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Predictors of Dementia

Insulin, Fatty Acids and Vascular Risk Factors

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ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2012

ISSN 1651-6206
ISBN 978-91-554-8248-0
urn:nbn:se:uu:diva-164528

Dissertation presented at Uppsala University to be publicly examined in Enghoffssalen, Akademiska sjukhuset ing 50 bv, Uppsala. Wednesday, February 15, 2012 at 13:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English.

Abstract
Rönnemaa, E. 2012. Predictors of Dementia: Insulin, Fatty Acids and Vascular Risk Factors. Acta Universitatis Upsaliensis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 734. 69 pp. Uppsala. ISBN 978-91-554-8248-0.

Identification of modifiable risk factors for Alzheimer's disease (AD) is crucial in order to diminish suffering from this devastating disease. The aim of this thesis was to investigate if different aspects of glucose metabolism, insulin, fatty-acid composition or other vascular risk factors predict the future development of AD and dementia.

This thesis is based on the Uppsala Longitudinal Study of Adult Men (ULSAM) cohort, which started in 1970. A total of 2322 men at age 50 were examined with focus on vascular risk factors. The cohort was re-examined at ages 60, 71, 77, 82 and 88. Incident diagnoses of AD, vascular dementia, other dementias and cognitive impairment were assessed in 2005–2010.

The risk of AD was increased in subjects with lower early insulin response measured with both an intravenous glucose tolerance test at 50 years and an oral glucose tolerance test at 71 years of age. The presence of vascular risk factors such as hypertension, obesity, hypercholesterolemia and smoking increased the risk of future vascular dementia but not of AD. Furthermore, saturated fatty acids at midlife were inversely associated with risk of AD. No evidence of a protective effect of omega-3 fatty acids against dementia was found.

The susceptibility allele, *APOE* ε4, was the strongest individual risk factor. *APOE* ε4 carriers with vascular risk factors had the greatest risk of developing dementia. Low insulin response was a risk factor for AD mainly in *APOE* ε4 non-carriers.

Disturbances in insulin and glucose metabolism, vascular risk factors and fatty acids are linked differentially to the pathogenesis of AD and vascular dementia. These observations should be considered when future clinical approaches are planned to prevent and postpone the onset of dementia.

Keywords: dementia, Alzheimer's disease, vascular dementia, insulin secretion, diabetes, fatty acids, epidemiology, vascular risk factors

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ISSN 1651-6206
ISBN 978-91-554-8248-0
urn:nbn:se:uu:diva-164528 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-164528)

“I’m flyyyiing! I’m flyyiing!!!”

(*Little pig*)

To Staffan

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List of papers

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

I Rönnemaa E., Zethelius B., Sundelöf J., Sundström J., Degerman-Gunnarsson M., Berne C., Lannfelt L., Kilander L. (2008) Impaired insulin secretion increases the risk of Alzheimer's disease. *Neurology* 71(14):1065-1071

II Rönnemaa E., Zethelius B., Sundelöf J., Sundström J., Degerman-Gunnarsson M., Lannfelt L., Berne C., Kilander L. (2009) Glucose metabolism and the risk of Alzheimer's disease and dementia. *Diabetologia* 52(8):1504-1510.

III Rönnemaa E., Zethelius B., Lannfelt L., Kilander L. (2010) Vascular risk factors and dementia: 40-year follow-up of a population based cohort. *Dementia and Geriatric Cognitive Disorders* 31(6):460-466.

IV Rönnemaa E., Zethelius B., Vessby B., Lannfelt L., Byberg L., Kilander L. (2011) Serum fatty acid composition and the risk of Alzheimer's disease: a longitudinal population based study. Submitted to *European Journal of Clinical Nutrition*.

Contents

Abbreviations

Aβ	Amyloid-beta
AD	Alzheimer’s disease
ADDTC	Alzheimer's Disease Diagnostic and Treatment Centers
AGE	Advanced glycation end product
AIR	Acute insulin response
APOE	Apolipoprotein E
APP	Amyloid precursor protein
BMI	Body mass index
CDK5	Cyclin dependent kinase-5
CI	Confidence interval
CT	Computed tomography
DHA	Docosahexaenoic acid
DM	Diabetes mellitus
DSM IV	Diagnostic and Statistical Manual of Mental Disorders IV
EIR	Early insulin response
EPA	Eicosapentaenoic acid
FA	Fatty acid
GSK-3	Glycogen synthase kinase-3
HOMA-IR	Homeostasis model assessment of insulin resistance
HR	Hazard ratio
IAPP	Islet amyloid polypeptide
IDE	Insulin degrading enzyme
IR	Insulin resistance
IVGTT	Intravenous glucose tolerance test
MRI	Magnetic resonance imaging
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
n-3 FA	Omega-3 polyunsaturated fatty acid
OGTT	Oral glucose tolerance test
PC	Plasma phosphatidylcholine
OR	Odds ratio
RR	Relative risk
SD	Standard deviation
ULSAM	Uppsala Longitudinal Study of Adult Men
VAD	Vascular dementia

Introduction to the epidemiology of dementia

Alzheimer's disease (AD) is the most common cause of dementia and its origin is likely to result from an interaction between genetic susceptibility and environmental risk factors. Interventions that could prevent or postpone the onset of dementia would have a major effect on public health.

The aim of this thesis was to investigate if different aspects of glucose metabolism, insulin, fatty-acid composition of serum lipids or other vascular risk factors predict the future development of AD and other dementia subtypes in the Uppsala Longitudinal Study of Adult Men (ULSAM) cohort.

Definitions of dementia, Alzheimer's disease and vascular dementia

Dementia is a syndrome that is defined by permanent (lasting at least six months) impairments in memory and at least one other cognitive function that are severe enough to cause significant impairment in social function or activities of daily living. Symptoms should represent a significant decline from a previous level of functioning. These most established criteria from 1994 are from the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) [1].

The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders working group (NINCDS-ADRDA) criteria were presented in 1984 [2]. These landmark criteria for AD include a specification that the onset of dementia should be insidious and that there is a lack of other systemic or brain diseases that may account for the progressive memory and other cognitive deficits. According to the NINCDS-ADRDA criteria, a definite diagnosis of AD is only to be made when there is histopathological confirmation of the clinical diagnosis.

Vascular dementia (VAD) is a heterogenous syndrome. A history of clinical stroke and/or presence of vascular disease (cortical infarcts, lacunar infarcts, strategically located subcortical infarcts, severe subcortical white matter disease or a combination of these) support a vascular rather than a degenerative cause for the cognitive impairment. According to the State of California Alzheimer's Disease Diagnostic and Treatment Centers (ADDTC) criteria [3], there should be a temporal relationship between the stroke and

dementia onset for a probable diagnosis. Typical neurological signs and vascular lesions in brain imaging support the diagnosis.

Prevalence, incidence and prognosis

There is considerable variation in the prevalence and prognosis of dementia and AD in different studies, which is probably caused by different population samples and diagnostic criteria. The prevalence of dementia is very low before the age of 60, but increases exponentially with increasing age. The crude prevalence of dementia is approximately 5% for individuals over 65 years and increases up to 20% for those over 80 years of age. For those over 90-95 years, the prevalence could be as high as 40-50% [4].

There are approximately 140 000 persons in Sweden with dementia and approximately 25 000 new cases of dementia occur every year. It has been estimated that the incidence of dementia in Sweden will dramatically increase after 2020 as the number of individuals older than 80 years increases. The most common dementia subtypes are AD (60-70%) and VAD (10-20%). However, the neuropathology of dementia in later life is often a mixture of Alzheimer pathology and vascular brain damage [5]. Women have a higher prevalence than men [6].

The prognosis for dementia depends on the underlying disease and age at diagnosis. On average, people with AD live eight years past their diagnosis, with a range from one to 20 years. The patients gradually develop memory loss, comprehension and language problems, visuospatial impairments, behavioural changes and loss of independence in activities of daily living. Long-term institutional care is often needed. At present, no therapies can reverse the progression of AD. Two different types of drugs, cholinesterase inhibitors and the NMDA receptor antagonist memantine, provide temporary improvement in cognitive functions for some patients with AD and may ameliorate disease progression.

The symptoms of VAD begin in most cases more abruptly than those of AD. The clinical course of VAD may be static, partially remitting or progressive. Impaired gait, incontinence, dysexecutive problems or mood and personality changes are often present early in the course. Symptoms may progress stepwise with the occurrence of new strokes. Treatment focuses on minimising the risk of further vascular events i.e. smoking cessation, aspirin therapy and treatment of hypertension. Cholinesterase inhibitors have been tested in clinical trials but the effect seems to be less than in AD.

Etiology

Alois Alzheimer was the first to describe the deposition of senile plaques and neurofibrillary tangles in the cerebral cortex of a demented patient in 1906. The content in the plaques was identified as a peptide named amyloid-beta (Aβ) in 1984 [7]. Aβ aggregates to produce senile plaques. According to the amyloid cascade hypothesis, this would initiate further pathological events, including the formation of neurofibrillary tangles containing hyper-phosphorylated forms of the tau-protein and disruption of synaptic connections, which would lead to a reduction in neurotransmitters, neuronal death and dementia [8]. However, later studies have observed that soluble, oligomeric and even intracellular Aβ, rather than insoluble forms of Aβ in plaques, have toxic effects [9].

VAD occurs from hypoxic-ischemic injury in the brain. A liberal definition includes all forms of cerebral vascular brain injury that leads to dementia. On the other hand, cognitive decline may predict stroke [10]. The major clinical variants are dementia due to subcortical lacunes and white matter changes, multi-infarct dementia, and dementia due to a single strategic infarct [11]. Other cerebrovascular causes include vasculitis, subdural hematoma and subarachnoid hemorrhage.

Risk factors

The dementia diagnostic work in ULSAM was initiated in 2006. The same year SBU (Swedish Council on Health Technology Assessment) published a review on dementia diseases and risk factors (**Table 1**). The number of publications on risk factors for dementia is continuously increasing and has nearly doubled during the past five years.

Table 1. Summary of the main proposed risk factors for Alzheimer's disease and other types of dementia according to the SBU report in 2006 [6]:

AD	**Increased risk**	**Decreased risk**
Strong evidence	*APOE* ε4 allele	---
Intermediary strong evidence	Family history, hypertension at midlife	Education, active leisure time
Limited evidence	High cholesterol at midlife, occupational exposure	Modest alcohol intake, anti-hypertensive drugs
Other dementias	**Increased risk**	**Decreased risk**
Strong evidence	---	---
Intermediary strong evidence	Family history, hypertension at midlife, diabetes, *APOE* ε4	Education, active leisure time, antihypertensive drugs
Limited evidence	Occupational exposure	Modest alcohol intake

Genetics and Family history

Getting old is a major risk factor for all dementias. Late onset sporadic AD, i.e. with onset after the age of 65, accounts for over 95% of AD cases. It is assumed to be polygenic and multifactorial. First-degree relatives of AD patients have an increased risk of developing the disease. However, only one gene polymorphism is known to influence disease development: apolipoprotein E (*APOE*) [12]. About 25% of the Swedish population inherit at least one copy of the *APOE* ε4 allele. This increases their risk of developing AD by up to four times, but it is neither necessary nor sufficient for the disease. The mechanism by which *APOE* alleles modulate Aβ accumulation in late onset AD is not clear. One of the suggested mechanisms is that it binds to Aβ with high avidity and regulates its clearance from the brain [13].

There are rare cases of families where mutations in the amyloid precursor protein (*APP*) gene or the two presenilin genes (*PSEN*-1 and *PSEN*-2) lead to AD. People with mutations in these genes may develop AD already before the age of 50 and the disease has autosomal dominant inheritance. These risk genes account for less than 0.1% of AD cases.

Twin studies have revealed that non-genetic risk also plays an important role, as there are monozygotic twin pairs in which only one individual has dementia [14]. Despite the fact that both family history and the *APOE* ε4

allele are associated with increased levels of cardiovascular risk factors, the association between VAD and the *APOE* ε4 allele is not clear.

Vascular risk factors

Several population-based middle aged cohorts started in the 1960s and 1970s in order to study risk factors for cardiovascular disease. Many of these studies have since mid-1990s, after more than 30 years of follow-up, assessed cognitive function and identified incident cases of dementia among the participants [15]. As a result we have information about the longitudinal associations of vascular risk factors to the risk of cognitive disorders.

High systolic blood pressure, hyperlipidemia, hyperglycemia, diabetes and markers of inflammation have all been associated with dementia and cognitive impairment but the results are far from conclusive. It is still not clear if some of these factors have a direct impact on the development of the specific neuropathologic lesions in AD; if they increase the risk of non-specific cognitive deterioration in general or if their effect is mediated by cerebrovascular lesions.

The risk factors for VAD are in many cases the same as traditional risk factors for stroke. Besides the above mentioned factors, clinical vascular diseases such as coronary artery disease, peripheral arterial disease, chronic kidney disease and atrial fibrillation are also risk factors for stroke and have been associated with cognitive impairment. The characteristics of risk factors for dementia may change with age as both the levels of risk factors and their treatment change.

Lifestyle factors

The association between low education and dementia is supported by a majority of studies, but very few studies have investigated whether this association may be attributed to lifestyle factors that co-vary with low education and low socioeconomic status, such as overweight and a sedentary lifestyle. Healthy diets, the prevention of nutritional deficiencies, modest alcohol intake and physical, cognitive and social activities are possible modifiable protectors against the development and progression of dementia. However, in most cases, these results are from observational studies, and need validation from large randomised clinical trials in older persons [6]. Smoking has effects on both the cardiovascular system and neurons and increases the risk of cognitive decline in most studies. Depression, psychological stress and head trauma have been proposed as risk factors for AD but these possible associations remain less explored.

Diabetes, insulin secretion and insulin resistance

Defects in insulin secretion and insulin action are the major abnormalities behind the development of type 2 diabetes mellitus (DM) [16]. For type 2 DM to develop, both defects must exist: for example, the majority of overweight individuals are insulin resistant, but only those with an inability to increase pancreatic beta-cell production of insulin sufficiently develop DM.

A glucose tolerance test, where blood glucose levels are determined after administration of glucose, is used to diagnose impaired glucose tolerance and DM. An intravenous glucose tolerance test (IVGTT) eliminates variations due to gastro-intestinal factors. A widely used procedure in clinical practice is the more physiological oral glucose tolerance test (OGTT). A two hour postload glucose level of at least 11.1 mmol/L is used as the reference standard for diabetes diagnosis. Because plasma glucose and insulin responses during this test reflect the ability of pancreatic beta-cells to secrete insulin, the 30 minutes ratio of change in plasma insulin and glucose has been used as an index of beta-cell function [17].

Insulin resistance is a condition in which normal amounts of insulin act inadequately in fat, muscle and liver cells. The gold standard for investigating and quantifying insulin resistance is the "euglycemic insulin clamp" [18], which measures the amount of glucose necessary to compensate for an increased insulin level without causing hypoglycemia. The test is rarely performed in clinical care, but is used in medical research. Insulin is infused at a constant rate through a peripheral vein. In order to compensate for the insulin infusion, glucose is infused to maintain normal blood glucose levels. The rate of glucose infusion is determined by checking the blood sugar levels every 5 to 10 minutes. This rate during the last 60 minutes of the two hour test determines the insulin sensitivity.

Both impaired secretion as measured by low acute insulin response to a glucose load and peripheral insulin resistance analysed by euglycemic insulin clamp technique have been shown to be independent predictors of worsening glucose tolerance and DM [19, 20].

Cross sectional case-control studies

The relationship between insulin and AD has been of great interest since the 1980s. AD patients had elevated fasting insulin levels compared to healthy elderly persons in a Swedish study with OGTT from 1983 [21]. These findings might be secondary to alterations in physical activity or adiposity, i.e. inactivity and inadequate diet may be consequences, rather than causes of dementia. One previous study using clamp technique found decreased insulin sensitivity in AD patients compared to controls, while another study did not [22, 23]. There were no differences in glucose and insulin responses measured with OGTT or IVGTT between the AD patients and controls in these two studies. In the population-based Rotterdam study, an increase in OGTT postload insulin was associated with decreased cognitive function only in women [24]. However, in a later study, demented patients had both higher fasting glucose and insulin levels (insulin resistance) than healthy controls and this was not influenced by differences in gender, adiposity, nutritional status, serum lipids or hypertension. The impact of physical activity was not studied [25].

Longitudinal population studies started at midlife

The different population characteristics and significant results of a set of previous longitudinal studies starting at midlife are illustrated in **Table 2**. In the Honolulu-Asia Aging study, midlife abnormalities in glucose tolerance were associated with increased risk of VAD but not with AD. The number of subjects with diabetes at midlife was too small to draw any conclusions about possible associations [26]. In another study from the same cohort with baseline late in life, DM was associated with all-cause dementia and especially VAD [27]. In a later report from this cohort, the risk of dementia was significantly increased at both extremes of insulin distribution [28].

Survival bias may be a significant limitation of studies with decades of follow-up. In the Israeli Ischemic Heart Disease study [29], subjects with diabetes had a higher prevalence of dementia than non-diabetic subjects. However, only 2% of the cohort participants had diabetes and follow-up data were only obtained for 5% of these diabetic subjects 35 years later. The high drop-out rate was mostly due to death before the follow-up assessment. As in most of the studies, diabetes diagnoses after baseline were not taken into account.

In the Kaiser Permanente Medical Care Program of Northern California, subjects with diabetes were more likely to get a diagnosis of dementia or memory impairment [30, 31]. A computerised out-patient diagnoses system made this large study possible, but the diagnoses obtained electronically

from chart diagnoses may be insensitive and the criteria vary widely. Furthermore, dementia subtypes were not studied.

Higher insulin levels at midlife were associated with a faster cognitive decline in two previous studies. The clinical significance of this decline remains unclear as the follow up of 4-6 years during midlife is too short to study dementia as an end-point [32, 33].

Longitudinal population studies started late in life

The different population characteristics and significant results of a set of previous longitudinal studies on dementia with measurements of glucose metabolism late in life are illustrated in the **Table 3.** Many of the longitudinal studies where both DM and dementia were assessed after 65 years of age also included subjects who were over 80 years at baseline, resulting in a wide baseline age spread. As dementia incidence dramatically increases with age, all characteristics of the older individuals of the cohort may be interpreted as risk factors for dementia if the associations are not properly age-adjusted. Some of the studies are very large. However, only a limited number have diabetes and a limited number of these have dementia. These low numbers are often not discussed as limitations of the reliability. All studies summarised in **Table 3** used clinical evaluation to set the dementia diagnoses.

In the Washington Heights-Inwood Columbia aging project no relation was found between DM and AD. However, the authors stress the significant association between DM and the composite outcome of AD and cognitive impairment. In the same study, there was a clear association between diabetes and stroke-associated dementia. Fasting insulin was measured in a subsample. Those with higher fasting insulin levels had an increased risk of AD and total dementia and declined in memory-related tests over time [35].

Diabetes increased the risk of AD in the Rotterdam study in a very heterogeneous age-group with a short follow-up [36]. The participants in the Religious Orders Study underwent annual clinical evaluations during a mean period of 5.5 years. DM was associated with an increased risk of AD and a decline in perceptual speed but not in other cognitive systems [37]. In the Cardiovascular Health Study Cognition Study, DM increased the risk of dementia but the association with AD was significant only in *APOE* ε4 carriers [38].

Table 2. Longitudinal studies measuring diabetes mellitus (DM), glucose or insulin at midlife and dementia.

Study Subjects (n)	Age at entry (years)	Follow-up time (years)	Exposure (n)	Outcome (n) *Method used*	Significant results
Honolulu-Asia Aging study [26] n=3774	45-68	25	Glucose tolerance (OGTT) Diabetes *n*=259	AD n=? VAD n=? *Clinical evaluation*	No
Israeli Ischemic Heart Disease study [29] n=1892	40-65	35	Diabetes n=42	No cognitive impairment n=1408 Dementia n=309 *Telephone testing*	DM and AD OR 2.8 (1.4-5.7)
Radiation Effects Research Foundation Adult Health Study from Hiroshima [34] n=1774	>30 Mean 43	30	Diabetes n=?	AD n=51 VAD n=38 Dementia n=114 No dementia n =1660 *Clinical evaluation*	DM and AD OR 4.4 (p<0.01)
Kaiser Permanente Medical Care Program of Northern California [31] n=8845	40-44	27	Diabetes n=972	Dementia or memory impairment n=721 *Database diagnoses*	DM and dementia HR 1.5 (1.2-1.8)
Nurses' Health Study, USA [32] n=121700	30-55	4	Fasting insulin n=1416	Cognitive decline *Telephone testing*	Hyperinsulinemia greater decline p for trend 0.04
Atherosclerosis Risk in Communities, USA [33] n=15732	45-64	6	Fasting insulin, HOMA insulin resistance n=7148	Cognitive decline n=7148 *Cognitive testing, delayed word recall*	Hyperinsulinemia greater decline p=0.01 Insulin resistance greater decline p=0.02

Table 3. Longitudinal studies measuring diabetes mellitus (DM), glucose or insulin late in life and dementia.

Study Subjects	Age at entry (years)	Follow-up time (years)	Exposure (*n*)	Outcome (*n*)	Significant results
Honolulu-Asia Aging study [28] n=2568	71-91	5	Fasting insulin n=2568	Dementia n=244	Low insulin and dementia HR 1.5 (1.1-2.1) High insulin and dementia HR 1.5 (1.1-2.3)
Honolulu-Asia Aging study [27] n=2574	73-81	3	DM n=900 Glucose tolerance from 2 hour OGTT	AD n=50 VAD n=34 Dementia n=128	DM and VAD RR 2.3 (1.1-5.0) DM and dementia RR 1.5 (1.0-2.2)
Washington Heights-Inwood Columbia aging project [35] n=1262	>65 70-82	4	DM n=252 Fasting insulin n=683	AD n=157 Dementia with stroke n=36	DM and VAD HR 4.2 (2.2-8.3) Hyperinsulinemia and AD HR 2.1 (1.5-2.9)
Religious Orders Study, USA [37] n=824	>55 68-80	5	DM n=127	AD n=151	DM and AD HR 1.7 (1.1-2.5)
Framingham study [39] n=2210	63-77	13	DM n=202	AD n=237 VAD n=32 Dementia n=319	No

Kungsholmen project [41] n=1301	>75 76-86	6	DM n=114 Blood glucose n=1248	AD n=260 VAD n=49 Dementia n=260	DM and VAD HR 2.6 (1.2-6.1) DM and dementia HR 1.5 (1.0-2.1)
Kungsholmen project [42] n=1248	<75, 76-86	9	Diabetes n=75 Undiagnosed DM n=42 Borderline DM n=47	AD n=320 VAD n=47 Dementia n=420	Inter alia: DM and VAD HR 3.2 (1.2-8.6) Undiagnosed DM and AD HR 3.3 (1.2-9.0)
Rotterdam study [36] n=6370	>55 64-82	2	Diabetes n=692	AD n=81 Dementia n=126	DM and AD RR 1.9 (1.2-3.1) DM and dementia RR 1.9 (1.3-2.8)
Canadian Study of Health and Aging [40] n=5574	mean 74	5	Diabetes n=503	AD, VAD, Dementia	DM and VAD RR 2.0 (1.2-3.6)
The Cardiovascular Health Study Cognition Study [38] n=2547	69-79	5	Diabetes n=320	AD n=207 VAD n=58 Dementia n=411	DM and dementia HR 1.4 (1.0-2.0)
Origins of variance in the Old-Old in Goth-enburg [43] n=702	>80	6	Diabetes n=31	AD n=105 VAD n=50 Dementia n=187	DM and VAD RR 2.5 (1.4-4.8)

Neither the Framingham study nor the Canadian Study of Health and Aging found that DM increased the risk of all-cause dementia or AD [39, 40]. The Kungsholmen project found a significant association between DM and VAD but not AD [41]. A later study from the same cohort suggested, however, that uncontrolled DM increases the risk of AD [42]. Diabetes was a risk factor for VAD but not for AD in a study from Gothenburg, "Origins of variance in the Old-Old" where the participants were older than 80 years. In this study, diabetes onset date was used in the Cox proportional hazards analysis instead of dementia diagnosis date [43].

In conclusion, the relationship between DM and AD has been studied in several large cohort studies; some did find an association, others did not. Diabetes is associated with increased risk of stroke that may lead to vascular dementia. Only few studies examined insulin abnormalities and risk of dementia. The differences in age at baseline and length of follow-up may partly explain the conflicting results of the different studies as well as other characteristics of the study population and study design.

Neuroimaging and neuropathology

In the 541 participants of the Honolulu Aging MRI study, the risk for both hippocampal atrophy and lacunes/infarcts was twice as high in subjects with type 2 DM [44]. Those with the longest duration of diabetes, those taking insulin, and those with diabetes complications, respectively, had relatively more pathologic brain changes in MRI [44]. DM was associated with hippocampal and amygdalar atrophy regardless of vascular pathology in an MRI study of 506 participants from the Rotterdam Study [45]. A total of 62 participants underwent IVGTT and MRI in the University of Kansas Brain Aging Project. Glucose and insulin were not related to brain volume or cognitive performance in nondemented individuals. Contrary to the original hypothesis, increased peripheral insulin was associated with reduced AD- related brain atrophy in MRI, better cognitive function, and reduced dementia severity [46].

As many studies with neuroimaging link DM with AD-related brain atrophy, the neuropathologial evidence is not convincing. A retrospective postmortem immunocytochemical and histofluorescent study did not find increased AD pathology in diabetic subjects compared with age-matched control subjects [47]. The Religious Orders Study (233 subjects) found a relation between DM and cerebral infarctions but not between DM and AD pathology at autopsy [48]. Similar findings were reported from a neuropathological study of 196 nursing home residents. The demented individuals without DM had a greater Aβ load while demented individuals with DM had

more microvascular infarcts [49]. Another study of 385 subjects, which controlled for age at death, dementia severity and presence of the *APOE* ε4 allele, indicated that subjects with DM had significantly fewer neuritic plaques and neurofibrillary tangles than those without DM [50]. Another study of 248 subjects concluded that insulin in combination with oral diabetes medication was associated with less AD neuropathology [51]. In accordance with previous studies, a recent study from Finland concluded that individuals with DM were less likely to have Aβ plaques and tangles but more likely to have cerebral infarcts after all adjustments [52].

One important limitation of autopsy studies is that the time of autopsy may differ greatly from the time of the clinical presentation of dementia, as the dementia disease duration may be over 10 years. By the time of autopsy the persons may have developed other neuropathological lesions than at the time of diagnosis.

Fatty acids

As treatment options for dementia are still very limited, knowledge of whether the risk of dementia can be reduced, for example by a healthy diet, is extremely important. Approximately 34% of the energy in the Swedish diet is from fat.

Omega-3 polyunsaturated fatty acids (n-3 FAs) contribute only to <1% of the energy intake but have long been known to be important for neurodevelopment [53]. The main types of n-3 FAs in the diet are α-linoleic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaoeic acid (DHA). The main sources of EPA and DHA are fish and other marine foods. ALA is found in flaxseed oil as well as rapeseed and soybean oil.

Approximately 14% of the energy in Swedish diet is from saturated fat. Palmitic acid and stearic acid are the most common saturated FAs. Examples of foods containing a high proportion of saturated FAs include animal fats such as cream, cheese, butter, fatty meats and chocolate.

Cross sectional case-control studies

Impaired cognitive functioning has been associated with lower n-3 FA levels in blood or plasma [54, 55]. Lower n-3 FA levels have also been reported in patients with dementia and AD [56-58]. As dementia itself affects dietary habits, a longitudinal prospective study design is more suitable for investigating causal associations than cross-sectional epidemiological studies.

Longitudinal population studies using dietary questionnaires

One way to estimate fatty acid composition is dietary intake registration. In the Rotterdam and Framingham studies [59, 60], the n-3 FA consumption was quantified using dietary questionnaires but no significant association with dementia and AD later was found after approximately ten years of follow-up. In contrast, in a stratified random sample of the Chicago Health and Aging Project, both total intake of n-3 FAs and of DHA were associated with reduced risk of AD four years later [61]. Most cohort studies have focused

on fish consumption to estimate n-3 FA intake without considering other dietary items. The food frequency questionnaires are known to underestimate for example saturated FA intake [63]. Subjects may also alter their usual diet during the recording period. Biomarkers measured directly in the blood, such as serum esterified FAs, are more objective and probably more accurate in evaluating the true dietary fat quality than e.g. food frequency questionnaires [62].

Longitudinal population studies measuring levels of fatty acids in serum/plasma

The characteristics of a set of previous longitudinal studies are illustrated in **Table 4**. In the Framingham study [60], higher plasma phosphatidylcholine (PC) DHA was associated with lower risk of dementia but not AD. In the Three City Study [64], higher EPA but not DHA was associated with lower risk of dementia. The Canadian Study of Health and Aging [65] did not find significant associations. One short article from 1999 reported an association between low DHA in the blood and AD in an unspecified population [66].

Neuropathology

Results from post-mortem studies on FAs in the brain are not conclusive. Several studies examined DHA content in membrane phospholipids in the frontal cortex or in the parahippocampal region. Some did find lower DHA in AD patients compared to controls in specific areas [67, 68], others did not [69-71].

Table 4. Longitudinal studies measuring lipids late in life and dementia.

Study population	Age at entry	Follow-up (years)	Exposure (n)	Outcome (n)	Significant results
Framingham Study [60] n=899	71-81	9	Serum PC: DHA, EPA, ALA	AD n=71 Dementia n=99	DHA and dementia HR/SD 0.53 (0.29-0.97)
Three City Study [64] n=1214	69-84	4	Plasma triglycerides: EPA, DHA, total n-3	Dementia n= 65	EPA and dementia HR/SD 0.69 (0.48-0.98)
Canadian Study of Health and Aging [65] n=665	74-86	5	Plasma phospholipids: DHA, EPA total n-3	AD n= 105 Dementia n= 149	No
"American subjects" [66] n=1188	Mean 75	10	Serum PC: EPA, DHA	AD n=?	Low DHA 67% greater risk of AD ($p<0.05$)

Other vascular risk factors

Hypertension

Hypertension is present if the blood pressure is persistently above 140/90 mmHg. The prevalence of hypertension increases with age. In the ULSAM cohort, 20% of the participants had hypertension or anti-hypertensive treatment at 50 years of age, compared to 32% at 70 and 51% at 83 years of age. In a previous report from ULSAM, hypertension at 50 years of age was associated with low results on cognitive tests at 70 years of age [72].

Alzheimer's disease

A recent meta-analysis identified 19 population studies that investigated the association between blood pressure and incident AD [73]. A significant harmful effect of hypertension or high systolic blood pressure was found for example in the NEDICES study in Spain, the Kungsholmen project and the North Karelia/ FINMONICA study. A protective effect of hypertension was found in the Three City Study, the Cache County and the Ibadan Dementia project. The summary estimates did not suggest a clear association between blood pressure and AD.

Four of these previous studies measured baseline blood pressure at midlife. The Honolulu-Asia Aging study reported a J-shaped relation where those with low (<110 mmHg) or high (>160 mmHg) blood pressure had an increased risk of AD. In the North Karelia/ FINMONICA study individuals with high (>160 mmHg) blood pressure had markedly increased AD risk whereas this association was less apparent in the other two studies.

The data on hypertension in late life is not consistent, either. Hypertension or higher systolic blood pressure had a protective effect in 11 and harmful effect in 6 populations but in most studies the results were not significant.

Vascular dementia

High blood pressure has long been known to be a powerful predictor of stroke and cerebrovascular disease [74]. A recent meta-analysis strongly supported the hypothesis that people with hypertension have a significantly higher risk for developing vascular dementia [75]. The association between hypertension and incident VAD was not significant in six longitudinal studies [76-81] but the summary estimate was significant (OR 1.6, $p<0.0001$). A similar association between hypertension and the risk of prevalent VAD was

found in four out of five cross-sectional studies [82-86]. Four additional longitudinal studies [87-90] support the connection between hypertension and VAD.

Obesity

Body mass index expressed in weight (kg) per height squared (m^2, BMI) is widely used as an indirect measure of adiposity in epidemiologic studies. Overweight is usually defined as BMI 25-30 and obesity as BMI >30. In ULSAM cohort, the mean BMI was 25.0 at 50 years of age, increasing to 26.3 at 70 and decreasing to 25.7 in the participants at 88 years of age. Around 10% were obese, the highest percentage at 70 years of age.

A recent meta-analysis identified 15 populations that studied the association between body mass index and incident AD and VAD [91] and data from at least one population has been published since then [92].

Alzheimer's disease

AD patients with no obvious deficit in food intake may still lose weight and on average weigh >20% less than persons without dementia [93]. Adiposity may both influence or be influenced by brain structures and functions.

One study that measured BMI at midlife found an association between underweight and increased risk of AD whereas two studies found an association between midlife overweight and AD. In totally eleven studies at late life, higher BMI was associated with increased risk of AD in two and decreased risk in six studies [92].

Vascular dementia

A recent twin study found an association between higher BMI at midlife and the risk of incident VAD [92]. BMI in late life had no significant association with VAD in four studies. High BMI is associated with elevated risk for cardiovascular disease. However, obese and overweight patients had paradoxically significantly better early and long-term survival rates after a stroke compared to those with normal BMI in a recent study [94].

All-cause dementia

Obesity in midlife increased the risk of dementia in two of three studies. A J-shaped relation has been observed, such that a BMI <20 and BMI >22.5 increase hospitalization for dementia [95]. The results concerning obesity in late life and risk of dementia from seven different studies vary [92].

Hypercholesterolemia

According to European guidelines from 2007, the desirable total serum cholesterol is <5.0 mmol/l and LDL cholesterol <3.0 mmol/l and even lower in patients with DM. In the ULSAM cohort, mean serum cholesterol was 6.9 mmol/l at 50 years of age, decreasing to 5.8 mmol/l at 70 and 5.2 mmol/l for participants at 82 years of age. Cholesterol-lowering drugs were developed in the late 1980s and their use is widespread, atorvastatin being the best-selling drug in history.

Alzheimer's disease, all-cause dementia

The relationships between total serum cholesterol, AD and dementia were investigated in a meta-analysis (2008) of 9 prospective studies [96]. Midlife serum total cholesterol was associated with an increased risk of AD in two Finnish studies [97, 98] and in the Kaiser Permanente Medical Care Program of Northern California, a large study based on medical records in 2009 [99]. No such association was found in the Framingham cohort [100] or in a recent study of Swedish women in Gothenburg [101]. A decline in serum total cholesterol levels was associated with the development of dementia and AD in the Honolulu Aging study [102].

Findings regarding late-life measurements of cholesterol vary as well but most of the studies did not find evidence supporting an association between high late life total cholesterol and AD [81, 90, 103]. One study had contradictory findings: high cholesterol in late life was associated with decreased dementia risk [104].

Vascular dementia

High level of cholesterol, particularly LDL cholesterol, is a well-established risk factor for developing coronary artery disease and stroke [74]. Midlife serum total cholesterol was associated with an increased risk of VAD in The Kaiser Permanente Medical Care Program of Northern California [99]. One study found a weak association between late-life total cholesterol and VAD [81]. The number of cases with VAD was low in studies showing no association [98, 102].

Smoking

In the ULSAM cohort, 51% of the participants smoked at 50 years of age, compared to 21% at 70 and only 6% at 82 years of age. These numbers illustrate the potential survival bias that complicates the study of all vascular risk factors as predictors of dementia. Smokers are more likely to die before reaching advanced age of for instance lung cancer or myocardial infarction.

Furthermore, smokers in older cohorts may represent survival elite less prone to developing dementia or other conditions.

Two independent meta-analyses on smoking in late life provided summary estimates showing that smokers had an increased risk of AD, VAD and all-cause dementia compared to former and never smokers [105, 106]. Most of the studies examined three smoking statuses: current, former and never smokers but a few used pack years to determine lifetime tobacco exposure.

Alzheimer's disease and all-cause dementia

In the Honolulu Asia Aging Study, the number of cigarettes smoked at midlife was associated with an increasing risk of AD and Alzheimer-type neuropathology up to heavy smoking levels. Stratification by *APOE* genotype did not change these associations [107]. In contrast, smoking was associated with AD and dementia only in individuals with the *APOE* ε4 allele in a Finnish population [108]. The same author found that only heavy smoking in midlife was associated with AD and dementia in another population [109]. Five of eight studies on smoking at late life and AD reported a significantly increased risk in two meta-analyses [105, 106].

Vascular dementia

Smoking is known to be a strong risk factor for stroke [74]. The well-known effects on the cardiovascular system are mediated generally by oxidative stress and inflammation leading to endothelial injury and atherosclerosis. Two studies have reported an association between smoking and VAD [109, 110] but others did not find significant associations [107, 111, 112]. The number of cases with VAD was generally low in all studies.

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Material and methods

Study population

This thesis is based on the ULSAM cohort. All men born in 1920-1924 living in the county of Uppsala were invited to participate in a health examination at age 50. The survey was carried out between April 1970 and October 1973. Of the 2841 invited men, 2322 (82%) participated at the baseline investigation. The men were invited to follow-up examinations at age 60, age 71, age 77 and age 82, and the data has been completed with annual updates on mortality using a national register.

In this thesis, baseline data from the investigations at age 50 and age 71 was used. All participants in the first investigation (at age 50) were invited to a reinvestigation at age 71. During the intervening 20 years 422 men had died and 219 had moved out of the Uppsala region. Of the 1681 men invited, 460 did not participate in this follow-up, leaving 1221 men aged around 71. (participation rate 73%). The reinvestigation was carried out between August 1991 and May 1995.

Baseline examinations at age 50 (study I, III, IV)

A total of 1792 subjects were tested with an intravenous glucose tolerance test (IVGTT). Postload blood glucose concentrations between 20 and 60 minutes were used for calculation of intravenous glucose tolerance. The peak insulin response was expressed as the mean of the serum insulin concentrations determined at four and six minutes. The acute insulin response (AIR) was calculated as the difference between the peak and the basal value [113].

Glucose metabolism

Insulin resistance (IR) was estimated using the Homeostasis Model Assessment (HOMA) and HOMA-IR index was calculated as fasting insulin x fasting blood glucose/22.5 [114]. Diabetes mellitus was defined as fasting blood glucose ≥6.1 mmol/l or use of pharmacological treatment for diabetes.

Fatty acid composition

For analysis of the fatty acid composition of the serum cholesterol esters, serum was extracted with a hexane-isopropanol solution (1+4) (Hara and Radin 1978). Cholesterol esters were separated from the extract by thin layer chromatography before inter-esterification (acidic methanol at 85°C, 2 h) (Stoffel et al 1959), and free cholesterol liberated in the reaction was removed by an aluminium oxide column to avoid contamination of the gas liquid chromatography column. The percentage composition of methylated fatty acids 14:0 to 22:6 was determined by gas chromatography (a 25 m NB-351 silica capillary column, i.d. 0.32 mm, phase layer 0.20 mm) with use of a flame ionisation detector and with helium as carrier gas. Every 25th sample was a serum control pool. The precision of the between-series analysis (n=35) varied from 2% (large peaks) to 10% (smaller peaks) and between successive gas chromatography runs (n=17) from 0.2 to 5% (CV).

Vascular risk factors

Blood pressure was measured in the right arm after 10 minutes rest in the recumbent position with mercury manometers. Height (without shoes) was measured in cm and weight (in undershorts) in kg. Body mass index (BMI) was calculated as weight divided by height (in metres) squared. Determinations of serum cholesterol concentrations were performed on a Technicon Auto Analyzer type II (Rush et al 1971) in 1981-82 on serum samples that had been stored in liquid nitrogen since 1970-73. Smoking status (current smoker or non-smoker) was assessed by interview questions.

Educational level

Educational level (elementary school, secondary school or university studies) was assessed by a questionnaire.

Baseline examinations at age 71 (study II, III)

Glucose metabolism

An oral glucose tolerance test (OGTT) was performed by measuring the concentrations of plasma glucose and insulin fasting, 30 and 120 minutes after ingestion of 75 g of anhydrous dextrose. The early insulin response (EIR) was calculated as the ratio of the increments in plasma insulin and plasma glucose during the first 30 minutes of the OGTT (5). The fasting concentrations of insulin, proinsulin and 32-33 split proinsulin were analysed. Analyses were carried out at the Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, UK [113, 115, 116]. Insulin sensitivity was determined using the euglycaemic insulin clamp. It was performed according to De Fronzo et al with slight modification [18]. Insulin- mediated glucose disposal rate, representing insulin sensitivity, was calculated as the

amount of glucose taken up during the last 60 minutes of the clamp procedure. The oral glucose tolerance test and the euglycaemic insulin clamp took place on separate days within one week.

Diabetes mellitus was defined as a fasting plasma glucose >7.0 mmol/l or the use of pharmacological treatment for diabetes.

Vascular risk factors

Blood pressure was measured twice in the right arm with the subject in the supine position after resting for 10 minutes. The mean of the two values was used. BMI was calculated as at age 50. Cholesterol concentration was analysed in serum and in the isolated lipoprotein fractions by enzymatic techniques using IL Test Cholesterol Trinders's Method and IL Test Enzymatic-colorimetric Method for use in a Monarch apparatus (Instrumentation Laboratories, Lexington, USA). Smoking status (current smoker or non-smoker) was assessed by interview questions.

APOE ε

APOE ε4 was genotyped by minisequencing in 860 of the baseline 71 participants.

Follow-up and assessment of dementia

All available ULSAM participants' medical records were reviewed with 31st December 2005 as the censor date in studies I, II and IV. In study III, the censor date was 1st January 2010. The outcome of interest was time to diagnosis of dementia. AD was diagnosed according to the NINCDS-ADRDA and to the DSM-IV criteria [1, 2]. VAD was diagnosed according to the ADDTC criteria [3]. Further, we identified other types of dementia: AD+VAD, dementia with Parkinson's disease, Lewy body dementia and frontotemporal dementia. Cases of dementia without neuroimaging and without sufficient clinical details in the medical records to set a specific dementia subtype diagnosis were classified as unspecified dementia. Status post stroke with major motor sequelae and/or aphasia precluding neuropsychological testing and/or inferring a major impact on daily functions, stable over time, was not classified as dementia.

Cognitive impairment was defined as evidence of significant and persistent cognitive decline although not severe enough (or not sufficiently documented severity) to interfere with social function and activities of daily living. All these cases, together with unspecified dementia, AD and VAD are included in the group "any dementia or cognitive impairment" in studies I and II. Instead of including cognitive impairment, all-cause dementia was used as one endpoint in studies III and IV.

Statistical methods

Logarithmic transformation was performed to achieve normal distribution for the non-normally distributed variables. The prognostic values for a one standard deviation change in a continuous variable on the dementia incidence were investigated using Cox proportional hazards. Proportional hazards assumptions were confirmed by Schoenfeld´s tests or visually examining Nelson-Aalen curves. Non-linear relations were excluded by examining incidence rates in quartiles of the independent variables. All analyses were adjusted for age at entry and observations were censored at death, date of migration out of Uppsala county, date of dementia diagnosis or at the censor date. The statistical software package STATA 8.2 (Stata Corporation, College Station, USA) was used in all analyses in studies I and II and STATA 11 in studies III and IV.

All analyses were performed as crude and adjusted for age, educational level and other cardiovascular risk factors as potential confounders for development of dementia. In the participants with data on *APOE* ε4 genotype, the associations for AD were stratified for *APOE* ε4 status in secondary analyses.

In studies I and II, the analyses were repeated in non-diabetic subjects to avoid confounding in baseline measurements of diabetes or diabetes treatment on observed associations. In study IV, a logistic regression analysis was performed in the subgroup of participants still alive in 2006 to eliminate the effect of competing risk by death. The potential competing risk problem from mortality was further studied by the method of Fine and Gray [117] and by cumulative incidence curves [118].

Results

Study I

A total of 394 persons developed dementia or cognitive impairment of whom 102 had pure AD and 57 pure VAD during a median follow-up period of 32 years (from 50 years of age until 31st December 2005).

Prediction of Alzheimer's disease

In crude Cox proportional hazard analyses, low AIR was associated with a higher risk of AD and the association remained significant even after adjustment for systolic blood pressure, serum cholesterol, BMI and education level.

A cumulative AD incidence in groups with AIR below and above the median is presented in **Figure 1**. Low AIR was associated with higher risk of AD even after adjusting for fasting serum insulin or HOMA-IR, indicating that impaired AIR is a predictor for AD, independently of insulin sensitivity.

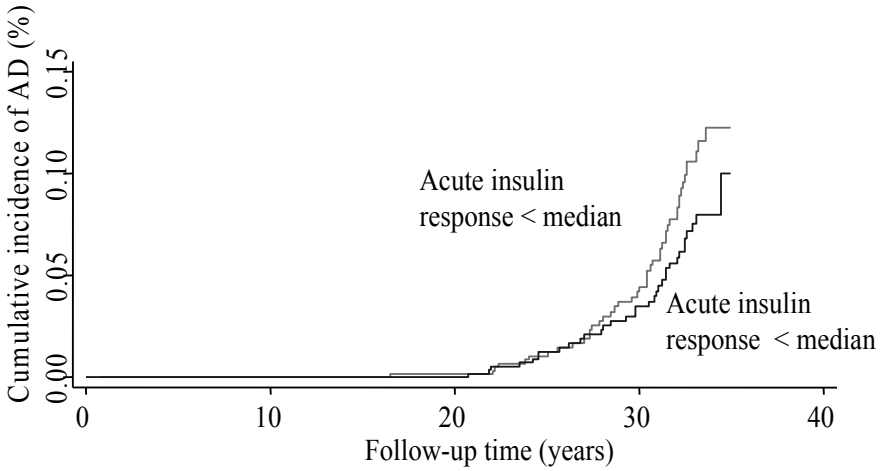

Figure 1. Cumulative incidence of AD in groups with acute insulin response below and above the median.

APOE ε4 allele carriers had an increased risk for developing AD compared to non-carriers [HR 2.6 (95%CI 1.6-4.2)]. There was an interaction between AIR and *APOE* ε4 status (p= 0.002). The association between AIR and AD was significant only in subjects without *APOE* ε4. In subjects with *APOE* ε4, however, high fasting serum insulin and HOMA-IR were associated with increased risk of AD.

Prediction of vascular dementia

APOE ε4 genotype did not affect the risk of VAD. A low glucose disappearance rate was associated with a higher risk of VAD but not for AD. HOMA-IR was significantly associated with higher risk of VAD in the non-diabetic subsample.

Prediction of any dementia or cognitive impairment

APOE ε4 carriers had an increased risk for developing any dementia or cognitive impairment compared to *APOE* ε4 non-carriers [HR 1.8 (95%CI 1.4-2.4)]. High fasting serum insulin and HOMA-IR, glucose intolerance and low AIR were all associated with a higher risk of all-cause dementia and cognitive impairment in non-diabetic subjects. However, only low AIR remained as a significant risk factor in the multiple adjusted models.

Study II

A total of 257 persons developed dementia or cognitive impairment, of whom 81 had AD and 26 VAD during a median 12-years follow-up period (from 71 years of age until 31st December 2005).

Prediction of Alzheimer's disease

Low EIR was associated with a higher risk of AD in Cox proportional hazard analyses. Adjustment for age, diabetes, systolic blood pressure, serum cholesterol, body mass index, smoking status and education level reinforced this association. We examined the AD incidence rate in quartiles of EIR and no obvious deviation from linearity was observed. The lowest risk was observed in the highest quartile of EIR. A cumulative incidence of AD in groups with EIR below and above the median is presented in **Figure 2**. High EIR was significantly associated with lower risk of AD also after adjusting for insulin sensitivity [HR for 1 SD decrease 1.3 (95%CI 1.0-1.6)]. The crude association between impaired insulin secretion and AD was somewhat stronger in the subsample free from diabetes than in the whole cohort [HR

1.4 (95%CI 1.1-1.8), unadjusted]. Insulin sensitivity measured with the euglycaemic insulin clamp was not associated with the development of AD.

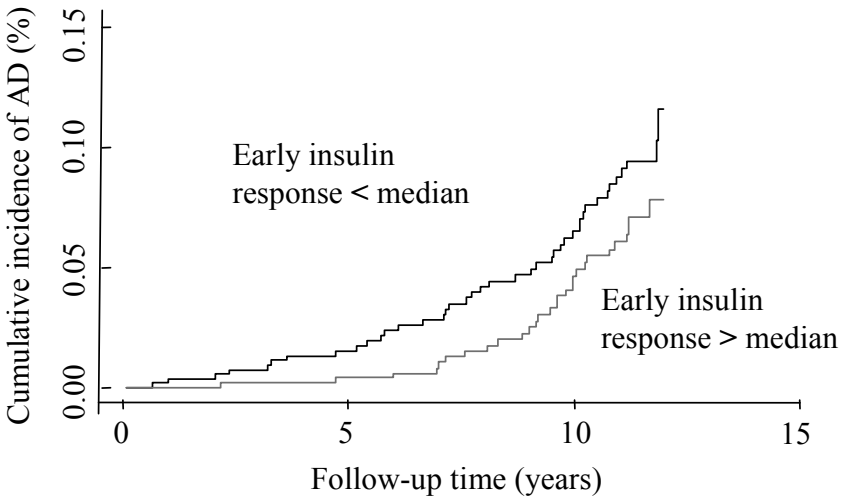

Figure 2. Cumulative incidence of AD in groups with early insulin response below and above the median.

The presence of the *APOE* ε4 allele increased the risk of developing AD [HR 2.4 (95%CI 1.5-3.9)]. We found a strong association between impaired EIR and the development of AD in *APOE* ε4 non-carriers [HR 1.63 (1.08-2.55) p=0.02] in the subsample free from diabetes. The association was weaker and no longer significant in *APOE* ε4 carriers [HR 1.18 (0.79-1.76)].

Prediction of vascular dementia

Low insulin sensitivity was associated with a higher risk of VAD. This association did not remain significant in the multivariable adjusted model. Neither low EIR nor *APOE* ε4-genotype was associated with the risk of VAD.

Prediction of any dementia or cognitive impairment

Low EIR, high fasting plasma glucose, 2-h plasma glucose at the OGTT and DM were all significantly associated with a higher risk of the composite outcome of any dementia and cognitive impairment. DM was a strong risk factor contributing to an elevated risk of 63% in the unadjusted model.

Study III

A total of 349 persons developed all-cause dementia of whom 127 had pure AD and 81 VAD during a maximum 40-years follow-up period (from 50 years of age until 1st January 2010. Other dementias included mixed dementia (n=17), frontotemporal dementia (n=6), Parkinson's disease dementia or Lewy body dementia (n=18) and unspecified dementia (n=100). Forty-four persons suffered from a stroke with sequelae where the application of dementia criteria was not possible. A quarter of the study participants (551 out of 2268) were still alive in 2010.

Midlife risk factors for dementia

In Cox proportional hazards analyses, midlife vascular risk factors did not increase the risk of pure AD. Vascular risk factors (fasting glucose, systolic blood pressure, BMI, cholesterol and smoking) were associated in a dose-dependent manner with VAD (p for trend <0.001) and all-cause dementia (p for trend 0.002). High midlife systolic blood pressure and smoking modestly increased the risk of VAD and all-cause dementia.

Late-life risk factors for dementia

Vascular risk factors at 71 years of age did not affect the future risk of pure AD but a dose-dependent increase of the risk of vascular dementia was obvious in spite of a limited number of cases (p for trend 0.01). Inclusion of persons with status post stroke with sequelae to the vascular dementia group further strengthened the associations. High systolic blood pressure and fasting plasma glucose significantly increased the risk of all-cause dementia. When the incidence rates were examined in quartiles, a tendency to a non-linear J-shaped association was found for BMI as the second quartile, (BMI 23-25) had the lowest hazard ratio for dementia [HR 0.9 (95% CI 0.6-1.4)] and the fourth quartile (BMI >27) the highest [HR 1.5 (95% CI 1.1-2.3)].

In the subgroup with *APOE* ε4 status information, HRs for *APOE* ε4 carriers compared to *APOE* ε4 non-carriers was 2.6 (95%CI 1.7-3.9) for AD, 1.7 (95%CI 0.9-3.3) for vascular dementia and 2.3 (95%CI 1.7-3.0) for all-cause dementia. There was a clear additive effect of multiple vascular risk factors on all-cause dementia in *APOE* ε4 non-carriers. *APOE* ε4 carriers without any of the vascular risk factors had a similar risk of developing dementia as *APOE* ε4 non-carriers with several risk factors (**Figure 3**). Smokers without the *APOE* ε4 allele had a tendency to an increased risk of dementia which was not observed among smokers carrying the allele in spite of similar mortality rates.

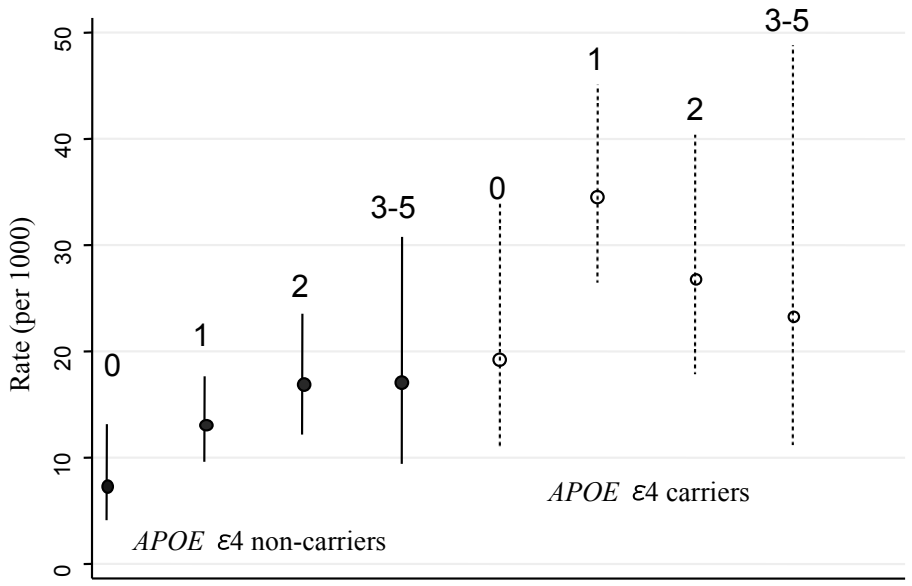

Figure 3. The number of vascular risk factors (0, 1, 2 or 3-5) at age 71 and incidence rate of all-type dementia in *APOE* ε4 non-carriers and *APOE* ε4 carriers. Lines indicate 95% confidence intervals.

Study IV

The proportions of fatty acids in serum cholesteryl esters were estimated in 2009 participants at baseline. A total of 213 participants developed all-cause dementia out of which 91 were pure AD during a maximum 35-year follow-up period (from 50 years of age until 31st December 2005).

Prediction of Alzheimer's disease

Subjects with incident AD had lower baseline myristic (14:0), palmitic (16:0), stearic (18:0) and oleic (18:1) acids proportions as well as a higher proportion of linoleic acid (18:2n-6), than those who did not develop dementia. Even among the cohort survivors in 2006, subjects with AD had lower myristic and palmitic acid than the non-demented subjects. There were no differences in the serum proportions of baseline n-3 FAs between the subjects with incident AD and dementia-free subjects in the cohort.

In Cox proportional hazards analysis, higher proportions of saturated FAs were associated with a decreased risk of AD. A similar tendency was observed for higher oleic acid and lower linoleic acid proportions. The risk of AD in quartiles of palmitic acid is presented in **Figure 4**. Consideration of age, educational level, body mass index, smoking, hypertension, diabetes and hyperlipidemia as potential confounders only marginally altered the

hazard ratios. In Cox proportional hazards analyses, the serum proportions of n-3 FAs were not associated with AD.

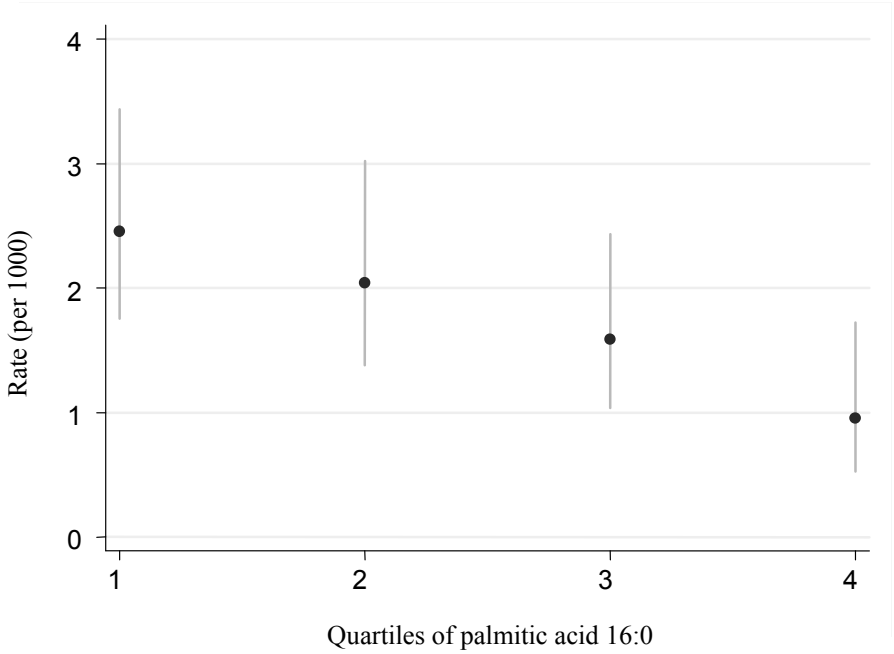

Figure 4. Incidence rates of AD by quartiles of palmitic acid (16:0) proportions. Lines indicate 95% confidence intervals.

In the subgroup of survivors up to the age of approximately 85 years, the results from logistic regression analysis were similar to Cox proportional hazards analysis above, regardless of whether the subjects with other dementias than AD and stroke with cognitive sequelae were excluded. The competing risk regression analysis according to Fine and Gray gave a similar profile [palmitic acid, HR for 1SD increase 0.7 (95% CI 0.6-0.8)]. In further competing risk analyses, the closeness of the cumulative incidence curves for AD and the Kaplan-Meier failure curve and the fact that the curves behaved similarly when divided by the median of individual FAs indicated that an approximation to Cox regression did not introduce major bias.

Prediction of vascular dementia

A total of 79 men had developed VAD or significant cognitive impairment after a stroke. There were no significant associations between serum FAs and this group. There was a tendency to an FA pattern which was different from that of AD: HR 1.2 (95% CI 0.9-1.4) for palmitic acid, HR 0.9 (95% CI 0.7-1.1) for linoleic acid.

Prediction of all-cause dementia

No significant associations between FA proportions and all-cause dementia were found: HR 1.0 (95% CI 0.8-1.1) for palmitic acid, HR 0.9 (95% CI 0.8-1.1) for linoleic acid and HR 1.0 (95% CI 0.9-1.2) for DHA.

In a separate analysis comparing *APOE* ε4 carriers (n=287) and *APOE* ε4 non-carriers (n=632), no significant differences were found between groups in the associations between serum proportions of FAs and either AD or all-cause dementia.

Discussion

Strengths and weaknesses

ULSAM is a large population-based cohort from the same age group with long follow-up and a careful classification of the dementia subtypes. The detailed baseline characterization of glucose metabolism, insulin secretion as well as serum FA composition at midlife is unique. To our knowledge, the ULSAM cohort is the largest population that has been examined with the euglycaemic insulin clamp method, the criterion standard for measurement of insulin sensitivity.

Insulin secretion was measured with two different methods, IVGTT and OGTT at two different occasions. An association between impaired EIR and AD both in study I and II suggests that this risk marker is stable at both middle and higher age. The associations between impaired EIR and dementia were stronger in men without diabetes, eliminating the possibility that the diabetic state or its treatment at baseline influenced our results. However, the participants may have developed diabetes and received treatment later during the follow-up.

The risk of dementia associated with smoking, obesity and high blood pressure is suggested to vary substantially with age [119-121]. Only a few other studies have had the possibility to study vascular risk factors in the same population both in midlife and late-life like in study III. Midlife serum FAs have previously been studied in relation to cognitive decline but not to dementia like in study IV. The competing risks were considered by different methods.

The ULSAM participants might have been healthier at baseline than those who did not participate. The participants were also likely to be treated more efficiently than the average population at that time [122]. At 71 years 35% of the participants were on medication for hypertension, 9% for hypercholesterolemia and 6% for diabetes. This could partly explain why the effect of vascular risk factors on cognition in the ULSAM study was somewhat weaker than in previous studies on average [123]. Any treatment effects on diabetes, hypertension, hypercholesterolemia, weight loss and smoking cessation after baseline were beyond the scope of this study.

APOE information in ULSAM was available only in a subgroup, all of them participants at age 71 limiting the power of these sub-analyses. Women have an increased risk of developing AD [124]. ULSAM as a very homoge-

neous sample representing men, standardized for age, limits the possibility of generalising the results to women or other age and ethnic groups. On the other hand, there is no confounding by these factors. Population studies are always carried out on a selected population, usually from one country and cultural group. It is not certain that these results can be generalised even to 50- and 71-year old men living presently in Uppsala as the risk factors may be different for a new generation.

Assessment of dementia

There is an absence of highly reliable consensus-based diagnostic criteria for cognitive impairment, AD and VAD. The available criteria have not been uniformly applied in previous studies and the methodology of dementia assessment varied greatly in the studies reviewed in this thesis.

All ULSAM participants were invited to cognitive testing at age approximately 72, 77 and 82 years. The responder rates were 50-80%. Testing at ages 70 and 77 included the MMSE and the Trail Making tests A and B. The tests used at age 82 were the MMSE and the 7-Minute Screening Test including clock drawing, enhanced cued recall, verbal fluency and temporal orientation [125]. The diagnoses were not based on these test results but participants with low performance were referred to the Uppsala Geriatric Memory Clinic for a follow-up, even though not all of them responded to this. Some previous studies based the dementia diagnosis on the medical records only and some used different types of standardised telephone interviews. In most of the studies, there was an initial screening across the whole population followed by a more detailed work-up in patients suspected of cognitive impairment.

Persons who develop cognitive impairment are less likely to attend follow-up examinations [126]. Therefore, an active screening is not enough to obtain complete follow-up of the population at risk. This is a limitation of many previous longitudinal studies on predictors of dementia. In this study, all ULSAM participants' medical records available were reviewed, first with 31st December 2005 as the censor date. Later, the review was extended to 1st January 2010. A similar strategy was used in the Rotterdam study where general practitioners and mental health care institutes cooperated in the study. Of subjects who could not be re-examined in person (due to death or refusal) medical files were studied.

In this study, the standardised review covered all in- and out-patient clinics including the Uppsala Geriatric Memory Clinic where persons may directly consult for memory problems, all hospitalisations at the Uppsala University Hospital including the Geropsychiatric Department and all primary care facilities, including community nursing homes and dementia group homes. Practically all medical care for the participants was provided in these

settings. The original diagnostic work-up of subjects with specific dementia subtype classification included a detailed medical history, relevant cognitive testing, physical examination, laboratory tests and in most cases neuroimaging to rule out other systemic or brain diseases that might account for the deficits. However, there is always a possibility that some participants with cognitive impairment did not seek medical care or help from the community. Further, to make a specific dementia subtype diagnosis can be difficult in a clinical situation [127]. Even in autopsy studies, various combinations of mixed pathologies are often seen and these do not always correlate with the clinical picture.

Brain imaging was obtained from all participants in few previous studies whereas in some studies imaging was added if needed on clinical grounds or was not performed at all. In ULSAM, brain imaging was performed when needed on clinical grounds in 89/102 (87%) of the AD cases, in 50/57 (88%) of the VAD cases and in 299/394 (76%) of all cases with any cognitive impairment.

In ULSAM, we aimed to make as strict diagnostic work-up as possible and this resulted in a number of diagnoses with unspecified dementia. The group "any dementia or cognitive impairment" was very heterogeneous in studies I and II. That is why we decided to use all-cause dementia (excluding mild cognitive impairment) as the endpoint in studies III and IV.

To standardise the possible differences between the original work-ups the records of all possible cases of dementia and cognitive impairment were reviewed by two experienced geriatricians, independently of each other and blinded to the baseline data. In case of disagreement, a third experienced geriatrician reviewed the case and the diagnosis was determined by majority decision. In most previous studies the diagnosis of dementia was also established by a consensus committee.

Epidemiological aspects

Study design

As dementia in itself affects lifestyle and dietary habits, conclusions on incidence rates or risks are difficult to make in a cross-sectional study. In case-control studies on dementia, one problem is to define controls. For instance, the persons with dementia could have more vascular risk factors due to a sedentary lifestyle than healthy persons of the same age. However, compared with all individuals born at the same time, we could find out that the persons with dementia actually lived longer and had less vascular risk factors than many others. This is important as we aim to find treatable factors that could postpone the disease.

Given the high incidence of dementia in persons over 80 years, the Cox regression analysis was preferred instead of logistic regression. In these

analyses, the length of time to reach the endpoint is of primary importance, rather than whether or not the subject reaches the endpoint. Furthermore, the non-demented subjects will be censored at the time of death. Our major outcome measure was time to dementia diagnosis. However, the date of diagnosis will always be an approximation because AD has an insidious course and because people seek medical care and get a diagnosis at different stages of the disease.

We do not know if the persons who moved away from the county of Uppsala would eventually have developed dementia later or if those who died during the follow-up would have developed dementia if they had not died of another cause. However, these persons were not known to have developed dementia before their censor date and this time is incorporated in the Cox proportional hazards regression model which was used in the survival analysis. Other methods which do not consider censored data could give misleading results.

As in any epidemiological study, our results are observational without absolute knowledge of causality. For instance, it is theoretically possible that those men with impaired insulin secretion already had genetic or other factors increasing the susceptibility to later dementia development, i.e. they were in an early preclinical phase of the disease (reverse causation).

We stated a priori that a complete description of our material regarding glucose and insulin metabolism, FAs and other vascular risk factors was important. Multiple independent analyses increase the risk of false positive results. For insulin secretion, however, two different baselines, 50 and 71 respectively, and two different methods, IVGTT and OGTT respectively, showed congruent results increasing the reliability.

Confounding, misclassification, publication bias

A confounder is associated with the exposure and has an effect on the outcome but it is not on the causal pathway of interest. The significance of individual vascular risk factors is difficult to interpret without adjustments for possible confounders. There is no consensus on the variables that should be considered in epidemiological studies on dementia. In our studies, we present the results crude and in multi-adjusted models. We adjusted for age and educational level in all four studies and also for different vascular risk factors in studies I, II and IV. However, we did not consider several other possible confounders, such as marital status, income level, occupation, alcohol consumption, depression, physical and cognitive activity.

Assigning a dementia diagnosis is both crucial and subtle in any clinical epidemiological study. The difficulty in the assessment of dementia diagnoses is discussed in the previous chapter.

It has been found that statistically significant results are three times more likely to be published than a null result. The field of dementia epidemiology research is also likely to suffer from this phenomenon called publication

bias. As a result, the meta-analyses on modifiable dementia risk factors may overestimate the true association. It is important that even negative results are published as in studies III and IV.

Competing risk

The population at risk will be transformed into a population of survivors due to the occurrence of any disease causing death over time. This phenomenon, known as competing risk, may lead to spurious associations if not taken into account, especially in studies where the outcome of interest generally occurs at old age. Due to competing risk, it may seem that a strong risk factor for death, such as smoking or atherosclerosis-associated fatty acid composition, protects from dementia [128].

In ULSAM, the FA composition characterised by high levels of palmitoleic and oleic acids and low intake of linoleic acid is previously shown to be associated with mortality [129] and stroke [130], thus competing with AD as an outcome. In study IV, higher levels of saturated serum fatty acids were unexpectedly associated with a lower risk of AD. Therefore, we sought to further investigate the effect of competing risk by death. Cumulative incidence curves suggested that approximation to the Cox model was reasonable. Furthermore, associations in a subgroup consisting of 85-year-old survivors were nearly identical to those in the total group, something telling against competing risk being the only explanation for our findings. Similar conclusions were drawn when competing risk from mortality was taken into account by the method of Fine and Gray. The methodology of calculating the risk of developing diseases in geriatric populations is continuously investigated and new models are introduced [131].

Possible connections

As our study is an epidemiological study, we can only speculate on the possible mechanisms behind associations between insulin secretion, serum fatty acids, hypertension, obesity, cholesterol, smoking and AD. A selection of interesting results from preclinical studies is presented in this chapter.

Insulin and the brain

AD and type 2 DM are both frequent disorders in the elderly. Impaired insulin signalling may affect neuronal repair mechanisms, energy metabolism, and glucose utilisation. Misfolding of proteins, aggregation of amyloid peptides and hyperphosphorylated proteins are present in both diseases. [132]

Insulin receptors and insulin-signalling proteins are widely distributed throughout the central nervous system, especially in the cerebral cortex and hippocampus. These control metabolism and brain function and associated disturbances might be of both genetic and environmental origin. There are

multiple isoforms of insulin-signalling proteins that may lead to more than 1800 potential combinations of signalling pathways [133]. Consequently, it is difficult to judge the importance of one single protein which is often the focus in mechanistic studies.

In AD, there is an accumulation of Aβ in the brain. In DM, islet amyloid polypeptide (IAPP) deposits are found in pancreatic beta-cells. IAPP and Aβ have a 90% structural similarity which suggests similar physiological roles [134]. The IAPP has toxic effects and its formation and progression may be concomitant with worsening beta-cell function in diabetes [135]. In AD, Aβ have toxic effects. [136].

The role of insulin in the pathophysiology of AD has been extensively studied by a group led by Dr. Suzanne Craft. Interestingly, there seems to be a connection between insulin and Aβ. Soluble Aβ oligomers bind to neurons, triggering oxidative stress and downregulation of plasma membrane insulin receptors. This leads to synapse deterioration which can be prevented by insulin and insulin sensitising drugs in cultures of hippocampal neurons [137]. In another report, insulin infusion reduced plasma Aβ-levels in normal adults but increased Aβ levels in AD patients [138]. According to one interesting study, intranasal insulin treatment for 21 days in patients with AD and amnestic mild cognitive impairment improved cognition and increased plasma Aβ40/42 ratio [139].

Chronic hyperinsulinemia appears to stimulate Aβ secretion and inhibit the extracellular degradation by competing with insulin-degrading enzyme IDE [140]. IDE cleaves both Aβ and insulin. IDE deficient mouse models resulted in >50% decreased Aβ degradation in the brain and insulin degradation in the liver [141]. The IDE expression was reduced in the hippocampus of AD patients [142]. IDE was also genetically associated with AD in a Finnish population [143] and in Swedish and UK case-control series [144] .

There are no representative animal models for the sporadic type of AD, i.e. AD not linked to a specific gene. Streptozotocin selectively destroys the insulin producing pancreatic beta-cells and is used to induce diabetes-like symptoms in animals. Interestingly, when streptozotocin is injected directly into the brain, it impairs brain glucose and energy metabolism, decreases acetylcholine levels in the hippocampus and induces impaired learning and memory. The animal develops AD-typical brain lesions and exhibits neurodegeneration [145].

The activation of insulin receptors may not only affect Aβ but also trigger tau-phosphorylation. This involves glycogen synthase kinase-3 (GSK-3) activation. Overactivity of GSK-3 could not only mediate the hyperphosphorylation of tau protein but also increase the production of Aβ and inflammation [146]. Another molecular mediator that could link diabetes and AD is proline-directed serine-threonine protein cyclin dependent kinase 5 (cdk5). Overactivation of cdk5 triggers progressive neurodegeneration and neurofibrillary tangle formation in mice [147]. Cdk5 plays a role in the loss

of beta-cell function in type 2 DM [148]. In a genome-wide association study, a variant in cdk5 influenced specifically insulin response [149]. Novel drugs that reduce Cdk5 activity or inhibit GSK-3 are possible targets in future therapies [150]. For example, leptin inhibits GSK-3 and reduces extracellular Aβ intracellular tau phosphorylation [151].

Insulin signalling plays a role in synaptic plasticity. Insulin and insulin receptor density decrease with aging [152] and in AD compared to controls of the same age [153]. The insulin receptors are desensitised in DM but also in the brain of AD patients. AD-type neurodegeneration can be produced experimentally by selectively impairing insulin/ insulin growth like factor function together with increasing oxidative stress [145].

The ageing brain can be damaged by toxic effects of hyperglycaemia, such as accumulation of advanced glycation end products (AGEs), oxidative stress and microangiopathy. AGEs are a heterogenous group of molecules that accumulate in various tissues during normal ageing and at an accelerated rate in DM as well as in AD [154]. AGEs exist in senile plaques and neurofibrillary tangles [155]. In diabetes, AGEs contribute to the common complications retinopathy, nephropathy and neuropathy. Serum or CSF levels of AGEs have been suggested to be potential biomarkers for early detection of AD [156].

Dietary fat quality

FA composition of plasma cholesterol esters (measured in ULSAM) reflects the dietary fat quality during the past 7-14 days [157]. FA composition in erythrocyte membranes or in adipose tissue mirrors the diet during past months and years. It is unknown how FA biomarkers reflect the FA uptake in the brain in humans. The uptake of different lipid classes varies as well [158].

Studies on animal models and cell cultures have shown that n-3 FAs are neuroprotective during development and ageing. In animal studies, the composition of fatty acids in the neuronal membranes reflects the diet [159]. Omega-3 FAs serve as energy substrates and major components of cell membranes. They may also have antioxidant and anti-inflammatory properties. DHA is a primary component of membrane phospholipids in the brain. The brain's need of n-3 FAs is predominantly met by the blood delivery due to the limited synthesis in the brain. DHA is also synthesised endogenously through a process of desaturation and elongation of its precursor n-3 fatty acids; ALA and EPA [160]. The ordinary Swedish diet contains in most cases a sufficient amount of n-3 FAs for the brain's need. Consequently, ULSAM participants were not likely to have a marked n-3 FA deficiency. This might explain the lack of association between n-3 FAs and AD in study IV.

Unexpectedly, the subjects with higher proportions of saturated fatty acids had lower AD risk in study IV. The finding is the opposite of experimen-

tal animal studies where chronic ingestion of saturated fats increased Aβ and resulted in blood-brain-barrier dysfunction [161]. Alterations of FA composition and FA-binding proteins may alter membrane fluidity and signal transduction, and consequently be involved in neuronal dysfunction in AD. Neuronal membranes with a larger proportion of saturated fatty acids may hypothetically be more rigid and thus possibly less accessible for gamma-secterase cleavage which is required to produce Aβ [162].

APOE ε4 status could modulate neuronal plasticity by a receptor-mediated uptake of lipids [163]. Some studies have found n-3 FAs to have a protective effect on cognition only in *APOE* ε4 non-carriers [55, 164]. In our population, no significant differences were detected which, however, may be due to the smaller sample sizes limiting the power.

Hypertension

The mechanistic evidence on the possible association between high blood pressure and AD pathology is limited. Uncontrolled hypertension predicted the level of neuritic plaques and neurofibrillary tangles in the Honolulu Asia Aging Study [165]. In AD transgenic mice, the regulation of cerebral blood flow is impaired and episodes of hypotension or hypertension may result in fluctuations in cerebral blood flow that may contribute to neuronal dysfunction. Cerebral ischemia may alter amyloid processing or clearance in the brain according to animal studies [73].

Obesity

Adipose tissue secretes several hormones such as leptin which have been shown to reduce the extracellular Aβ load and tau phosphorylation in neuronal cells in animal models. Leptin was associated with a reduced incidence of AD in the Framingham cohort [166]. Leptin expression is lower in visceral fat than subcutaneous fat [167]. Waist-hip ratio might display the metabolic condition in overweight better than BMI as abdominal obesity and visceral fat are more correlated with vascular risk [168]. One recent study found that central obesity, but not BMI, was related to a higher risk of AD [169]. Other potential mechanisms also include inflammation as adipose tissue also secretes inflammatory cytokines.

Cholesterol

Cholesterol might theoretically increase the activity of the β- or γ-secretase cleavages that generate Aβ from *APP*. The application of cholesterol-lowering drugs to *APP* overexpressing human embryonic cells has also been shown to decrease β-cleavage. Once Aβ has been produced, the cholesterol level could influence its aggregation state. On the other hand, cholesterol depletion has adverse effects on dendrite growth and axonal branching [170].

Smoking

The mechanism through which smoking would increase the risk of AD is not known but may include oxidative stress and inflammation. Neuroimaging studies suggest that smoking may accelerate cerebral atrophy and cerebral white matter lesions. Importantly, quitting smoking may reverse this development [171]. In contrast, nicotine may also stimulate cholinergic pathways in the brain aiding learning and memory [172].

Aggregation of vascular risk factors

The aggregation of vascular risk factors may have a greater impact on the development of dementia than the individual factors *per se* [30, 173]. The different vascular risk factors may act in synergistic or additive manners in the development of cognitive disease as they do in vascular disease. In clinical practice, a cluster of cardiovascular risk factors in the same patient is the rule, rather than the exception. The metabolic syndrome is a condition characterised by abdominal obesity, elevated blood pressure, hyperglycemia and dyslipidemia. It has different definitions and is also known as insulin resistance syndrome. The metabolic syndrome has been associated with AD in cross-sectional studies [174, 175]. In the Honolulu Aging Study, metabolic syndrome (calculated from seven factors representing the syndrome) at midlife was, however, associated with VAD, but not with AD [176]. Similarly, metabolic syndrome increased the risk of VAD in the Italian Longitudinal Study on Ageing [177]. In contrast, the metabolic syndrome was not associated with an increased dementia risk in the multiethnic Washington Heights-Inwood Columbia aging project cohort [178].

APOE

The *APOE* ε4 allele is the most important genetic risk factor for sporadic late-onset AD. It might act like a pathological chaperone, providing a suitable structure for Aβ monomers and oligomers to stick to. *APOE* ε4 carriers have lower Aβ concentrations in cerebrospinal fluid and greater burden of amyloid plaques than non-carriers in neuropathological studies.

However, atypical early-onset AD patients seldom carry the *APOE* ε4 allele. They are probably predisposed to brain vulnerability by a different mechanism [179]. In ULSAM, the association between impaired early insulin response and AD was stronger in *APOE* ε4 non-carriers as well as the association between vascular risk factors and dementia. Similarly, many other studies have found different risk patterns in populations depending on the *APOE* ε4 genotype. APOE ε4 classification could be useful in the development of therapeutic interventions.

Intervention studies

While waiting for the novel treatments directly targeting Aβ and tau pathology, large-scale randomised controlled trials are needed to test other disease-modifying strategies to maintain cognitive function in individuals at risk for decline and to identify factors that may delay the onset and progression of AD.

The effect of glucose lowering on cognitive function was recently determined by the ACCORD-MIND trial [180]. The intensive-treatment group with HbA1C less than 6.0 had a greater mean total brain volume but the cognitive outcomes were not different from the control group. The intervention occurred too late in the course of diabetes to have any effect on early insulin response. Moreover, there was an increased mortality in the treatment group!

The double-blind, placebo-controlled Systolic Hypertension in Europe (Syst-Eur) trial investigated the effect of long-term antihypertensive therapy on 3902 patients with baseline systolic blood pressure between 160 and 219 mmHg. The treatment group had a 55% reduced risk of dementia during following 2-4 years [181]. This study was interrupted due to increased mortality in the placebo group. In the Study on COgnition and Prognosis in the Elderly (SCOPE), effective antihypertensive therapy reduced cognitive decline incidence in patients with systolic blood pressure between 166 and 179 and slightly impaired cognitive function [182].

A suggested association between cholesterol and dementia has led to the anticipation that lipid-lowering drugs, statins, could prove useful in treating or preventing AD. The permeability of statins across the blood-brain barrier is disputed. A randomised controlled trial from 2010, the LEADe study evaluated the efficacy of atorvastatin in patients with mild to moderate AD. Treatment for 18 months was not associated with significant clinical benefit [183]. Other randomised controlled trials have also failed to show an effect of cholesterol lowering on cognition [184, 185].

More than ten clinical trials have examined the effect of DHA supplementation, alone or together with other n-3 FAs, on cognition during 3-12 months. The study subjects have been healthy elderly, nursing home residents, patients with mild cognitive impairment or patients with AD. In two studies, improvement in cognitive tests was noticed after DHA supplementation in subjects with mild cognitive impairment [186, 187] and healthy subjects [188]. However, several studies with healthy subjects [189, 190] or AD patients [186, 191-193] did not find an effect, except for one study in dementia nursing home residents in Japan from 2001 [194]. A recent large placebo-controlled double-blind intervention study (n=2911) showed no effect of dietary doses of n-3 fatty acids on global cognitive decline in coronary heart disease patients [195].

Prolonged randomised clinical trials on all vascular risk factors and unhealthy behaviours, i.e. smoking are complex to perform. In addition to the ethical issues of randomising persons to such interventions, a randomised clinical trial testing this relationship is infeasible because of the long follow-up period and large sample size required. In an observational longitudinal cohort study, the health behaviours of the participants might be more close to people in the general population compared with the greater selection bias and artificial behaviours often induced by the conduct of randomised intervention trials. However, a recent paper declared that a 10-25% reduction in seven risk factors (diabetes, midlife hypertension, midlife obesity, smoking, depression, cognitive inactivity or low educational attainment, and physical inactivity) could potentially prevent as many as 1.1-3.0 million AD cases worldwide [196]!

Consequently, some ongoing projects will have a try. In Holland, a large randomised trial called PreDIVA recruits 3700 elderly subjects for a 6-year follow-up. The aim is to assess whether nurse-led intervention including intensive treatment of hypertension, hypercholesterolemia, diabetes and reducing overweight, smoking cessation, and stimulating physical exercise in primary care decreases the incidence of dementia and reduces disability [197]. The Finnish FINGER study for prevention of dementia and memory disorders will involve about 1,200 participants who will be assigned to intensive counselling (exercise, nutritional guidance, cognitive training and reduction of vascular risk factors) and standard health counselling groups. An intensive counselling intervention and detailed monitoring and reduction of cardiovascular disease risks is expected to reduce memory loss markedly and delay the onset of dementia.

Future

An enormous worldwide scientific effort is currently being made to find diagnostic tools and novel treatments for preclinical AD that reverse the process starting with toxic β-amyloid species leading to neuronal death. Even if this work will be successful, other pathologies than AD still lead to dementia in the population. In this future scenario, the impact of possible modifiable risk factors for dementia might be completely different. As there are no representative animal models for late onset sporadic AD, clinical human research is obligatory.

To be able to detect AD in the preclinical stage is a prerequisite for many possible future treatments. The newly suggested National Institute on Aging and the Alzheimer's Association workgroup (NIA-AA) criteria for preclinical AD are based on biomarkers of brain amyloidosis and neuronal injury that are detected in cerebrospinal fluid or with advanced neuroimaging for

earlier and more precise diagnosis. However, these criteria are at present for purposes of research only, and need further validation [198].

ULSAM

The ULSAM cohort has collected plenty of observations and data for further research on dementia risk factors, for example variables from examinations of atherosclerosis by carotid ultrasound, physical activity and condition, muscle strength, nutrition, and life quality. Studies on neuropathology and cerebrospinal fluid markers are ongoing. The modern proteomics and metabolomics methods may reveal new interactions between genetic and environmental risk factors for dementia. The latest investigation of the ULSAM men at 88 years of age was completed in December 2009 and focused on predictors for successful ageing.

Ethical aspects on successful ageing

The aim of future intervention strategies is to increase the chance of retaining a highly functional brain in late life. It is likely that this also promotes a longer life. As age is the strongest risk factor for dementia, the result may as well be an increase in dementia prevalence as we already generally have seen with an increased life expectancy.

All specialties in medicine strive for better survival i.e. cardiologists work hard to prevent the patients from dying in heart attacks. On the other hand, if all myocardial infarctions would have a deadly outcome, the prevalence of dementia would drop dramatically. In Sweden, marked cognitive decline is often regarded as a limiting factor and people with severe dementia are usually not subject to advanced life support.

In future epidemiological studies in geriatric populations, choosing the endpoint is an interesting, partly ethical question that opens a new field of important clinical research. Are we striving for the longest autonomous, dementia-free life expectancy? Or the longest life expectancy with the highest self-reported quality of life? Or the longest life expectancy in persons who live in a nursing home?

Conclusions

In this community-based cohort of Swedish men, a low early insulin secretion was associated with an increased risk of AD. Similar associations were found using measurements from the IVGTT at 50 years of age and from the OGTT at 71 years of age. Low insulin sensitivity at 71 years of age was not associated with a higher risk of AD. This knowledge may have implications for prevention and treatment of AD - in particular the timing and nature of the intervention.

In contrast to experimental studies, saturated serum fatty acids were inversely associated with risk of AD. No evidence of a protective effect of n-3 fatty acids against AD or all-cause dementia was found. These results remained essentially unchanged if competing risk from mortality was taken into account.

No associations between hypertension, BMI, cholesterol, smoking and the risk of AD were found. High blood pressure measured in midlife or late-life increased the risk of all-cause dementia. Individuals with both an *APOE* ε4 allele and vascular risk factors had the greatest all-cause dementia risk. In this context it is important to bear in mind that a large group of individuals develop AD without an extensive burden of vascular risk factors earlier in life. Brain ageing begins relatively early but the dementia incidence increases dramatically only after 75 years of age. We need to identify factors that may initiate the disease process and contribute to its progression. Early insulin response could be one of these markers.

According to the US National Institutes of Health State-of-the-Science statement in 2010, there is no evidence of sufficient scientific quality supporting the association of any modifiable factor with reduced risk of AD [199]. However, we should still aim for a healthy lifestyle and treat vascular risk factors to prevent other late-life chronic diseases including heart disease, stroke and VAD. There is strong evidence that the *APOE* ε4 allele and a family history of AD are associated with higher risk of AD but the strongest at present known risk factor for AD and other dementias is high age.

Summary in Swedish

Sammanfattning på svenska

Alzheimers sjukdom (AD) och vaskulär demens är de vanligaste demenssjukdomarna som innebär stort lidande för patienter och anhöriga samt stora kostnader för samhället. Därför är det angeläget att försöka identifiera påverkbara riskfaktorer för demens. Avhandlingens mål är att studera hur glukosmetabolism, insulin, fettsyresammansättning och andra vaskulära riskfaktorer är kopplade till framtida utveckling av AD och vaskulär demens.

Avhandlingen baserar sig på ULSAM (Uppsala Longitudinal Study of Adult Men) kohorten som startades 1970. Totalt 2322 män i 50 års ålder undersöktes med fokus på vaskulära riskfaktorer. Kohorten undersöktes åter vid 60, 71, 77, 82 och 88 års ålder. De män som insjuknade i demens till och med 2010 identifierades noggrant.

Risken för AD var ökad hos män med lågt tidigt insulinsvar som mättes både med intravenös glukostoleranstest vid 50 samt med oral glukostoleranstest vid 71 års ålder. Vaskulära riskfaktorer; hypertoni, övervikt, högt kolesterol och rökning ökade risken för vaskulär demens, men inte för AD. Högre andel saturerade fettsyror i blodet var associerat med lägre risk för AD. Det fanns ingen evidens för att omega-3 fettsyror skulle skydda mot demens.

Förutom hög ålder var genvarianten *APOE* ε4 den starkaste riskfaktorn för AD i ULSAM. Individer med både *APOE* ε4 och vaskulära riskfaktorer hade den största risken för att utveckla demens. Lågt tidigt insulinsvar var en riskfaktor huvudsakligen hos män som inte var bärare av *APOE* ε4.

Dessa resultat tyder på att störningar i glukosmetabolism, insulinsekretion, fettsyror och vaskulära riskfaktorer är relaterade på olika sätt i uppkomsten av AD och vaskulär demens. Fynden har potentiella kliniska tillämpningar i att förebygga eller senarelägga AD och vaskulär demens i framtiden.

Summary in Finnish

Yhteenveto suomeksi:

Alzheimerin tauti ja verisuoniperäinen dementia ovat tavallisimmat dementoivat sairaudet. Ne aiheuttavat suurta kärsimystä potilaille ja heidän omaisilleen sekä suuria kustannuksia yhteiskunnalle. Riskitekijät, joiden vaikutuksesta dementia puhkeaa ja etenee, ovat paljolti epäselviä. Tämän väitöskirjatyön tarkoituksena oli saada selville miten sokeriaineenvaihdunta, insuliini, rasvahapot ja muut verisuonisairauksien riskitekijät liittyvät Alzheimerin taudin ja verisuoniperäisen dementian ilmaantumiseen.

Väitöskirja pohjautuu ULSAM (Uppsala Longitudinal Study of Adult Men) tutkimukseen joka alkoi vuonna 1970. Yhteensä 2322 50-vuotiasta miestä tutkittiin tarkasti keskittyen verisuonitautien riskitekijöihin. Miehet tutkittiin uudellen 60, 70, 77, 82 ja 88 vuoden iässä. Dementiaan sairastuminen selvitettiin tarkasti vuoteen 2010 asti.

Riski sairastua Alzheimerin tautiin oli suurempi miehillä, joiden insuliinin erityksen ensivaihe oli heikentynyt. Tulokset olivat samanlaiset, kun insuliinin eritys mitattiin joko suun kautta tai suonensisäisesti tehdyssä sokerirasituskokeessa. Korkea verenpaine, ylipaino, korkea seerumin kolesteroli ja tupakointi altistivat verisuoniperäiselle dementialle mutta eivät Alzheimerin taudille. Ne miehet, joilla seerumin rasvahapoista suurempi osuus oli tyydyttyneitä, oli alhaisempi Alzheimerin taudin riski. Tyydyttymättömät omega-3 rasvahapot eivät suojanneet Alzheimerin taudilta.

Ikääntyminen ja *APOE*-geenin ε4 alleeli olivat Alzheimerin taudin tärkeimmät riskitekijät ULSAM-tutkimuksessa. Miehet, joilla oli sekä *APOE* ε4 alleeli ja verisuonitaudin riskitekijöitä oli korkein riski sairastua dementiaan. Heikko insuliinin eritys oli riskitekijä erityisesti miehillä, joilla ei ollut *APOE* ε4 alleelia.

Sokeriaineenvaihdunta, insuliini ja verisuonisairauksien riskitekijät vaikuttavat Alzheimerin taudin ja verisuoniperäisen dementian puhkeamiseen todennäköisesti eri tavoin. Väitöskirjan tuloksilla on merkitystä pyrittäessä tulevaisuudessa ehkäisemään Alzheimerin tautia ja verisuoniperäistä dementiaa.

Acknowledgements

I wish to express my sincere gratitude to all who have contributed to this thesis and to all who have supported and encouraged me during these years, in particular to:

My principal supervisor Lena Kilander: You are both the brain and the muscle behind this work. What a great fun we had together!

My co-supervisor, Lars Lannfelt, who offered me a job at Geriatrics in 2003 and has generously, friendly and understanding supported me since then.

My co-supervisor Björn Zethelius: You always provided valuable comments on my work and shared your extensive knowledge about epidemiology and diabetes.

Johan Sundelöf, my co-author. You made my way easy by doing it all before me.

Malin Degerman-Gunnarsson, my co-author, for the diagnostic work in UL-SAM. You, Kristin and the Others who shared the finest room at the institution with me: I am grateful for all small and big talks that many times did not concern this research at all.

Johan Sundström, my co-author, for valuable advice, for introducing me to Cox-analysis and giving me my first do-file!

Christian Berne, my co author, for the idea of paper I and II and for valuable, friendly comments.

Liisa Byberg, my co-author, for a super STATA course and all statistical advice in paper IV.

Bengt Vessby, my co-author, for valuable ideas and comments on paper IV.

My clinical tutors Inga Sjögren, Bernice Wiberg and Per-Erik Andersson for all warm support and guidance during these years.

All my past and present co-workers and colleagues at the Uppsala University Hospital, Department of Geriatrics and Memory Clinic. You have worked hard in taking care of the geriatric patients while I have been writing this thesis. A special thanks to my Chiefs Elina Alvarez and Margareta Falkeborn.

All friends and colleagues at DH 13B, Geriatrics and Clinical Nutrition, for many laughs, pleasant lunch and coffee breaks.

Rawya Mohsen, Ewa Warensjö Lemming and Vilmantas Giedraitis: past and present data managers of the ULSAM cohort, for taking exellent care of the ULSAM database, always friendly and quick responding.

David Ekstrand for English proofreading.

Dear ALF, for grants that made this work possible.

All ULSAM co-workers during these four decades and

!!All brave and faithful Uppsala-men, participants in the ULSAM study!!

All my relatives and friends both in Finland and Sweden as well as friendly neighbours. Especially all the best girls for thrills: those who travel with me the world around, take me to gympa and singing Ursulas.

My mother Anna-Maija who has always been there for support and who has taken care of me and the rest of the family when needed. My father Tapani for all enthusiasm in research and guidance in papers I and II.

Aura and Artur: for songs, smiles and deep thoughts about beyblades and flying pigs: for giving my life a new meaning.

"*If I speak in the tongues of men or of angels, but do not have love, I am only a resounding gong or a clanging cymbal. If I have a faith that can move mountains, but do not have love, I am nothing*." You are everything, Staffan.

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Editor: The Dean of the Faculty of Medicine

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