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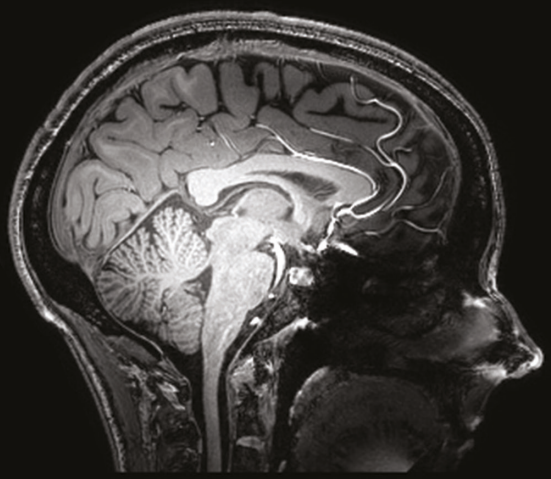

Risk factors for development of neurocognitive disorders

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Title
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Abstract

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Paper I: arterial stiffness was not cross-sectionally related to cognitive performance or presence of cerebral microbleeds, and microbleeds did not affect cognitive performance in cognitively healthy elderly (n=208, mean age 72 years). There was a trend towards an association between arterial stiffness and white matter hyperintensities.

Paper II: increased levels of triglycerides and cholesterol in midlife (mean age 54 years) were independently associated with AD biomarkers (ß-amyloid and tau) 20 years later, in 318 individuals who were cognitively healthy at follow-up (mean age 73 years).

Paper III: higher physical activity in midlife (assessed twice, mean age 58 and 63 years) was independently associated with reduced risk of incident vascular dementia (n=300) during 14 years of follow-up in a population-based cohort (n=20 639). No association between physical activity and incident all-cause dementia (n=1375) or AD (n=834) was found.

Paper IV: ultrasound markers of atherosclerosis measured in midlife (mean age 58 years) were associated with incident vascular dementia (n=109) and all-cause dementia (n=462), but not with AD (n=285) during 20 years of follow-up in a population-based cohort (n=6103). In a cognitively healthy subcohort (n=330) midlife atherosclerosis (mean age 54 years) was associated with cerebral small vessel disease, but not with AD biomarkers at follow-up (mean age 73 years).

These findings suggest that midlife dyslipidaemia may be directly related to brain AD pathology, whereas arterial stiffness, physical activity, and atherosclerosis seem to be primarily related to cerebrovascular disease.

Key words:
vascular risk factors, dementia, Alzheimer's disease, ß-amyloid, tau, white matter hyperintensities

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To my family

Table of Contents

List of original papers

I. **Gustavsson A-M**, Stomrud E, Abul-Kasim K, Minthon L, Nilsson P M, Hansson O, and Nagga, K. Cerebral Microbleeds and White Matter Hyperintensities in Cognitively Healthy Elderly: A Cross-Sectional Cohort Study Evaluating the Effect of Arterial Stiffness. *Cerebrovasc Dis Extra, 2015. 5(2): p. 41-51.*

The final, published version of this article is available at http://www.karger.com/?doi=10.1159/000377710.

II. Nagga K*, **Gustavsson A-M***, Stomrud E, Lindqvist D, van Westen D, Blennow K, Zetterberg H, Melander O, and Hansson O. Increased midlife triglycerides predict brain beta-amyloid and tau pathology 20 years later. *Neurology, 2018. 90(1): p. e73-e81.*

The final, published version of this article is available at https://n.neurology.org/content/90/1/e73.long

III. Hansson O*, Svensson M*, **Gustavsson A-M***, Andersson E, Yang Y, Nägga K, Hållmarker U, James S, and Deierborg T. Midlife physical activity and incidence of vascular dementia and Alzheimer's disease dementia. *Manuscript*.

IV. **Gustavsson A-M**, van Westen D, Stomrud E, Engström G, Nägga K*, and Hansson O*. Dementia incidence and biomarker findings in relation to midlife atherosclerosis. *Manuscript.*

*Equal contributions

Abstract

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These findings suggest that midlife dyslipidaemia may be directly related to brain AD pathology, whereas arterial stiffness, physical activity, and atherosclerosis seem to be primarily related to cerebrovascular disease.

Populärvetenskaplig sammanfattning

Riskfaktorer för utveckling av kognitiva sjukdomar

Bakgrund

Sjukdomar som drabbar hjärnan med påverkan på tankeförmågor, intellekt och funktionsnivå kallas demens eller kognitiva sjukdomar. I takt med stigande medellivslängd ökar andelen äldre i vår befolkning och då ålder är den starkaste riskfaktorn för demens utgör kognitiva sjukdomar ett stort globalt hälsoproblem.

Vaskulära riskfaktorer som högt blodtryck och höga blodfetter i medelåldern anses öka risken för de vanligaste demenssjukdomarna, Alzheimers sjukdom och vaskulär demens. Även ökad kärlstelhet och ateroskleros (åderförkalkning) tycks kunna påverka hjärnförmågor negativt. Vi vet dock fortfarande inte tillräckligt om hur dessa faktorer påverkar de bakomliggande förändringarna i hjärnan, som är olika beroende på vilken sjukdom som ligger till grund för demenssyndromet.

För att fördjupa kunskapen om detta är det avgörande att studera de olika sjukdomarna specifikt, och inte utgå från symtomdiagnosen demens. Tack vare utvecklingen av biomarkörer (biologiska sjukdomsmarkörer) kan vi numera även studera patologiska förändringar i hjärnan i tidiga sjukdomsstadier, innan kognitiva symptom etablerats. Biomarkörer för Alzheimers sjukdom (β-amyloid och tau) kan mätas med ryggvätskeprov och PET-kameraundersökningar och markörer för kärlsjukdom i hjärnan (t ex mikroblödningar och vitsubstansskador) kan påvisas med magnetkamera.

Målsättning

Målet med den här avhandlingen är att bättre karakterisera olika riskfaktorers roll i utvecklingen av demenssjukdomar. Det är exempelvis ännu inte klarlagt ifall vaskulära faktorer påverkar Alzheimerförändringar i hjärnan direkt eller om sambandet beror på ökad förekomst av vaskulära skador i hjärnan som i sin tur gör att symptomen förstärks. Vi har därför undersökt samband mellan vaskulära riskfaktorer och biomarkörer för både Alzheimers sjukdom och vaskulära hjärnskador i en grupp kognitivt friska äldre. Dessutom har vi studerat om riskfaktorer påverkar insjuknande i olika demenssjukdomar i en stor befolkningsstudie.

Resultat

I den första studien såg vi att det fanns ett visst samband mellan stelare kärl och förekomst av vitsubstansskador i hjärnan. Kärlstelhet påverkade däremot inte förekomst av mikroblödningar eller resultat på kognitiva tester.

Den andra studien visade att höga blodfetter i medelåldern ökade risken för Alzheimerförändringar i hjärnan 20 år senare, men i den fjärde studien fann vi *inga*

samband mellan ateroskleros i medelåldern och Alzheimerförändringar. Däremot var ateroskleros associerat med småkärlssjukdom i hjärnan 20 år senare.

I den tredje studien studerade vi samband mellan fysisk aktivitet och insjuknande i demens, och resultaten visade att fysisk aktivitet verkar skydda mot insjuknande i vaskulär demens men inte Alzheimers sjukdom. Likaså fann vi i den fjärde studien att ateroskleros i medelåldern ökade risken för insjuknande i vaskulär demens men inte Alzheimers sjukdom.

Slutsats

Sammantaget indikerar studierna att blodfettrubbningar i medelåldern kan vara en riskfaktor för utveckling av Alzheimers sjukdom. Däremot verkar kärlstelhet, låg fysisk aktivitet och ateroskleros främst vara riskfaktorer för kärlrelaterade hjärnförändringar och vaskulär demens.

Betydelse

Det finns ännu inget sätt att bota demens men om insjuknandet kan skjutas upp med bara några år kan många demensfall undvikas genom att ett stort antal individer aldrig hinner utveckla symptomgivande kognitiv svikt. Detta kan delvis uppnås genom bättre kontroll av riskfaktorer. Våra studier bidrar till att öka förståelsen kring vilka faktorer som är involverade i utvecklingen av kognitiva sjukdomar. Mot bakgrund av den demografiska utvecklingen med alltfler äldre är detta ett viktigt forskningsområde, såväl ur ett patient-anhörigperspektiv som ur ett samhällsekonomiskt perspektiv.

Abbreviations

AD	Alzheimer's disease
Aβ	β-amyloid
APOE	Apolipoprotein E, when italic referring to the gene
CI	Confidence interval
CSF	Cerebrospinal fluid
CV	Cardiovascular Cohort
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, fifth Edition
HR	Hazard ratio
IMT	Intima Media Thickness
MDCS	Malmö Diet and Cancer Study
MDCS CV	Cardiovascular Cohort of the Malmö Diet and Cancer Study
MMSE	Mini Mental State Examination
MRI	Magnetic Resonance Imaging
OR	Odds ratio
P-tau	Phosphorylated tau
PET	Positron Emission Tomography
PWV	Pulse Wave Velocity
SD	Standard deviation
VCI	Vascular Cognitive Impairment
WMH	White Matter Hyperintensities

Introduction

Neurocognitive disorders and dementia

Major neurocognitive disorder is a modern name for dementia, introduced by the American Psychiatric Association in the latest version of the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) [1]. This denomination implies that the syndrome is both neurologic and cognitive, as opposed to the older name. The term dementia originates from Latin and literally means "a being out of one's mind". Consequently, the newer term should preferably be used since it is more neutral and accurate. However, the implementation of new terminology is often slow and I use the older term in my papers. In the title of the thesis I aim for the modern approach, but within the papers and the thesis the old and shorter term is more commonly used. In any case, the terms are used interchangeably in this thesis.

Cognition, or cognitive function, refers to our mind and its abilities to interpret and interact with our surroundings.

In the Oxford dictionary, cognition is defined as

> "The mental action or process of acquiring knowledge and understanding through thought, experience, and the senses" [2].

In Mosby's Medical Dictionary, cognitive function is defined as

> "An intellectual process by which one becomes aware of, perceives, or comprehends ideas. It involves all aspects of perception, thinking, reasoning, and remembering" [3].

Major neurocognitive disorder or dementia is a syndrome diagnosis, and can shortly be defined as verified cognitive decline affecting the ability to be independent in everyday activities. Being a syndrome diagnosis, refers to the fact that it is based on clinical symptoms and does not take biological background or pathophysiology into account. The full DSM-5 criteria for major neurocognitive disorder are summarised in table 1.

Table 1. Major neurocogitive disorder
Diagnostic criteria for major neurocognitive disorder according to DSM-5 [1].

A. Evidence of significant cognitive decline from a previous level of performance in one or more cognitive domains (complex attention, executive function, learning and memory, language, perceptual-motor, or social cognition) based on:
1. Concern of the individual, a knowledgeable informant, or the clinician that there has been a significant decline in cognitive function; and
2. A substantial impairment in cognitive performance, preferably documented by standardized neuropsychological testing or, in its absence, another quantified clinical assessment.
B. The cognitive deficits interfere with independence in everyday activities (i.e., at a minimum, requiring assistance with complex instrumental activities of daily living such as paying bills or managing medications).
C. The cognitive deficits do not occur exclusively in the context of a delirium.
D. The cognitive deficits are not better explained by another mental disorder (e.g., major depressive disorder, schizophrenia).

Dementia is a major health issue affecting an increasing number of individuals worldwide due to medical advances and improved longevity [4]. As of today, around 40 million individuals are estimated to have dementia [5], with prevalence numbers around 6-7% in individuals over 65 years old in western Europe [6].

Historically, dementia was not necessarily considered a disease but was sometimes believed to be part of normal ageing. This is evident from the formerly used diagnosis 'senile dementia', which was part of the ninth edition of the International Classification of Disease (ICD-9) used in Sweden until 1997. The word senile originates from senex, which is Latin for old. In Swedish everyday language, this was called "åldersdemens" (dementia due to age), thus implying that the dementia syndrome was *caused* by old age. Dementia is definitely age-related [7], where the risk increases exponentially with age. Based on current incidence curves [8], one may argue that at some age point (> 110 years), dementia prevalence will reach 100%. Nevertheless, studies on 90- and 100-year olds, clearly show that cognitive abilities and independence can be maintained throughout advanced age considering that 50-60% of individuals aged 90+ do *not* fulfil dementia criteria [9, 10]. Yet, age alone cannot be considered the sole cause of dementia. Instead the term age-related cognitive decline is of relevance, since abilities like psychomotor speed, complex attention, executive function, and memory seem to deteriorate with age [11].

Due to research advancements, knowledge regarding the underlying pathologies causing dementia has increased. In order to offer optimised care and symptomatic treatment, physicians now strive to characterise the aetiology behind the dementia syndrome. This is a fundamental part of the clinical routine in all tertiary centres, specialising in neurocognitive disorders, but it is also increasingly acknowledged in primary care settings.

Alzheimer's disease (AD)

AD was first described in the early 1900's, by the German psychiatrist and neuropathologist Alois Alzheimer. Today, AD is considered to be the principal cause of dementia worldwide, accounting for 50-70% of cases [12]. The neuropathologic alterations first noticed by Dr Alzheimer and described in his publication in 1907 [13], was later identified as plaques of amyloid [14] and neurofibrillary tangles of tau [15]. Still today, they constitute the pathologic hallmarks of AD.

The amyloid hypothesis is the leading theory on the formation of these pathological changes behind the disease. In short, increased production and accumulation of the protein β-amyloid (Aβ) leads to formation of toxic oligomers and deposition of plaques. This induce tau phosphorylation and accumulation as intracellular tangles, synaptic and neuritic injury, eventually causing neurodegeneration and dementia [16-18]. The amyloid hypothesis relies on the identification of inherited gene mutations, affecting the amyloid precursor protein (APP) or the protease involved in cleavage of Aβ (presenilin 1 or 2), thereby causing the familial form of AD [17]. Further, the APP gene is localised on chromosome 21 and individuals with trisomy 21 thereby have three copies of this gene and almost inevitably develop AD neuropathology [18]. It is now suggested that the initial assumption of linear causality may be too simple, considering the increasingly recognised complexity of dementia development, especially in the sporadic form. The dual pathway hypothesis suggests that Aβ and tau pathology may be driven by separate mechanisms, instead of tau being a downstream effect of Aβ [19]. Further, several genes associated with AD have been identified in genome wide association studies (GWAS), where alternative processes suggested to be involved in AD pathology include the immune system as well as lipid, synaptic, and cell membrane processes [20, 21]. There are also proposals that Aβ-pathology may be a downstream effect of other putative risk factors and not necessarily the causative factor [22].

Diagnostic criteria

Both Aβ and tau can be measured reliably in vivo using cerebrospinal fluid (CSF) or positron emission tomography (PET), and alterations appear up to 20 years before onset of cognitive symptoms [23-25]. Thereby, it is now possible to diagnose individuals with prodromal or preclinical AD [23], which leads to important research opportunities, particularly for disease-modifying treatment trials that need to aim at preclinical stages in order to halt pathology before irreversible neurodegeneration and dementia are established. Further, these AD biomarkers add important prognostic and etiological information in clinical routine [26, 27]. Biomarkers are introduced as the basis in updated research criteria for AD [28], but in the clinical setting the diagnosis still relies on clinical presentation with key symptoms of gradual impairment in short-term memory, learning, orientation, and word finding [29]. Similarly, the DSM-5 criteria for AD (summarised in table 2) do not consider biomarkers.

Table 2. Major Neurocognitive Disorder Due to Alzheimer's Disease
Diagnostic criteria for Major Neurocognitive Disorder Due to Alzheimer's Disease according to DSM-5 [1].

A. The criteria are met for major neurocognitive disorder.
B. There is insidious onset and gradual progression of impairment in one or more cognitive domains (for major neurocognitive disorder, at least two domains must be impaired).
C. Criteria are met for either probable or possible Alzheimer`s disease as follows: Probable Alzheimer´s disease is diagnosed if either of the following is present; otherwise, possible Alzheimer`s disease should be diagnosed:
1. Evidence of a causative Alzheimer`s disease genetic mutation from family history or genetic testing.
2. All three of the following are present:
a. Clear evidence of decline in memory and learning and at least one other cognitive domain (based on detailed history or serial neuropsychological testing).
b. Steadily progressive, gradual decline in cognition, without extended plateaus.
c. No evidence of mixed etiology (i.e., absence of other neurodegenerative or cerebrovascular disease, or another neurological, mental, or systematic disease or condition likely contributing to cognitive decline).

It is still not known what triggers the abnormal accumulation of Aβ and tau in the majority of cases, known as sporadic or late-onset AD. Further, cerebral amyloid pathology is common also in cognitively normal individuals [25] and it is sometimes proposed that familial and sporadic AD constitute different disease entities [22]. Sporadic AD is generally considered a multifactorial disease likely driven by both environmental and genetic factors [12]. Several risk factors for AD have been identified (discussed in later), but none of them proving to be a major causative initiator.

Vascular cognitive impairment (VCI) and vascular dementia

Cerebrovascular pathology has long been recognised to contribute to cognitive decline und up until the 1960's, dementia was generally thought to be caused by cerebral atherosclerosis [30]. Today, vascular dementia is considered to be the second most common form of dementia, accounting for around 15% of cases, though prevalence numbers vary considerably [31]. During the last 20 years, efforts have been made to formulate and harmonise definitions and diagnostic criteria for cognitive impairment with an assumed vascular aetiology. Still, the terminology is somewhat diverse. In 2003, the term vascular cognitive impairment (VCI) was introduced as an umbrella term to include all forms of underlying vasculopathy presumed to cause cognitive impairment, ranging from multi-infarct and post-stroke dementia to subcortical ischemic vascular disease and silent or lacunar infarcts [32]. In short VCI can be defined as a syndrome with cognitive impairment in combination with evidence of clinical stroke or subclinical vascular brain injury where the most severe form can be termed vascular dementia [33]. VCI thus applies to both dementia (major neurocognitive disorder) and mild cognitive impairment (minor neurocognitive disorder), where independence in everyday activities is preserved.

The principal feature of vascular dementia is disruption of normal brain function through ischemia or haemorrhage, in turn causing cognitive impairment. The pathophysiology is rather complex, involving athero- and arteriolosclerosis, amyloid

angiopathy, and other vasculopathies as well as the cardiovascular system affecting the brain via hypoperfusion and cardiac embolism [34]. A genetic condition called cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) was described in the 1990's [35] and is known to cause cognitive impairment. CADASIL is caused by mutations in the NOTCH3 gene [36], leading to a specific non-atherosclerotic, amyloid-negative angiopathy primarily involving small arteries of the brain [35-37]. Generally, the cerebral vasculopathy manifests as various parenchymal alterations visualised at neuropathological examination or in vivo by neuroimaging, using either computer tomography (CT) or magnetic resonance imaging (MRI).

Categorisation

As mentioned, vascular dementia can be categorised in many different ways, based on different classifications. A perhaps simplified but clinically useful divide is based on individuals presenting with large or small vessel disease, mainly contributing to cortical or subcortical forms, respectively. Large vessel disease generally cause dementia through clinically overt cerebral infarcts or haemorrhages, severe or strategic enough to cause persistent cognitive impairment [34]. The cognitive abilities affected depend on location of the stroke. It usually presents quite dramatically and develops in a stepwise manner, where new vascular insults give rise to overt deterioration. Small vessel disease is often subtle or insidious in its presentation, where multiple minor events can pass by unrecognised as silent infarctions. The cognitive deficits are caused by white matter lesions, microinfarcts, lacunar infarcts, and microbleeds [34, 38], mainly resulting in processing speed deficits and executive dysfunction [39]. Often, large and small vessel disease co-occur, and it is increasingly recognised that both forms are involved in cortical and subcortical manifestations [34].

Table 3. Vascular terminology
Concepts involved in vascular disease pathology.

Denominations
***Athero*sclerosis –** atheromatous wall thickening and plaque formation
***Arterio*sclerosis –** hardening of medium or large size arteries
***Arteriolo*sclerosis –** hardening of arterioles or small arteries

Small vessel disease

The term cerebral small vessel disease can be used in different contexts (clinically, neuropathologically, and in neuroimaging) with somewhat varying definitions, but ultimately it encompasses any pathological process affecting small vessels of the brain [38]. Since the actual vessels are rather difficult to image, the term generally refers to the parenchymal manifestations detectable on CT and/or MRI such as white matter hyperintensities (WMH) or lesions, lacunar infarcts, and microbleeds [40]. The pathophysiology behind these visible brain lesions is not fully understood, but the main aetiologies are arteriolosclerosis and cerebral amyloid angiopathy, whereas CADASIL

and inflammatory disorders constitute rare origins [38]. *Arteriolosclerosis* (table 3) is considered to be related to age, hypertension, and other vascular risk factors leading to wall thickening and hyalinisation [41]. Further, distal manifestations of *atherosclerosis* may also contribute. Together, these alterations primarily lead to ischemic lesions through chronic diffuse hypoperfusion (i.e. WMH) or acute localised hypoperfusion (i.e. lacunar infarcts). Blood brain barrier damage and inflammation may also contribute. Cerebral amyloid angiopathy refers to deposition of Aβ in the cerebral vessel walls, and is associated with both large cerebral haemorrhages and microbleeds but also with white matter lesions and microinfarcts [38].

Diagnostic criteria

There are no currently accepted neuropathological criteria to confirm a clinical diagnosis of vascular dementia, mainly due to pathological heterogeneity as well as inconsistencies between infarct number and volume in relation to cognitive impairment [31, 33]. This can in part be explained by the importance of location, where infarctions of cognitively important areas bear greater significance, referred to as strategic infarcts [31]. Unfortunately, the criteria applied in different centres also vary substantially [42]. Instead, diagnostic criteria rely on the clinical presentation, were a diagnosis preferably should be verified by evidence of significant cerebrovascular pathology on neuroimaging [1, 33, 43]. The DSM-5 criteria for major vascular neurocognitive disorder are summarised in table 4.

Table 4. Major Vascular Neurocognitive Disorder
Diagnostic criteria for Major Vascular Neurocognitive Disorder according to DSM-5 [1].

A. The criteria are met for major neurocognitive disorder.
B. The clinical features are consistent with a vascular etiology, as suggested by either of the following:
1. Onset of the cognitive deficits is temporally related to one or more cerebrovascular events.
2. Evidence for decline is prominent in complex attention (including processing speed) and frontal-executive function
C. There is evidence of the presence of cerebrovascular disease from history, physical examination, and/or neuroimaging considered sufficient to account for the neurocognitive deficits
D. The symptoms are not better explained by another brain disease or systemic disorder.
Probable vascular neurocognitive disorder is diagnosed if one of the following is present; otherwise possible vascular neurocognitive disorder should be diagnosed:
1. Clinical criteria are supported by neuroimaging evidence of significant parenchymal injury attributed to cerebrovascular disease (neuroimagingsupported).
2. The neurocognitive syndrome is temporally related to one or more documented cerebrovascular events.
3. Both clinical and genetic (e.g., cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) evidence of cerebrovascular disease is present.
Possible vascular neurocognitive disorder is diagnosed if the clinical criteria are met but neuroimaging is not available and the temporal relationship of the neurocognitive syndrome with one or more cerebrovascular events is not established.

All-cause dementia

Other forms of neurocognitive disorders that are included in the term all-cause dementia mainly comprise Lewy Body dementias [44] and frontotemporal dementia [45].

Lewy body dementias refer to both Parkinson's disease dementia and dementia with Lewy bodies, and are characterized by neuronal inclusions of α-synuclein into Lewy bodies. The two forms also share clinical features such as spontaneous parkinsonism, visual hallucinations, fluctuating awareness/cognition, and sleep disturbances, with the only prominent difference being the earlier debut of motor disturbances in Parkinson's disease dementia. Prevalence numbers vary substantially, where dementia with Lewy bodies may account for up to 23% of dementia cases [44].

Frontotemporal dementias are characterised by progressive personality changes, disinhibition, and executive disturbances (behavioural variant) or language impairment (semantic variant or non-fluid variant). Neuropathologically, temporal and frontal regions degenerate due to three main proteinopathies; tau, TDP-43, and FUS. Prevalence numbers are low in the general population, but frontotemporal dementias account for a substantial part of early-onset cases (before <65 years of age) [45].

In reality, the dementia syndrome commonly develops due to mixed aetiologies where at least two different neuropathologies co-exist [46-50]. The younger the age of onset, the higher the probability of one single disease. In geriatric medicine, one may argue that pure forms can be considered exceptions, and the task for clinicians is to define the *primary* contributor to the dementia syndrome in order to offer patient-centred care.

Risk factors and biomarkers

Definitions

The World Health Organization defines a risk factor as

> "Any attribute, characteristic or exposure of an individual that increases the likelihood of developing a disease or injury" [51].

A more detailed definition can be found in a dictionary for epidemiology as follows

> "An aspect of personal behaviour or life-style, an environmental exposure, or an inborn or inherited characteristic, that, on the basis of epidemiological evidence, is known to be associated with health-related condition(s) considered important to prevent" [52].

The term risk factor can include fixed factors, variable factors, modifiable factors, and causative factors [53]. A fixed factor does not change over time, like date of birth or a genetic variant, whereas variable factors, like blood pressure or cholesterol, *do* change

over time and can be modifiable. Further, risk factors can be causative, meaning that they *directly* increase the probability of the event occurring, but the term risk factor does not necessarily imply causality.

The terms risk marker and risk factor are often used interchangeably. However, a risk marker emphasises that no causal link has been established. Further, risk marker is often used to address a *measurement*, whereas a risk factor has a broader meaning.

A biological marker (biomarker) can be defined as

> "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." [54]

In this thesis, when I use the term biomarker I refer to an objective measure that indicates presence of a certain pathology. The biomarker is then used as endpoint, and represents a hallmark of a specific disease. Further I use the term risk marker when the objective measure reflects a pathological process that is used for prediction, and not as endpoint. A risk marker is not self-reported, but preferably measured (i.e. by ultrasound). Finally, I use the term risk factor as a broader definition, including not only risk markers but also lifestyle related factors that are of a more subjective origin (i.e. self-reported). Risk factors are always used as predictor variables, to study associations to the outcome of interest. In this thesis, the term risk factor does not imply causality but merely represent an estimation, either measured or reported, that is statistically related to an increased risk of disease or disease-related pathology.

Age and apolipoprotein E (APOE)

Age is by far the strongest risk factor for dementia, regardless of underlying diagnosis. The second most important *known* risk factor for sporadic AD is the ε4 allele of the *APOE* gene. APOE is a protein involved in lipid metabolism, directing transportation and distribution of lipids between cell types and tissues [55] such as clearance of cholesterol from plasma [56]. There are three main isoforms of APOE, known as E2, E3, and E4, with differences in amino acid composition which seem to affect the functional properties of the protein to some extent [55]. The different isoforms are coded by different alleles, known as ε2, ε3, and ε4. We all carry two *APOE* alleles, with ε3 being the most common (50-90%). Carrying one ε4 (heterozygous) or two ε4 alleles (homozygous) increase the risk of developing AD [55-57] by around three and twelve times respectively [55]. Further, the age of onset decreases with ε4 carrier status [57]. On the contrary, carrying the ε2 allele seem to protect against AD [55]. Further, *APOE* ε4 has also been shown to increase risk of Lewy body dementias [58] and vascular dementia [59], though evidence is not as profound as for AD [55].

APOE ε4 has been suggested to be a causal risk factor for AD under the hypothesis that APOE4 increases Aβ accumulation and/or impairs Aβ clearance, in comparison to the

other protein isoforms [55]. Other theories, imply that the link between *APOE* ε4 and AD is mediated via lipid metabolism [55, 60] or tau phosphorylation and aggregation [55]. There are also evidence proposing that *APOE* ε4 carriers are more susceptible to other AD risk factors, such as high cholesterol [61] and hypertension [62], compared to non-carriers.

Vascular factors

There is a general consensus that vascular risk factors, such as hyperlipidaemia, hypertension, obesity, diabetes, smoking, and physical inactivity are risk factors for dementia in general but also for AD specifically, and not just vascular dementia [12]. However, one may argue that it is still not established if these vascular factors are directly related to the neuropathological changes known to be involved in AD or if the increased risk is conveyed thorough concomitant cerebrovascular pathology accelerating the disease into a symptomatic state. This issue is of relevance in order to conduct individualised risk factor control aiming at primarily preventing the disease of interest.

Epidemiological studies with clinical diagnosis as outcome

Several large cohort studies have reported that *midlife* hypercholesterolemia [63, 64], *midlife* hypertension [63, 65], and *midlife* obesity [66, 67] increase AD risk. Nevertheless, other studies failed to find significant associations to AD, even when midlife assessments and long follow-up periods were evaluated [68-72]. Diabetes is also associated with increased risk of AD [73, 74], though again there are contradictory results as well [75, 76].

Smoking and physical inactivity are sometimes also classified as vascular risk factors, though they are primarily lifestyle related. Smoking has been associated with increased AD risk [77, 78], though other studies report no such association [70, 79]. The association between physical activity and dementia has been assessed in numerous prospective cohort studies [79-95] and physical inactivity has been associated with AD in some observational studies with longer follow-up (> four years) [80, 84, 93, 96], but no significant effect was detected in others [79, 81, 82, 89, 90, 94]. Exercising may act protectively across the lifespan, and not only in midlife, but long follow-up is of relevance in order to account for reverse causality where the cognitive deterioration may result in a more sedentary lifestyle and not vice versa.

Based on meta-analyses [97-104] and systemic reviews [105-107], the prevailing view is that vascular risk factors increase risk of AD, though some reviews are more hesitant [108, 109].

Treatment and prevention

Regarding treatment effects, antihypertensives and statins have been found to reduce risk of incident dementia in observational studies but no effect has been proven in

pooled results from intervention trials [110, 111]. In many cases, *midlife* (40-65 years of age) risk factors seem to convey the greatest risk, and since the association between blood pressure, cholesterol, and weight in relation to dementia risk appears to reverse during the life course [97, 106, 107, 112-114], it can be debated that treating these risk factors in late life (>75 years of age) will not give the desired effect. Therefore, the lack of treatment effect may be due to study design. A recent publication do report a *decrease* in age-specific AD prevalence [6], also confirmed in others [12], which is generally considered to be attributed to higher education and treatment of vascular risk factors in midlife. This supports the potential of preventing dementia by intervening against modifiable risk factors [115].

Relation to AD biomarkers

As described above, the current evidence regarding vascular risk factors and AD is mainly based on epidemiological cohort studies, using dementia incidence assessed as clinical diagnosis as outcome. Recently, studies on associations to AD biomarkers have emerged, which can be argued to be a more direct and objective estimation than clinical diagnosis. The largest study on 942 individuals found that midlife dyslipidaemia was significantly associated with increased Aβ deposition in late-life, as opposed to other midlife risk factors (physical inactivity, obesity, smoking, diabetes, hypertension) [116]. On the contrary, midlife obesity, smoking, diabetes, and hypertension were all associated with neurodegeneration in AD signature regions, assessed as cortical thickness on MRI [116]. In another study on 322 individuals, only midlife obesity was independently associated with late-life Aβ deposition, and none of the other midlife assessments (smoking, hypertension, diabetes, and high cholesterol) [117]. In cross-sectional studies, diabetes was not associated with Aβ PET [118, 119] nor CSF-Aβ [118], but instead with lower cortical thickness, CSF-tau [118], and hypometabolism measured with FDG-PET [119] which may indicate that diabetes is primarily associated with other pathologies than Aβ per se. On the contrary, when vascular risk factors have been assessed as a quantitative score, an elevated score has been associated with increased Aβ deposition, both longitudinally [117] and cross-sectionally [120]. The combined results are thus inconclusive at this point.

Genetic studies

Besides *APOE*, several other genes encoding lipid metabolism are associated with AD [20, 21, 121], well in line with the notion that hyperlipidaemia increases AD risk. No other "vascular" pathways have been identified to be associated with AD in genome wide associations studies [20, 21].

Protective factors

Education has consistently been associated with dementia, where low education confers an increased risk of AD and dementia [122]. Higher education is thought to be protective under the cognitive reserve hypothesis, proposing that individuals with higher IQ,

education, or occupational attainment tolerate accumulation of grater brain pathology before developing manifest cognitive disease [122, 123]. Further, intellectually stimulating leisure activities and social engagement have been proposed to act protectively [123]. As previously discussed, physical activity has also been suggested to decrease dementia risk [102, 104], as has light to moderate alcohol intake [124].

Novel vascular risk markers

The abovementioned factors can be considered traditional vascular risk factors, meaning that they have been known to be associated with risk of cardiovascular disease since the 1960's [125], further confirmed in large multicentre studies of more recent date [126]. In the past decades novel risk markers have emerged, aiming to detect subclinical signs of vascular disease. Two such markers include pulse wave velocity (PWV) and carotid intima media thickness (IMT). These markers provide quantitative measures and enable assessments of vasculopathy before overt vascular disease has been established.

Pulse wave velocity (PWV)

PWV can be measured in different vascular regions, but the carotid-femoral (aortic) measure is considered to be gold standard [127] and is the assessment primarily addressed in this thesis. PWV estimates arterial stiffness, which can be argued to be an objective measure of known and unknown factors influencing vascular properties thus rendering the arteries stiffer.

The pathophysiology involves collagen deposition and degeneration of elastic fibres [127, 128], leading to progressive stiffening likely reflecting arteriosclerosis [33] (separate from atherosclerosis, table 3, page 17). The elasticity of the arteries is important in order to carry pulse waves optimally, and proximal arteries, such as aorta and the carotid arteries, are elastic whereas distal arteries generally are muscular. It is primarily the elastic arteries that are subjected to progressive stiffening as an inevitable effect of ageing [128]. Besides age, arterial stiffness is strongly related to blood pressure [129], whereas other vascular risk factors may be of less importance [129], though evidence do converge [130].

PWV and risk prediction

PWV has been shown to predict future cardiovascular events such as myocardial infarction and stroke [131] independently of traditional vascular risk factors [132], thereby suggesting that the measure contribute additive information.

Besides cardiovascular disease, elevated PWV is independently associated with cerebral small vessel disease visualised as WMH, both cross-sectionally [133-138] and longitudinally [139]. Associations to lacunar/subcortical infarcts [133, 138, 140, 141], silent infarcts [134], and microbleeds [133, 138, 140, 141] are less well established, though pooled estimates in a meta-analysis (also including brachial-ankle measures)

found higher PWV to be significantly associated with microbleeds as well as cerebral infarcts [142].

Results regarding PWV and cognitive function vary [134, 136, 138, 143-147], both in terms of significant associations and affected cognitive domains. Considering that the field has been extensively studied and has rendered heterogenous results, it is not easily summarised. Overall, the current evidence is suggestive of an independent association between higher PWV and worse cognitive function, as proposed in systemic reviews [142, 148, 149], and in more recent publications [138, 143]. However, the reported effect sizes are rather small and several studies did not find PWV to be significantly associated with dementia [145, 150, 151], though a recent study report contradicting results in a small population where 60% developed dementia during follow-up [152].

Carotid intima media thickness (IMT) and plaques

IMT is measured with ultrasound, and can be estimated in the common carotid artery, the carotid bifurcation or bulb, and in the internal carotid artery. In the sonogram, the thickness of the innermost layers, tunica intima and media, of the arterial wall is measured [153]. Additionally, carotid plaques are usually assessed during the ultrasound procedure and are characterised by a focal structure protruding into the arterial lumen [154]. Plaques predominantly appear in areas of turbulence such as the bifurcation, as opposed to the common carotid artery where the hemodynamic conditions are substantially less plaque prone [155, 156]. Estimation of IMT in the common carotid artery is thus preferred, since it is best measured in plaque-free areas [154].

Atherosclerosis is the main pathophysiology behind arterial wall thickening, where lipids and fibrous material accumulate into foam cells (cholesterol-containing macrophages) and fatty streaks in the vessel. Gradually these alterations can develop into plaques of various complexity, including calcification and ulceration, which ultimately can occlude the blood flow via blood clots or through total lumen obstruction by the plaque itself [157]. In general, IMT is considered to be a measure of atherosclerosis [156], but IMT also reflects non-atherosclerotic remodulation such as medial hypertrophy [154, 158]. Carotid plaques are sometimes considered a more accurate measure of atherosclerosis, than IMT [158, 159].

IMT, plaques, and cardiovascular risk prediction

A meta-analysis of several population-based cohorts (including the Malmö Diet and Cancer Study) conclude that IMT is a predictor of future myocardial infarction and stroke in age- and sex adjusted models [160]. The risk prediction remains significant when the traditional vascular risk factors are accounted for [161] using the Framingham Risk Score (age, sex, cigarette smoking status, blood pressure, antihypertensive medication use, total cholesterol level, high-density lipoprotein cholesterol level, and presence of diabetes mellitus) [162]. However, the added value over traditional risk

prediction is limited, and therefore IMT is not recommended in clinical risk assessments regarding myocardial infarction or stroke [161].

Carotid plaques also predict future cardiovascular events [159], and have been proposed to be a better predictor of coronary artery disease than IMT [158].

Regardless of the clinical utility in cardiovascular risk prediction, IMT and presence of plaques can be used as markers or indicators of subclinical atherosclerosis in order to study associations to different diseases or disease mechanisms.

IMT, plaques, and cognitive disease

In terms of relation to cognition and dementia, increased IMT has been associated with cognitive decline [163-167], whereas no association was found between carotid plaques and cognition [163, 167]. The major longitudinal studies assessing relation between IMT and plaques and incident dementia are summarised in table 5. In short, higher IMT was associated with all-cause dementia [168-170] and AD [169, 170] in most, but not all [171], studies. IMT was not associated with incidence of vascular dementia in neither of the two large studies (the Rotterdam study and the three-city study) assessing it as a separate outcome [169, 171]. On the contrary, carotid plaques were associated with vascular dementia in the tree-city study, but not in the Rotterdam study, whereas none of these found plaques to be associated with dementia in general nor AD. However, the small Baltimore study found bilateral, but not unilateral, plaques to be associated with dementia but not AD.

Table 5. Atherosclerosis and dementia
Assoiations between IMT and palques and incident dementia in longituinal studies.

Cohort	Total number	Age, baseline Follow-up	Main results Dementia	AD	VaD
Cardiovascular Health Study	2539	74 (65-97) years Mean 5.4 years	n=376* IMT +	n=236* IMT (+)	n=20* n/a
Rotterdam study	6647	69 ± 9 years 2-9 years	n=678 IMT + Plaque /	n=476 IMT + Plaque /	n=78 IMT / Plaque /
Three-city study	6025	73 ± 4.8 Mean 5.4 years	n=421 IMT / Plaque /	n=272 IMT / Plaque /	n=83 IMT / Plaque +
Baltimore Longitudinal Study of Aging	364	74± 8.3 Mean 7 years	n=60 IMT + Plaque (+)	n=53 IMT + Plaque /	n/a

*individuals with prevalent or incident stroke excluded from analyses (n=259)
+ denote a significant postive association. / denote no associaiton (non-significance). n/a = not applicable, VaD = vascular dementia

Likewise, results regarding the association between IMT, plaques and cerebral small vessel disease are also conflicting. Several studies found higher IMT to be significantly associated with WMH [172-174], contradicted by another study that found plaques, but

not IMT, to be associated with WMH and lacunar infarcts [175]. So far, only one small (n=34) cross-sectional study has assessed the relation between IMT and AD biomarkers, and this study revealed no association [120].

AD biomarkers

β-amyloid (Aβ)

As discussed previously, Aβ and tau are considered to be pathological hallmarks of AD [176]. Beginning in the 1990's, quantification of Aβ in CSF was initiated. At first APP was measured [177], but later it was concluded that decreased levels of the 42 amino acid form of Aβ (Aβ42) and low Aβ42/40-ratio provided the best diagnostic accuracy [26]. A decade later, visualisation of amyloid was developed using Pittsburgh Compound-B (PiB) positron emission tomography PET [178]. Later, other tracers like ^{18}F-flutemetamol were developed [26], and these imaging biomarkers have since proven to be equally reliable as CSF measures in detecting Aβ pathology [179-181]. PET ligands mainly bind to aggregated amyloid, and the decrease in CSF Aβ42 has been shown to reflect aggregation as well [26, 180].

Tau

Quantification of total and phosphorylated tau (p-tau) in CSF also begun in the 1990's, where increased levels were found in AD patients [182, 183]. Total-tau levels increase in response to cortical axon damage in general, and is thus elevated in other brain disorders such as stroke, vascular dementia, frontotemporal dementia, and other neurodegenerative diseases. Certain isoforms of p-tau indicate better specificity and can be used to differentiate between AD and other neurocognitive disorders [27]. Recently, PET tracers that accurately detect tau tangles and filament-tau containing neurites have also been developed [184], further improving the diagnostic potential in discriminating AD pathology from other neurodegenerative lesions in vivo [185].

Clinical utility

As of today, the use of CSF Aβ42, Aβ42/40-ratio, total-tau, and p-tau are part of the diagnostic work-up at specialist units in Sweden, often referred to as Memory Clinics. Together, these biomarkers enable diagnostic sensitivity and specificity for AD of around 85-90% in predementia stages [176, 186]. Considering that multi-pathological alterations are prevalent in the elderly [46-50], it may not be possible to identify a biomarker with complete accuracy. However, in individuals with mild cognitive symptoms where all three CSF AD biomarkers are negative, there is a high negative predictive value virtually excluding AD [176]. This is a major diagnostic advantage in the clinical setting, and in the near future PET imaging of tau may prove to increase diagnostic accuracy even further [185].

Biomarkers for cerebral small vessel disease

The use of CT was initially implemented in dementia diagnostics primarily to exclude treatable causes of cognitive impairment, such as brain tumours or normal pressure hydrocephalous. However, neuroimaging has gradually become increasingly important in characterisation of the underlying brain disorder and is useful both to assess regional atrophy and vascular lesions.

Biomarkers for cerebral small vessel disease refers to the parenchymal lesions visual on neuroimaging, as previously described. The most commonly described manifestations include WMH, also known as white matter lesions, small subcortical infarcts, lacunar infarcts, perivascular spaces, and microbleeds [40]. All of these changes can be evaluated on MRI, using different sequences, whereas CT scans do not allow for full identification (i.e. microbleeds are not detectable).

All of these neuroimaging markers of cerebral small vessel disease are strongly age-related and are therefore prevalent among elderly. Prevalence of microbleeds range between 10-15% general populations [187-190] and WMH were present in 95% of individuals in two separate population-based cohorts over 60-65 years old [191, 192]. Presence of low grade WMH can thus be considered completely normal in aged populations. However, increasing WMH load is associated with cognitive decline [39] and dementia incidence [39, 193], as are microbleeds [190, 194, 195].

Since neuroimaging is commonly performed, often with other objectives than cognitive investigations, the significance of these commonly occurring findings can be hard to interpret on the individual patient-level. Attempts have been made to create combined rating scales, but the sensitivity and specificity for dementia is often poor [196]. However, once cognitive impairment is determined, biomarker evidence of small vessel disease indicates that cerebrovascular disease contribute to the syndrome - either as the primary origin or as an additive component.

Rationale

In light of these often diverse and sometimes contradictory findings regarding the role of vascular disease and related risk factors in development of cognitive impairment and dementia, there is a need to further study these proposed associations. The emergence of dementia biomarkers provides a possibility to better characterise the links between the two by using direct measures of brain pathology known to be involved in dementia development. Ultimately, sound evidence of an association relies on reproducibility that indicates consistency between different study populations as well as coherence between different assessments reflecting the studied biological processes. These considerations illustrate the rationale for this thesis.

Aims

The overall aim of this thesis is to extend the knowledge regarding factors suggested to be involved in the development of neurocognitive disorders.

More specifically, the aims are

- assess the consequences of MRI findings of cerebral small vessel disease on cognitive function
- study if traditional vascular risk factors and novel vascular risk markers are associated with biomarkers of cerebrovascular pathology or AD in cognitively healthy elderly, thus assessing preclinical stages of dementia
- assess if previously reported findings regarding risk factors for neurocognitive disorders or dementia can be reproduced in a large population-based setting

Methods

Here follows an overview of the different study populations and methods used in the studies. Generally, a more detailed description can be found in the separate papers.

Study populations

All participants originate from the prospective population-based Malmo Diet and Cancer Study (figure 1). It was initiated in the early 1990's with the aim to study diet and cancer, as is evident from the cohort name, but also to function as a source to test emerging hypotheses in other research contexts [197]. Parallelly, a cardiovascular cohort was introduced to study carotid artery disease [198, 199]. The cardiovascular cohort was later invited to a reinvestigation, and cognitive screening tests were added to the study protocol. This led to the recruitment of a cognitively healthy cohort who agreed to take part in the Swedish BioFINDER study. Consequently, all cohorts share the same baseline protocol.

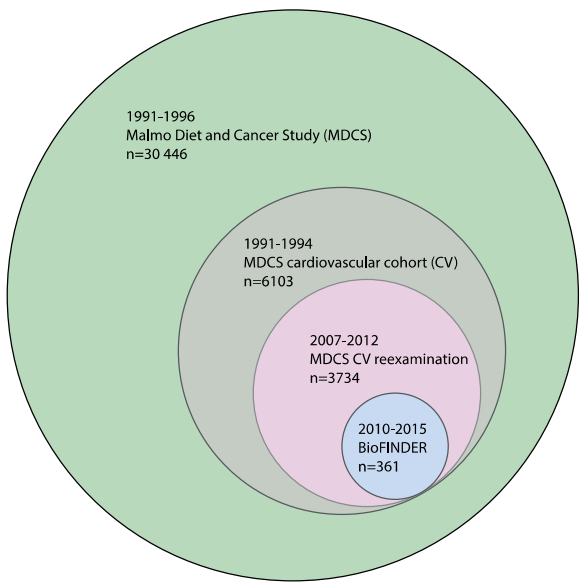


Figure 1. Study populations
All cohorts stem from the same population-based study.

Malmo Diet and Cancer Study (MDCS) baseline

Between 1991 and 1996 Malmö inhabitants aged 44 to 74 years were invited to the prospective Malmo Diet and Cancer study (MDCS). Selection was based on the population register, and in total 74 138 individuals were originally identified for recruitment. The only a priori exclusion criteria were mental retardation or language problems. Invitation was made via public advertisement and personal letters (up to three letters per individual). The major reasons for non-participation were no response (n=21 817), refusal (n=16 942), death/move (n=3017), and exclusion (n=1975) due to language or mental retardation [200].

At baseline, a questionnaire was handed out, blood pressure, height, and weight were measured, and blood samples were collected and stored in a blood bank. The questionnaire was self-administered and assessed education, physical activity, tobacco and alcohol use, medical history, current health, and medication use. Other information was also gathered, such as dietary assessments, but these aspects are not covered in this thesis.

From the background population (n=74 138), 30 446 individuals (41%) were recruited and are included in the MDCS data set.

MDCS five-year reinvestigation

Between 1997 and 2001, the same questionnaire that was administered at baseline was sent out to all MDCS participants. 22 369 participants (equivalent to a participation rate of 73% of the original cohort) responded to the questionnaire a second time and were thus part of the five-year reinvestigation (figure 2). No other measurements were performed at this point.

MDCS cardiovascular cohort (CV) baseline

The cardiovascular cohort (n=6103) is a subpopulation of the MDCS, initiated to study the epidemiology of carotid artery disease [198, 199]. The MDCS CV constitute a random sample of participants entering the MDCS 1991-1994 (figure 1 and 2). The baseline study protocol was extended and also included carotid ultrasound. Further, quantification of fasting blood glucose and lipid levels were done.

MDCS CV reinvestigation

The cardiovascular cohort was invited to a reinvestigation that took part between 2007 and 2012 (figure 1 and 2). During the follow-up period of 16.7 ± 1.5 years (mean ± SD), 1036 (17%) of the baseline cohort were deceased and 143 (2,3%) emigrated. 3734 individuals took part in the reinvestigation, which resulted in an attendance rate of 76% of the surviving baseline population.

The reinvestigation protocol involved a questionnaire, covering the same aspects as the baseline questionnaire, renewed blood sampling, as well as height, weight, and blood pressure measurements. Carotid ultrasound was repeated and measurement of pulse wave velocity was added to the study protocol. Further, in 2008 the protocol was

extended to also include the cognitive screening tests Mini-Mental State Examination (MMSE) [201] and A Quick Test of cognitive speed (AQT) [202].

The cognitively healthy cohort of the Swedish BioFINDER study

Beginning in 2009, participants were recruited to the BioFINDER study from the ongoing MDCS CV reinvestigation (figure 2). Eligibility was based on >60 years of age, MMSE score of >27 points, and no subjective cognitive impairment. Individuals fulfilling these criteria were invited to take part in the BioFINDER study and if positive, they were referred to the Memory clinic for a thorough assessment before final inclusion. This second clinical evaluation was performed by trained physicians and included renewed detailed medical history, physical examination covering both neurologic and psychiatric status, clinical dementia rating (CDR), and extended cognitive testing. In total, 437 individuals underwent a second evaluation and 76 individuals were excluded due to the following exclusion criteria; 1) CDR > 0, mild cognitive impairment or dementia, 2) presence of significant neurologic or psychiatric disease (e.g. clinically diagnosed stroke, Parkinson's disease, multiple sclerosis, major depression, alcohol abuse), or 3) unwillingness or failure to fulfil inclusion criteria at the second assessment. Inclusion terminated when the predefined cohort size was arrived at, resulting in 361 participants (figure 1 and 2).

The Swedish BioFINDER study is a prospective study including four different cohorts followed longitudinally with assessments biannually. Apart from the cognitively healthy cohort, it consists of the following cohorts; 1) mild cognitive symptoms (mild cognitive impairment or subjective cognitive decline), 2) clinical dementia, and 3) Parkinsonian disorders. Since this thesis only include the *cognitively healthy cohort* of the Swedish BioFINDER study, it is usually referred to as the BioFINDER cohort.

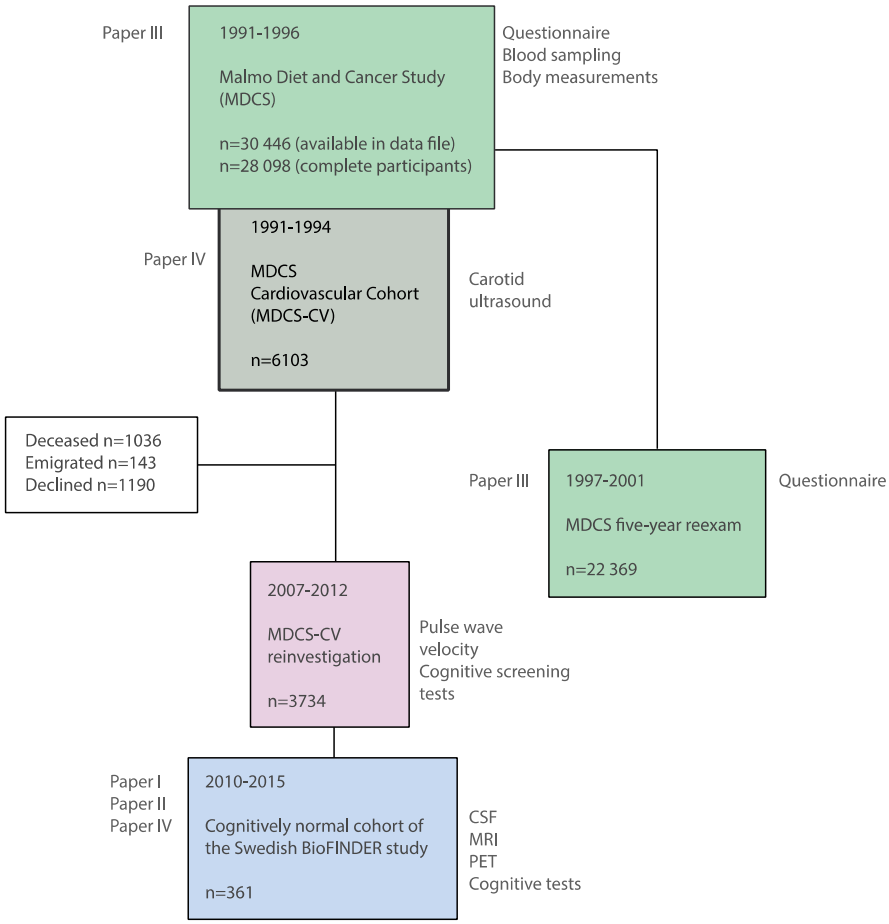


Figure 2. Flow diagram of the different cohorts

Paper I

Paper I include the 208 cognitively healthy participants enrolled in the BioFINDER study who had undergone MRI by the time of the image analyses, performed in 2013.

Paper II

In paper II, the study population constitutes the 318 cognitively healthy participants in the BioFINDER study who had available data on CSF biomarkers and midlife laboratory tests (derived from the MDCS CV baseline).

Paper III

The study population in paper III comprise all MDCS participants who provide data on physical activity at *both* baseline and the five-year reinvestigation, and who were non-demented at the reinvestigation (n=20 639).

Paper III also includes another prospective cohort called the Vasaloppet cohort and an experimental animal model. These cohorts are *not* included in this thesis, since my main responsibility was the MDCS cohort. Briefly, the Swedish Vasaloppet study included Vasaloppet skiers (n=197 685) and frequency-matched controls from the general population (n=197 684), denoted non-skiers. The AD mouse model consisted of 30 female mice co-expressing five familial AD mutations (5xFAD), three APP mutations and two Presenelin 1 mutations, and thereby rapidly developing severe Aβ pathology.

Paper IV

The MDCS CV baseline population (n=6103) constitute the study population in paper IV. Further, all participants with available CSF data in the cognitively healthy BioFINDER cohort (n=330) was included as a subcohort. Both cohorts share the same baseline data (figure 1 and 2).

Predictors

Pulse wave velocity – arterial stiffness (paper I)

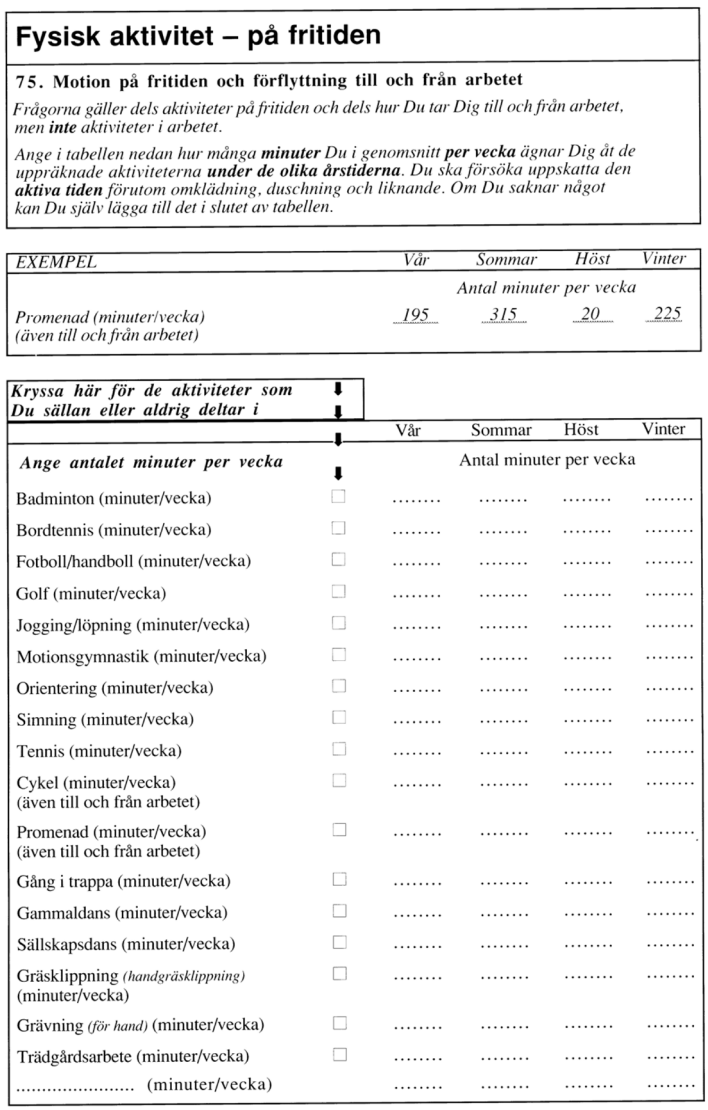
Arterial stiffness was measured as carotid-femoral PWV at the MDCS reinvestigation (2007-2012). Using a specific device (SphygmoCor, Atcor Medical, Australia), pulse waves from the femoral and carotid arteries were measured consecutively. The velocity (metres/second) of the pulse wave was computed as the ratio between the carotid femoral path length (using standardised body surface measurements) and the carotid femoral transit time (using electrocardiogram) [127, 203]. In the statistical analyses, PWV was modelled continuously as a z-score, in order to present results per SD increase. Dichotomisations of PWV at the top quartile (75^{th} percentile), top quintile (80^{th} percentile), and top decile (90^{th} percentile) were also applied to test non-linear associations (not included in the published paper).

Lipids (paper II)

Serum lipid levels were measured after an overnight fast at the baseline visit in MDCS CV (1991–1994). Triglycerides, total cholesterol, high-density lipoproteins (HDL), and low-density lipoproteins (LDL) were quantified using standard clinical procedures at the University Hospital [199].

Physical activity (paper III)

Information on physical activity during leisure time was self-reported in two questionnaires, administered at the MDCS baseline (1991-1996) and at the five-year re-examination (1997-2001). Physical activity was estimated by calculating a score, where reported activities were graded with intensity codes (activity specific factors) and then multiplied with reported time of performance (minutes/week for all four seasons).



Fysisk aktivitet – på fritiden

75. Motion på fritiden och förflyttning till och från arbetet

*Frågorna gäller dels aktiviteter på fritiden och dels hur Du tar Dig till och från arbetet, men **inte** aktiviteter i arbetet.*

*Ange i tabellen nedan hur många **minuter** Du i genomsnitt **per vecka** ägnar Dig åt de uppräknade aktiviteterna **under de olika årstiderna**. Du ska försöka uppskatta den **aktiva tiden** förutom omklädning, duschning och liknande. Om Du saknar något kan Du själv lägga till det i slutet av tabellen.*

EXEMPEL	*Vår*	*Sommar*	*Höst*	*Vinter*
	Antal minuter per vecka			
Promenad (minuter/vecka) (även till och från arbetet)	*195*	*315*	*20*	*225*

Kryssa här för de aktiviteter som Du sällan eller aldrig deltar i	Vår	Sommar	Höst	Vinter
Ange antalet minuter per vecka	Antal minuter per vecka			
Badminton (minuter/vecka) ☐	.....	.....	.....	.....
Bordtennis (minuter/vecka) ☐	.....	.....	.....	.....
Fotboll/handboll (minuter/vecka) ☐	.....	.....	.....	.....
Golf (minuter/vecka) ☐	.....	.....	.....	.....
Jogging/löpning (minuter/vecka) ☐	.....	.....	.....	.....
Motionsgymnastik (minuter/vecka) ☐	.....	.....	.....	.....
Orientering (minuter/vecka) ☐	.....	.....	.....	.....
Simning (minuter/vecka) ☐	.....	.....	.....	.....
Tennis (minuter/vecka) ☐	.....	.....	.....	.....
Cykel (minuter/vecka) (även till och från arbetet) ☐	.....	.....	.....	.....
Promenad (minuter/vecka) (även till och från arbetet) ☐	.....	.....	.....	.....
Gång i trappa (minuter/vecka) ☐	.....	.....	.....	.....
Gammaldans (minuter/vecka) ☐	.....	.....	.....	.....
Sällskapsdans (minuter/vecka) ☐	.....	.....	.....	.....
Gräsklippning *(handgräsklippning)* (minuter/vecka) ☐	.....	.....	.....	.....
Grävning *(för hand)* (minuter/vecka) ☐	.....	.....	.....	.....
Trädgårdsarbete (minuter/vecka) ☐	.....	.....	.....	.....
..... (minuter/vecka)	.....	.....	.....	.....

Figure 3. Physical activity assessment
Description of how physical activity was reported in the MDCS questionnaire.

As can be seen in figure 3, participants stated the form of physical activity performed (for example walking, gardening, and/or running) and minutes per week the activity was done at every season (spring, summer, autumn, winter). The activity was then multiplied with an intensity factor, where heavier activities were graded with a higher factor. This generates a validated physical activity score [204] calculated as; the sum of number of minutes per week for all four seasons multiplied with the activity specific factor, for every activity stated.

The combined physical activity score was calculated as the sum of the scores from the two time points (MDCS baseline and five-year reinvestigation). The combined score was chosen since it can be hypothesised that individuals who report high physical activity at two occasions five years apart are more likely to have an effect of their lifestyle than individuals only reporting it on one occasion. In the statistical analyses, physical activity was modelled both continuously based on a z-score (per SD increase) and categorically based on tertiles of the total physical activity score (the 33rd and 67th percentile). This categorisation into three groups was done to see if there were any non-linear effects of physical activity, but also to try and minimise effects of extreme values. Further, it was done in order to facilitate comparison to the Vasaloppet cohort, where the population was categorised as skiers and non-skiers.

Intima media thickness and plaque – atherosclerosis (paper IV)

Ultrasound of the right carotid artery was performed at the MDCS CV baseline visit (1991-1994), and carotid IMT and plaques were measured by certified sonographers. A specially designed computer-assisted imaging system was used to estimate IMT in the common carotid artery, measured in millimetres (mm). In the statistical analyses, IMT was assessed both as a continuous measure and as a categorical measure, where individuals were grouped by IMT quartiles (the 25th, 50th, and 75th percentiles). This was done to evaluate non-linear, or threshold effects, and also for comparison to previous publications that generally model IMT categorically.

Carotid plaques were measured in a prespecified area of the carotid bifurcation, including 3 cm of the right common carotid artery and 1 cm of both the internal and external carotid artery. During the baseline data collection, a 3-graded scale was initially used (n=1600) but was later replaced by a 6-graded scale (n=4249).

We used two plaque definitions in the statistical analyses, one dichotomous (binary) and one categorical (by three classes). The categorisation was done in order to assess gradual effects. Presence of any carotid plaque was defined as a focal intima-media thickening of >1.2 mm. To construct the categorical variable, the 6-graded scale was converted to the 3-graded scale, and roughly modelled as no plaque (0) vs one plaque (1) or multiple plaques (2). A full description of the plaque score categorisation can be found in paper IV.

Outcomes

Cerebrovascular biomarkers (paper I, II, and IV)

MRI was used for detection of cerebrovascular disease, visualised as white matter hyperintensities, cerebral microbleeds, and lacunar infarcts. Participants in the BioFINDER study, underwent 3T MRI between 2009 and 2015. The study protocol comprised axial T2 fluid-attenuated inversion recovery (FLAIR), coronal magnetisation-prepared rapid gradient echo (MPRAGE) sequence, and coronal gradient-echo T2*- weighted images (GRE) or susceptibility weighted images (SWI). SWI were added to the study protocol in 2014, and therefore only performed in a subset of participants (n=70).

White matter hyperintensities

In paper I, WMH were rated visually on FLAIR images, according to Fazekas rating scale (table 6) [205].

Table 6. Fazekas rating scale
Description of how white matter hyperintensities were graded.

Periventricular hyperintensities (PVH)		Deep white matter hyperintensities (DWMH)	
0	Absence	0	Absence
1	Caps or pencil thin lining	1	Punctuate foci
2	Smooth halo	2	Beginning confluence of foci
3	Irregular, extending into deep white matter	3	Large confluent areas

WMH were classified as present (abnormal) in individuals who demonstrated a score of ≥2 in either periventricular or deep white matter regions. This categorisation was done since grade 1 WMH can be considered normal in this age group as only 4% in a large stroke-free population showed *no* signs of white matter abnormalities [192]. After this dichotomisation, presence of WMH (yes/no) was used as dependent variable in multivariable logistic regression models. This categorisation was also used to group individuals as WMH positive (+) or negative (-), in analysis of covariance.

When recruitment to the cognitively healthy BioFINDER cohort was completed, a volumetric estimation of WMH was performed. WMH volume was assessed using an automated tool [206], and calculated in millilitres (mL). This volume estimation was not available when Paper I was finalised.

In paper II, WMH volume was assessed as a continues measure (in mL) and used as dependent variable in linear regression models.

In paper IV, WMH volume was included as a measure of cerebral small vessel disease. In this context, WMH volume was dichotomised at the median (50th percentile). This

categorisation was done in order to get a more complete estimation of cerebrovascular disease, extending beyond the sole measure of WMH.

Cerebral microbleeds

Microbleeds were rated visually and defined as round, hypointense lesions (signal voids) with a maximum diameter of 10 mm. Ratings were performed on GRE images (n=207) in paper I and on either GRE (n=256) and SWI (n=63) in paper IV. In paper I, microbleeds were assessed binary (yes/no) and used as dependent variable in logistic regression models and used to group individuals as having microbleeds (+) or not (-) in analysis of covariance. In paper IV, presence of microbleeds were incorporated in the small vessel disease estimate.

Cerebral small vessel disease

In paper IV, we created a variable representing cerebral small vessel disease by combining estimations of WMH volume, microbleeds, and lacunar infarcts. WMH volume and microbleeds were assessed as described above. Lacunar infarcts were assessed visually on FLAIR and MPRAGE images by an experienced neuroradiologist, according to Wardlaw [40]. Small vessel disease was then defined as either WMH volume > median, presence of cerebral microbleeds, and/or presence of lacunar infarcts.

Cognitive test results (paper I)

Within the BioFINDER study, a set of cognitive tests were administered aiming to cover the major cognitive domains (table 7). The MMSE and the delayed word recall task have ceiling effects with a set maximum, whereas none of the other tests do. Reference values are not presented since these are age and education dependent for several tests.

Table 7. Cognitive tests witin the BioFINDER study
Short summary of included tests.

Test	Estimate	Cognitive domain
Mini-mental state examination (MMSE)	Number of correctly answered questions, 0-30 points	Global function
A quick test of cognitive speed (AQT)	Naming color and shape of 40 geometric symbols, seconds	Executive function, speed, and attention
Alzheimer's disease assessment scale (ADAS), delayed word recall	Number of words recalled, 0-10	Episodic memory
Symbol Digit Modalities Test (SDMT)	Number of decifered symbols in 90 seconds	Speed and attention
Stroop test	Naming word colour instead of reading the text, seconds	Executive function
Trailmaking test A (TmT-A)	Connect numbers in ascending order, seconds	Speed and attention
Trailmaking test B (TmT-B)	Connect numbers and letters in ascending and alternating order, seconds	Executive function
Letter S fluency	Number of words beginning with S in 60 seconds	Language fluency
Animal fluency	Number of animals in 60 seconds	Language fluency

Cognitive test results were modelled continuously, using the raw measure, in the statistical analyses.

AD biomarkers (paper II and IV)

Cerebrospinal fluid (CSF)

CSF was collected within the BioFINDER study (2010-2015), where Aβ42 and tau phosphorylated at Thr181 (p-tau) were quantified using INNOTEST ELISA. Aβ42 and Aβ42/p-tau ratio were classified as normal or abnormal based on cut-off values calculated with mixture models. Under the assumption that the data is a mixed sample of two different normal distributions, the model reveals a cut-off point at the intercept between the two. Both variables revealed a bimodal distribution suitable for establishing nonoptimized, unbiased cut-offs with mixture modelling. The estimated cut-offs were abnormal Aβ42 <500 pg/mL and abnormal Aβ42/p-tau ratio <7.7. The CSF Aβ42/p-tau measure presented the best sensitivity and specificity of all CSF and PET measures described in a previous publication [207].

Positron Emission Tomography (PET)

A subgroup of 139 individuals in the BioFINDER cohort additionally underwent ^{18}F-flutemetamol PET (2013-2105) as a measure of cerebral Aβ accumulation. The standardised uptake value ratio (SUVR) was the global composite tracer uptake, normalised for the mean uptake in the cerebellar cortex. A composite SUVR >1.42 was considered abnormal, based on mixture modelling.

To explore associations to AD pathology, Aβ42, Aβ42/p-tau ratio, and PET SUVR were dichotomised according to the described cut-offs, and used as dependent variables in the statistical analyses.

Clinical dementia diagnoses (paper III and IV)

All dementia diagnoses registered in the Swedish National Patient Register (NPR) on individuals in the MDCS baseline cohort were retrieved in 2014. The NPR covers inpatient care since 1987 and hospital-based outpatient care since 2001, with almost full coverage [208]. The diagnoses included in the NPR are derived from hospital charts, where both primary and secondary diagnoses are routinely registered by the treating physician according to the International Classification of Diseases (ICD).

Following the register outtake, all diagnoses were thoroughly reviewed in electronic charts by medical doctors at the Memory Clinic at Skåne University Hospital. Based on symptom presentation, cognitive test results, brain imaging (CT or MRI), and CSF analyses (when available), all diagnoses were assessed in accordance with DSM-5 [1] (summarised in table 1, 2, and 4). A diagnosis of mixed AD, or AD with concomitant cerebrovascular pathology, was used when the clinical presentation and neuroimaging

findings suggested that both pathologies significantly contributed to the disorder. In the statistical analyses, AD (both pure and with concomitant cerebrovascular disease), vascular dementia, and all-cause dementia were used as event variables.

Confounders

The covariates used to control for confounding in the different studies vary (table 8). The reason for this is that the different studies use different predictors and outcomes and thereby the rationale for covariate selection differ. Additionally, availability also affected covariate selection between studies since the different cohorts provide somewhat different data. Generally, covariate selection was based on previous publications assessing similar predictors and outcome. For example; mean arterial pressure, heart rate, and height were used as covariates in paper I, since these factors directly influence PWV [209] and are considered standard to adjust for in PWV assessments. In the remaining papers, systolic blood pressure and body mass index were instead used, since these are more commonly addressed in dementia research (discussed in the paragraph on risk factors). This approach was used both to address the theoretical grounds for confounding, thereby including vascular risk factors as covariates, and to simplify comparison to previous research.

Table 8. Covariates
Summary of covariates used in the differens studies.

Study	Model 1	Model 2	Model 3	Model 4
Paper I	Age and sex	+ mean arterial pressure, heart rate, height, weight, smoking, total cholesterol, lipid lowering medication, and blood pressure-lowering medication		
Paper II	Age	+ sex, *APOE* ε4, and education	+, intima-media thickness, systolic blood pressure, fasting blood glucose, and body mass index	+ cardiovascular disease, smoking, physical activity, and lipid-lowering medication (at follow-up)
Paper III	Age, sex, and education	+ smoking, systolic blood pressure, body mass index, alcohol consumption, diabetes, cardiovascular disease, blood pressure-lowering medication, lipid-lowering medication,and physically heavy work	+ *APOE* ε4 (in a sensitivity analysis)	
Paper IV	Age	+ sex, *APOE* ε4, and education	+ systolic blood pressure, body mass index, smoking, diabetes mellitus, blood pressure-lowering medication, lipid lowering medication, and stroke	

Due to reviewer preferences in paper II, we used rather extensive adjustments and thus needed to perform backward elimination procedures in order not to violate the plausible number of covariates. Generally, there is a rule of thumb saying a model can hold one covariate per 10 positive events in the dependent variable in order not to overfit the model thus losing power to detect true associations [210].

Statistics

Group differences were assessed with independent samples t-test, Mann Whitney U test, Pearson χ^2 test, or Fischer's exact test as appropriate. Continuous variables were converted to z scores to model the predictor per standard deviation increase instead of increase per measured unit. Z scores were calculated according to the formula $z = (x - \mu)/\sigma$ using the raw measure (x), mean (μ), and SD (σ). Interaction terms were assessed by simultaneously entering the two predictor variables separately and a variable consisting of their product in a regression model. Descriptions of how the different predictors (independent variables) and outcomes (dependant variables) were modelled are described in the sections on the respective predictors and outcomes.

Regression analyses

Main analyses were performed with multivariable regression models, to study associations between the predictors and outcomes while adjusting for possible confounders. Linear regression was used for linear or continuous dependent variables and logistic regression was used for binary or dichotomous dependent variables. Cognitive tests were also assessed using analysis of covariance, to compare mean values between groups.

Cox regression was used to explore associations to incident dementia, where time under risk and censoring need to be addressed. When follow-up duration varies between participants and when the event of interest (dementia) has not occurred in all participants by the end of the study, it is preferable to use a model that takes time under risk into consideration so that individuals are censored when they are no longer under risk (i.e. at end of follow-up or death).

Covariates were added in a stepwise manner in order to show both cruder associations (i.e. only age-adjusted) and then to test if a potential association was independent of other theoretically important factors. The conclusions were primarily drawn based on the fully adjusted model.

Underlying model assumptions were tested during data analyses. Examples include Schoenfeld residuals to verify the proportionality assumption in Cox regression models. Hosmer-Lemeshow test of goodness of fit and Cooks distances were checked in logistic regression models. Collinearity between covariates was assessed using variation inflation factors.

Incidence

Incidence is defined as the number of individuals developing a disease in a specified population during a given period [52]. Incidence stands in contrast to *incidence rate*, which denotes the rate at which new events occur in a population. Incidence rate is calculated as number of new events during the period divided by number of individuals at risk during this period, sometimes expressed as person-time incidence rate. In prospective population-based cohort studies, the study period often varies between participants. For example, in the MDCS, inclusion took part during five years (1991-1996) and follow-up ended simultaneously (2014), except when individuals were deceased or lost to follow-up when it terminated earlier. This means that the population and study period are not fixed. Therefore, age-specific person-time incidence rates are preferable when incidence needs to be compared between groups.

In papers III and IV, we only present simple incidence numbers describing the number of new cases. Thus, the terms "incidence of dementia", "dementia incidence", and "incident dementia" refer to the number of identified dementia cases developing during the study period (from baseline and onwards till end of follow-up) in the addressed study population. These simple incidence numbers do not account for individual follow-up time. Importantly, the varying follow-up and thereby the number of individuals at risk at every time-point, is accounted for in the statistical analyses (as described in the section on Cox regression).

Table 9. Characteristics

Characteristics of the full Malmö Diet and Cancer Study cohort and the different (sub)cohorts included in the four papers. Only baseline data is presented, in order to give an overview of the expected health selection successively occuring. The MDCS CV is a random sample of the full MDCS cohort.

Characteristics at baseline 1991-1996	Full MDCS cohort	**MDCS 5y cohort - study III**	**MDCS CV cohort - study IV**	**BioFINDER cohort - study I[a], II, and IV**
Number of participants	30 446	20 639	6103	330
Age	58.0 ± 7.6	57.8 ± 7.5 *	57.5 ± 5.9	53.8 ± 4.6 #
Sex, women	18 326 (60)	12 460 (60)	3531 (60)	198 (60)
Education:		*		#
Primary/elementary school (≤8 years)	11 971 (42)	8 159 (40)	2676 (46.3)	79 (24)
Secondary school/high school (9-12 years)	9965 (35)	7 449 (36)	1982 (34.3)	143 (44)
Higher education/university (≥13 years)	6563 (23)	5 001 (24)	1123 (19.4)	104 (32)
APOE ε4 carriers [b]	4845 (30)	3 306 (30)	1704 (30)	92 (28)
Smoking, current	8087 (28)	5 455 (26) *	1620 (28)	67 (21) #
Systolic blood pressure	141.1 ± 20.1	140.5 ± 19.6 *	141.4 ± 19.1	133 ± 16 #
Body mass index	25.8 ± 4.0	25.6 ± 3.9 *	25.8 ± 4.0	24.8 ± 3.5 #
Physical activity score	8085 ± 6647	8292 ± 6746 *	8137 ± 5923	8598 ± 5675 #2
Coronary disease or stroke	923 (3.0)	543 (2.6) *	147 (2.4)	0 (0) #
Diabetes	1380 (4.5)	790 (3.8) *	290 (4.8)	7 (2.1) #
Blood pressure-lowering medication	5279 (17)	3 568 (17)	1010 (17)	31 (9.4) #
Lipid-lowering medication	919 (3.0)	629 (3.0)	141 (2.3)	3 (0.9)
Dementia diagnoses at follow-up -2014				
Any dementia	2118 (3.8)	1 375 (3.5)	462 (3.5)	
Vascular dementia	531 (0.9)	300 (0.8)	109 (0.8)	
Alzheimer's disease dementia	1211 (2.2)	834 (2.1)	285 (2.1)	

Numbers are mean ± standard deviation or numbers (%). Dementia diagnoses are described as number of incident events with incidence rate per 1000 person-years in parenthesis. Group differences are estimated with independent samples t-test or χ^2.

* Significant difference (p<0.01) compared to *non-participants* from the full MDCS population. # Significant difference (p<0.05) compared to *non-participants* from the MDCS CV population. #2 denote significance estimated with Mann-Whitney U-test.

[a] In paper I, only the first 208 individuals were included but these are not separately presented in this table. [b] *APOE* data was available in 53% of the full MDCS cohort and in 94% of the cardiovascular cohort

Main results and discussion

Baseline characteristics for all cohorts are summarised in table 9.

Paper I

In this cross-sectional study, we investigated the relation between arterial stiffness (PWV) and biomarkers of cerebral small vessel disease in cognitively healthy elderly (n=208). Further, we assessed if arterial stiffness and MRI markers of small vessel disease affected cognitive performance in elderly with globally preserved cognitive abilities.

Results

Prevalence of cerebral small vessel disease in the study cohort is summarised in table 10. Mean age ± SD was 72 ± 4.8 years.

Table 10. Prevalence of cerebral small vessel disease in cognitively healthy elderly
Results from visual rating of MRI

MRI findings	Number (%)
Microbleeds	25 (12%)
Lobar microbleeds	22 (11%)
Deep microbleeds	3 (1.4%)
Multiple microbleeds	6 (3%)
Moderate or severe WMH*	65 (31%)

*Fazekas score 2-3 in either periventricular or deep white matter

PWV was not associated with microbleeds, and only partially associated with WMH (table 11). Dichotomising PWV at the top quartile (75^{th} percentile) did not reveal any significant associations to neither microbleeds nor WMH (analyses not included in the published paper). However, when PWV and WMH load > median was assessed in the whole population (n=320, post publication analyses) there was a significant association (OR per SD increase in PWV 1.4, 95% CI 1.02-1.93, adjusted for age, sex, *APOE* ε4, education, heart rate, mean arterial pressure, weight, height, smoking, total cholesterol, diabetes, use of lipid-lowering medication, and use of blood pressure-lowering medication).

Table 11. Arterial stiffness (PWV) and MRI markers of cerebral small vessel disease
Logistic regression models

PWV	Microbleeds		WMH	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Unadjusted	1.09 (0.63-1.88)	0.75	1.83 (1.24-2.72)	0.003
Model 1	0.88 (0.48-1.19)	0.69	1.58 (1.04-2.40)	0.03
Model 2	0.67 (0.31-1.44)	0.31	1.29 (0.81-2.08)	0.29

OR (95%CI) are per SD increse in PWV
Model 1 adjusted for age and sex. Model 2 + mean arterial pressure, heart rate, height, weight, smoking, total cholesterol, use of lipid-lowering medication, and use of blood pressure-lowering medication

Neither PWV nor microbleeds were cross-sectionally associated with cognitive performance. Individuals with WMH performed worse in one test of speed and attention (39 ± 8.1 vs 35 ± 7.8 points, p=0.049, adjusted for age, sex, and education). No other significant differences were found (table 4 in paper I).

Discussion

Arterial stiffness and microbleeds

When this paper was prepared, only two previous studies had assessed if arterial stiffness was associated with microbleeds. These studies also reported neutral results, both in the population-based Rotterdam cohort (n=1460) [133] and in a risk population (n=167) of hypertensive patients [140]. Our study supports these findings also in a selected population of healthy elderly. Subsequently, another population-based study (n=1255) replicated these findings [138], though yet another paper found a borderline significant association between higher PWV and microbleeds (adjusted OR 1.12, 95% CI 1.00-1.26, in the fully adjusted model) [141]. Due to sample size, we could not perform analyses based on number or location of microbleeds. It is hypothesised that lobar microbleeds are mainly due to cerebral amyloid angiopathy, whereas deep or subcortical microbleeds are mainly due to hypertensive vasculopathy [187]. In the Rotterdam study, higher PWV was associated with deep or infratentorial microbleeds, but only in a subgroup of individuals with uncontrolled hypertension [133]. One could argue that this is mediated by hypertension per se, and the PWV measure may not add much information since it is already established that PWV is highly blood-pressure dependent [129]. Indeed, no association between PWV and subcortical microbleeds was found in the whole population [133], and neither in the other population-based cohort [138].

Arterial stiffness and WMH

The lack of a robust association between PWV and WMH may be due to the small sample size, since there is rather convincing evidence of an association between the two in the literature [133-139]. This was indeed confirmed when we ran analyses in the full BioFINDER population (post publication). There is also a plausible biological association, since the brain is an organ with high blood flow it is sensitive to increased

pressure and flow. Structures in the deep, subcortical parts of the brain receive blood from arteries that arise directly from the circle of Willis and may thus be less able to damp excessive pulsatility [211]. This may in turn result in WMH through arteriolosclerosis and chronic diffuse hypoperfusion [38].

Small vessel disease and cognitive function

Our results regarding cognitive function were neutral, except for a marginally significant difference in one test. Considering the large number of statistical tests performed in the paper, the risk of type I error increases and the reported significance in this one test may be wrongfully inferred. Nevertheless, a growing literature suggests that WMH [39, 193], and (multiple) microbleeds [190, 194, 212] are in fact associated with cognitive performance and dementia.

Arterial stiffness and cognitive function

Since there was already a great body of evidence suggestive of an inverse association between PWV and cognitive function when we conducted the study, as summarised in a systemic review from 2014 [149], one may argue that our analyses on PWV and cognitive test results do not add significantly to the research field. Especially considering the cross-sectional design of the study, the small sample size, and the predefined inclusion criteria of being cognitively well-functioning. However, another review pointed out that there is evidence of publication bias in the field, where published studies reporting significant associations had relatively smaller sample sizes [142]. This possibly *overestimates* the association between PWV and cognition, and highlights the need to publish neutral or negative results.

Most of the large cohort studies assessing association between PWV and cognition do *not* include markers of cerebral small vessel disease in the statistical models [134, 138, 143-145]. When this was done in one study, it led to attenuation of the association [136]. One may therefore speculate that the observed associations between PWV and cognition may be mediated by cerebral small vessel disease, as recently proposed [141]. Further, no obvious publication bias was observed for the relation between PWV and cerebral small vessel disease [142]. This could possibly also explain our neutral results, since we studied a healthy population with relatively modest cerebrovascular burden.

Interpretation

Arterial stiffness is not associated with cerebral microbleeds, but the joint literature suggests an association with WMH though our results were not robust to full adjustments. The relation between arterial stiffness and WMH may, at least in part, mediate the reported association between arterial stiffness and cognitive function. Solitary microbleeds and moderate WMH do not necessarily affect cognitive performance in elderly that are otherwise well-functioning. This emphasise the fact that a dementia diagnosis can never be made based on an image, but truly requires the whole picture. Nevertheless, individuals presenting with signs of extensive cerebral small

vessel disease are at risk of developing cognitive impairment and should preferably be followed to monitor decline [39, 193].

Paper II

In this longitudinal study, we investigated if lipid levels in midlife were associated with Alzheimer pathology 20 years later in individuals who were cognitively healthy at follow-up (n=318).

Results

CSF and PET biomarkers revealed abnormal amounts of Aβ in 20% and indicated mixed Aβ/p-tau pathology in 16% of the cognitively healthy population. Mean age ± SD at baseline was 54 ± 4.7 years and mean follow-up time was 20 ± 1.6 years. Results are summarised in table 12. In short, midlife triglycerides were associated with both Aβ and Aβ/p-tau pathology at follow-up. Midlife cholesterol levels were associated with abnormal CSF Aβ/p-tau, but not with CSF Aβ and only partially with Aβ PET. LDL was partially associated with CSF Aβ/p-tau. No significant associations were found for HDL.

Table 12. Midlife lipids and late-life AD biomarkers
Logistic regression models (backward elimination, thus only showing results for variables with p<0.10)

	Abnormal Aβ42	**Abnormal Aβ42/p-tau**	**Abnormal Aβ PET***
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Triglycerides			
Model 1	1.39 (1.08–1.79)	1.54 (1.17–2.03)	1.80 (1.13–2.86)
Model 2	1.40 (1.08–1.81)	1.53 (1.15–2.02)	1.75 (1.07–2.84)
Model 3	1.41 (1.08–1.84)	1.53 (1.16–2.03)	1.75 (1.07–2.84)
Model 4	1.34 (1.03–1.75)	1.46 (1.10–1.93)	
Cholesterol			
Model 1		1.49 (1.10–2.01)	1.49 (0.99–2.24)
Model 2		1.40 (1.00–1.95)	
Model 3		1.41 (1.01–1.97)	
Model 4		1.44 (1.03–2.02)	
HDL			
Model 1			0.59 (0.34–1.03)
Model 2			0.61 (0.34–1.09)
Model 3			0.61 (0.34–1.09)
Model 4			
LDL			
Model 1		1.49 (1.10–2.01)	1.45 (0.97–2.15)
Model 2		1.38 (0.99–1.92)	
Model 3		1.39 (1.00–1.93)	
Model 4		1.45 (1.04–2.02)	

OR (95%CI) are per SD increse in lipid concentration

Model 1 adjusted for age. Model 2: + sex, APOE ε4, and education. Model 3 + intima-media thickness, systolic blood pressure, fasting blood glucose, and body mass index. Model 4: + cardiovascular disease, smoking, physical activity, and lipid-lowering medication (at follow-up). * PET was performed in 134 participants.

There was no significant interaction between triglycerides and *APOE* ε4, (interaction term p=0.11, p=0.45, and p=0.20 respectively for the different dependent variables), but since there is a plausible biological relation between the two, we performed analyses by *APOE* ε4 strata (table 13, not included in the published paper). The association between triglycerides and *pure* Aβ pathology was primarily found in *APOE* ε4 carriers. However, there was a trend towards an association between triglycerides and CSF Aβ/p-tau in *non-carriers* (table 13).

Table 13. Midlife triglycerides and late-life AD biomarkers stratified by *APOE* ε4 carrier status
Logistic regression models (backward elimination, thus only showing results for variables with p<0.10)

	Abnormal Aβ42		**Abnormal Aβ42/p-tau**		**Abnormal Aβ PET***	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
***APOE* ε4 carriers**						
	n=87				n=37	
Model 1	2.08 (1.17-3.71)	0.01			2.76 (1.09-6.97)	0.03
Model 4	2.57 (1.34-4.91)	0.004			2.93 (1.11-7.74)	0.03
Non-carriers			n=228			
Model 1			1.42 (1.03-1.96)	0.04		
Model 4			1.34 (0.96-1.86)	0.09		

OR (95%CI) are per SD increse in lipid concentration
Model 1 adjusted for age. Model 4: + sex, education, intima-media thickness, systolic blood pressure, fasting blood glucose, body mass index, cardiovascular disease, smoking, physical activity, and lipid-lowering medication (at follow-up)
* PET was performed in 134 participants.

Discussion

The objective of this paper was to see if midlife lipids were associated with AD-pathology, since epidemiological studies reported that midlife dyslipidaemia was a risk factor for clinically diagnosed AD. When we drafted this paper, the association between blood lipids and AD pathology in vivo had only been studied cross-sectionally, with inconsistent results [213-216]. Further, one autopsy study found total cholesterol, LDL, and triglycerides, measured 10 years earlier, to be significantly associated with neuritic plaques but not neurofibrillary tangles [217]. Another autopsy study found that late-life HDL, but not total cholesterol, was associated with neuritic plaques and neurofibrillary tangles. However, for midlife HDL (measured 20 years earlier in a subgroup, n=89) there was a trend towards an association with neuritic plaques, and a rather strong association with neurofibrillary tangles [218]. Hence, the relation between lipids and AD pathology was unclear.

Lipids, Aβ, and APOE ε4

During the finalisation of the manuscript, two separate papers on midlife vascular risk factors and Aβ PET were published. The larger of the two (n=942), found that midlife dyslipidaemia was associated with Aβ deposition [116], where elevated triglycerides were included in the definition. On the contrary, the other study (n=322) did not find hypercholesterolemia to be associated with Aβ PET. A very recent publication could

not replicate our finding regarding midlife triglycerides and Aβ, where no association was detected in a population of 122 women. Instead, mainly LDL and partly total cholesterol was associated with Aβ, but these associations were attenuated when *APOE* ε4 was added to the model. However, they found *APOE* ε4 carriers with high cholesterol and LDL to have 10-fold increased odds of Aβ pathology (but do not present any interaction statistics) [61]. As is shown in table 13, we also found *APOE* ε4 carriers to be more vulnerable to Aβ-pathology with higher triglyceride levels, though without a significant interaction.

APOE ε4 carriers seem to be predisposed to increased AD risk based on the effects of other known risk factors as well [219]. This was also found in relation to Aβ pathology, where a cumulative number of midlife risk factors increased the odds of Aβ positivity almost 10-fold in *APOE* ε4 carriers, whereas there was no significant risk increase for non-carriers. However, the authors of this study conclude that they did *not* prove a significantly elevated risk of Aβ deposition related to vascular risk factors in *APOE* ε4 carriers, based on the non-significant interaction [117]. However, in a cross-sectional study (n=118) there was a significant interaction between *APOE* ε4 and hypertension in relation to Aβ pathology, indicating that hypertensive *APOE* ε4 carriers had an increased risk of Aβ deposition [62]. On the contrary, a meta-analysis found more pronounced effects of smoking on AD risk in *non*-carriers, but the interaction term was not tested [101].

In conclusion, it is not yet established if there is a significant interaction between different lipids and *APOE* ε4 in relation to Aβ pathology, since it is possible that prevailing studies have been underpowered to detect such effect [117]. In our study, the interaction term of triglycerides**APOE* ε4 for CSF Aβ42 showed a trend towards significance (OR 1.69, 95% CI 0.88-3.25, p=0.11). Therefore, one could argue that these findings may be *suggestive* of an interactive effect, which then would support that a plausible link between dyslipidaemia and AD could involve both APOE4 and Aβ. As previously mentioned, APOE4 is involved in lipid metabolism and seems to increase Aβ accumulation and impair Aβ clearance [55]. The relation between APOE and Aβ is also supported by the finding that *APOE* ε4 is associated with Aβ pathology [116, 220] but not neurodegeneration, when Aβ was accounted for [116], nor tau [220]. Neurodegeneration seem to be more closely related to tau pathology than Aβ [221, 222]. Further, dyslipidaemia was not associated with neurodegeneration, and neurodegeneration may be an effect of other parallel pathologies than Aβ per se [116].

There are also potential links between dyslipidaemia and Aβ directly, such as lipids possibly influencing membranes and thus secretase-mediated Aβ [223] or that lipids could affect Aβ aggregation [224]. Therefore, it is possible that triglycerides relate to Aβ regardless of APOE4.

Interpretation

We confirm a relation between midlife dyslipidaemia and AD by showing that elevated midlife triglycerides and cholesterol are associated with AD biomarkers in cognitively healthy elderly.

Paper III

In this longitudinal study, we aimed to investigate if physical activity in midlife was associated with subsequent development of dementia and to study if there were any effect differences between dementia subtypes, assessed as clinically-derived diagnoses.

Results

Mean age was 57.8 ± 7.5 years at baseline, and 62.8 ± 7.5 at the 5-year reinvestigation. Participants were followed for a mean of 19.2 ± 3.8 years from baseline and 14.3 years ± 3.6 years from the 5-year reinvestigation. Based on the diagnostic review process 1375 individuals (6.7% of total study population) were diagnosed with dementia during the follow-up period, equal to an incidence rate of 3.5 per 1000 person-years. Out of all dementia cases, 300 (22%) were classified as vascular dementia and 834 (61%) as AD (including AD with concomitant cerebrovascular disease). Mean age at time of dementia diagnosis was 79.4 ± 6.0 years.

Higher physical activity was associated with reduced risk of incident vascular dementia, but not all-cause dementia nor AD (table 14). Results were similar when physical activity was modelled linearly (per SD increase) and categorically as three groups (table 14).

Excluding individuals developing dementia within five years from the second physical activity assessment rendered similar results (table 4 in paper III). Likewise, including *APOE* ε4 as a covariate did not alter any findings in the subgroup with available *APOE* ε4 data (n=10 971). There was no significant interaction between *APOE* ε4 and physical activity for any of the dependant variables (p=0.68 for AD, p=0.40 for vascular dementia, and p=0.32 for any dementia).

Table 14. Physical activity and incident dementia
Cox regression models

Physical activity	Alzheimer's dementia	Vascular dementia	Any dementia
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Per SD increase in combined score			
Model 1	1.03 (0.97-1.09)	**0.81 (0.72-0.93)**	0.96 (0.91-1.02)
Model 2	1.03 (0.97-1.10)	**0.84 (0.74-0.96)**	0.97 (0.92-1.02)
Per physical activity group (tertiles)			
Model 1	832 events	300 events	1373 events
Low (Reference)	1	1	1
Intermediate	1.01 (0.85-1.19)	0.87 (0.66-1.14)	0.99 (0.87-1.12)
High	1.04 (0.88-1.23)	**0.63 (0.48-0.84)**	0.90 (0.79-1.02)
Model 2	815 events	293 events	1341 events
Low (Reference)	1	1	1
Intermediate	0.98 (0.82-1.16)	0.91 (0.69-1.19)	0.98 (0.86-1.12)
High	1.03 (0.87-1.22)	**0.67 (0.50-0.90)**	0.90 (0.79-1.03)

Model 1 adjusted for age, sex, and education. Model 2 + smoking, systolic blood pressure, body mass index, alcohol consumption, diabetes, cardiovascular disease, blood pressure-lowering medication, lipid-lowering medication, and physically heavy work.

Results from additional cohorts

In paper III, results from another epidemiological cohort and an AD mouse model are also included (see appendix). Briefly, in the Swedish Vasaloppet study, the HR for dementia, AD, and vascular dementia were assessed for Vasaloppet skiers (n=197 685) vs matched controls from the general population (n=197 684). Vasaloppet skiers had lower risk of developing all-cause dementia and vascular dementia, but not AD, during follow-up. In the AD mouse model, brain levels of Aβ, synaptic proteins, and cognitive function were studied in an exercising group (n=16) exposed to 6 months of voluntary wheel running, compared to sedentary controls (n=14). No significant differences in these outcomes were noted between exercising and sedentary mice, except that *sedentary* mice had significantly *better* spatial memory compared to exercising mice.

Discussion

The objective to study the association between physical activity and dementia development in the MDCS arouse when the co-authors of paper III presented the results from the Vasaloppet cohort in combination with that of the mouse model. The lack of an association between exercise and incident AD and AD pathology was a bit controversial, and the field was extensively studied. When these results were confirmed in the MDCS cohort, we wanted to communicate these findings in a paper combining all three cohorts showing concurrent results based on different study setups.

As previously mentioned, numerous prospective cohort studies assessing physical activity and dementia have been published [79-95] and likewise several meta-analyses [102-104, 225]. This abundance aggravates the interpretation of the field since there is

considerable heterogeneity in assessments and results are often diverse. However, meta-analyses providing pooled estimates do support the notion that higher physical activity reduce risk of both dementia in general [102-104], and AD specifically [103, 104].

Effects of follow-up and reverse causality

If one exclusively looks at results from studies with at least *four years* of follow up that presents *separate results for AD*, several did not reveal *significant* associations between physical activity and incident AD [79, 81, 82, 89, 90, 94]. Some do [80, 84, 93, 96], and others report partly significant findings [86, 95]. The largest (n=10 308) population-based study on physical activity and dementia provide repeated physical activity assessments and reports that physical activity begin to decline up to *nine years* before diagnosis of dementia [85], thus emphasising the possible impact of reverse causality in studies with shorter follow-up. In this study, no association between midlife physical activity and dementia was found during 27 years of follow-up, but no separate analysis for dementia subtypes are presented [85]. Other studies that assess *midlife* measures of physical activity (mean age <65 at baseline) again report mixed results [83, 84, 89, 92, 95].

One meta-analysis tried to address the reverse causality paradigm by separately analysing studies with more than ten years of follow-up and found that these showed weaker (non-significant) protective effects of physical activity for both cognitive decline (RR 0.89, 95% CI 0.62-1.27) and dementia (RR 0.86, 95% CI 0.68-1.11). Importantly, this meta-analysis also found that publication bias may have influenced the findings since a large number of smaller studies showed larger-than-average effect [102]. Noteworthy, no separate publication has included as many participants as the MDCS, and the largest published study (n=10 308), which found no relation between physical activity and cognition nor dementia, [85] has not been included in any of the meta-analyses.

Vascular dementia

Fewer studies report separate results for vascular dementia, and the number of incident cases in the separate studies are rather low (between 27 and 54) [79, 83, 91, 93, 94, 96], except in one study with 213 cases [90]. Results are yet again mixed, and only two studies report significant associations between physical activity and vascular dementia [79, 91], whereas one more study reports a significant association with dementia with cerebrovascular disease [94]. However, a meta-analysis found a protective effect from a pooled estimate including 374 cases with vascular dementia [225], whereas a more recent meta-analysis did not [226].

Biological mechanism

Physical activity has been proposed to act protectively through improved cerebral perfusion and cognitive reserve [102], or via improved vascular health thus lowering cerebrovascular disease load [102, 227]. Animal studies have suggested that physical activity may reduce AD pathology specifically [228], but this was not confirmed in the

experimental part of paper III (appendix). Further, no longitudinal association has been found in humans assessing associations between physical activity and Aβ pathology measured with PET [116], and no short-term effects have been found on AD biomarkers in intervention studies [229]. However, one intervention trial proved that a multi-domain intervention, including physical activity, improved cognitive function in *at-risk* elderly [230]. Other intervention trials saw no effect on incident dementia [231] or cognitive function [232], but in the study with the longest follow-up (mean 6.7 years), the risk of developing non-AD dementia was significantly reduced, with a trend towards protection against vascular dementia specifically [231].

Interpretation

In conclusion, our results support an association between physical activity and vascular dementia. However, considering the extensive literature finding physical activity to reduce AD risk, our results do not necessarily provide evidence *against* an association between physical activity and AD due to methodological short-comings that may have influenced the results (mainly the lack of a complete dementia assessment and attrition/missing data, discussed later). Nevertheless, in favour of reporting neutral and non-significant results in order to reduce publication bias, this study provides results from a very large population-based cohort with many incident dementia cases. It also contributes data from midlife (reported twice five years apart) in combination with extended follow-up, thus enabling analyses accounting for reverse causality. All this can be argued to add considerably to the field, and also may *question* the relation between physical activity and AD, especially in the context of intervention studies failing to reduce dementia development [231].

Paper IV

In this longitudinal study, we aimed to investigate if ultrasound markers of atherosclerosis are associated with incident dementia in a population-based cohort (n=6103) during long-term follow-up. We further aimed to study if the same markers are associated with abnormal accumulation of Aβ and tau or small vessel disease in a subcohort (n=330) with no signs of cognitive impairment at follow-up.

Results

Ultrasound markers and incident dementia

During a mean follow-up of 20 ± 5.0 years, 462 individuals (7.6%) were diagnosed with dementia with an incidence rate of 3.5 per 1000 person-years. Out of all dementia cases, 285 (63%) were classified as AD (including AD with concomitant cerebrovascular pathology), and 109 (24%) as vascular dementia. Mean age at baseline was 57.5 ± 5.9 years and mean age at dementia diagnosis was 77.7 ± 5.8 years.

Midlife IMT was not associated with incident AD, but higher IMT in midlife was associated with increased risk of both vascular dementia and all-cause dementia (table 15). Results for categorical IMT were mainly concurrent (table 2, paper IV).

Presence of carotid plaques (any vs none) was not significantly associated with any dementia outcome, except with vascular dementia in the age-adjusted model (table 15). When plaques were modelled categorically, higher plaque score was significantly associated with vascular dementia and partly with all-cause dementia, but not with AD (table 3, paper IV).

Table 15. Atherosclerosis and incident dementia in a population-based cohort (n=6103)
Cox regression models

	Alzheimer's dementia	Vascular dementia	Dementia
IMT	HR (95% CI)	HR (95% CI)	HR (95% CI)
1 SD increase			
Model 1	1.08 (0.95-1.21)	**1.36 (1.16-1.59)**	**1.16 (1.06-1.27)**
Model 2	1.06 (0.93-1.21)	**1.40 (1.19-1.66)**	**1.17 (1.06-1.29)**
Model 3	1.05 (0.92-1.20)	**1.32 (1.10-1.57)**	**1.14 (1.03-1.26)**
Carotid plaque			
Model 1	1.08 (0.83-1.41)	**1.82 (1.13-2.94)**	1.23 (1.00-1.52)
Model 2	1.10 (0.84-1.45)	1.60 (0.97-2.66)	1.20 (0.96-1.50)
Model 3	1.09 (0.83-1.45)	1.46 (0.87-2.43)	1.15 (0.92-1.44)

Model 1 adjusted for age. Model 2: + sex, APOE ε4, education. Model 3: + systolic blood pressure, body mass index, smoking, diabetes mellitus, blood pressure-lowering medication, lipid lowering medication, and stroke.

Ultrasound markers and brain pathologies

In the cognitively healthy subcohort (n=330), CSF revealed abnormal Aβ42 in 75 participants (23%) and abnormal Aβ42/p-tau ratio in 52 participants (16%). Cerebral small vessel disease was present in 170 (53%) participants, either as WMH volume >median, lacunar infarcts (present in 12 participants), or cerebral microbleeds (present in 27 participants). Mean age at baseline was 53.8 ± 4.6 years and mean age at follow-up was 73.3 ± 5.0 years.

There was no independent association between midlife IMT and abnormal CSF Aβ42 and Aβ42/p-tau ratio 20 years later. Higher IMT was associated with cerebral small vessel disease (table 16).

Carotid plaques in midlife was not associated with any of the measured brain pathologies 20 years later (table 16), neither when categorical assessments were applied (table 5, paper IV).

Table 16. Atherosclerosis and biomarkers for AD and cerebral small vessel disease in cognitively healthy elderly (n=330)
Logistic regression models

	Abnormal Aβ42	Abnormal Aβ42/p-tau	Small vessel disease
	OR (95% CI)	OR (95% CI)	OR (95% CI)
IMT			
Model 1	**1.41 (1.02-1.95)**	1.45 (1.00-2.09)	**1.50 (1.10-2.03)**
Model 2	1.28 (0.89-1.84)	1.27 (0.85-1.89)	**1.52 (1.11-2.08)**
Model 3	1.28 (0.87-1.90)	1.35 (0.86-2.13)	**1.47 (1.05-2.06)**
Carotid plaque			
Model 1	1.20 (0.69-2.06)	1.06 (0.56-2.00)	1.00 (0.62-1.63)
Model 2	1.03 (0.57-1.85)	0.91 (0.47-1.78)	0.97 (0.59-1.58)
Model 3	1.05 (0.57-1.94)	0.94 (0.47-1.90)	0.99 (0.59-1.65)

Model 1 adjusted for age. Model 2: + sex, APOE ε4, education. Model 3: + systolic blood pressure, body mass index, smoking, diabetes mellitus, blood pressure-lowering medication, and lipid lowering medication. No individuals in the subcohort had stroke at baseline.

Discussion

This study was undertaken to study if the relation between vascular risk factors, here assessed as subclinical atherosclerosis, and AD is mediated through a direct effect of vascular factors on key AD pathology like Aβ and tau. We also studied associations with cerebrovascular pathology, as this may be an alternative way through which the relation could be mediated. Since we also had data on incident dementia in the population-based cohort from where the subgroup with MRI and CSF was recruited, we wanted to look at associations with clinically-derived dementia diagnoses as well.

Previous studies

As summarised in table 5 on page 25, available publications show diverging results regarding markers of atherosclerosis and incident dementia. Our findings are in line with most previous studies reporting higher risk of all-cause dementia with higher IMT, measured in late-life [168-170]. Results regarding the relation between atherosclerosis and AD are more diverging. In accordance with the three-city study [171], we did not find IMT to be associated with AD, as opposed to other previous publications [168-170]. Carotid plaques have generally not been associated with AD [169-171]. Clinically derived diagnoses encompass uncertainty and mixed pathologies are common [46-50], which may be an important factor contributing to these incoherencies. This may be of especially great importance in our study, where no standardised cognitive assessments were performed within the study protocol.

Other reasons for discrepancies between studies may be the IMT categorisation. Most studies found that individuals with IMT in the top quintile (20%) had increased risk of dementia and AD, but findings for continuous measures were either not reported [168, 169] or non-significant [170]. Carcaillon et al argue that this threshold effect may reflect plaques within the thickened IMT measure [171].

Atherosclerosis and AD pathology

The use of direct measures of AD pathology may clarify some of the queries raised when assessing clinically-derived diagnoses. Unfortunately, neuropathological studies assessing the relation between atherosclerosis and AD pathology also show diverging results [233, 234]. The one published study assessing IMT and Aβ in vivo, found no cross-sectional association [120]. Taken together, our study together with this previous report [120] and one neuropathological study [233] do not support an association between atherosclerosis and AD.

Interpretation

Midlife atherosclerosis may primarily relate to increased risk of dementia through cerebrovascular pathology and not AD pathology.

General discussion and future perspective

Main findings – added value

Vascular risk factors and dementia biomarkers

Despite the extensive literature addressing the role of vascular risk factors in dementia development, several issues remain to be elucidated. One of the unsolved questions concerns if vascular risk factors are associated with the specific brain changes occurring in AD development or if the risk increase is mediated via concurrent vascular brain pathology rendering the individual more vulnerable to AD pathology. By using biomarkers for AD and cerebrovascular pathology it is possible to better address this question since clinically derived diagnoses encompass diagnostic uncertainty and can be greatly influenced by mixed pathologies [46-50]. We found that higher lipid levels in midlife were indeed associated with direct measures of AD pathology, but not WMH, after long term follow-up. On the contrary, we did not find midlife atherosclerosis to be associated with Aβ or tau but instead with cerebral small vessel disease. Arterial stiffness was not assessed in relation to AD biomarkers, but our findings indicated a cross-sectional association with cerebrovascular disease.

Lipids

Based on our findings in combination with others, one may argue that hyperlipidaemia differ from other vascular risk factors. Firstly, there is a plausible relationship, where dyslipidaemia may exert its effects via APOE4 and Aβ [55, 235, 236]. Secondly there is genetic evidence that lipid metabolism is involved in AD [20, 21]. Thirdly, studies on direct measures of Aβ has found dyslipidaemia to be of primary significance [116]. Further, there is actually not as profound evidence for hypercholesterolemia as a risk factor for vascular dementia as there is for AD [64, 97], which possibly may be due to this effect not being conveyed through general vasculopathy.

Arteriosclerosis and atherosclerosis

Arterial stiffness can be considered to reflect arteriosclerosis, and one can speculate that these alterations primarily lead to cognitive decline through cerebral small vessel disease [141]. Our results support that increased pulse wave velocity may contribute to

development of WMH, but not microbleeds nor cognitive performance in cognitively healthy elderly. The diversity regarding results of arterial stiffening on cognitive function [142, 148, 149], and dementia [145, 150, 151], highlights the complexity of the brain and the multifactorial origin of dementia. The same accounts for studies assessing markers of atherosclerosis in relation to cognition and dementia [168-171], where we speculate that atherosclerosis contribute to dementia primarily through cerebrovascular alterations and do not directly affect Aβ or tau pathology. This can also be the case for arteriosclerosis, since PWV failed to prove a cross-sectional association between the gold-standard PWV measure (carotid-femoral) and AD pathology [237]. Regardless, these age-related vascular alterations most likely account for some of the decline in cognitive function known to occur with age and probably *contribute* to dementia development. This can also be conferred from the proposition that small vessel disease is a leading cause of cognitive decline and functional loss in the elderly [38].

Lifestyle and dementia

We could not confirm an association between midlife physical activity and AD in our large population-based sample. This may either be due to a true lack of an association or to methodological shortcomings discussed below. Regardless, we did find physical activity to be associated with vascular dementia, the second most common form of dementia, and this association was independent of several other vascular risk factors. Cerebrovascular disease is commonly found in elderly post-mortem and considering the importance of mixed pathologies in dementia development [46-50], the possibly protective effect of physical activity on vascular dementia is of great importance. Thereby, our findings can be considered to support the notion that lifestyle interventions may help reduce or postpone dementia development, irrespective of any direct effects on AD per se.

Methodological considerations

Recruitment bias

In epidemiology, recruitment bias can be expected and this mainly introduces bias by participants being generally healthier than non-participants [238]. This can in turn lead to wrongfully inferred results, but hopefully it predominantly leads to an *under*estimation of found associations since the event of interest probably occurs in fewer individuals within the study population as compared to the true population. A publication addressing recruitment bias in the MDCS found that non-participants had 2-4-fold higher mortality than participants [200], thereby suggesting health selection bias.

This recruitment bias is even more pronounced in the cognitively healthy BioFINDER cohort, as is evident in table 9, page 44. Since we aimed at investigating healthy elderly, this was anticipated and we believe that it is of importance to study associations in this setting in order to evaluate the relevance of risk factors in such populations. This complements studies carried out in memory clinic settings, thus focusing on diseased brains.

Attrition

In addition to recruitment bias, attrition inevitably occurs during follow-up in prospective cohort studies, and again, individuals adhering to the study protocol are generally healthier than dropouts. Especially cognition seems to be related to attrition [239]. As can be seen in table 9 on page 44, this is also true in the MDCS. Yet again, this introduces health selection bias. This is further demonstrated in table 17 where the proportion of individuals with dementia in the study cohort in paper III is compared to non-participants ("dropouts"). These individuals were excluded since they did not participate in the five-year reinvestigation and thus do not contribute repeated physical activity assessments, but they do provide data on incident dementia. As can be seen in table 17, non-participants were generally *less* physically active at baseline and had a *higher* dementia incidence rate. This may underestimate the association between physical activity and vascular dementia, and importantly *may* bias the finding that physical activity was not associated with all-cause dementia or AD.

Table 17. Attrition analysis in paper III
Incidence rate per 1000 person-years in the study cohort and non-participants in combination with som additional descriptive data

Dementia diagnoses at follow-up -2014	**Study cohort** n=20639	**Non-participants** n=9807
	Incidence rate per 1000 person-years	
Any dementia	3.5	4.7
Vascular dementia	0.8	1.5
Alzheimer's disease dementia	2.1	2.4
	Mean ± SD	
Age at baseline	57.8 ± 7.5	58.5 ± 7.8
Age at dementia diagnosis	79.4 ± 6.0	77.4 ± 6.9
Time from baseline to dementia diagnosis	15.4 ± 4.1	12.9 ± 5.6
Time from baseline to last follow-up	19.2 ± 3.8	16.5 ± 6.3
Physical activity score at baseline	8292 ± 6746	7532 ± 6344

Missing data

Another important possibility of bias is missing data. This partly revolves around the same issue as attrition since individuals with missing data are excluded from the statistical analyses even if they participate in the study, referred to as complete case analyses. If data is missing *completely* at random, it is not considered to induce bias (for example, if one in fifty blood samples are lost). However, in observational studies this is seldom the case, but instead data is usually missing based on some other observed factor, called missing at random (for example, obese individuals may be less likely to provide data on carotid IMT due to technical difficulties) [240]. If missing data fulfils this so called missing at random assumption, there are statistical methods that can be used to impute values based on observed data within the cohort (multiple imputation) [240]. To complicate matters more, data can also be missing *not* at random, meaning that missingness depends on *un*observed data thus making it difficult to account for using available study data. In the MDCS, data is generally missing at random which would make it suitable for multiple imputation. This is something that we consider to perform in papers III and IV in order to reduce the potential missingness bias.

Covariate selection

In epidemiology it is also of relevance to account for confounders in the statistical analyses, something that we were able to do to a reasonable extent considering the detailed study protocols. Yet, one could argue that we did not use the definition of a confounder correctly [241], and thereby wrongfully adjusted for factors not related to *both* the predictor and the outcome. For example, education has been suggested to be related to Aβ pathology [25], but is there really an association between education and blood lipid levels? If so, is this relation not attributed to some other factor, like diet, rather than education per se? Overall, considering the extensive study protocols, covariate selection was rather challenging in all papers. The pragmatic approach has thus been to use similar adjustments as in previous publications addressing the same question, thereby possibly using a suboptimal statistical approach.

Mediators

Further, no mediation analyses were performed, and it is possible that some of the factors included as confounders were in reality mediators. In contrast to a confounder, a mediator is a presumed causal consequence of the predictor [241]. For example, one could argue that the association between physical activity and vascular dementia is mediated by blood pressure, meaning that physical activity is not directly related to vascular dementia but instead physical activity affects blood pressure levels and hypertension is in turn related to dementia. This reasoning, seem rather plausible. However, based on previous literature, we could argue that blood pressure is associated with both physical activity [242] and dementia [105] and should then be treated as a confounder since we are not sure that blood pressure is the causal link between physical activity and dementia. Further, physical activity may exert additional effects on

dementia development, possibly mediated by other known or unknown factors. Indeed, in paper III, we found physical activity to be associated with vascular dementia regardless of numerous other factors, included as confounders. This may be interpreted as an additional effect of physical activity, not covered by these other factors. Nevertheless, *no* causal effect can be inferred from this observational study.

Covariate selection and statistical modelling are intricate issues without simple answers. Hopefully, the presentation of several models and stepwise adjustments with transparency about number of events and individuals in every model allows the reader to interpret the findings as correctly as possible, with all the insecurity that observational studies encompass. Performing multiple imputation may be a reasonable approach to validate the results further.

Dementia assessment

Individuals developing dementia during follow-up were identified via linkage to a hospital-based register. As opposed to many other prospective cohort studies, no structured cognitive assessment was performed on *all* study participants, and this implies that demented individuals most probably are included as non-demented participants. This may induce bias yet again. However, there is an advantage to using register-based diagnoses, namely that individuals are not lost to follow-up. In the MDCS, all registered diagnoses were thoroughly evaluated based on medical records and the diagnostic review showed that 80% were diagnosed in tertiary care, which suggests good diagnostic accuracy. Further, the use of register-based diagnoses enabled us to assess associations in a very large population-based cohort with many incident cases thus further enabling subtype analyses. This can be considered especially relevant for vascular dementia, since there are few cases with vascular dementia in many observational studies, consequently limiting the possibility to detect statistically significant associations. Thereby, our study set up also contributes methodological strengths to the research area.

Concluding remarks

Main conclusions

- Solitary microbleeds do not affect cognitive performance cross-sectionally in healthy elderly.
- Arterial stiffness is not associated with cerebral microbleeds in healthy elderly, but higher pulse wave velocity seems to be associated with white matter hyperintensities
- Higher levels of triglycerides and cholesterol in midlife are associated with increased risk of AD pathology in late-life, also in preclinical stages assessed in cognitively healthy elderly
- Physical activity in midlife is associated with reduced risk of vascular dementia, but the proposed association between physical activity and AD development could not be replicated
- Midlife atherosclerosis is associated with increased risk of vascular dementia and cerebral small vessel disease, but does not seem to be related to AD or related brain pathology

Major strengths

This thesis contributes to dementia risk factor research by combining data from a prospective population-based study with a subgroup of healthy elderly providing detailed assessments. Main contributions include:

- *midlife* risk factor assessments and *longitudinal* design (papers II-IV)
- the use of *biomarkers* for direct measures of dementia related pathology (papers I-II and IV)
- assessments of preclinical stages in order to study risk factors for *early* disease related events (papers I-II and IV)
- the use of novel vascular risk markers in order to explore effects of *subclinical* vascular alterations (paper I and IV)
- data on incident dementia in a very *large population* thus enabling subtype analyses and to test reproducibility of previous findings (paper III-IV)

Future perspective and implications

The implementation of dementia biomarkers is an important research advancement and the emergence of large cohort studies with biomarker data enables an extended understanding of previously suggested associations. Future studies may help to further decipher if vascular factors directly increase accumulation of Aβ and/or tau, as well as aid in revealing differences between associations with the two. Moreover, biomarkers

can improve diagnostic accuracy when clinical dementia diagnoses are the outcome of interest.

There is already evidence of a positive effect of risk factor control [115], and if dementia onset can be postponed many individuals will never develop symptomatic disease thus alleviating major suffering. Overall, continued research for identification of novel risk factors is of relevance, especially in the absence of disease-modifying treatment effects. Continued research regarding treatment is also of great importance since reasons for the failures in Aβ-modifying treatment trials may be the time of intervention in relation to disease stage. As the neuropathologic alterations of AD are believed to precede symptom onset by 20 years [23-25], trials now aim at targeting prodromal stages to halt the underlying neuropathology and thereby stop disease progress and preserve cognitive abilities.

Clinical implications

The clinical implication of this thesis is that risk factor control seems to be of relevance and may help reduce dementia development. Yet, our findings may not translate to the general population and we do not prove causality within our studies. Hence, continued research is needed and intervention trials addressing these issues will hopefully provide knowledge on prevention of neurocognitive disorders. These will also help us guide patients seeking to maintain intellectual abilities throughout advanced age.

The possible interaction between vascular risk factors and *APOE* ε4 is a research question of significance. If *APOE* ε4 carriers are more susceptible to the potential hazards of vascular risk factors, there may be clinical implications to screen for carrier status and pursue more intensive risk factor control in *APOE* ε4 carriers.

Research advancements can also help raise awareness and highlight the importance of cognitive medicine. Dementia is a global health priority and this stresses the need of improved elderly care. Still today, cognitive symptoms and complaints are often disregarded by health care professionals, both in primary care and in hospital-based care. This ignorance is truly unfortunate and probably occurs due to inadequate education and knowledge, resulting in diagnostic insufficiency. In turn, patients suffering from undiagnosed cognitive disease are deprived of optimised care and symptomatic medication. Old age ought not to be a reason to disregard symptoms of cognitive impairment, but rather constitutes a motive to actively investigate if cognitive dysfunction is present in order to accurately diagnose and manage dementia.

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