studies are to investigate associations between (1) pre-diabetes and diabetes and cognitive test results, (2) physiological levels of glucometabolic biomarkers and cognitive test results, (3) type 1 diabetes, cognitive ability and long-term morbidity and mortality in a historical cohort and (4) genetic risk markers of type 2 diabetes and dementia outcomes.

Methods

Studies I, II and IV are based on the population-based Malmö Diet and Cancer Study (MDCS) with 30 446 participants at baseline 1991–96 and 3 734 participants at follow-up 2007–12 (the cardiovascular cohort, MDCS-CV). Data from questionnaires, physical health examinations, blood samples, genome wide association study (GWAS) screening and national health registers were obtained. Dementia diagnoses were validated by physicians. Study III is based on the Southern Sweden Diabetes in Conscripts Study (SSDCS) consisting of 120 men with type 1 diabetes and 469 control men that were examined at baseline during 1953–59 and followed up until 2018 in national registers. Statistical methods in the thesis include multiple regression and Cox regression analyses for epidemiological associations and 2-sample Mendelian randomization (MR) analyses for causal associations.

Results

Study I. In the MDCS-CV pre-diabetes, diabetes, fasting glucose and 2-h glucose (post oral glucose tolerance testing), were cross-sectionally associated with lower Mini-Mental State Examination (MMSE) results (a test of global cognition used to screen for dementia), as well as with worse results in A Quick Test of cognitive speed (AQT).

Study II. In the MDCS-CV positive associations were found between insulin sensitivity, plasma glucagon and incretin levels on the one hand and cognitive test results on the other. Moreover, negative associations were found between insulin resistance and advanced glycation end products (AGEs) on the one hand and cognitive test results on the other.

Study III. In the SSDCS based on material ranging back to 1953, men with type 1 diabetes had dramatically higher incidence rates of mortality and cardiovascular events compared to men without type 1 diabetes in a control group. Higher cognitive ability at baseline (at 18 years of age) was associated with lower mortality in the control group, but not in the group with type 1 diabetes.

Study IV. In the MDCS a polygenic risk score (PRS) for type 2 diabetes was associated with all-cause dementia, mixed dementia and vascular dementia (VaD). Associations were stronger for non-carriers of the risk gene APOE- $\epsilon 4$ for Alzheimer's disease (AD). However, two-sample-Mendelian Randomization (MR) analyses could not confirm a causal link between type 2 diabetes and dementia.

Conclusions

In conclusion, we identified genetic risk markers associated with type 2 diabetes that were associated with vascular dementia (VaD) in a population-based cohort, but no causal association between diabetes and dementia was established. Pre-diabetes as well as diabetes were associated with adverse cognitive test results. Glucose levels, incretin levels, insulin resistance, glucagon and AGEs may be important biomarkers for the association between diabetes and cognition. Cognitive ability in early adulthood may predict risk of mortality in the general population. However, we could not identify this phenomenon in men with type 1 diabetes in a historic cohort where strong effects of cardiovascular mortality were present.

Thesis articles

- I. Dybjer E, Nilsson PM, Engström G, Helmer C, Nägga K. Pre-diabetes and diabetes are independently associated with adverse cognitive test results: a cross-sectional, population-based study. *BMC Endocr Disord.* 2018;18(1):91.
- II. Dybjer E, Engström G, Helmer C, Nägga K, Rorsman P, Nilsson PM. Incretin hormones, insulin, glucagon and advanced glycation end products in relation to cognitive function in older people with and without diabetes, a population-based study. *Diabet Med.* 2020;37(7):1157-66.
- III. Dybjer E, Dahl Aslan AK, Engström G, Nilsson ED, Nägga K, Nilsson PM, Hassing L. Type 1 diabetes, cognitive ability and incidence of cardiovascular disease and death over 60 years of follow-up time in men. *Diabet Med.* 2022;39(8):e14806.
- IV. Dybjer E, Kumar A, Nägga K, Engström G, Mattsson-Carlgrén N, Melander O, Hansson O. Polygenic risk of type 2 diabetes is associated with Vascular Dementia but not with Alzheimer's Disease: A Prospective Cohort Study. (Submitted manuscript)

Popular Science Summary

People suffering from diabetes have a two-fold risk of developing dementia compared to the general population. While cognitive impairment in diabetes was first studied in 1922, the association has not been well-established until recently. However, the reasons for the association are still not well understood. Both diabetes and dementia cause a great deal of disability and suffering, not to mention the considerable consequences for the economy. In this thesis, we present new findings regarding the association between diabetes and effects on cognition (that is, mental capacity that involves understanding, thought process and knowledge) and the risk of developing dementia. The results bring hope of finding new targeted interventions towards cognitive impairment in people with, or at risk of developing, diabetes.

In the first study, we show that pre-diabetes and raised levels of blood sugar in the general population can be associated with adverse cognitive test results (results of tests that can be used to diagnose dementia). The second study focuses on biomarkers (i.e. measurable substances) that are affected by glucose (sugar) metabolism, and their relation to cognitive test results. We found significant correlations between some of the biomarkers and cognitive test results, which could motivate further studies on these biomarkers to find possible treatments.

In the third study, we present historical data on type 1 diabetes prognosis as regards mortality and cardiovascular disease during 1953–2018 in Sweden's oldest cohort with type 1 diabetes. We also investigated the impact of cognitive ability in young adulthood on future health outcomes. We found that cognitive ability affected risk of mortality in a group without, but not in a group with, type 1 diabetes.

Finally, in the fourth study we employed Mendelian randomisation, a genetics-based method for establishing causal links. However, although we found that the summarised genetic risk of type 2 diabetes is associated with an increased risk of dementia in a population-based cohort, we could not establish a causal link.

The findings of these studies may serve as a basis for further studies to help identify individuals with diabetes who are at risk of developing cognitive impairment, but also, in the future, possibly even to provide these people with specific therapies.

Populärvetenskaplig sammanfattning

Personer med typ 2-diabetes (diabetes som oftast utvecklas under senare delen av livet) har en fördubblad risk att utveckla demens jämfört med den övriga befolkningen. Sambandet mellan sjukdomarna beskrevs första gången 1922, men har etablerats alltmer under senare år. Det finns däremot fortfarande mycket som är oklart kring vad sambandet beror på. Både diabetes och demens ökar i prevalens i dagens samhälle i takt med att befolkningen åldras, och båda är sjukdomar som orsakar mycket lidande. Att ha båda sjukdomarna samtidigt riskerar dessutom att leda till en snabbare försämring av hälsan och funktionsförmågan, bland annat genom sämre följsamhet till behandling. Med tanke på dessa aspekter, samt även de hälsoekonomiska konsekvenser som detta får, är det angeläget att vetenskapen når längre för att ta reda på vad sambandet mellan sjukdomarna beror på samt hur denna kombination av två sjukdomar bäst behandlas.

I denna avhandling presenteras olika aspekter av sambandet mellan diabetes och demens eller påverkan på kognition (dvs hjärnfunktioner som innebär förståelse, tänkande och kunskap). Den första studien berör hur även förstadier till typ 2-diabetes (pre-diabetes) kan vara associerade med sämre kognitiva testresultat. Detta skulle kunna innebära att personer som ännu inte utvecklat diabetes men riskerar att göra det skulle kunna ha nytta av förebyggande strategier. Den andra studien handlar om vilka ämnen/substanser i kroppen (biomarkörer) som kan vara påverkade vid diabetes och som också kan kopplas till nedsatt kognition.

I den tredje studien undersöker vi i stället om kognition (resultat på IQ-tester vid mönstring) kan förutsäga hälsoutfall vid typ 1-diabetes (diabetes som ofta börjar tidigt i livet). Studien ger också en historisk överblick av risken över tid för hjärtkärlsjukdom och död vid typ 1-diabetes under perioden 1953–2018.

Slutligen, i det fjärde delarbetet, använde vi oss av mendelsk randomisering. Detta är en modern genetik-baserad metod för att etablera orsakssamband. Här fann vi ett samband mellan demens (speciellt vaskulär demens) och genetiska variationer kopplade till risk för typ 2-diabetes. Dock kunde vi inte visa att detta samband var ett orsakssamband.

Resultaten i dessa studier kan bidra till att i framtiden identifiera personer med diabetes som riskerar att insjukna i demens, och kanske även till att anpassa behandlingen för dessa personer.

Abbreviations

AGE	Advanced glycation end products
AD	Alzheimer's disease
APOE-ε4	Apolipoprotein E-ε4
AQT	A quick test of cognitive speed
BMI	Body mass index
c-f PWV	Carotid-femoral pulse wave velocity
CI	Confidence interval
CVE	Cardiovascular events
CVM	Cardiovascular mortality
CT	Computer tomography
DPP4	Dipeptidyl-peptidase-4
GIP	Glucose-dependent insulintropic peptide
GLM	General linear model
GLP-1	Glucagon-like peptide-1
GWAS	Genome-wide association study
HbA _{1c}	Hemoglobin A _{1c}
HOMA-IR	Homeostatic model assessment of insulin resistance
HR	Hazard ratio
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
ISI	Insulin sensitivity index
LD	Linkage disequilibrium
MAF	Minor allele frequency
MCI	Mild cognitive impairment
MDCS	The Malmö Diet and Cancer Study
MMSE	Mini-mental state examination
MR	Mendelian randomization
MRI	Magnetic resonance tomography
NFG	Normal fasting glucose
NGT	Normal glucose tolerance
OGTT	Oral glucose tolerance test
PRS	Polygenic risk score
SAF	Skin-autofluorescence
SD	Standard deviation
SNP	Single nucleotide polymorphism
SSDCS	The Southern Swedish Diabetes in Conscripts Study
SVD	Small-vessel disease
VaD	Vascular dementia
WMH	White matter hyperintensities

Context of this thesis

There is a need for more studies on different aspects of the association between impaired glucose metabolism and cognitive ability. For instance, little is known about the role of glucometabolic biomarkers for the association. Studies that use validated dementia endpoints instead of register-based diagnoses, as well as genetic data for use in Mendelian randomization analyses to examine the causality hypothesis are also needed.

The PhD project was originally outlined by Peter M Nilsson, Gunnar Engström and Katarina Nägga, as a collaborative project between the Internal Medicine Research Group and the Clinical Memory Research Unit in Malmö, which both belong to the department of Clinical Sciences in Malmö, Sweden.

The data from the Malmö Diet and Cancer Study were available to me from the start of the PhD project, apart from the data on validated dementia diagnoses, which was just being collected at the time. The baseline data were collected by Gerd Östling and Margaretha Persson at Skåne University hospital and the data management was carried out by Anders Dahlin. The baseline data of the Southern Sweden Diabetes in Conscripts Study were collected by Sven E Nilsson and transcribed into digital format by Anna Dahl. I participated in the application process of follow-up data from the National Board of Health and Welfare in collaboration with Linda B Hassing during 2018. Data management was carried out by Caddie Zhou at the National Diabetes Registry (NDR).

The data in this thesis were analysed by myself, apart from the creation of the polygenic risk scores from genetic data and the Mendelian randomisation analyses in Study IV, which were done by Atul Kumar.

I have received supervision from Peter Nilsson (my main supervisor until his partial retirement in 2021) and Gunnar Engström (my current main supervisor) at the Department of Clinical Sciences in Malmö, Lund University, and Linda B Hassing (my co-supervisor) at the Department of Psychology in Gothenburg. Katarina Nägga, Clinical Memory Research Unit, Malmö, has also played an important role in all four studies, and Cathérine Helmer at ISPED, Bordeaux University supervised my work while analysing parts of Study I-II in Bordeaux during 2015.

I have also collaborated with the Clinical Memory Research Unit at the Department of Clinical Sciences in Malmö, Lund University and with the National Diabetes Registry in Gothenburg.

Introduction

The population of older people worldwide is growing. Between 2015 and 2050, the proportion of people over 60 years is estimated to almost double from 12% to 22% (1). As a consequence of the increase in life expectancy, diseases that are common among the older population will be prevalent. Diabetes and dementia are two examples of such diseases. Although recent epidemiological data suggests a moderate slowdown or decrease in the incidence of both diabetes (2) and dementia (3) in most countries, the prevalence of both diseases has increased dramatically during the past decades worldwide (4) (5). Around 50 million people worldwide currently have dementia, and there are 10 million new cases every year (6). It is one of the major causes of disability among older people. Diabetes also causes a great deal of suffering as complications include serious damage to the heart, blood vessels, eyes, kidneys and nerves (7). The prevalence of diabetes is 422 million worldwide, and 1.5 million die of the disease every year (7).

During the past decades, there has been a growing body of evidence that diabetes and cognitive impairment or dementia are associated (8), and dementia is now considered as one of the known complications of diabetes. Only the most recent textbooks on diabetes, however, include information on this fact, which is why many clinicians are still unaware of it. Furthermore, little is still known about the underlying reasons for the association. It is also unclear whether diabetes actually causes cognitive decline and dementia, or whether common risk factors shared between the two diseases are instead responsible for the association.

Diabetes mellitus

The medical term diabetes mellitus is taken from the Greek word *diabetes*, meaning ‘to pass through’ and the Latin word *mellitus*, meaning sweet (9). The group of disorders named diabetes, or diabetes mellitus, have in common that blood sugar, or blood glucose, levels are raised. Ancient Greek, Indian and Egyptian civilizations discovered that the urine of certain patients was sweet to taste, which led to the term eventually

being developed by Apollonius of Memphis around 250 to 300 BC. The role of the pancreas in the regulation of blood sugar was discovered by Mering and Minkowski in 1889. In 1922, the hormone insulin was discovered by Banting, Best and Collip, leading to development of treatment for the disease for the first time in history (9). Common symptoms in untreated diabetes are frequent urination, thirst, fatigue, weight loss and increased appetite (7). Long-term complications include macrovascular complications (i.e. adverse effects on large blood vessels in the body) such as cardiovascular disease (heart failure, ischemic heart disease), and microvascular complications (adverse effects on small blood vessels) such as diabetic kidney disease (DKD), diabetic retinopathy and neuropathy. These complications lead to increased mortality, blindness, kidney failure and decreased quality of life for those affected (10).

Apart from type 1 and type 2 diabetes, other less common forms include gestational diabetes, Maturity Onset Diabetes of the Young (MODY) and Latent Autoimmune Diabetes in Adults (LADA).

Type 1 diabetes

Previously known as childhood-onset diabetes or insulin-dependent diabetes, type 1 diabetes is characterised by deficient insulin production by the pancreas (7), resulting from an auto-immune destruction of insulin-producing beta cells (11). The cause of this process is not known today, but it is hypothesised that it results from a combination of genetic and environmental factors, for example infectious diseases triggering an immune response in the body (11). The prognosis is in general more severe than in type 2 diabetes. Lifetime expectancy is still substantially shorter than in the general population, although treatment has improved in recent years (12).

A side theme in this thesis, discussed in Study III, is the long-term prognosis of type 1 diabetes. Historically, the largest relative improvements in prognosis occurred before the 1980's when insulin treatment was gradually refined. More ambitious treatment goals were implemented after results from the Diabetes Control and Complications Trial (DCCT) study were published in 1987, resulting in better health outcomes (13) (14). Data on incidence of complications and mortality from before these decades worldwide are, however, scarce. In Study III, such data are presented for the oldest cohort of men with type 1 diabetes that exists in Sweden.

Type 2 diabetes

The most common form of diabetes is type 2 diabetes, which most often debuts in adulthood. Raised blood glucose values are in general due to a combination of deficient insulin production in the pancreas and insulin resistance in the body tissues (7), making it difficult for glucose to be transported into the cells. The pathophysiology is complex and still under investigation. Recent research suggests that, since the disease is highly heterogenous, a new classification method should be used which divides diabetes into five different phenotypes (autoimmune, insulin-related, obesity-related, ageing-related and sex-related) (15).

The risk of developing type 2 diabetes increases with age, obesity and physical inactivity. Excess weight and in particular abdominal fat are factors associated with increased insulin resistance (16). There is a strong hereditary component associated with the risk of developing type 2 diabetes, but only a small proportion of the underlying genetic risk variants have so far been discovered (17). The standard treatment of type 2 diabetes consists of management of lifestyle factors as well as pharmacological treatment.

Type 2 diabetes is diagnosed through analysis of blood samples, i.e. blood glucose or hemoglobin A_{1c} (HbA_{1c}, a value that represents long-term blood sugar levels during 2-3 months), and/or presentation of symptoms. Diagnostic criteria are, as specified by the American Diabetes Association (ADA), a fasting plasma glucose ≥ 7 mmol/L, 2-h glucose (2 hours after intake of 75 grams of glucose after overnight fasting) ≥ 11.1 mmol/L, HbA_{1c} ≥ 48 mmol/mol or a random plasma glucose value of ≥ 11.1 mmol/L in a patient with classic symptoms. In the absence of symptoms, at least two of the above listed results within the diabetic range, from blood samples taken on different days, are needed to determine the diagnosis (16).

Pre-diabetes

Pre-diabetes is associated with a 70% risk of developing diabetes during the rest of the life course (18). The condition is characterised by the presence of insulin resistance and elevated blood glucose levels that do not quite reach the threshold of diabetes. The definition is having one of or a combination of the following: (1) impaired fasting glucose (IFG), i.e. fasting plasma glucose 5.6-6.9 mmol/L, (2) impaired glucose tolerance (IGT), i.e. 2-h glucose 7.8-11.0 mmol/L and/or (3) HbA_{1c} 39-47 mmol/mol.

It is debated whether more should be done to screen people who may have pre-diabetes, as implementation of lifestyle interventions in those affected could result in an improvement of their general health and quality of life in addition to a lower risk of

manifest diabetes (19). According to the ADA, screening for pre-diabetes should be considered in adults with a first-degree relative with diabetes, high-risk ethnicity (African American, Latino, Asian American, Native American or Pacific Islander), hypertension, hypercholesterolemia, a history of cardiovascular disease, polycystic ovarian syndrome, obesity, physical inactivity, or other conditions associated with insulin resistance (16).

Cognitive ability and cognitive testing

The word ‘cognition’ comes from the Latin verb *cognoscere*, meaning ‘to know’ or ‘to learn about’ (20). Abilities that are studied in cognitive research (in contrast to behavioural research), and that can be examined through cognitive testing, include thinking, memory, perception, problem solving, intelligence, reasoning, language and creativity. Intelligence, a more specific cognitive ability, is the capacity to think in the abstract, reason, problem-solve, and comprehend (21).

Different categories of cognitive tests are used for different purposes. Intelligence tests with g-factor (g = general intelligence) as a summary measure (22) are used in clinical practice, but also for the purpose of military recruitment. G-factor has been linked to neuronal function in biological studies, and is positively correlated with many brain functions (23).

Screening tests of dementia, a heterogenous set of disorders that can affect different cognitive domains, usually test a wider set of cognitive abilities than just intelligence or problem-solving, for instance memory and orientation. The most widely used cognitive screening tests for detecting cognitive impairment in adults are the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA) and the Mini-cog. These all take less than 15 minutes to perform and are tests of global cognitive ability. The MMSE tests orientation, registration, attention, calculation, memory and language (24). The test is in general more sensitive in detecting Alzheimer’s disease (AD) than vascular dementia (VaD) and mild cognitive impairment (MCI) (25). It is often desirable in clinical practice to test attention and executive functioning, as these domains affect the ability to drive, and are also impaired in early forms of several types of dementia. There are specific tests to assess these domains, for instance the Stroop test (26) and the Trail making test (27) used internationally, as well as A Quick Test of Cognitive Speed (AQT) mainly used in Sweden (28). The MoCA test includes a clock-drawing and a trail-making task to assess these abilities (29). There is also a separate Clock Drawing Test (CDT) (30) that is often used in combination with the MMSE.

Cognitive ageing, mild cognitive impairment and dementia

According to a recent meta-analysis, normal cognitive ageing is characterised by nearly linear declines in speed, and accelerating declines in memory and reasoning (31). The word ‘dementia’ comes from the Latin word *demens* meaning ‘out of one’s mind’. It is an umbrella term for several diseases that affect memory, other cognitive abilities and behaviour, that interfere with a persons’ ability to maintain daily activities. Advancing age is the strongest known risk factor for dementia, but dementia is per definition not a part of normal ageing (6).

There is currently a risk score that can be used for future prediction of dementia, the Cardiovascular Risk Factors, Ageing and Dementia (CAIDE) risk score, which takes into account age, sex, educational level, systolic blood pressure, BMI, total cholesterol, physical activity and the *APOE-ε4* genotype (32). It can be noted that many of these are common risk factors or risk markers for diabetes, although diabetes is not included in the score due to lack of current evidence on whether diabetes causes dementia or not.

Mild cognitive impairment (MCI) is a clinical term that describes a mental state that occurs along a continuum between normal ageing and dementia, and that in some cases could be reversible through lifestyle intervention (33). However, not all people with MCI develop dementia.

Alzheimer’s disease (AD)

The most common form of dementia is Alzheimer’s disease, which represents 60-70% of the cases (6). The initial phase is characterised by deficits in the ability to encode and store new memories. At later stages there are more progressive changes in cognition and behaviour (34). The underlying pathophysiological process is neurodegeneration, i.e. death of nerve cells, resulting in brain atrophy (see **Figure 1**). This is caused by changes in amyloid precursor protein (APP) cleavage and production of the APP fragment beta-amyloid ($A\beta$) as well as hyperphosphorylated tau protein aggregation (34). Several contributing factors to this process have been suggested, including metabolic, vascular, and inflammatory changes, as well as comorbid pathologies (34). There is currently no cure, although symptomatic treatment can have a modest effect on the progression of cognitive decline (34).

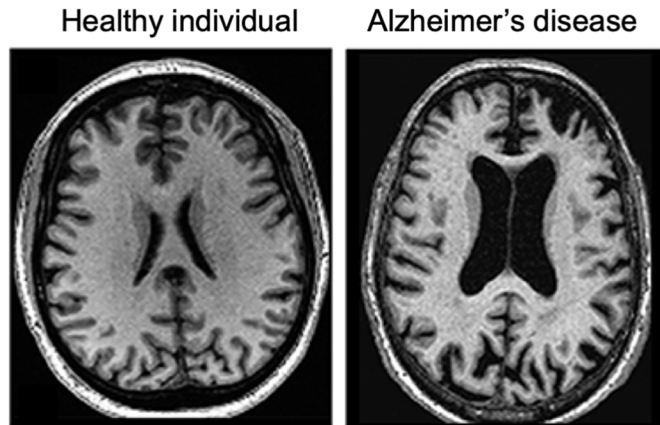


Figure 1.

Cerebral atrophy in Alzheimer's disease (right) compared to the brain of a healthy individual (left) on brain magnetic resonance imaging (MRI) scans.

Image credit: Ferreira D, Pereira JB, Volpe G, Westman E. Subtypes of Alzheimer's Disease Display Distinct Network Abnormalities Extending Beyond Their Pattern of Brain Atrophy. *Frontiers in Neurology*. 2019;10 (35). Reprinted with permission. This is a cropped version of the original figure.

Vascular dementia (VaD)

Vascular dementia (VaD) is the second most common cause of dementia and accounts for 15% of the cases (36). Clinical features are heterogenous, but are often characterised by loss of executive function, and milder memory loss as compared with AD (37). Atherosclerotic and cardioembolic diseases combined are the most common types of vascular brain injury that cause VaD. Cerebral small-vessel disease (CSVD) is the pathophysiological underlying condition in most cases, and is characterised by arteriolosclerosis (stiffening of small arteries), lacunar infarcts, cortical and subcortical microinfarcts and diffuse white matter changes involving myelin loss and axonal abnormalities (38). In both VaD and AD global brain atrophy and focal atrophy of the medial temporal lobe develops (38). However, the mechanism linking AD and VaD has not yet been identified (36). There are also several uncertainties regarding the relationship between cerebrovascular pathology and cognitive impairment, and also regarding the disease classification and diagnostic criteria (36). There is no curative treatment for VaD. Anticholinesterase inhibitors and memantine can, however, have modest effects on symptoms (39), and treatment of cardiovascular risk factors could potentially postpone the onset or even reverse disease progression (40) (41). **Figure 2** illustrates typical white matter lesions in CSVD.

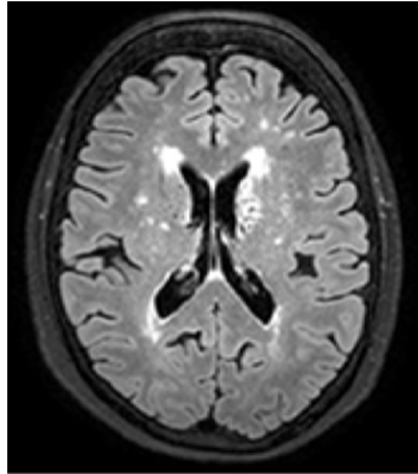


Figure 2.

Brain magnetic resonance imaging (MRI) scan of a brain with white matter hyperintensities (white areas), typical for cerebral small vessel disease (SVD), a common feature in people with vascular dementia.

Image credit: Sudre CH, Moriconi S, Rehwald R, Smith L, Tillin T, Barnes J, et al. Accelerated vascular aging: Ethnic differences in basilar artery length and diameter, and its association with cardiovascular risk factors and cerebral small vessel disease. *Frontiers in Cardiovascular Medicine*. 2022;9. (42) Reprinted with permission. This is a cropped version of the original figure.

Diabetes and effects on cognitive ability

The first reported study on associations between diabetes and cognitive ability was carried out by the American research scientists Walter Richard Miles and Howard Root in 1922. They had observed how patients with diabetes seemed to suffer from memory problems more often than other people, and proved this by administering cognitive tests on patients with and without diabetes (43). Since then there has been a growing body of evidence showing a clear epidemiological association between diabetes and cognitive impairment or dementia (8) (44).

Type 1 diabetes and cognitive ability

Type 1 diabetes is associated with moderate effects on cognitive functioning already in childhood (45) (46). The effects are most clear in children with early-onset diabetes (diagnosis during the first 4-7 years of life) (47). Type 1 diabetes can affect several cognitive domains (47), and has been associated with lower IQs in children (48) as well as with poor school results and low employment in young adults (49). Neuropathological processes could include gliosis, demyelination and altered

osmolarity (48). Both hyperglycaemia and hypoglycaemia episodes have been associated with reductions in grey matter volume in neuro-imaging studies (50), but further studies are needed.

In adults, type 1 diabetes is associated with a slowing of mental speed and a diminished mental flexibility, whereas learning and memory are often spared (51). These cognitive decrements are, however, in general less pronounced than in people with type 2 diabetes (52). Few studies have investigated long-term effects of type 1 diabetes on cognition, and the strength of the association with dementia in late life is somewhat unclear (53). One study estimated the relative risk (RR) of dementia due to type 1 diabetes to be 1.65 compared to people without type 1 diabetes (54).

Pre-diabetes, type 2 diabetes and cognitive ability

Type 2 diabetes is associated with mild cognitive decrements throughout life, as well as a two-fold risk of dementia (55). Cognitive domains that are often affected include verbal memory, information processing speed, attention and executive function (55). One study showed that the average cognitive performance of people with type 2 diabetes was around the 35th to 40th percentile of the general population (56). This is a small, and not necessarily clinically relevant, difference. The fact that the effects on cognition correlated with diabetes are relatively evenly distributed in different age groups, and that effect sizes are similar for different disease durations, may mean that this is a separate process from the development of dementia. However, lower cognitive ability may increase vulnerability to developing dementia (55), so the effect could be additive.

In people with MCI, diabetes is associated with a 1.5-3.0 times increase in the conversion rate to dementia (57) (58). It is likely that the conversion rate is also higher in people with pre-diabetes and MCI, compared with people with MCI without diabetes (55). The prevalence of MCI among people with type 2 diabetes was estimated to be 45% in a meta-analysis from 2021 (59).

It has not been firmly established until recently that diabetes is associated with an increased risk of dementia. A meta-analysis from 2012 summarised findings of longitudinal studies from 2006 to 2012, and concluded that diabetes is associated with a two-fold risk of dementia, and more precisely with a relative risk (RR) of 2.48 (95% confidence interval (CI) 2.08-2.96) for vascular dementia and a RR of 1.46 (1.20-1.77) for Alzheimer's disease (AD)

Possible reasons for the association between diabetes and negative effects on cognitive ability

Although an epidemiological association between diabetes and cognitive decrements or dementia has been established, the underlying mechanisms are largely unknown, and common risk factors could partly confound the association (60) (61) (62) (63). However, many studies have found associations irrespective of adjustment for such factors (lifestyle, demographics and health-related factors). Possible pathophysiological pathways behind the association include (a) negative effects of diabetes and associated biomarkers on vascular tissue, and (b) negative effects of these biomarkers on nerve cells. It is also possible that shared genetic risk variants for the two diseases could be partly responsible for the association. **Figure 3** shows an overview of this hypothesis.

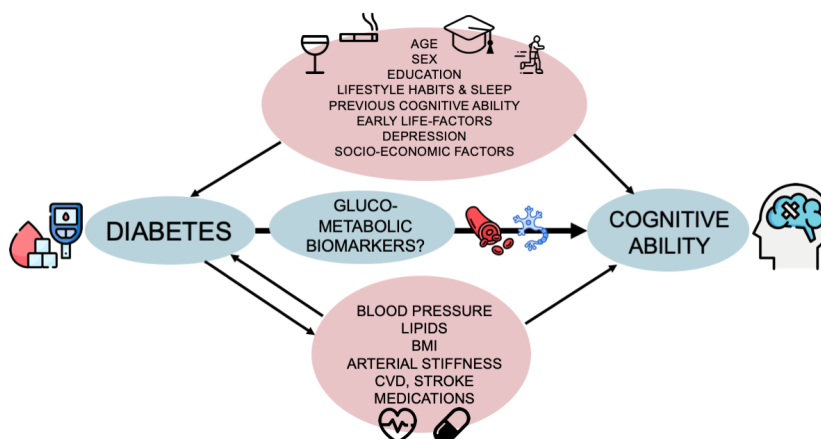


Figure 3.

An overview of the association between diabetes and cognitive ability, potential confounding factors and possible mediators.

BMI=Body Mass Index, CVD=Cardiovascular Disease

Vascular pathophysiological pathways

Other complications of diabetes, such as retinopathy, diabetic kidney disease, and atherosclerosis, are caused by damage to small and large blood vessels. It is therefore likely that this phenomenon also plays a significant role in the brain. Studies have for instance linked diabetic retinopathy to prevalence of cerebral small-vessel disease (CSVD) as well as cognitive impairment, indicating the possibility of microvascular damage in both organs simultaneously (64). Effects on microvascular structure may be caused by detrimental effects of hyperglycaemia, as in other organs (65). Adverse effects

of glucose metabolism on large vessels, i.e. causing both atherosclerosis (66) and arterial stiffness (67) (68), could also be a reason for the increased risk of CSVD. Furthermore, it has been hypothesised that disturbed blood-brain barrier (BBB) function (69) and neurovascular coupling (i.e. cross-talk between vascular tissue and other brain cells) (70) could play a role.

The two most consistent MRI findings in people with type 2 diabetes are slight brain atrophy (cortical and sub-cortical), and small subcortical (lacunar) infarctions (55). Brain atrophy has also been associated with duration of diabetes. White matter hyperintensities (WMH) and cerebral microbleeds have also been highlighted as characteristic for diabetes, but less consistently. While lacunar infarctions, WMH and cerebral microbleeds are all typical manifestations of CSVD, brain atrophy can be the result of both neurodegeneration and vascular damage to the tissue (71).

Links to Alzheimer's disease

Diabetes, obesity and cardiovascular disease are today considered as risk factors for the development of Alzheimer's disease (AD). It is, however, debated whether they contribute to the neuropathological process or not. In a study, people that had signs of AD post-mortem had a lower likelihood of having had diabetes and cardiovascular disease than those without AD-signs, suggesting that these factors are not likely to contribute to AD (72). On the other hand, mechanistic studies on biomarkers have proposed possible pathways, including oxidative stress, mitochondrial dysfunction, inflammation, damage to the BBB and negative effects on neuronal plasticity (73). Insulin resistance may also inhibit protective effects of insulin action on these processes (73). Furthermore, hyperglycaemia can induce both insulin resistance and accumulation of advanced glycation end products (AGEs), as described more below in the section on biomarkers (74).

Figure 4 shows a hypothetical overview of mechanistic pathways between diabetes and cognitive decline.

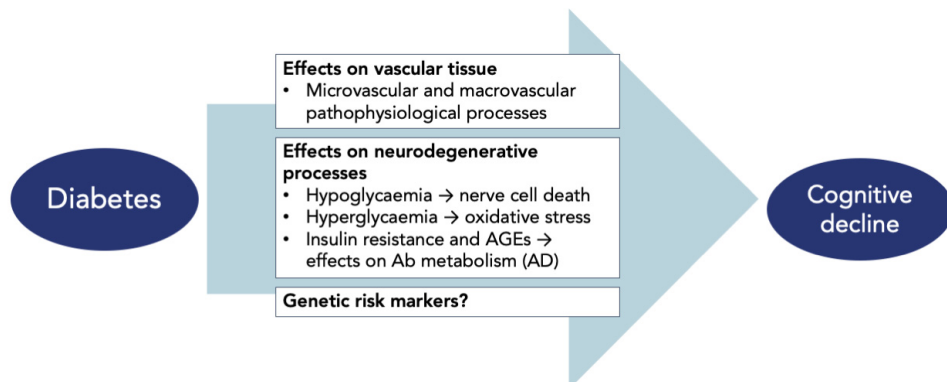


Figure 4.

An overview of different mechanistic hypotheses behind the association between diabetes and adverse effects on cognition.

AGE = Advanced glycation end products, Ab = Amyloid beta, AD = Alzheimer's Disease

Role of previous cognitive ability, socio-economic factors and lifestyle factors

Could the association between diabetes and dementia be due to confounding factors, such as shared risk factors? Occupation, low level of education and socio-economic status have for instance all been linked both to type 2 diabetes and dementia. However, a review recently stated that out of these factors, there is only enough evidence to conclude that educational level is linked to dementia risk (75). Furthermore, smoking, low level of physical activity, obesity and dietary factors such as intake of more trans-fat and saturated fat, have all been associated with the risk of cognitive decline (75) (76) (77) (78) (79), as well as with the risk of diabetes (80).

Another question is whether previous cognitive ability could modify the risk of cognitive impairment in diabetes. A reverse causation hypothesis has been proposed, i.e. the possibility of poor cognitive ability causing lack of compliance to treatment and therefore worse diabetes prognosis (81). Population-based studies have also shown that higher intelligence can protect against adverse health outcomes, for instance mortality (82) (83) and coronary heart disease (84) (83, 85), but also hyperglycaemia later in life (86) (87), which indicates that this is a factor to be considered also in people with diabetes.

Furthermore, cognitive ability but also the risk of cardiovascular disease and diabetes, can be affected by early life-factors. Such factors include both genetic factors,

intrauterine factors (e.g. low birth weight, poor foetal growth and short gestational age) as well as factors during the post-natal period and early childhood (e.g. psycho-social factors, poor nutrition and poor education) (88) (89) (90) (91) (92).

It has been shown that people with type 2 diabetes who are hypertensive are at greater risk of developing dementia and cognitive impairment than those without hypertension (93). Moreover, hypertension seems to affect the risk of cognitive decrements most at pre-diabetic stages (94). Other risk factors that could be associated with cognitive decline in diabetes are obesity and high blood lipid levels, but studies have been inconsistent (55). The effects of diet are also unclear, but one study showed that lower intake of saturated or trans fat and higher intake of polyunsaturated fat may reduce cognitive decline in people with type 2 diabetes (77).

Role of biomarkers of glucose metabolism

There are several biomarkers involved in glucose metabolism that have been associated with effects on cognitive ability.

Glucose

Studies have shown that both hyperglycaemia (glucose and HbA_{1c} levels) (78, 95) and repeated episodes of hypoglycaemia (96) are associated with negative effects on cognition, although all studies have not been consistent (97). This could be due to hyperglycaemia causing oxidative stress in both nerve cells and vascular tissue (98) and hypoglycaemia causes neuroglucopenia and nerve cell death (99).

Insulin

Insulin levels are chronically raised in type 2 diabetes as a result of high glucose levels over time. Insulin resistance has been negatively associated with cognitive test results, but a genetic study could not prove causality in the association (100). On the contrary, insulin has been shown to stimulate neuronal growth and inhibit apoptosis in nerve cells (101). Insulin resistance in the brain may disturb these neuroprotective effects (73), a phenomenon that has been identified in brain tissue of patients with AD (102). Furthermore, it has been hypothesised that insulin resistance in the brain may cause glycation of Amyloid beta (Ab) plaques and formation of neurofibrillary tangles (103).

Glucagon

Glucagon is a hormone that counteracts the effects of insulin. It is secreted from alpha-cells in the pancreas as a result of hypoglycaemia, leading to an increase in glucose levels. Glucagon has not yet been studied in relation to cognitive ability. Its receptors have

been found in the brain, but their function is unclear (104), and only trace amounts have been detected in the central nervous system (CNS) (105). It is not either known whether the hormone crosses the blood-brain barrier or not.

Incretin hormones

Incretin hormones are released from the small intestine after food intake, and thereafter stimulate the glucose-dependent insulin secretion from the pancreas. Two such hormones are glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1), which both cross the blood brain barrier (BBB) (106). GLP-1 receptor activation in different parts of the brain (hypothalamic nuclei, hindbrain nuclei, hippocampus and mesolimbic reward circuitry) can lead to reduced food intake and body weight (107). This is one of the benefits of GLP-1 analogue treatment (a type of anti-diabetic drug), in particular for people that are overweight or obese. Animal studies have also shown that GLP-1 analogue treatment may prevent cognitive decline (106) (108). There are some mechanistic hypotheses as to why. These include inhibition of oxidative stress, neuronal apoptosis, neuroinflammation and neurotoxicity, as well as inhibition of amyloid beta (Ab) and tau protein (AD-related biomarkers) (109). Randomised clinical trials are now being carried out on humans (110-113). A mouse model also showed that GIP analogues had neuroprotective properties on hallmarks of AD such as improved memory function, synaptic function and a reduced number of amyloid plaques (114). An overview of the effects of incretin hormones, as well as insulin and glucagon is illustrated in **Figure 5**.

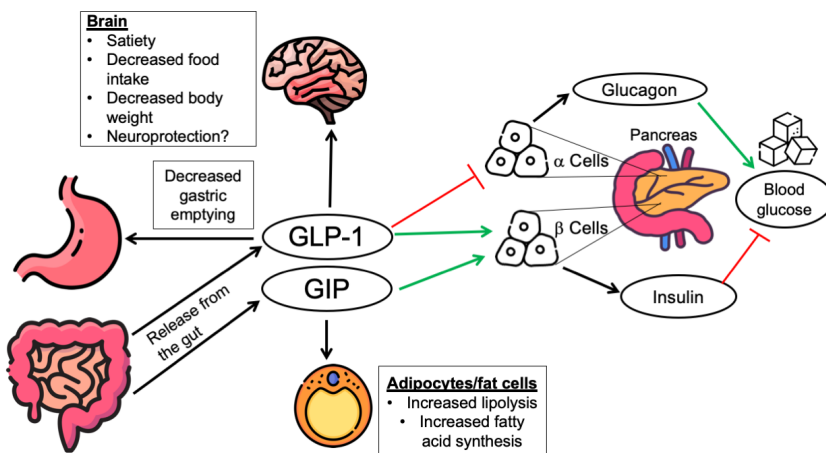


Figure 5.

Metabolism of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). For GLP-1 there is a well-established gut-brain axis as regards effects on satiety. It has also been proposed that GLP-1 has neuroprotective effects.

Advanced glycation end products

Advanced glycation end products (AGEs) are waste products of glucose metabolism that consist of lipids and proteins with attached glucose molecules. They can accumulate in different organs as a result of long-standing hyperglycaemia and inflammation (115). Raised levels of AGEs have been associated with AD, and have been hypothesised to contribute to AD-pathological processes such as glycation of Ab and phosphorylation of tau (116). Reduced dietary intake of AGEs has also been shown to prevent cognitive decline in older adults (117).

Role of systemic inflammation

Alongside vascular and metabolic pathways, systemic inflammation may play a role in causing cognitive decline in diabetes. People with diabetes have a proinflammatory state with higher levels of circulating inflammatory mediators than in the general population (118). Inflammation can also predict incident type 2 diabetes (119) as well as cognitive decline in type 2 diabetes (120) according to studies. Furthermore, most of the metabolic processes described in the previous section are also driven by inflammation. It is therefore possible that inflammation both has a separate role in causing cognitive decline in diabetes, as well as taking part in these metabolic processes.

Role of genetic risk variants

There is a possibility that genetic factors may influence the association between type 2 diabetes and risk of dementia. Studies on associations between known genetic risk variants of type 2 diabetes and the incidence of dementia are needed. Furthermore, Mendelian randomisation (MR) is a method in which causality can be investigated, through the use of genetic risk markers as instruments in the inference with an outcome.

Genetic terminology

To determine which variations in our DNA that are associated with certain traits such as diabetes, genome-wide association studies (GWAS) can be used to find such so-called genetic risk markers (121). A genetic risk marker that consists of a variation of a single base pair on the DNA strand is called a single nucleotide polymorphism (SNP). Hundreds of thousands of SNPs are analysed in GWAS studies for associations with the trait of interest. Those with genome wide significance, i.e. with $p < 5 \cdot 10^{-8}$ for the association with the trait, are considered risk variants for the trait. These are then validated in replication studies.

Genetics of type 2 diabetes

Type 2 diabetes is a highly hereditary condition and having one parent with type 2 diabetes is associated with a 40% risk of developing the condition (122). Although around 250 SNPs have been discovered using GWAS, these explain only a small proportion, around 10%, of the heritability (123). This is often referred to as a ‘missing heritability’ problem and is likely to be due to common variants that have small effects and have not yet been detected, and possibly also rare variants that are not well identified (123).

Polygenic risk scores

The summarised known genetic risk of a disease or a trait can be calculated from adding together the weights (effect size of the association with the trait) of the SNPs found in GWAS studies, into a so-called polygenic risk score (PRS) (124). In a PRS study, summary statistics from a GWAS study (a base data set, ideally the largest available genetic study) on known genetic risk variants (SNPs) can be used to identify the corresponding variants in a target data set (i.e. the scientist’s own data set), in relation to an outcome. As well as SNPs with genome wide significance, it is also possible to include lower-frequency risk alleles in the scores (with less stringent p-value thresholds).

Some PRS studies on type 2 diabetes (or associated traits such as insulin resistance) and prediction of dementia have been carried out, but with few significant results (125) (87). There is also a need for studies with validated dementia outcomes, as most studies previously have used register-based data, which, regarding dementia in particular, can be unreliable (126).

Mendelian Randomisation

Mendelian randomisation (MR) is a method for improved investigation of causal links between a trait and an outcome. The method builds on the fact that the genetic risk markers of the trait are not sensitive to environmental factors, nor to reverse causation. Only genetic variants that are strongly associated ($p < 5 \times 10^{-8}$) with the exposure variable (the trait) are included and used as instrumental variables in the analysis to predict the outcome (127). In a study with an exposure and an outcome, an instrumental variable is a third variable that can affect the outcome variable of interest but only through the exposure variable (128). **Figure 6** illustrates the principles behind MR analyses. The causal effect of A (exposure) on B (outcome) is based on the assumption that the relationship between G (genetic variants) and A is insensitive to confounders (U), and that causal links between G and B (with G as an instrumental variable) therefore reflects the causal effect of A on B (129).

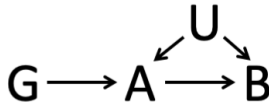


Figure 6.

Principle of Mendelian randomisation (MR). G is genetic variants, A is the putative causal trait (risk factor, in this case diabetes), B is the outcome of interest (in this case dementia), and U represents confounders.

In contrast to PRS analyses mentioned above, MR is designed to separate the causal effect between exposure and outcome from confounding. There are certain limitations of MR which must be considered when interpreting the results. These include (130):

- (1) *Pleiotropic effects*, i.e. that polymorphisms may have several phenotypic effects associated with disease. Such effects can be unknown although the MR-Egger method tries to account for them.
- (2) *Confounding* by other polymorphisms in linkage disequilibrium (i.e., that are largely correlated) with the polymorphism under study.
- (3) The possibility of *lack of suitable polymorphisms* for studying modifiable exposures of interest
- (4) *Canalization*, i.e. the buffering of the effects of genetic variation during development.

MR studies on whether type 2 diabetes is causally related to dementia have so far not been able to find causality in this association (60) (61) (62) (63). More studies are needed, especially using validated dementia endpoints with high diagnostic precision.

Rationale

There are still many gaps in our knowledge about the link between diabetes and cognitive ability or dementia.

One important question is whether pre-diabetes is associated with clinically relevant cognitive decrements. To determine this could lead a greater focus on preventive strategies, such as lifestyle interventions, at earlier stages of impaired glucose metabolism.

Another question is: Are there any glucometabolic biomarkers that are associated with worse or better cognitive performance in the population? There is a lack of population-based studies on for instance incretin hormones and their association with cognitive test results, as most studies so far have studied associations between these hormones and cognitive ability in rodents. Clinical trials are also currently investigating possible neuroprotective effects of incretin-based medications in people with diabetes (110-113), but whether these biomarkers are also correlated with cognition in the general population is unclear.

It has been proposed that cognitive ability early in life can modify the risk of future health outcomes in the general population (82) (83) (84) but it is unclear whether it affects the prognosis of diabetes.

Furthermore, it is not known whether any genetic risk markers associated with type 2 diabetes are also associated with dementia. More knowledge in this field could in the future help to develop and target treatment strategies and to target them at those with diabetes who are at greatest risk of developing dementia.

Finally, it is also unclear whether the association between type 2 diabetes and dementia is of a causal nature or not. The few studies that have been conducted have not found any causal inference (60) (61) (125) (63), but more are needed, especially with refined dementia endpoints.

Aims

The general aim of this thesis was to investigate the impact of diabetes and impaired glucose metabolism on cognitive performance and on the risk of dementia in a population-based setting.

The specific aims addressed in the four studies were:

1. To investigate associations between pre-diabetes and diabetes on the one hand and cognitive test results on the other, and also between fasting and 2-h glucose levels measured during the oral glucose tolerance test (OGTT) and cognitive test results
2. To investigate relationships between physiological levels of glucometabolic biomarkers and cognitive test results in a population-based setting
3. To examine associations between type 1 diabetes in young men, diagnosed before the age of 18, and long-term morbidity and mortality, and to investigate whether cognitive ability plays a role in long-term morbidity and mortality risk
4. To investigate associations between genetic risk markers of type 2 diabetes and validated dementia diagnoses, and to investigate possible causal associations between exposure and outcome through Mendelian randomisation analyses.

Methods

Study populations

The Malmö Diet and Cancer Study

Recruitment at baseline and representability

The Malmö Diet and Cancer Study (MDCS) is a population-based prospective cohort study from the city of Malmö in Southern Sweden. The baseline cohort was formed during 1991–96. The background population consisted of 74 138 people and was defined as all men in Malmö born 1923–45 and all women born 1923–50. The reason for including more women was because one of the original study aims was to study incidence of breast cancer. Participants were invited via letter or through responding to a newspaper advertisement.

In **Figure 7**, a flow chart of the study is presented. In this thesis, Study IV is based on the participants of the MDCS baseline study for which blood samples were drawn, and for which genetic data was later obtained, i.e. 30 446 people. The baseline population with full data including health examination, dietary registration and questionnaire data included 28 098 people. Reasons for non-participation have previously been described for these participants (131). The participation rate was 41% of the eligible participants identified in national registries, and 2/3 were women. Exclusion criteria were severe intellectual disability or language difficulties (N= 1 975 excluded for these reasons).

Since the participation rate was relatively low, a study was carried out to examine the representability of the participants, where the cohort characteristics were compared with a health survey on the background population (the Health Situation in Malmö '94 survey, HSM:94). The results showed that the MDCS was comparable to the HSM:94 population as regards socio-demographic structure, smoking and obesity. However, cardiovascular risk factors and mortality were less prevalent in the MDCS than in this comparative cohort, indicating that they were more prevalent in non-participants of the MDCS (131).

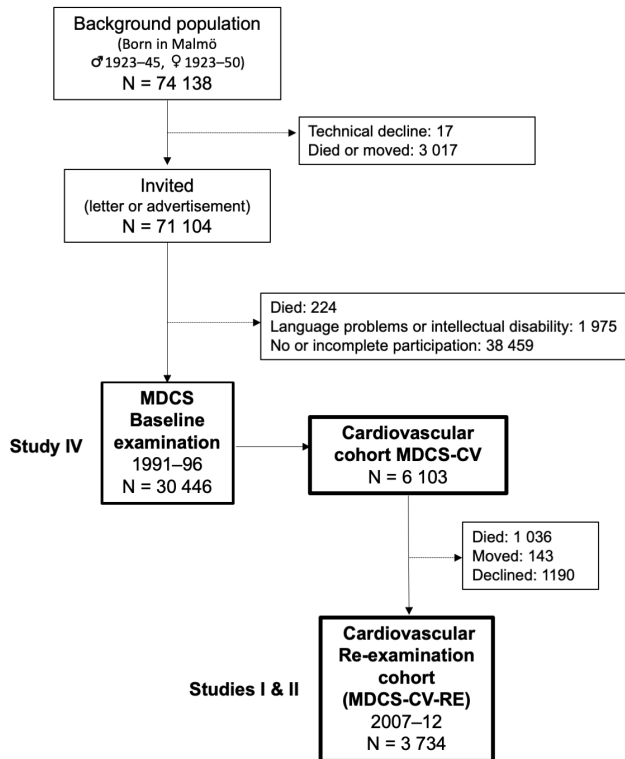


Figure 7.

The Malmö Diet and Cancer Study (MDCS). Flow-chart of recruitment to the baseline examination (1991–96) and to the Re-examination cohort 2007–12 (MDCS-CV-RE). Studies I and II are based on the MDCS-CV-RE, and Study IV on MDCS baseline data. The background population consisted of women in Malmö born 1923–50 and men born 1923–45. Recruitment from the background population was carried out first through a newspaper advertisement to which 5 500 responded. After that, the remaining population was invited via letter.

Cardiovascular sub-cohort

Out of the participants that entered the MDCS between November 1991 and February 1994 a random 50% were invited to participate in a study on the epidemiology of carotid artery disease (132). This sub-study, the Malmö Diet and Cancer Study Cardiovascular Cohort (MDCS-CV), comprised 6 103 participants.

Re-examination of the Cardiovascular sub-cohort

During 2007–12, the MDCS-CV participants were followed up for further health examinations (MDCS-CV Re-Examination). Out of 6 103 participants at baseline, 4 924 had not died or moved from Malmö, and were thus invited, out of which 3 734 attended the examination (133). A study compared baseline characteristics between attendees and non-attendees at follow-up, and found that the prevalence of

smoking and diabetes was higher in non-attendees than in attendees (133). Studies I and II in this thesis are based on the MDCS-CV cohort.

The Southern Sweden Diabetes in Conscripts Study (SSDCS)

The Southern Sweden Diabetes in Conscripts Study (SSDCS) is the oldest study of type 1 diabetes in Sweden. Data at baseline was collected by Sven E Nilsson during 1959–61. A flow-chart of the cohort is presented in **Figure 8**.

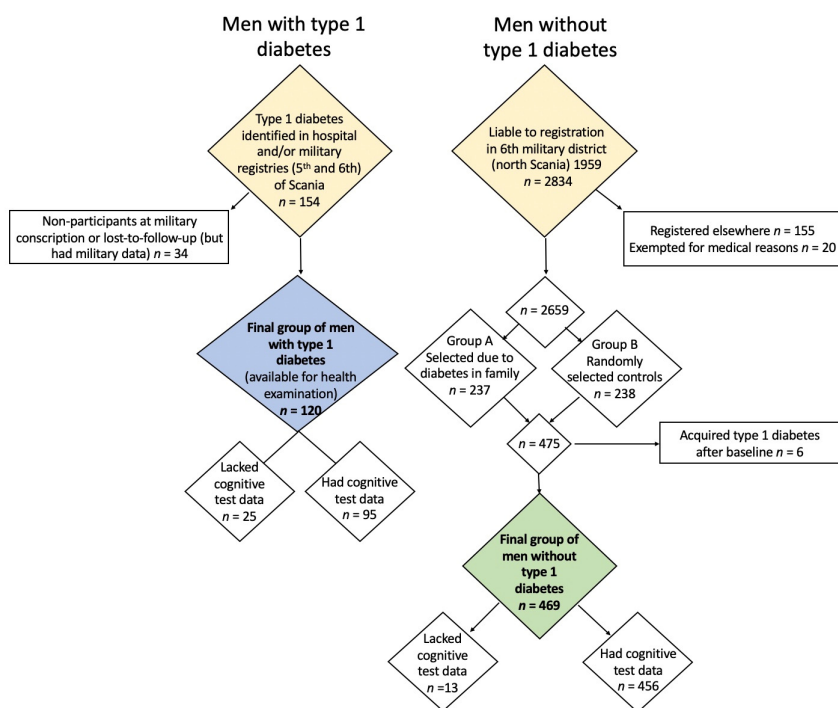


Figure 8. The Southern Sweden Diabetes in Conscripts Study (SSDCS). Flow-chart of participants with and without type 1 diabetes, reasons for non-participation or exclusion and information on availability of cognitive test data within each group.

Type 1 diabetes group

A group of 154 men with type 1 diabetes born during 1934–43 were identified in hospital registers from the whole of the province of Scania, Southern Sweden. Military conscription data on height, weight and cognitive test results at 18 years of age were also obtained for 139 of these men. All the 154 men were invited to a physical health examination six months after inclusion in the study, out of which 120 participated. Due to the delay that arose during the collection of these data, the 34 remaining

participants were lost to follow-up. However, military data for these individuals were available in summarised format (mean weight, height and cognitive test score). As it was assumed that hardly any children with diabetes would have been unidentified by the healthcare system in Scania, the group of 120 men was approximated to represent 80% of all men with type 1 diabetes in this area of the studied age group.

Control group

The aim of the original study by Sven E Nilsson was to study hereditary factors of type 1 diabetes (134). For this reason, two control groups were formed, one with and one without a family history of diabetes. A written inquiry was sent out to all men in the same school year in the northern half of the Scania province, to identify men with a family history of diabetes (defined as having a second degree relative). Those responding positively were included in the group with a family history of diabetes (n = 237). A control group (n = 238) without a family history of diabetes was also selected randomly from the same military district. The background population for this military district was 2 834 men liable to registration, out of which 20 did not attend for health reasons and 155 were registered elsewhere. In Study III, the two control groups were studied as a whole, apart from six men who were excluded due to acquiring type 1 diabetes, leaving a total of 469 men. These men were born in 1941 except for 23 who were born 1939–40 or 1942–43.

Follow-up

All participants in the study were followed up until 2016 as regards medical diagnoses and until 2018 as regards mortality data, as described in the section on data that follows.

Data and assessment tools

MDCS: Data at baseline

Questionnaire

The invited participants who attended the MDCS baseline examination filled in a questionnaire during their first visit. This included questions on education, occupation, physical activity, social network, use of tobacco and alcohol, current health, medical history, medications, as well as diseases in close relatives.

Physical examination and blood sampling

Blood pressure, height, weight and body composition were measured during the first visit to the lab. Blood samples were also drawn at baseline. Of relevance for the studies in this thesis, fasting glucose, HbA_{1c} as well as genotype were analysed. Genotyping was carried out in blood samples of all participants when technically possible (n=29 451). The blood samples were analysed using the Illumina GSA v1 genotyping array.

Validated dementia diagnoses

National Patient Register (NPR) diagnoses of dementia from the baseline examination until 31st December 2014 were obtained (n=2 206). These diagnoses covered 99% of all inpatient medical diagnoses, and from 2001 also outpatient diagnoses (135), although not diagnoses from primary care. The diagnoses were then validated by trained physicians at the Memory Clinic, Skåne University Hospital in Malmö. Details of the validation procedure have been described (136). In brief, the diagnoses were determined through assessment of medical records, including information on symptoms, cognitive test results, Computer Tomography (CT) and Magnetic Resonance Imaging (MRI) data (available for 86%) and, for 38%, also cerebrospinal fluid (CSF) biomarkers. The number of dementia cases after validation was 2 039 out of which 598 were classified as Alzheimer's disease (AD), 510 as vascular dementia (VaD), 578 as mixed dementia (AD+VaD) and 353 as other forms of dementia.

Clinical type 2 diabetes

Clinical type 2 diabetes at baseline (as defined in Study IV) was determined through combining information from different data sources, as the type of diabetes was not specified in the MDCS baseline questionnaire. The data sources included the Diabetes 2000 Register, the National Diabetes Register (NDR), the local HbA_{1c} registry, the Swedish Cause of Death Register, the Swedish National Patient Register (NPR), the Swedish Prescribed Drug Register and the Alla Nya Diabetiker I Skåne (ANDIS) regional Register. We also included information on diabetes from the MDCS as well as the cohort study the Malmö Preventive Project (data from blood samples, self-reported diabetes and drug use). Type 2 diabetes at baseline was classified as (a) having a diabetes diagnosis in any of the sources, (b) not having other specified types of diabetes and (c) not having self-reported insulin treatment as only diabetes treatment at baseline.

MDCS: Re-examination data

Questionnaire

A self-administered questionnaire was provided at follow-up including information on educational level, physical activity, smoking habits and alcohol habits. Educational level was classified as ≤ 10 years, 11-12 years or > 12 years of school. Physical activity was categorised into 'sedentary spare time', 'moderate exercise' and 'regular exercise'. Smoking habits were categorised into 'never-smoker', 'former smoker' and 'current smoker'. Alcohol intake was recorded as number of standard units per week. In our studies, we re-categorised this information into 'no consumption', 'consumption below risk level', and 'consumption above risk level' (> 9 standard alcohol units per week for women or > 14 for men according to Swedish guidelines).

Carotid-femoral pulse wave velocity

Using applanation tonometry (SphygmoCor, Atcor Medical, Australia), carotid-femoral pulse wave velocity (c-f PWV) was measured. This is a gold-standard method of assessing the level of arterial stiffness (137).

Physical examination and blood sampling

Body measurements (height, weight, hip- and waist), heart rate (HR) and blood pressure were measured at follow-up. Oral glucose tolerance testing (OGTT) was carried out, for which blood samples were drawn after overnight fasting and two hours after intake of 75 grams of glucose (with an exception for people with diabetes who only took a blood test at fasting). Plasma glucose, serum insulin, plasma glucagon, serum glucose-dependent insulinotropic peptide (GIP) and plasma glucagon-like peptide-1 (GLP-1) were also measured both at fasting and at two hours. The laboratory analyses were carried out at the Department of Clinical Chemistry, Skåne University Hospital, Malmö. The laboratory methods are described in **Table 1**.

Table 1. Laboratory methods of biomarkers analysed in the MDCS cohort.

ANALYSIS	LAB METHOD/SYSTEM	COMMENTS
Plasma glucose	HemoCue glucose system (HemoCue AB, Ångelholm, Sweden)	
Serum insulin	Dako ELISA kit (Glostrup, Denmark)	Minimum detection level 3 pmol/l, intra- and interassay coefficients of variation 5.1–7.5% and 4.2–9.3%. Haemolytic blood samples excluded: n = 190 at fasting, n = 205 at 2h.
Plasma glucagon	RIA GL-32K (Merck Millipore, Darmstadt, Germany)	Minimum detection level 18.5 pg/ml, intra- and interassay coefficients of variation 3.6–6.2% and 8.7–14.7% respectively.
Serum GIP	Millipore Human GIP Total ELISA #EZHGIP-54K (Merck Millipore)	Minimum detection level 1.65 pmol/l, intra- and interassay coefficients of variation 3–8.8%, and 1.8–6.1% respectively. Haemolytic blood samples excluded: n = 194 at fasting and n = 188 at 2h.
Plasma GLP-1	Radio-immunological analysis of intact GLP-1 and the metabolite GLP-1 9-36. N-terminally specific guinea pig anti-GLP-1 antiserum (Linco Research, St Charles, MO, USA) used for intact GLP-1 and C-terminally directed antiserum (code no. 89390) used for total GLP-1.	Minimum detection limit 1 pmol/l, intra- and interassay coefficients of variation 6.0% and 1.5%, respectively.

Insulin resistance and insulin sensitivity calculation

To calculate the level of insulin resistance, the homeostatic model assessment of insulin resistance (HOMA-IR) formula (138) was used:

$$\text{HOMA} - \text{IR} = \frac{\text{fasting glucose} * \text{fasting insulin}}{22.5}$$

To assess the level of insulin sensitivity we also used the formula the insulin sensitivity index ($\text{ISI}_{0-120 \text{ min}}$) which has been proven to more accurately reflect euglycaemic hyperinsulinaemic clamp measurements (139). First, glucose uptake m in mg/min is calculated, using glucose values at 0 min and 120 min and body weight (BW):

$$m = 75000mg + \frac{(0 \text{ min glucose} - 120 \text{ min glucose}) * 0.19 * BW}{120 \text{ min}}$$

Next, the metabolic clearance rate (MCR) is calculated through division of m with the mean plasma glucose value (MPG), i.e. (0 minute value+120 min value)/2.

$$\text{MCR} = m/\text{MPG}$$

Finally, the insulin sensitivity index is calculated using MCR and the mean serum insulin value (MSI), i.e. (0 minute value+120 min value)/2 (139).

$$ISI_{0-120min} = MCR/\log(MSI)$$

Advanced glycation end products (AGEs)

Advanced glycation end products (AGEs) were estimated for a sub-population of 454 randomly selected participants using skin-autofluorescence, measured by using an AGE reader, as shown in **Figure 9** below (140). Skin reflectance (per cent reflected fluorescent light) was also calculated as it may affect the results and should therefore be adjusted for in analyses.



Figure 9.

AGE-reader used to measure skin-autofluorescence, to estimate the level of advanced glycation end products (AGEs) in the skin.

Image credit: Ubelien de Weerd, Diagnostics, Netherlands.

Categories of glucose metabolism

Categories of normal or impaired glucose metabolism were created as follows using WHO 2006 criteria for OGTT measurements:

- Normal Glucose Tolerance (NGT): fasting glucose < 6.1 mmol/l and 2-h glucose < 7.8 mmol/l)
- Pre-diabetes:
 - Impaired Fasting Glucose (IFG): 6.1mmol/l ≤ fasting glucose < 7.0 mmol/l, or:
 - Impaired Glucose Tolerance (IGT) 7.8 mmol/L ≤ 2-h glucose < 11.1 mmol/L
- Type 2 diabetes: fasting glucose ≥7.0 mmol/L or 2-h glucose ≥11.1 mmol/L. People with self-reported diabetes or glucose-lowering medication at follow-up were also included in this category.

A sub-group of long-term diabetes was also created (diagnosis since baseline, determined through self-reported diagnosis, medication, HbA_{1c} or fasting glucose).

Cognitive testing

Cognitive testing sessions were carried out in collaboration with the Memory Clinic in Malmö, Skåne University Hospital. The tests included the Mini-Mental State Examination (MMSE) and A Quick Test of cognitive speed (AQT).

The MMSE is a widely used global cognitive screening test, which includes questions on orientation, memory, naming ability, ability to follow instructions, attention and ability to copy pentagons (141). It has been validated in Swedish populations (142)

The AQT is a test of attention and processing speed, and is used as a screening test to detect early stages of dementia. The test involves naming colour, shape and then both colour and shape of a set of geometrical figures on time, as shown in **Figure 10** (28) (143).

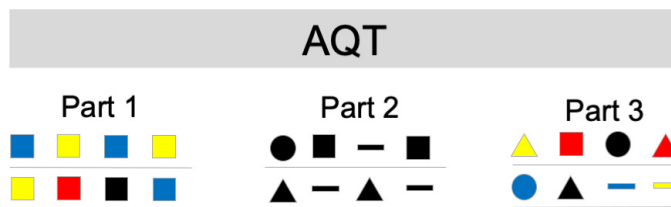


Figure 10.

The principle of A Quick Test of cognitive speed (AQT). (This is not the original version of the test designed by Pearson Education, Inc. TX, USA.) The test is timed and comprises naming (1) colour (2) type of geometric shape and (3) colour and type of shape for each shape at a time. The cognitive domains that are tested are processing speed and attention and executive function (the latter tested in part 3 only).

SSDCS: Data at baseline

Baseline data was collected during 1959–61 (134). Conscription data was obtained from military registers. The participants were also interviewed and examined six months after the conscription testing sessions. Body measurements as well as socio-demographic information were obtained. Education was classified as elementary school or less (≤ 7 years); secondary school or folk high school (8–11 years); or higher education (≥ 11 years). Socio-economic status was defined by the occupation of the father and was classified into three social groups (3 = highest status). From military registers, body measurements, muscle strength and cognitive test data were also available from conscription testing sessions during 1953–61, when the participants were 18 years old.

Cognitive test results

The cognitive tests used were based on the American ‘Army General Classification Test’ and previous versions of the intelligence test used for conscription examination in

Sweden (143). They included sub-tests on logic/general intelligence, verbal tests of synonym detection, tests of visuospatial/geometric perception and technical/mechanical skills with mathematical/physics problems. These were graded from 1-9, a standard-nine (stanine) scale, and then re-calculated into a summarised g-factor (a widely used measurement of general intelligence (22)), also with scale 1-9.

SSDCS: Data during follow-up

Data from national registers administered by the National Board of Health and Welfare were collected. Individuals in the cohort were identified through their Swedish personal identity numbers. The outcomes during follow-up included mortality data from the Swedish Cause of Death Register (from the start of the register in 1961 until the end of 2018), and medical diagnoses from the National Patient Register (from the start of the register in 1964 until the end of 2016). The latter had full national coverage from 1987 and onwards (144). The diagnoses were classified according to the International Classification of Diseases (ICD) system established by the WHO, versions 7-10. Both the Cause of Death Register and the National Patient Register have an estimated validity of over 90% (145) (135).

Statistical Methods

Software

Statistical analyses were performed using SPSS versions 22-25 for Macintosh, with Process version 3.4 for mediation analyses (Study II). R studio version 1.4.1717 and PLINK 2 were also used in Study IV. Figures in Study IV are designed using Prism 9 for MacOS.

Data preparation

In Study I and II, multiple imputation with five consecutive imputations was used to estimate missing data values in covariates. To minimise ceiling effects in the variable MMSE (cognitive test data, Study I-II), we used a validated normalising transformation method (146). In all studies, continuous covariates in the analyses were re-calculated into natural logarithmic values when needed to achieve normal distribution. We also re-calculated the biomarker lab data (Study II) into standardised values. Reasons for

exclusion of participants from the studies are shown in **Table 2**. Quality control (QC) was performed for the genetic data (see Study IV for more details).

Polygenic Risk Score Calculation

For Study IV, we created Polygenic Risk Scores (PRS) of genetic variants (SNPs) associated with (a) type 2 diabetes, (b) fasting glucose (c) fasting insulin and (d) HbA_{1c}. First, summary statistics from large publicly available datasets with such information were downloaded (17). To ensure that the data was not overloaded with correlated SNP sets, linkage-disequilibrium (LD) clumping was performed (see Study IV for details). The PRS was determined for each individual by summing up the effective number of alleles (0, 1, 2) of the SNPs weighted by the natural logarithm of their respective odds ratio (OR). The default formula for PRS calculation in PLINK, where i is the base dataset and j is the target dataset, is:

$$PRS_j = \frac{\sum_i^N S_i * G_{ij}}{P * M_j}$$

where the effect size of SNP i is S_i , the number of effect alleles observed in sample j is G_{ij} , the ploidy of the sample is P (is generally 2 for humans), the total number of SNPs included in the PRS is N , and the number of non-missing SNPs observed in sample j is M_j . If the sample has a missing genotype for SNP i , then the population minor allele frequency multiplied by the ploidy ($MAF_i * P$) is used instead of G_{ij} (147).

Seven PRS scores (PRS 1-7) were hereby created, using different p-value thresholds for each score: $p = 0.05$ (PRS 1), $p = 5 \times 10^{-3}$ (PRS 2), $p = 5 \times 10^{-4}$ (PRS 3), $p = 5 \times 10^{-5}$ (PRS 4), $p = 5 \times 10^{-6}$ (PRS 5), $p = 5 \times 10^{-7}$ (PRS 6) and the GWAS-level significance thresholds of $p = 5 \times 10^{-8}$ (PRS 7). The reason for doing this is that the optimal p-value threshold is unknown, why this method is often used in genetic studies (147).

Descriptive analyses (Study I-IV)

In Studies I, II and IV Chi-square tests were performed to compare frequencies of data in categorical variables between groups of different gluco-metabolic categories. For continuous variables, one-way between groups analyses of variance (ANOVA) were used in Study I (comparison of three groups) and independent-samples t-tests were used in Study II and IV. In Study III we first calculated means and standard deviations of continuous variables and proportions of categorical variables across the whole study sample. We then used logistic regression models with type 1 diabetes as dependent

variable to calculate differences in the variables between the group with and without diabetes.

Main analyses

Study I

General linear model analyses (GLM) were performed to compare adjusted mean cognitive test results in participants with normal glucose tolerance (NGT) as a reference group and groups with pre-diabetes and diabetes. Linear trends across categories were also investigated in multiple regression analyses. Post-hoc analyses were performed with diabetes categorised into short-term or long-term diabetes. We adjusted for age, sex, education, physical activity, smoking habits and alcohol habits in *Model 1* (possible confounding factors that can affect both risk of diabetes and cognitive decline). In *Model 2* (adjustment for cardiovascular risk factors), we adjusted for factors in *Model 1* and additionally systolic blood pressure (SBP), heart rate (HR), carotid-femoral pulse wave velocity (c-f PWV), waist circumference, total cholesterol, and anti-hypertensive, lipid-lowering and glucose-lowering drug treatment. Interactions between covariates and effect on the association between diabetes and cognitive test results were tested in multiple regression analyses. We then stratified for these variables in further GLM analyses when interaction terms were significant. A sensitivity analysis excluding participants with stroke was also performed.

We then carried out multiple regression analyses to examine associations between fasting- and 2-h glucose levels and cognitive test results, using the same adjustment models. These analyses were also performed for the group without diabetes.

Study II

Multiple regression analyses were performed with biomarkers (standardised) as exposure variables and cognitive test results (MMSE, AQT) as outcomes. The results were then stratified for diabetes/no diabetes, and in a supplementary analysis also into more detailed groups (NGT at both baseline and follow-up, pre-diabetes at follow-up, diabetes at follow-up but not at baseline and diabetes since baseline). Covariates in the adjustment models were as follows: *Model 1*: Adjustment for age, sex, education, physical activity, smoking habits and alcohol habits. *Model 2*: Adjustment for factors in *Model 1* and systolic blood pressure, waist circumference, total cholesterol, and anti-hypertensive, lipid-lowering and diabetes drug treatment.

Mean cognitive test results for quartiles of each biomarker were then visualised in box-plots. In further multiple regression analyses, we explored interactions of diabetes on

the associations between biomarkers and cognition, in models equivalent of *Model 1*. Biomarkers that were significantly correlated with cognitive test results were then analysed as mediators in mediation analyses with diabetes as exposure and cognitive test results as outcome.

Study III

We investigated the impact of type 1 diabetes at baseline on risk of future negative outcomes (mortality, cardiovascular mortality, cardiovascular events and diabetes complications), through univariate Cox regression analyses with type 1 diabetes as predictor of outcomes. Age was used as time scale, to compensate for group differences in year of birth. The participants were followed for a maximum of 77 years, so that the length of the follow-up period would be the same for cases and controls. A propensity score adjusted supplementary analysis was also performed to account for possible socio-economic differences between the groups (see Study III).

We then tested interactions between cognitive ability at baseline and associations between diabetes status and health outcomes, through Cox regression analyses with type 1 diabetes, g-factor (binary variable, 0=1-5, 1=6-9), and type 1 diabetes*g-factor as covariates. We also stratified for type 1 diabetes to assess which group was most affected by g-factor.

Kaplan-Meier curves were created using log-rank tests, also with age as time scale, to visualise incidence of outcomes over time in groups with and without diabetes, subgrouped into high or low to medium g-factor (i.e. 4 groups).

Study IV

Epidemiological associations between clinical type 2 diabetes at baseline and time until first dementia event or censoring were investigated in Cox regression analyses, adjusted for age, gender and education in *Model 1*, and these factors as well as smoking, alcohol consumption, physical activity, SBP, BMI, ApoB/ApoA-ratio, history of CVD (stroke or coronary event), use of anti-hypertensive medication and use of lipid-lowering treatment in *Model 2*. An interaction model including *APOE-ε4* status (0 or 1-2 alleles), equivalent of *Model 1*, was also performed, as well as an analysis stratified for *APOE-ε4* carriership.

To test whether SNPs in PRS 1-7 of type 2 diabetes were correlated to clinical type 2 diabetes, binary logistic regression analyses were performed.

We then investigated associations between PRS 1-7 of type 2 diabetes, fasting glucose, fasting insulin and HbA_{1c} as exposure variables and validated dementia endpoints as outcome variables in Cox regression analyses. We adjusted for age, gender and

education in *Model 1*, and these factors and additionally *APOE-ε2* and *APOE-ε4* genotype (0, 1 or 2 alleles) in *Model 2*. We also tested interactions between *APOE-ε4* status (0 or 1-2 alleles), and PRS 1-7 for type 2 diabetes on the association with dementia, and performed stratified analyses (equivalent of *Model 2*). A sensitivity analysis using MAF $\geq 1\%$ was also performed.

We also carried out 2-sample MR analyses using the same reference dataset (17) to test whether type 2 diabetes shared genetic effects with any of the dementia subtypes. (We did not carry out MR for the exposure variables fasting glucose, fasting insulin or HbA_{1C}, as there were not enough known SNPs for these traits with genome wide significance.) The MR test was based on 243 independent variants (used as instrumental variables) of type 2 diabetes with a genome-wide significant association at $p < 5 \times 10^{-8}$.

Different mathematical models of Mendelian randomization (MR) were used to calculate the causal effect between exposure (type 2 diabetes) and outcome (dementia types). These included the methods ‘simple mode’ and ‘weighted mode’ where the most common effect size of the causality is calculated, where the latter means that weights are assigned to each instrumental variable (148), ‘weighted median’ (the weighted median value of the causal effect of all the genetic variants), ‘inverse variance weighted’ (where a summarised effect size is approximated through a formula often used in meta-analyses (149)), and ‘MR Egger’, where the size of estimated pleiotropy is also accounted for (149).

The conventional MR methods (all above except MR Egger) are based on that certain instrumental variable assumptions are satisfied. These are (1) that the genetic variants of the trait are associated with the risk factor (trait), (2) that each genetic variant is independent of confounders of the association between exposure and outcome (pleiotropic effects), and (3) that if the risk factor was kept constant, intervention on the genetic variant would not have an effect on the outcome.

The MR Egger method is designed to estimate the pleiotropic effect and to separate this from the direct causal effect between exposure and outcome (149). This is done by calculation in three steps including a test for directional pleiotropy, a test for causal effect and then an estimate of the causal effect. This estimate of the causal effect is tested under an assumption called the Instrument Strength Independent of Direct Effect (InSIDE) assumption, which assumes that the pleiotropic effects are independently distributed from the genetic associations with the risk factor (149).

Additional analyses

For the purpose of interpreting the findings in this thesis, some additional analyses were carried out. Multiple regression analyses were performed to analyse explained variance of pre-diabetes and diabetes (as in Study I) by cognitive test results. Linear regression analyses were also performed to calculate explained variance of clinical type 2 diabetes by PRS for type 2 diabetes (as in Study IV).

To explore whether our exposure and outcome variables were stable over time or not, we tested how well fasting glucose at baseline correlated with fasting glucose at follow-up for the MDCS-RE participants, through calculating the proportions of people with normal fasting glucose (NFG), IFG and fasting glucose over the threshold of diabetes that stayed within the same category or changed category at follow-up.

Next, to determine whether cognitive test results correlated with dementia diagnoses, mean cognitive test results within participants with or without dementia within the MDCS-CV-RE were calculated, and independent samples t-tests were performed to determine significant differences between groups.

Finally, a Kaplan-Meier analysis with a log-rank test was performed, to visualise the epidemiological association (unadjusted) between clinical type 2 diabetes and all-cause dementia, as defined in Study IV.

Table 2. Overview of study-specific methods in this thesis.

STUDY	COHORT & TYPE OF STUDY	SAMPLE SIZE (N)	EXCLUDED	EXPOSURE	OUTCOME	STATISTICAL ANALYSES
I	MDCS-CV-RE Cross-sectional	2994	289 Non-scandinavian, 113 no country of birth, 439 missing data	Pre-diabetes, type 2 diabetes fasting glucose, 2-h glucose (post-OGTT)	Cognitive test results (MMSE, AQT)	Multiple linear regression analyses
II	MDCS-CV-RE Cross-sectional	3001	289 Non-scandinavian, 113 no country of birth, 331 missing data	Insulin, glucagon, incretins (fasting and 2-h) & AGEs in sub-population, n=454	Cognitive test results (MMSE, AQT)	Multiple linear regression analyses
III	SSDCS Prospective cohort study of case-control study at baseline (longitudinal)	120 type 1 diabetes, 469 controls	5 controls with type 1 diabetes	Type 1 diabetes & cognitive ability at baseline	Mortality, cardiovascular mortality, cardiovascular events and diabetes complications	Cox regression analyses
IV	MDCS baseline Longitudinal & Mendelian randomisation	29 139	995 no blood sample, 72 missing data (blood sample number or age), 240 failed QC	Genetic risk markers of type 2 diabetes	Validated dementia diagnoses	Cox regression analyses & Mendelian randomization

AGE = Advanced glycation end products , AQT = A Quick Test of cognitive speed, MDCS-CV-RE = Malmö Diet and Cancer Study Cardiovascular Cohort Re-examination, MMSE = Mini-Mental State Examination, QC = Quality control

Ethics

Ethical approval

The MDCS was approved by the Ethical Committee of Lund University, Sweden (MDC baseline examination LU-51-90, MDC Re-examination Dnr. 532/2006). Written informed consent was obtained from all study participants. The SDCSS was approved by the Regional Ethical Committee in Linköping (Dnr 2011/15-31) and in Gothenburg (Dnr 2017/461-32). Written informed consent was obtained from all participants. All procedures performed in the MDCS and SDCSS were in accordance with the Declaration of Helsinki as revised in 2008.

Ethical considerations

Considerations regarding cognitive testing

There are always certain ethical problems to consider when performing cognitive tests. For example, when screening people from a population-based cohort, there is a risk of over-diagnosis, as well as a risk of possible negative feelings among those with poor cognitive test results that would not otherwise have got themselves tested, i.e. did not feel troubled by their symptoms. Another aspect is the possibility that certain people with impaired cognition were not completely informed. However, all participation was voluntary, and we have concluded that the benefits outweigh the negative aspects. For example, all people with sub-normal cognitive test results were referred straight to the Memory Clinic in Malmö for further assessment.

Risk of misinterpretation of the results

Most results in this thesis are applicable to patient groups of today, but it is important to remember that the future might be different as the prognosis of both type 1 and type 2 diabetes is improving over time. For example, in Study III, prognostic data on type 1 diabetes are presented for a group of patients that lived during a time period when different treatment options were available to the ones available today. This should be remembered when interpreting these results.

Results

In this section, the main results of the Studies I-IV are presented. For more details, see the individual published papers (Study I-III) and manuscript (Study IV) included in this thesis.

Characteristics of Study Populations

MDCS participants (Study I, II and IV)

MDCS participants were on average 58 years at baseline (1991–94, Study IV), and 72 years at follow-up (sub-population, MDCS-CV-RE, 2007–12, Studies I-II). The proportion of women was 60% both at baseline and follow-up.

In Study I-II (MDCS-CV-RE participants), cognitive test results were in general lower and cardiovascular risk factors more prevalent in categories of pre-diabetes and diabetes, but there were no significant differences between the groups in educational level or smoking status.

In Study IV (MDCS baseline participants), the prevalence of all-cause dementia was 11% in participants with type 2 diabetes compared to 7% in participants without type 2 diabetes. *APOE-ε2* and *APOE-ε4* genotype was not significantly different between these groups.

SDCSS participants (Study III)

The group of men with type 1 diabetes had higher average cognitive ability compared to the group without type 1 diabetes at baseline (mean g-factor 5.24 compared to 4.51, $p < 0.001$). There were no significant differences in educational level or socio-economic status between the groups. The 34 participants that were lost to follow-up at baseline had the same mean g-factor (general cognitive ability) as the included participants.

Pre-diabetes, type 2 diabetes and cognitive test results (Study I)

Adjusted mean cognitive test results in categories of NGT, pre-diabetes and diabetes respectively are shown in **Table 3**. Pre-diabetes and diabetes were associated with slightly lower cognitive test results as compared to the group with NGT. This applied both to MMSE results (global cognition, 1.9 or 2.0 normalised MMSE points/100 difference between the NGT group and groups of pre-diabetes and diabetes respectively), and to AQT results (processing speed, attention and executive function, 3.0 and 5.2 seconds slower in total test time for groups of pre-diabetes and diabetes respectively compared to NGT). This was found in *Model 1* that was adjusted for age, sex, education, smoking, alcohol consumption and physical activity. When additionally adjusting for cardiovascular risk factors in *Model 2*, differences in cognitive test results between the categories and trends across the categories were in general non-significant. In post-hoc analyses, the difference in results for a group with long diabetes duration (since baseline) was greater, i.e. 5.7/100 MMSE points ($p < 0.01$) and 17.8 seconds of AQT test time ($p < 0.001$).

There was a significant interaction between age and physical activity on the associations between the glucometabolic categories and AQT results in *Model 1*. Stratified analyses showed that the relationship between diabetes and poor AQT results was stronger in individuals that are older or less physically active.

Table 3. General linear models (GLM) of adjusted mean cognitive test results of groups with NGT, pre-diabetes and diabetes.

	Model 1, n=2994	Model 2, n=2994
MMSE (points/100, normalised)		
NGT	80.4 (79.7-81.1)	80.0 (79.3-80.8)
Pre-diabetes	78.5 (77.6-79.4)**	78.3 (77.4-79.2)**
Diabetes	78.4 (77.2-79.5)**	79.8 (78.3-81.2)
P for trend across categories	0.001	0.143
AQT (time in seconds)		
NGT	130.8 (129.5-132.2)	131.8 (130.3-133.1)
Pre-diabetes	133.8 (132.0-135.5)*	134.0 (132.2-135.8)
Diabetes	136.0 (133.8-138.2)*	133.2 (130.5-135.9)
P for trend across categories	<0.001	0.125

* $p < 0.05$, ** $p < 0.01$ of difference in mean test results between NGT and each other category.

Model 1: Adjusted for age, sex, education, physical activity level, smoking habits and alcohol consumption.

Model 2: Adjusted for factors in *Model 1* and cardio-metabolic risk factors: Systolic blood pressure, heart rate, c-f PWV, waist circumference, total cholesterol levels and medications (anti-hypertensive, anti-diabetic and lipid-lowering treatment).

Biomarkers of glucose metabolism and cognitive test results (Studies I-II)

Glucose

As shown in Study I, associations between fasting and 2-h glucose as exposure, and MMSE and AQT results as outcome for the whole study population are presented in Table 4. All associations were significant in adjustment *Model 1*, indicating that higher glucose levels at fasting and at two hours were correlated with worse cognitive test results. When adjusting for cardiovascular risk factors in *Model 2*, there was a significant correlation between 2-h glucose and MMSE scores, but not for the other combinations of exposure and outcome variables.

We then tested the same associations in all participants *without* diabetes in the study sample. The associations in *Model 1* were significant also in this set of analyses, and for most combinations of exposure and outcome variables in *Model 2*.

Table 4. Multiple linear regression analyses of linear relationships between fasting and 2h-glucose respectively and cognitive test results. Unstandardised regression coefficients (B) and p-values for the associations are shown.

	Model 1		Model 2	
	B	p	B	p
ALL PARTICIPANTS				
Fasting glucose (n=2991)				
MMSE total score	-5.325	<0.001	-2.720	0.135
AQT total score	0.087	<0.001	0.034	0.188
2h-glucose (n=2671)				
MMSE total score	-2.147	0.012	-1.787	0.046
AQT total score	0.033	0.006	0.023	0.072
PARTICIPANTS WITHOUT DIABETES				
Fasting glucose (n=2484)				
MMSE total score	-8.323	0.001	-5.860	0.030
AQT total score	0.098	0.004	0.035	0.360
2h-glucose (n=2433)				
MMSE total score	-2.961	0.005	-2.563	0.019
AQT total score	0.042	0.004	0.030	0.046

Model 1: Adjusted for age, sex, education, physical activity level, smoking habits and alcohol consumption.

Model 2: Adjusted for factors in *Model 1* and cardio-metabolic risk factors: Systolic blood pressure, heart rate, c-f PWV, waist circumference, total cholesterol levels and medications (anti-hypertensive, anti-diabetic and lipid-lowering treatment).

AQT = A Quick Test of cognitive speed, MMSE= Mini-Mental State Examination

Insulin, glucagon, GIP, GLP-1 and AGEs

In Study II, cross-sectional associations between biomarkers of glucose metabolism (insulin, glucagon, GIP, GLP-1 and AGEs) were tested in MDCS-CV-RE participants. Table 5 presents associations between these biomarkers and cognitive test results of MMSE (normalised score 1-100 points) and AQT (measured in time). The results are presented as mean cognitive test result per 1 SD increment of each biomarker.

Table 5. Multiple regression analyses of changes in mean cognitive test results (MMSE and AQT results) per 1 standard deviation (SD) increment of each biomarker or index (n=3001). Biomarkers with significant associations with cognitive test results are highlighted in yellow.

	N	Model 1		Model 2	
		Means per 1 SD incr	p	Means per 1 SD incr	p
A. MMSE (points/100, normalised)					
S-Insulin 0 min (pmol/L)	2843	-0.475	0.07	-0.251	0.42
S-Insulin 120 min (pmol/L)	2525	-0.461	0.10	-0.437	0.16
HOMA-IR	2841	-0.734	0.006	-0.427	0.19
ISI (0-120 min)	2396	0.822	0.004	0.820	0.010
P-Glucagon 0 min (pg/mL)	2991	0.289	0.28	0.534	0.05
P-Glucagon 120 min (pg/mL)	2668	0.596	0.026	0.640	0.017
S-GIP 0 min (pmol/L)	2840	0.050	0.85	0.299	0.27
S-GIP 120 min (pmol/L)	2525	0.581	0.040	0.603	0.034
P-GLP-1 0 min (pmol/L)	2956	-0.544	0.033	-0.469	0.07
P-GLP-1 120 min (pmol/L)	2525	0.585	0.038	0.606	0.033
Skin autofluorescence (AU)*	454	-1.459	0.030	-1.235	0.07
B. AQT (seconds)					
S-Insulin 0 min (pmol/L)	2828	0.003	0.47	-0.001	0.80
S-Insulin 120 min (pmol/L)	2510	0.007	0.07	0.008	0.07
HOMA-IR	2826	0.007	0.08	0.001	0.87
ISI (0-120 min)	2382	-0.010	0.018	-0.010	0.025
P-Glucagon 0 min (pg/mL)	2975	0.006	0.13	0.002	0.56
P-Glucagon 120 min (pg/mL)	2653	0.002	0.52	0.001	0.70
S-GIP 0 min (pmol/L)	2826	0.002	0.57	-0.001	0.73
S-GIP 120 min (pmol/L)	2510	-0.001	0.80	-0.001	0.89
P-GLP-1 0 min (pmol/L)	2940	0.008	0.022	0.008	0.037
P-GLP-1 120 min (pmol/L)	2510	-0.001	0.81	-0.001	0.90
Skin autofluorescence (AU)*	454	0.012	0.18	0.010	0.29

Model 1: Adjusted for age, sex, education, smoking, alcohol consumption and physical activity.

Model 2: Adjusted for age, sex, education, smoking, alcohol consumption, physical activity, systolic blood pressure, waist circumference, total cholesterol, anti-hypertensive treatments, lipid lowering treatment and diabetes treatment.

* Analyses with skin autofluorescence are also adjusted for skin reflectance.

AQT= A Quick Test of cognitive speed, AU = arbitrary units, GIP= glucose-dependent insulinotropic peptide, GLP-1 = Glucagon-like peptide-1, HOMA-IR= homeostatic model of insulin resistance, ISI = insulin sensitivity index, MMSE= Mini-Mental State Examination.

Biomarkers that were significantly associated with cognitive test results were, in summary:

- Negatively associated with cognitive test results: HOMA-IR, fasting plasma GLP-1, AGEs (skin autofluorescence)
- Positively associated with cognitive test results: ISI_{0-120 min}, 2-h plasma glucagon, 2-h serum GIP, 2-h plasma GLP-1

We also performed the same analyses but only including participants without diabetes. All significant associations in the Table above were also significant when tested in this group, apart from results regarding 2-h GLP-1.

Out of the strongest significant associations that were found, one SD increment of each biomarker corresponded to approximately 2 normalised MMSE score points difference or one second difference in AQT time. The effect sizes corresponded to 4 years of age difference as regards MMSE results (Beta = -0.50, $p < 0.001$) and 10 years as regards AQT results (Beta = 0.010, $p < 0.001$), adjusted for *Model 1*.

Type 1 diabetes, cognitive ability and risk of incident cardiovascular disease and mortality (Study III)

Long-term prognosis of men with type 1 diabetes

In Study III, we analysed incidence of cardiovascular morbidity and mortality in a group with type 1 diabetes and a control group, with participants born 1934–41 who were followed from age 18 until 2018. First, unadjusted analyses were performed. The results are presented in **Table 6**. The group of young men with type 1 diabetes, compared with the control group, had a 4.6 times increased HR of all-cause mortality, 5.6 times increased HR of cardiovascular mortality and 4.0 times increased HR of cardiovascular events during follow-up. See also the Kaplan-Meier curves presented in **Figure 12** for visualisation of incidence of events over time.

Table 6. Univariate Cox proportional hazards modelling of years from birth to first event with type 1 diabetes as predictor of the events (unadjusted analysis). Hazard ratios (HR) and confidence intervals (CI) as well as p-values for the associations are shown.

Total cohort (N = 589)	HR (95% CI)	p
All-cause mortality	4.62 (3.56-5.60)	<0.001
Cardiovascular mortality	5.60 (3.27-9.57)	<0.001
Cardiovascular events (Acute myocardial infarction or stroke)	3.97 (2.79-5.64)	<0.001
Acute myocardial infarction	4.17 (2.72-6.37)	<0.001
Stroke	3.68 (2.22-6.09)	<0.001
Heart failure	4.98 (3.11-7.98)	<0.001



Figure 11. Swedish military conscription testing of stamina in the 1960's. Image credit: Armémuseum, Stockholm.

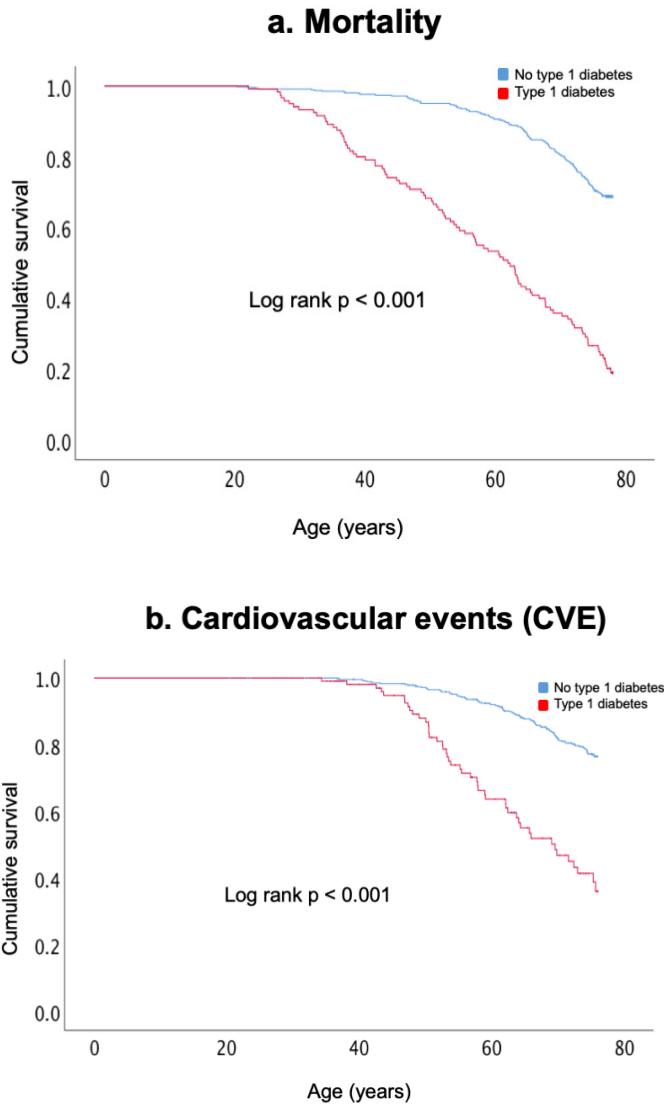


Figure 12. Kaplan-Meier curves with log-rank tests of (a) mortality and (b) cardiovascular events (CVE) over time in men with and without type 1 diabetes.

Impact of cognitive ability on morbidity and mortality

In Table 7, an interaction model is presented where we examine the impact of cognitive ability on morbidity and mortality. There was a significant effect of cognitive ability in the whole study group on all-cause mortality (HR 0.59, 95% CI 0.39-0.91), but not on the other outcomes. There was also a significant interaction between type 1 diabetes and g-factor in relation to this outcome ($p = 0.015$). When we stratified for type 1 diabetes in a Cox regression analysis of cognitive ability as a predictor of all-cause mortality, higher cognitive ability was significantly associated with a lower risk of mortality within the control group (HR 0.59, 95% CI: 0.39-0.90), but not within the group with type 1 diabetes (HR 1.19, 95% CI: 0.76-1.86). See also Kaplan-Meier curves in Figure 13 for visualisation of incidence rates of outcomes for groups with and without type 1 diabetes, further grouped into low to normal or high cognitive ability.

Table 7. Multivariable Cox proportional hazards modelling of years from birth to first event with type 1 diabetes, cognitive ability at 18 years of age (g-factor), and interaction between diabetes and g-factor as predictors of the events. Hazard ratios (HR) and p-values for the estimates are shown.

Total cohort (N= 551)	HR (95% CI)	p
All-cause mortality		
Diabetes	3.79 (2.63-5.47)	<0.001
g-factor	0.59 (0.39-0.91)	0.015
Diabetes x g-factor		0.023
Cardiovascular mortality		
Diabetes	3.66 (1.65-8.13)	<0.001
g-factor	0.40 (0.14-1.14)	0.086
Diabetes x g-factor		0.800
Cardiovascular events^a		
Diabetes	3.89 (2.41-6.29)	<0.001
g-factor	0.72 (0.45-1.17)	0.186
Diabetes x g-factor		0.788
Acute myocardial infarction		
Diabetes	3.41 (1.88-6.19)	<0.001
g-factor	0.69 (0.38-1.24)	0.213
Diabetes x g-factor		0.325
Stroke		
Diabetes	4.72 (2.48-8.99)	<0.001
g-factor	0.81 (0.41-1.60)	0.547
Diabetes x g-factor		0.349
Heart failure		
Diabetes	5.07 (2.72-9.46)	<0.001
g-factor	0.71 (0.35-1.43)	0.334
Diabetes x g-factor		0.879
Diabetes complications^b		
g-factor	1.11 (0.75-1.65)	0.590

g-factor (1 = higher cognitive ability)

a: Cardiovascular events = Acute myocardial infarction or stroke

b: Complications only analysed within diabetes group

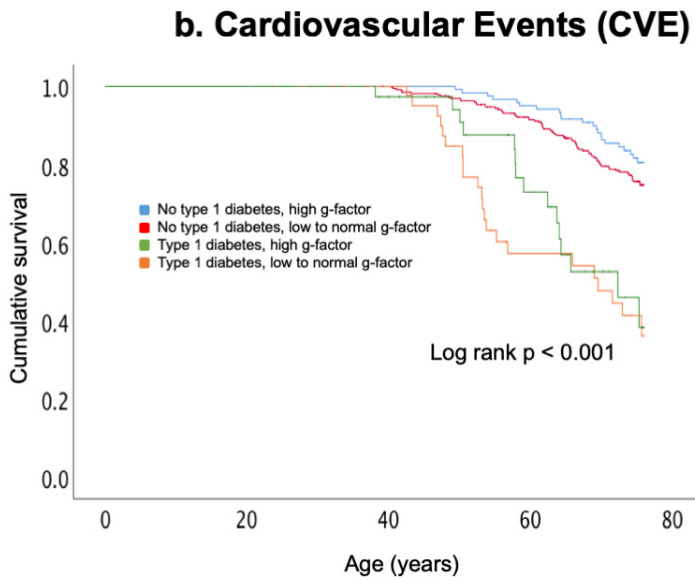
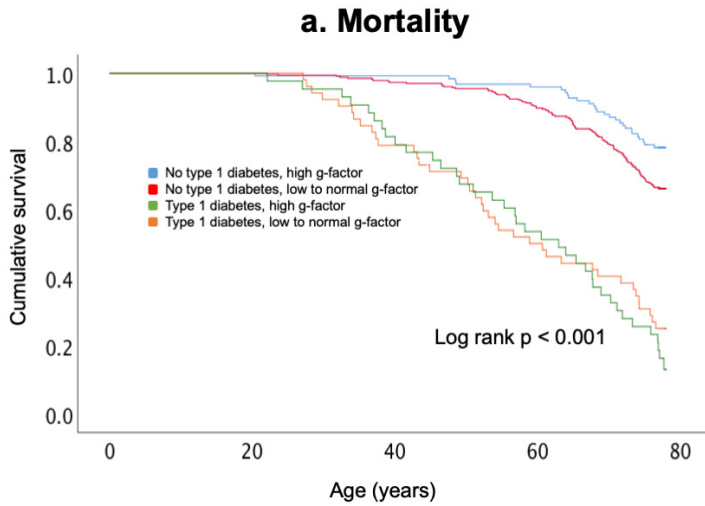


Figure 13. Kaplan Meier curves with log rank tests of (a) mortality and (b) cardiovascular events (CVE) among men with or without type 1 diabetes, sub-grouped into high or low to medium cognitive ability.

Clinical type 2 diabetes and incident dementia (Study IV)

In Study IV, participants with clinical type 2 diabetes at baseline had a 1.46 times higher HR of all-cause dementia, a 1.61 times higher HR of mixed dementia and a 1.84 times higher HR of VaD after full adjustment (including covariates age, sex, education and *APOE* $\epsilon 4$ status), but not a higher risk of AD, than those without type 2 diabetes. The results are shown in Table 8.

Table 8. Epidemiological associations between type 2 diabetes and dementia in the MDCS baseline cohort calculated through Cox proportional hazards modelling of years from birth to first dementia event with type 2 diabetes as predictor. Analyses are presented for the total cohort and then stratified for *APOE* $\epsilon 4$ -genotype (0 or 1-2 alleles). Hazard ratios (HR), 95% confidence intervals (CI) as well as p-values for the associations are shown.

	Model 1 HR (95% CI)	p	Model 2 HR (95% CI)	p
Total cohort (n = 29,139)				
All-cause dementia	1.61 (1.34-1.92)	<0.001	1.46 (1.20-1.77)	<0.001
Mixed dementia	1.83 (1.32-2.54)	<0.001	1.61 (1.12-2.30)	0.010
VaD	2.25 (1.67-3.03)	<0.001	1.84 (1.32-2.58)	<0.001
AD	1.16 (0.79-1.70)	0.464	1.26 (0.84-1.89)	0.272
No <i>APOE</i>-$\epsilon 4$ (n = 20,359)				
All-cause dementia	1.99 (1.58-2.52)	<0.001	1.83 (1.42-2.36)	<0.001
Mixed dementia	2.23 (1.42-3.48)	<0.001	2.00 (1.23-3.27)	0.006
VaD	2.44 (1.67-3.56)	<0.001	2.16 (1.43-3.26)	<0.001
AD	1.85 (1.07-3.20)	0.027	1.82 (1.00-3.31)	0.049
<i>APOE</i>-$\epsilon 4$ (n = 8,780)				
All-cause dementia	1.26 (0.94-1.68)	0.117	1.16 (0.84-1.60)	0.369
Mixed dementia	1.45 (0.87-2.40)	0.152	1.22 (0.69-2.16)	0.488
VaD	1.96 (1.17-3.27)	0.010	1.59 (0.87-2.92)	0.136
AD	0.90 (0.52-1.56)	0.704	1.09 (0.62-1.91)	0.774

Model 1: Adjusted for age, sex and education

Model 2: Adjusted for age, sex, education, smoking, alcohol consumption, physical activity, SBP, BMI, ApoB/ApoA-ratio, history of CVD (stroke or coronary event), use of anti-hypertensive medication and use of lipid-lowering treatment.
AD = Alzheimer's Disease, VaD = Vascular Dementia

Genetic risk of type 2 diabetes and incident dementia (Study IV)

Figure 14 shows results of Cox regression analyses with PRS 1-7 (with different p-value thresholds as described in the Methods section) for type 2 diabetes (standardised) as exposure and time to first dementia event as outcome. There were no significant associations between any of the PRS for type 2 diabetes and dementia endpoints in Model 1 (adjusted for age, sex and education). In Model 2 (adjusted for age, sex,

education, *APOE-ε2* and *APOE-ε4*) however, PRS 1 and 2 were significantly associated with all-cause dementia (HR of 1.11 for both PRSs, Bonferroni corrected p-value 3.9×10^{-3} and 3.6×10^{-3} respectively). PRS 1, 2, 3 and 4 were also significantly associated with mixed dementia with the strongest association for PRS 2 (HR 1.18, Bonferroni corrected p 3.3×10^{-4}). No significant associations were found between PRS for type 2 diabetes and AD, but all were significantly associated with risk of VaD, out of which PRS 2 showed the strongest association with a HR of 1.28 (Bonferroni corrected p-value 9.6×10^{-5}).

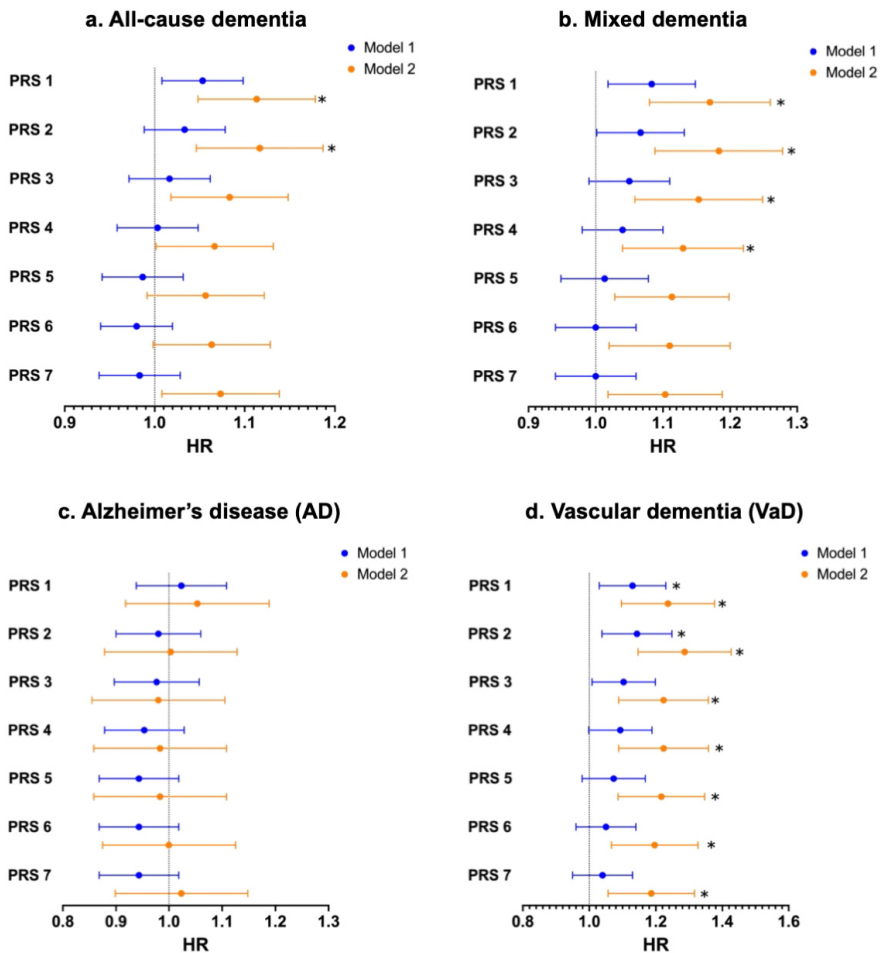


Figure 14. Multivariable Cox regression analyses of associations between SNP's of type 2 diabetes (PRS 1-7) and incident (a) all-cause dementia, (b) mixed dementia, (c) Alzheimer's disease (AD) and (d) vascular dementia (VaD). Significant associations after Bonferroni correction are marked with an asterisk.

Model 1: adjusted for age, sex and education

Model 2: adjusted for age, sex, education, ApoE $\epsilon 2$ and ApoE $\epsilon 4$ genotype

Furthermore, there was a significant interaction between *APOE*- ϵ 4 status (0 or 1-2 alleles) and the associations between (a) all-cause dementia and PRS 1-2 and (b) for VaD and PRS 1-7 (*Model 2* with interaction term). A stratified analysis, as shown in **Figure 15**, showed that for non-carriers of *APOE*- ϵ 4, higher PRS score 1, 2, 3 and 7 significantly increased the risk of all-cause dementia and all PRS-scores increased the risk of VaD (average HR per SD of PRS-score 1.21, $p < 0.002$). For carriers of *APOE*- ϵ 4, none of these associations were significant.

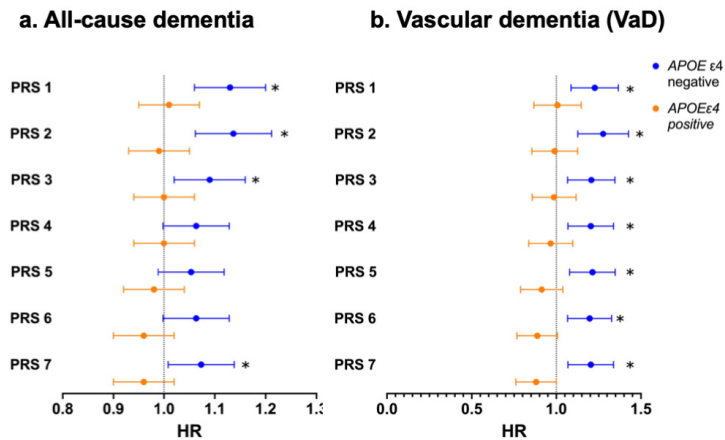


Figure 15. Cox regression analyses stratified for *APOE* ϵ 4-genotype (as colour coded: *APOE*- ϵ 4 positive or *APOE*- ϵ 4 negative): Associations between SNP's of type 2 diabetes (PRS 1-7) and incident (a) all-cause dementia and (b) vascular dementia (VaD), adjusted for age, sex and education. Significant associations after Bonferroni correction are marked with an asterix.

Causal associations between type 2 diabetes and dementia (Study IV)

No causal associations were found when using different Mendelian randomisation methods with type 2 diabetes (using 243 SNPs with genome wide significance as instrumental variables) as exposure, and dementia types as outcome variables, as shown in **Table 9**.

Table 9. Mendelian randomisation analyses using different mathematical methods of calculating the effect size of causality, with type 2 diabetes as exposure (243 SNPs with genome wide significance) and dementia types as outcome.

Outcome and MR method	Beta	SE	p
All Cause Dementia			
MR Egger	-0.08	0.2	6.77E-01
Weighted median	0.003	0.1	9.75E-01
Inverse variance weighted	-0.04	0.1	6.14E-01
Simple mode	0.07	0.2	6.90E-01
Weighted mode	0.04	0.1	7.12E-01
Mixed Dementia			
MR Egger	0.02	0.2	9.17E-01
Weighted median	0.09	0.1	3.71E-01
Inverse variance weighted	0.01	0.1	9.40E-01
Simple mode	0.09	0.3	7.05E-01
Weighted mode	0.09	0.2	5.19E-01
AD			
MR Egger	-0.15	0.2	5.36E-01
Weighted median	-0.13	0.1	1.24E-01
Inverse variance weighted	-0.11	0.1	2.59E-01
Simple mode	-0.11	0.2	6.24E-01
Weighted mode	-0.11	0.1	3.89E-01
VaD			
MR Egger	0.06	0.2	7.80E-01
Weighted median	0.15	0.1	2.89E-01
Inverse variance weighted	0.1	0.1	2.75E-01
Simple mode	-0.11	0.4	7.61E-01
Weighted mode	0.1	0.2	6.10E-01

Additional results

To add some general information about central variables and associations presented in this thesis, some additional analyses were carried out.

Variance in cognitive test results explained by pre-diabetes and diabetes

In **Table 10**, explained variance (R^2) of cognitive test results by pre-diabetes and diabetes (newly diagnosed or long-term, i.e. since the MDCS baseline examination) are shown. The explained variance was in generally low as regards the associations with significant ($p < 0.05$) results. Moreover, the variance in cognitive test results was only explained by 0.1-0.3% of the variance in pre-diabetes and short-term diabetes and 0.9% of long-term diabetes. In *Model 2* (after cardiovascular adjustment), the associations were non-significant or had very low explained variance.

Table 10. Multiple regression analyses with R²-values(explained variance) with categories of glucose metabolism as exposure and cognitive test results (MMSE and AQT results) as outcome (normal glucose tolerance, NGT = reference), for the MDCS-RE population as defined in Study I (n=2994).

	Model 1, n=2994			Model 2, n=2994		
	B	R ²	p	B	R ²	p
MMSE (normalised, points/100)						
NGT	0	-	-	0	-	-
Pre-diabetes	-1.882	0.0031	0.001	-1.734	0.0025	0.004
Short-term diabetes*	-1.443	0.0012	0.049	-0.122	<0.001	NS
Long-term diabetes**	-5.717	0.0037	<0.001	-3.178	<0.001	NS
AQT result (time in s)						
NGT	0	-	-	0	-	-
Pre-diabetes	0.022	0.0021	0.007	0.017	0.0012	0.042
Short-term diabetes*	0.025	0.0016	0.018	0.007	<0.001	NS
Long-term diabetes**	0.128	0.0090	<0.001	0.097	0.0036	<0.001

Model 1: Adjusted for age, sex, education, physical activity level, smoking habits and alcohol consumption

Model 2: Adjusted for factors in *Model 1* and for systolic blood pressure, heart rate, c-f PWV, waist circumference, total cholesterol levels and medications (anti-hypertensive, anti-diabetic and lipid-lowering treatment)

*Short-term diabetes = newly diagnosed at the re-examination.

**Long-term diabetes = diagnosis since baseline, at least 11 years earlier.

AQT = A Quick Test of cognitive speed, c-f PWV = carotid-femoral pulse wave velocity, MMSE = Mini-Mental State Examination, NGT= Normal Glucose Tolerance

Variance in type 2 diabetes explained by PRS for type 2 diabetes

The variance of type 2 diabetes that was explained by PRS for type 2 diabetes was in general around 1% or lower, as shown in Table 11.

Table 11. Binary logistic regression analyses of polygenic risk scores (PRS) for type 2 diabetes as exposure and clinical type 2 diabetes as outcome. Odds ratios (OR) with corresponding p-values are shown. R² values for the associations are also presented (calculated through linear regression analyses).

Polygenic Risk Score	P-value threshold for PRS	OR incident T2D	p	R ²
PRS 1	5,00e-02	1.65	<0.001	0.009
PRS 2	5,00e-03	1.78	<0.001	0.012
PRS 3	5,00e-04	1.76	<0.001	0.012
PRS 4	5,00e-05	1.71	<0.001	0.010
PRS 5	5,00e-06	1.68	<0.001	0.010
PRS 6	5,00e-07	1.66	<0.001	0.009
PRS 7	5,00e-08	1.59	<0.001	0.008

Cognitive test results in participants with and without dementia

In Table 12, mean cognitive test results are shown for participants of the MDCS-RE with or without dementia during the follow-up period. It must, however, be noted that cognitive testing sessions were held during 2007–12, whereas dementia was diagnosed from the baseline (1991–96) at the earliest until 2014 at the latest.

Table 12. Independent samples t-test of mean cognitive test results across groups with and without dementia.

	N	Mean (SD)	P for difference between groups
MMSE score (points/30)			<0.001
No dementia	3163	28.2 (1.68)	
Dementia	166	25.8 (3.22)	
AQT total test time (s)			<0.001
No dementia	3148	135.7 (29.8)	
Dementia	162	176.9 (62.4)	

Fasting glucose measurements at baseline and follow-up

In clinical practice, two blood glucose values are needed to determine a diagnosis of diabetes in most cases. In the MDCS-RE, we only had one measurement of fasting glucose per individual and point of time. For this reason, we investigated how glucometabolic status varied over time (between baseline and follow-up) if only defined by fasting glucose (i.e. not the same categorisation method used in Study I). As diabetes treatment affects glucose measurements, treated participants were defined as a separate category in this analysis. In Table 13, proportions of MDCS-RE participants that were in the same fasting glucose category at baseline and follow-up are shown.

Table 13. Categories based on fasting glucose levels or anti-diabetic treatment at baseline, and proportions that converted to the same or to other categories at follow-up, in the MDCS-CV-RE population.

Category at baseline	Category at follow-up	N (%)
NFG, n = 2558	NFG	1702 (67)
	IFG	694 (27)
	Diabetes (untreated)	153 (6.0)
	Diabetes (treated)	8 (0.3)
IFG, n = 40	NGT	7 (18)
	IFG	13 (33)
	Diabetes (untreated)	18 (45)
	Diabetes (treated)	2 (5)
Diabetes (untreated), n = 5	NGT	0 (0)
	IFG	1 (20)
	Diabetes (untreated)	2 (40)
	Diabetes (treated)	2 (40)
Diabetes (treated), n = 281	NFG	15 (5.3)
	IFG	14 (5.0)
	Diabetes (untreated)	24 (8.5)
	Diabetes (treated)	228 (81)

NFG= Normal fasting glucose, IFG= impaired fasting glucose (i.e. pre-diabetic range)

Incident dementia among MDCS participants with or without type 2 diabetes

To further visualise the associations presented in Study IV of clinical type 2 diabetes and dementia risk over time, a Kaplan-Meier curve with a log rank test is presented in Figure 16.

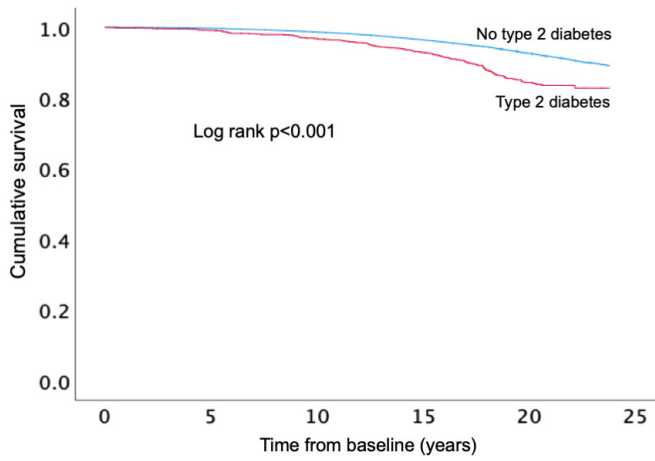


Figure 16. Kaplan-Meier curve with log-rank test of incident dementia between the MDCS baseline examination 1991–96 until dementia diagnosis or censoring, or until 2014 at the latest, for groups with or without clinical type 2 diabetes at baseline.

Discussion

This thesis addresses many different aspects of the association between impaired glucose metabolism and diabetes on the one hand and cognitive ability and dementia risk on the other. We show that cognition is affected already at pre-diabetic stages in Study I (150) and that physiological levels of different biomarkers of glucose metabolism in the population correlate with cognitive test results in Study II (151). In Study III we found that there was no effect of baseline cognition on outcomes in a group of men with type 1 diabetes living during a period with sub-optimal treatment (152). Finally, in Study IV we show that there are genetic risk markers of type 2 diabetes that are associated with validated dementia endpoints during follow-up, but our findings did not support the hypothesis that type 2 diabetes was causally associated with dementia (153).

Interpretation of findings

Pre-diabetes, type 2 diabetes and cognitive ability

In Study I, we found that average cognitive test results were lower in groups of pre-diabetes and diabetes than in a group with normal glucose tolerance (NGT). The differences in results were so small that they cannot be regarded as clinically relevant. This is in line with current knowledge that diabetes and impaired glucose metabolism are associated with mild cognitive decrements across the life span, corresponding to small differences that are not clinically relevant in the population (55). The results also show that a longer diabetes duration is associated with lower test results. Of the cognitive domains we studied the most affected ones were processing speed, attention and executive function. This is also in line with findings in other studies (154), and there is not enough evidence yet to know exactly which cognitive tests are the most suitable to use for people with diabetes (155).

Biomarkers of glucose metabolism and effects on cognition

Glucose

In Study I, we found correlations between fasting glucose and 2-h glucose levels and cognitive test results in the general population, and in people without diabetes. This supports findings of previous studies (156) (157) (158) that suggest that glycaemic control itself is correlated with cognitive outcomes. Interestingly, we also found a continuous relationship between glucose levels and cognition in the general population. However, further studies are needed to determine whether sub-clinical levels of elevated blood glucose and pre-diabetes can affect cognitive ability.

Underlying mechanisms behind the association may be oxidative stress caused by hyperglycaemia both in nerve cells and vascular tissue (98). It may also be the case that underlying genetic susceptibility affects the association, but we could not find any support for this in Study IV, where we investigated whether polygenic risk of HbA_{1c} and fasting glucose were associated with dementia. Some other studies using Mendelian randomisation have, however, found significant causal associations between glucose or HbA_{1c} and dementia (159) (160) (63).

It is unclear whether better glucose control could prevent cognitive decline in diabetes. One randomised clinical trial (ACCORD) that investigated this matter could not observe any such effects (161). However, the study may have been biased due to discontinuation of the placebo treatment arm. Therefore, more interventional studies are needed to investigate the effects of glucose levels on cognition. The promising results from the Finnish FINGER study imply that lifestyle intervention is a possible way forward to prevent cognitive decline in patient groups at risk (162).

Insulin

As we had hypothesised, peripheral insulin resistance correlated negatively with cognitive ability in the population, and higher insulin sensitivity was associated with better cognitive test results in Study II. The insulin level in blood was also positively correlated with cognitive test results in people without diabetes. In Study IV, a polygenic risk score for fasting insulin was negatively associated with dementia (all-cause dementia, mixed dementia and Alzheimer's disease), although not after Bonferroni correction for multiple associations. Although these results must be interpreted with caution, this could imply what mechanistic studies have already shown, which is that higher insulin levels are beneficial for neuronal activity (101), that insulin resistance could disturb these neuroprotective effects (73), and potentially also increase the risk of AD (102) (103). However, more studies are needed to see whether CSF levels of insulin correlate with cognitive test results, as peripheral levels do not

reflect this exactly, and could also be confounded by concomitant insulin resistance (which is often accompanied by hyperinsulinemia), which is instead negatively associated with cognitive function.

Glucagon

Glucagon levels correlate positively with cognitive test results in Study II. This is as far as we know a novel finding. More studies are needed to investigate the mechanistic actions of this hormone in the brain. However, there are some hypotheses. One is that high glucagon levels increase glucose availability in the brain. Only trace amounts of glucagon have so far been found in the CNS (105), but it is possible that the hormone crosses the BBB under certain conditions when the permeability is increased (such as in diabetes or neurodegenerative processes). As glucagon has similar properties to GLP-1, it could also have similar positive effects on nerve cells, such as receptor activation leading to an intracellular cAMP/PKA-dependent mechanism that enhances synaptic transmission (163).

GIP

Post-load levels of GIP were associated with better cognitive test results in Study II. This is in line with the fact that animal studies have shown that GIP could have neuroprotective effects (106). The association was dependent on diabetes status, according to an interaction analysis. Possible benefits from GIP treatment (GIP and GLP-1 dual agonists) on diabetes in general are currently being investigated (164), but their effect on cognitive ability is yet unknown. A potential confounding factor is also that there are inter-regulatory effects between GIP, GLP-1 and glucagon (165).

GLP-1

There were negative associations between fasting levels of GLP-1 and cognitive ability, but positive associations between 2-h GLP-1 and cognitive ability. Our hypothesis was that GLP-1 is neuro-protective. The negative finding of fasting GLP-1 could potentially be due to the fact that fasting levels of incretins often are elevated as a consequence of impaired glucose metabolism and insulin resistance. The positive finding of 2-h GLP-1 is, however, in line with animal studies that have shown neuroprotective properties of GLP-1 agonist treatment (106). It remains to be discovered whether such drug treatment in humans could prevent cognitive decline, as studies on DPP4-inhibitors that elevate GLP-1 levels have not yet been able to prove this (166) (108). A new clinical trial is currently evaluating whether those with early AD could benefit from GLP-1 agonist treatment (110).

AGE

As some studies have previously shown (167) (168) AGE-levels correlate negatively with cognitive test results in Study II. The associations are also significant for people with pre-diabetes, and this could imply that they affect cognition at early stages of impaired glucose metabolism. AGEs and their receptors (RAGE) could affect blood-brain-barrier function contributing to the pathophysiological process of AD (169), but more studies are needed.

Role of potential confounding factors

In Study I (on pre-diabetes and diabetes) and II (on biomarkers of glucose metabolism), we adjusted for common risk factors for both diabetes and dementia, such as demographics and lifestyle. These factors attenuated the associations slightly, but the results were still significant. When additionally adjusting for cardiovascular factors, pre-diabetes and diabetes were in most cases not significantly associated with cognitive test results (Study I). This could indicate that the associations are not independent of cardiovascular risk factors, and it is thus likely that they are mediating factors.

However, in Study II, most associations between biomarkers ($ISI_{0-120\text{ min}}$, HOMA-IR, glucagon, GIP and GLP-1) survived adjustment for cardiovascular factors, which could imply that these factors are associated with cognitive ability through mechanisms that are not dependent on cardiovascular factors. Associations between AGEs and cognition were however not significant, which could be due to lower statistical power in this analysis, but also possibly due to the fact that AGE is associated with atherosclerosis (170).

In Study I, we also found in a stratified analysis that the association between diabetes and AQT results (processing speed, attention and executive function) was stronger in people who were older or less physically active. Previous studies show that physical activity can have positive effects both on glycaemic control (171) and on cognitive ability (172), which is in line with this finding. This could imply that it is useful to determine whether lifestyle interventions including increased physical activity could mitigate diabetes-associated cognitive impairment.

Previous cognitive ability is also a potential confounding factor between diabetes and cognitive impairment. This is something which has not been considered much previously. We could not investigate this factor in relation to dementia or cognitive impairment in diabetes in any of the studies, but we do investigate the impact of previous cognitive ability on morbidity and mortality in Study III. In the whole cohort (men with and without type 1 diabetes), there was an association between cognition in early adulthood and risk of mortality, but no such association was found in the group

with type 1 diabetes. The reason, apart from possible lack of statistical power, could be that effects of suboptimal treatment of type 1 diabetes during this historical period outweighed the effects of cognitive ability on the prognosis. There is a need for studies on larger and later-born cohorts of patients with type 1 diabetes in order to investigate such effects, as well as the possible effects on dementia and cognition later in life.

Genetic risk markers of type 2 diabetes and dementia risk

We found associations between PRS of type 2 diabetes and incident all-cause dementia, mixed dementia and vascular dementia. A PRS generated using a weighted score of $p < 0.005$ for the included SNPs in relation to type 2 diabetes had the lowest p-value in relation to dementia in most of the models. This suggests that the combined estimated effect of these variants might be partly responsible for the development of dementia.

Associations between both clinical type 2 diabetes and dementia, as well as genetic risk of type 2 diabetes and dementia, were stronger in non-carriers of *APOE-ε4* than in carriers. This is in contrast to a meta-analysis that reported that carriers are more vulnerable to diabetes-associated dementia of all forms including vascular dementia (173). It is possible that our results are partly influenced by less statistical power in the subgroup of *APOE-ε4* carriers. Another possibility is that carriers of *APOE-ε4* have a higher risk of Alzheimer's disease than non-carriers and are therefore exposed to a relatively lower risk of diabetes-associated dementia.

Causality in associations between type 2 diabetes and dementia?

In Study IV, no causal associations between type 2 diabetes and dementia were found in 2-sample MR analyses. The significance of these analyses is, however, limited in the sense that a negative finding cannot be securely interpreted as an absence of effect. Although the finding was negative, it is in line with other studies where no causality has been found between type 2 diabetes and dementia (60) (61) (62) (63).

In other words, we could not find any support for the hypothesis that diabetes causes dementia. It is, however, possible that risk factors for diabetes (such as genetic factors, lifestyle factors and cardiovascular comorbidities) instead could be responsible for causing or modifying the risk of dementia. People that have these risk factors would therefore be more likely to develop both diabetes and dementia.

Another hypothesis is that there are some causal links between diabetes and dementia, but due to the complexity of both diseases, it is difficult to pinpoint these links through

these methods. Elements of the diabetes phenotype could, in other words, be responsible for causing elements of the dementia phenotype. For example, other MR studies have found causal associations between fasting glucose and risk of dementia (159) (160), between HbA_{1c} and impaired visual memory (61) and between insulin resistance and AD (159) (63), but more studies are needed.

Type 2 diabetes is part of the metabolic syndrome which includes the phenotypes insulin resistance, obesity, atherogenic dyslipidaemia and hypertension (174). Studies have also shown causal links between hypertension (175) and obesity (176) on the one hand and dementia on the other, indicating that these may be strong underlying factors behind the association.

Long-term morbidity and mortality outcomes in men with type 1 diabetes

In Study III, we present data from a historical cohort of men with type 1 diabetes and a control group born 1934–43, followed from baseline 1953–61 until 2018. Type 1 diabetes was associated with a 4.6 times higher hazard ratio of mortality and 4.0 times higher hazard ratio of cardiovascular events (heart attacks or stroke) than controls. These data reflect a historic time period characterised by a less than optimal treatment of type 1 diabetes. The prognosis of type 1 diabetes has improved considerably since then, thanks to better treatment goals and new technology including continuous glucose measurements (CGM), smart apps and insulin pumps. The results are therefore unlikely to be applicable to younger age groups of today with type 1 diabetes.

Methodological considerations

Study design

Two studies in this thesis (I and II), were cross-sectional. Hence, the results do not prove causality between exposure and outcome. The two other studies in the thesis (III and IV), were longitudinal, using survival statistics (Cox regression). This has the advantage that temporality in the association between exposure and outcome suggests the possibility of causal inference.

One study also used Mendelian randomisation, although, as stated, the power was not sufficient to deduce a negative result. Mendelian randomisation can, when sufficient power is present and all assumptions are fulfilled, show true causality for an association, through the assumption that genes are not susceptible to changes caused by

confounding factors or reverse causality. However, the quality of the evidence in a MR study is dependent on the instrumental variable assumptions (129).

The first assumption is that the genetic variants of the trait are associated with the trait/risk factor (diabetes). We show that this is true in the Supplement of Study IV (PRS of type 2 diabetes in relation to clinical type 2 diabetes), and in Additional Results in this thesis summary. However, the explained variance of clinical type 2 diabetes by PRS for type 2 diabetes was relatively low (Table 11, Additional Results). This could be due to the knowledge gap about type 2 diabetes genetics (i.e. that known genetic factors only explain a small proportion of the heritability).

The second assumption is that each genetic variant is independent of confounders of the association between exposure and outcome. This is also called pleiotropic effects. We cannot rule out that there are such effects, although we tried to minimise the impact of such effects through use of the MR Egger method.

The third assumption is that if the risk factor (diabetes) was kept constant, intervention on the genetic variant would not influence the outcome. Applied to this study, this would mean that the genetic factors of type 2 diabetes are not causally related to dementia. It is possible that there are such effects in this study, which is why we must interpret the findings with caution.

Sources of error

In this section the potential sources of error that could have impacted the results in this thesis are discussed.

Systematic error

Systematic error means lack of internal validity, and this relates to problems in how the study was conducted. The three categories of systematic error are (1) selection bias, (2) information bias and (3) confounding (177).

(1) Selection bias

The MDCS had relatively low participation rates (41% at baseline). On the other hand, the health survey that was conducted in Malmö, however, showed that the cohort was representative of the population in Malmö as regards socio-demographic structure, smoking and obesity, although cardiovascular factors were more prevalent in non-participants. Possible selection bias due to individuals that were lost to follow-up between the examinations must also be considered, as those who died between the examinations had a worse risk factor profile at baseline than the attendees at follow-up

(133). This may overall imply that the studies I, II and IV are at risk of underestimating the effect of diabetes on cognition. The fact that the associations between glucose levels or diabetes and cognitive test results in Study I corresponded to less than 1% of the variance in cognitive test results, in contrast to 10% of the variance explained by HbA_{1c} in a meta-analysis (97), may partly depend on this, but also on the study being population-based.

The SSDCS population that is described in Study III had full coverage of liable men with type 1 diabetes at baseline, but 34 men were not followed up. These men had equal cognitive ability at conscription as attendees, and very little difference in height and weight. The non-participation rate was 6% in the group without diabetes. While the 'healthy respondent effect' is not likely to be an issue in this study, mortality rates were high in the group with diabetes during the follow-up period, which is a factor to take into consideration when interpreting the results of other outcomes.

There was also a difference in geographical coverage (urban-rural) between cases and controls. This may have been the reason why the men with type 1 diabetes on average had higher baseline cognition than the control group. However, when adjusting for a propensity score taking socio-economic background into account, the results were essentially unchanged.

(2) *Information bias*

Information bias arises when information collected about or from study subjects is erroneous (177). *Non-differential misclassification* is when there is the same risk of misclassification for exposure among those with or without an outcome, or vice versa. In this thesis, this would mean misclassification of diabetes (or related variables) or dementia/cognitive test results due to lack of precision in measuring or assessing these variables. This would not be expected to vary depending on health status. It is likely that the risk of non-differential misclassification of diabetes and pre-diabetes is low in Study I as oral glucose tolerance testing (OGTT) is a gold standard method with high validity (178). However, some studies have argued that even this method is associated with a risk of false positive diagnosis (179).

The MMSE has ceiling and floor effects, meaning that high scores may not reflect absence of cognitive impairment and low scores may not necessarily reflect the presence of severe cognitive impairment (142), why some individuals in Studies I-II may have been misclassified. Furthermore, unlike the AQT test, the MMSE does not measure processing speed which is often negatively affected early in patients with diabetes (154). Dementia diagnoses in Study IV, however, were validated and had a high diagnostic

precision (136). National registry data (Study III, health outcomes) also had high validity (145) (135).

Differential misclassification can occur when different groups (e.g. exposed or unexposed) have a different risk of being misclassified depending on their health status. Some information used in covariates in our studies was self-reported (physical activity, alcohol consumption, smoking habits and use of medications), which could lead to this kind of bias.

(3) Confounding

A factor that is a risk factor for the studied outcome, and at the same time associated with the exposure variable, is a confounder. We adjusted for the main known confounding factors of the association between diabetes and cognition in Studies I, II and IV, i.e. demographics, lifestyle and different cardiovascular factors (which also could act as mediators, as previously discussed). In Studies I and II a limiting factor was that data on *APOE* $\epsilon 4$ genotype was not yet available to us. Other factors that it would have been optimal to be able to adjust for are inflammation, depression, sleeping problems, diet and socio-economic status (SES). However, both diet and SES are difficult to summarise as variables and are often collinear with other factors in the adjustment models.

Random error

Major sources of random error in epidemiological studies are measurement errors and errors related to random variations in the study sample. To minimise these effects, it is important to use a large enough study population, and to minimise the risk of measurement errors. Studies I, II and IV are relatively large population-based studies. There may have been some random error in lab analyses and cognitive testing, but this is unlikely to have greatly biased the results. In Study III, the study sample was smaller, but we do not believe this significantly impacted the risk of measurement errors in the variables, owing to the methods used (high reliability of type 1 diabetes diagnosis and cognitive test results at military conscription).

Reverse causality and critical periods

In cross-sectional studies such as Studies I and II, we cannot rule out the possibility that effects of reverse causality may be present. In longitudinal studies (III-IV), the analyses can, however, show that exposure came before outcome. Some studies have proposed that there may be reverse causality in general in the association between diabetes and cognition (86) (87). It is a limitation that we could not account for effects of previous cognitive ability on later cognitive functioning and dementia in Studies I,

II and IV. Furthermore, dementia can develop during a long pre-clinical phase of around 20 years (180). Hence it is possible that some effects of (pre-) diabetes on cognitive outcomes may be attributed to cognitive changes rather than to diabetes itself.

Generalisability or external validity

As discussed, the MDCS was likely to have been influenced by health selection bias. Given this many aspects of the cohort were also representative of the background population (the population of older adults in Malmö, Sweden). The magnitude of the observed effects in Study I, II and IV can be generalised to populations with similar characteristics, although some associations may have been slightly underestimated. We also note that two thirds were women in the MDCS, and that the educational level was moderate. The genetic associations may moreover not apply to other ethnicities or populations from other parts of the world, as this was a cohort of mainly white European ancestry.

The SSDCS is not generalisable to younger populations of today with type 1 diabetes, but is instead attributable to older people with type 1 diabetes who have survived during the period of the study. Since this time period, the average educational level in Scania has risen, the prevalence of smoking has decreased and treatment of type 1 diabetes has improved. It may also be possible that the results are not applicable to future cohorts, as improving trends in health outcomes and cognition evolve over time (e.g. the Flynn effect where a tendency towards higher IQ in the population is seen over time (181)).

Strengths and limitations

Some strengths and limitations have been discussed, but here follow some more specific strengths and limitations for each study. These are also summarised in **Table 14**. It should also be mentioned that a limitation of this thesis is that we did not include any neuro-imaging data in any of the studies. On the other hand, we were able to include genetic data and incretin levels measured in many individuals, which are important strengths.

Studies I and II

A strength of these studies was that the sample size was relatively large and that we were able to perform oral glucose tolerance testing (OGTT) on a large number of people, as well as to analyse incretin levels. Few studies have measured incretins in such large

population-based cohorts. We were also able to adjust for extensive cardiovascular risk factors including carotid-femoral pulse-wave velocity (c-f PWV), a measure of arterial stiffness.

A limitation of Studies I and II was that there was a time delay of a few months between blood samples of OGTT and the cognitive assessment sessions due to staff shortage and logistics. Furthermore, we were not able to administer more extensive neuropsychological tests to so many participants. As mentioned, the MMSE itself has many limitations. On the other hand, the AQT test is more sensitive in detecting early cognitive impairment. Although our adjustment models were extensive, we could not adjust for *APOE* $\epsilon 4$ status, inflammation, sleeping patterns or depression, which would have been optimal.

Study III

This study had the exceptionally long follow-up time of 65 years from baseline to the end of follow-up. There is also little data worldwide on type 1 diabetes from the decades preceding the 1970s worldwide.

A limitation of Study III is that only men were included and that the sample size was small. Moreover, 34 men with type 1 diabetes were not followed up. Furthermore, data from national registers was not available during the earliest part of the studied time period. These registers started during the early 1960s, and the Patient Register started on a national scale in 1987.

Study IV

The most important strength of Study IV was that the dementia endpoints were validated by physicians at the Memory Clinic in Malmö, through careful assessment of medical records, neuro-imaging data and CSF biomarkers. This is unique, as most other studies previously have used only register-based diagnoses.

A limitation is that the time period of data on validated dementia ended in 2014 for logistic reasons. The other limitations of the MDCS baseline cohort have been mentioned and also apply to this study.

Table 14. Strengths and limitations of Studies I-IV

	Strengths	Limitations
Study I	<ul style="list-style-type: none"> • Large, population-based • OGTT performed for a large number of people • Adjustment for extensive cardiovascular risk factors including pulse-wave velocity (c-f PWV) 	<ul style="list-style-type: none"> • MDCS – low participation rate 41% at baseline • Time delay between blood samples and cognitive assessments • MMSE: ceiling and floor effects. Not a very sensitive test. • Inability to adjust for <i>APOE-ε4</i>, inflammation, sleep and depression
Study II	<ul style="list-style-type: none"> • Large, population-based • Measurements of insulin, glucagon and incretins in many people • Measurement of AGEs 	<ul style="list-style-type: none"> • MDCS – low participation rate 41% at baseline. • Time delay between blood samples and cognitive assessments • MMSE: See above. • Inability to adjust for <i>APOE-ε4</i>, inflammation, sleep and depression
Study III	<ul style="list-style-type: none"> • Long follow-up time • One of the oldest existing cohorts of type 1 diabetes • High validity of register-based diagnoses 	<ul style="list-style-type: none"> • SSDCS: Small study (N = 551) • 34 people with diabetes lost to follow-up • Data from national registers not available before their start (1961) • No primary care diagnoses among outcomes
Study IV	<ul style="list-style-type: none"> • Validated dementia endpoints by physicians • GWAS data for large cohort 	<ul style="list-style-type: none"> • MDCS – low participation rate 41% at baseline • Not significant power to interpret negative Mendelian randomisation finding

AGE = Advanced glycation end products, *APOE ε4* = Apolipoprotein E ε4, GWAS = Genome wide association study, MDCS = Malmö Diet and Cancer Study, MMSE = Mini-Mental State Examination, OGTT = oral glucose tolerance test

Conclusions

- I. Pre-diabetes and diabetes, as well as higher glucose levels within the normal range, were cross-sectionally associated with lower cognitive test results in a population of older adults in Sweden. This adds to the body of evidence that impaired glucose metabolism affects cognitive ability at an early stage.
- II. In a cross-sectional population-based study, associations were found between biomarkers of glucose metabolism and cognitive test results. Higher insulin sensitivity index ($ISI_{0-120 \text{ min}}$), 2-h plasma glucagon, 2-h serum glucose-dependent insulintropic peptide (GIP) and 2-h plasma glucagon-like peptide-1 (GLP-1) were positively associated with cognitive test results, whereas higher insulin resistance (HOMA-IR), fasting plasma GLP-1 and Advanced glycation end products (AGEs) were negatively associated with cognitive test results. Longitudinal and interventional studies are needed to explore possible neuro-protective or adverse effects of these biomarkers.
- III. Cognitive ability in early adulthood was not associated with long-term prognosis of type 1 diabetes in a study of men of 18 years of age during the 1950's followed until 2018. The inability to detect such effects could be due to strong effects of mortality in this group, since this study concerns a period with less than optimal treatment of diabetes. However, high cognitive ability was associated with fewer cardiovascular events over time in a group without diabetes. This study also presents trends in mortality and cardiovascular disease in Sweden's oldest cohort of type 1 diabetes.
- IV. A polygenic risk score (PRS) of type 2 diabetes was associated with an increased risk of all-cause dementia, mixed dementia and vascular dementia (VaD) but not with Alzheimer's disease (AD). Mendelian randomisation analyses could, however, not find any support for a causal association between type 2 diabetes and dementia, in line with other studies. This does, however, not rule out the possibility that elements of the diabetes phenotype cause elements of the dementia phenotype. An alternative explanation for the epidemiological association could be shared risk factors, for instance adverse lifestyle, hypertension and obesity.

Clinical Implications

The findings of this thesis contribute to current knowledge in the research field of diabetes-associated cognitive impairment in ways that are clinically relevant. Studies on pre-diabetes associated with cognitive outcomes have not been consistent so far, and Study I contributes to this research field, as a large population-based study. It is also an interesting fact to consider as a clinician that high levels of glucose, but still within the normal range, could be associated with impaired cognitive ability. When more is known about the mechanisms of early brain changes in pre-diabetes, and more lifestyle intervention studies are carried out, it will be possible to know what would be the most effective way of preventing further cognitive deterioration in this patient group.

Incretin treatment as a possible neuroprotective therapy for people with diabetes is an important current focus within the pharmaceutical industry. Study II on glucometabolic biomarkers in the population adds to the evidence that incretin levels correlate positively with cognitive test results. The other biomarkers in this study also need to be further investigated. For example, intranasal insulin treatment has also shown promising results in preventing early AD (182), but more studies focused on people with diabetes are needed.

The results of Study III are relevant for clinicians working with patients in older age groups who have survived type 1 diabetes for many decades. Treatment possibilities are different nowadays, and hence the findings of this study are not likely to be applicable to younger age groups. The fact that impaired cognitive ability in early adulthood can be a risk factor for worse health outcomes in the general population is also relevant for health professionals to be aware of.

Polygenic risk of type 2 diabetes was associated with all-cause dementia, mixed dementia and vascular dementia (validated dementia diagnoses) in Study IV. This implies that certain people with type 2 diabetes may, due to their genetic background, be more prone to develop diabetes-associated dementia. This knowledge, and other genetic studies that address this research question, may in the future lead to targeted preventive strategies.

Future Perspectives

There are still many unanswered questions regarding the association between glucose metabolism and cognitive ability. The results in this thesis could serve as a basis for future studies in several areas.

First, more studies are needed on the mechanisms of brain changes in diabetes or pre-diabetes. There are for instance some studies on whether the loss of function of the neurovascular coupling unit could contribute to both pathophysiological processes (70). More studies are needed on the possible neuroprotective properties of insulin, glucagon and incretin levels, and on whether AGEs are responsible for changes in the brain in diabetes. It would for instance be of value to analyse the biomarkers in the cerebrospinal fluid, and to determine possible associations with effects on cognitive function.

Genetic factors also need further investigation, for instance whether *APOE-ε4* carriership modifies the risk of dementia in diabetes or not. Larger studies with more statistical power are also needed in order to carry out Mendelian randomisation analyses on other diabetes-associated traits (such as selected biomarkers) and their effect on dementia outcomes.

It remains to be discovered whether anything can be done to prevent diabetes-associated cognitive impairment. Effects of multidomain lifestyle interventions in older adults were investigated in the Finish Geriatric Intervention (FINGER) study. There were positive effects on cognitive test results after two years, but the study was not targeted at people with diabetes specifically (183). However, known risk factors for dementia including diabetes-related factors, summarised as CAIDE score, were also reduced during the trial period for the intervention group (162). The interventions included changes in diet, exercise, cognitive training and vascular risk management (183). Lifestyle intervention to prevent cognitive decline in diabetes is a poorly studied area, and the few studies that have been carried out have been unable to prevent cognitive decline through weight loss (184) or through exercise and diet modifications (185). There are however some ongoing trials (186).

Some studies have investigated the effect of anti-diabetic medications on cognitive outcomes, but more are needed. Metformin treatment was associated with slower

cognitive decline in an Australian observational study (187), but not in the randomised Diabetes Preventive Project from the US (185). Intensive treatment, in the ACCORDION MIND study, was not associated with better cognition or brain MRI findings after a trial period (although loss to follow-up may have contributed to the results) (161).

Since animal studies have shown possible neuro-protective effects of GLP-1, DPP4-inhibitors (that stop GLP-1 degradation) and GLP-1 analogues have recently been investigated for this objective in randomised clinical trials. Two studies on linagliptin (a DPP4-inhibitor) have been unsuccessful in preventing cognitive decline (166) (108), but in 2020, positive effects of dulaglutide (a GLP-1 analogue) on cognitive test results were seen for the first time (188). There are also some ongoing clinical trials investigating neuroprotective effects of both DPP4-inhibitors, GLP-1 analogues and the new anti-diabetic drug category SGLT2-inhibitors (110-113).

The American Diabetes Association (ADA) guidelines of 2022 state that physicians should consider screening adults 65 years and older with diabetes for cognitive impairment (189). They also recommend simplifying treatment and preventing hypoglycaemia as much as possible in patients with diabetes who already have signs of cognitive impairment (189). It would also be of value to determine which patient groups are most at risk of cognitive impairment among people with type 2 diabetes. As mentioned, type 2 diabetes is highly heritable, but the known genetic variants only stand for 10% of the heritability. Hence family history may be a more useful risk factor to consider in clinical practice.

Furthermore, more research is needed to analyse all aspects of cognitive dysfunction among patients with type 1 diabetes, as the phenomenon is more studied in type 2 diabetes.

Acknowledgements

During these six years of part-time PhD work, parallel to clinical work and to having two children, it has been a long journey! It would, however, never have been possible, or completed to the end, without the people around me.

First, I would like to thank **Peter M Nilsson**, who was my main supervisor from 2016 until his partial retirement in 2021, for all his help and patience with me throughout this time period. Peter, I am so grateful to you for introducing me to the world of research. Despite my ups and downs, you have helped me to maintain the spark for this work with your enthusiasm. You have helped me to develop my critical thinking and to understand the world of research and the value of networking, and you have been a true inspiration and motivator this whole time. Without you I would not have had so many interesting international experiences in France, Spain, Portugal, the Netherlands and Denmark. And you have also been a support in helping me find the right way in my career.

I also want to thank my present main supervisor **Gunnar Engström** for all his support and help during this time period. Gunnar, you have patiently taught me so many things about statistics and epidemiology, and you have, like Peter, also helped me with valuable career advice and helped me find good opportunities for the future. I always feel relieved when having talked to you about how to solve a theoretical problem, because you always have an answer that puts everything into place.

My gratitude also to **Linda Hassing**, my co-supervisor and local contact in Gothenburg where I have had access to an office at the department of Psychology. Linda, thank you for all your theoretical help and kind support. It has meant a lot to have you close by and to be able to talk to you now and again. You have also taught me a lot about the beauty of keeping things simple.

I would also like to thank the other co-authors to the papers. **Katarina Nägga**, thank you for your contribution to all four studies, and for your kindness, wise input and encouraging comments. **Anna Dahl**, thank you for your valuable help with Study III, your input really improved the study a lot. **Patrik Rorsman**, thank you for your pre-clinical insights that helped the interpretation of the findings in Study II. **Atul Kumar**, thank you for your invaluable contribution as a joint first author of Study IV, for

helping me understand the genetic world and for your kindness in general. **Oskar Hansson**, **Olle Melander** and **Niklas Mattsson-Carlgren**, thank you for making Study IV possible and for your important advice and guidance. I also want to thank **Mikael Gottsäter** and **Linda Johnsson** for practical advice and help at the very beginning of my PhD studies. Thank you **Erik Nilsson** for introducing me to your grandfather **Sven Nilsson**'s data material. His work will be remembered through this study and probably several more to come.

I would also like to thank **Margaretha Persson** and **Gerd Östling** for all their work in organising and collecting data for the MDCS cohort, to **Anders Dahlin** for data management and to the team of physicians at the Malmö Memory clinic for their work with the validation procedure of dementia diagnoses. I also want to thank **Anna-Märta Gustavsson** for help with introducing me to these data.

Cathérine Helmer and **Christophe Tzourio**, thank you for all your help at the University of Bordeaux in 2015, where I learned the basics for my future PhD work. Thank you **Geert Jan Biessels** at Utrecht University and **Coen Stehouwer** at Maastricht University and both of their research teams for a very fulfilling research visit in 2016.

I also thank the team at the National Diabetes Registry in Gothenburg, where I have spent some time, for all their contributions, especially to **Ann-Marie Svensson** with whom I had many interesting discussions. Ann-Marie passed away last year and will be missed by many people. Thank you also to **Soffia Gudbjörnsdottir**, **Ia Svensson** and **Caddie Zhou** for welcoming me to NDR and making my time there so nice.

Finally, I thank my family for putting up with me throughout this time period. A special thanks to my partner **Emil Westerlund** for being so supportive, to my little ones **Klara** and **Kasper**, as well as my mother **Miranda Hinde**, my dad **Peter Dybjer**, my mother's partner **Richard Watson**, my sister **Hannah Dybjer** and my brother **Paul Dybjer**. Thank you also to family members who kindly helped to proof-read and improve the English in some of my work: **Kate McCullough**, **Hugh McCullough**, **Francis Hinde**, **Miranda Hinde**, **Peter Dybjer** and **Emil Westerlund**. Thank you to my granny **Hester Hinde** and grandad **Robert A. Hinde** who are no longer with us, and to **Joan Stevenson-Hinde**, who is still there supporting me.

Funding

All studies were funded by the Research Council of Sweden (Grant K2011-65X-20752-04-6), the Anders Pålsson Foundation, the Ernhold Lundström Foundation, the Regional Agreement on Medical Training and Clinical Research (ALF) between Skåne County Council and Lund University, the Swedish Alzheimer's Foundation (Alzheimerfonden), the Diabetes Fund (Diabetesfonden) and research grants from Region Skåne. The baseline investigations in Study III were supported by grants to Sven E Nilsson from the Swedish Diabetes Foundation and the (Novo) Nordic Insulin Foundation, Gentofte, Denmark. Entering the raw data of Study III into SPSS was supported by a grant from Region Jönköping to Anna K Dahl Aslan.

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Diabetes, Cognitive Ability and Dementia

Diabetes and dementia are increasing in prevalence worldwide, and both diseases cause a great deal of suffering for those affected as well as for the people around them. It has recently been established that people with diabetes run a two-fold risk of developing dementia compared to the general population, but there is not enough knowledge about why the two diseases are associated. This thesis aims to investigate some important aspects of the association, such as potential biomarkers and genetic factors of importance. The results serve as a starting point for further research with a view to identify



people with diabetes who are at greater risk than others of dementia, and to provide these individuals with suitable treatment.

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