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The impact of vitamin D, insulin, midlife work-related stress, and the "CAIDE Dementia Risk Score" on cognitive performance and structural brain changes

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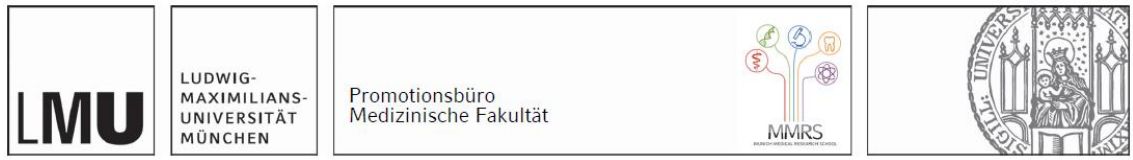

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List of abbreviations

25(OH)D	25-hydroxyvitamin D
AD	Alzheimer's disease
APOE	Apolipoprotein E
Aβ	β-amyloid
CAA	Cerebral Amyloid Angiopathy
CAIDE	The Finnish Cardiovascular Risk Factors, Aging, and Dementia Study
CSF	Cerebrospinal Fluid
DLB	Dementia with Lewy bodies
FINGER	The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability
FTLD	Frontotemporal Lobar Degeneration
HOMA	Homeostatic Model Assessment
MCI	Mild cognitive impairment
MRI	Magnetic resonance imaging
NFT	Neurofibrillary tangles

List of publications

List of publications This doctoral thesis is based on the following original papers:

I- **Hooshmand B**, Lökk J, Solomon A, Mangialasche F, Miralbell J, Spulber G, Annerbo S, Andreasen N, Winblad B, Cedazo-Minguez A, Wahlund LO, Kivipelto M. Vitamin D in relation to cognitive impairment, cerebrospinal fluid biomarkers, and brain volumes. *J Gerontol A Biol Sci Med Sci. 2014 Sep;69(9):1132-8.*

II- **Hooshmand B**, Rusanen M, Ngandu T, Leiviskä J, Sindi S, von Arnim CAF, Falkai P, Soininen H, Tuomilehto J, Kivipelto M. Serum Insulin and Cognitive Performance in Older Adults: A Longitudinal Study. *Am J Med. 2019 Mar;132(3):367-373.*

III- Sindi S, Kåreholt I, Solomon A, **Hooshmand B**, Soininen H, Kivipelto M. Midlife work-related stress is associated with late-life cognition. *J Neurol. 2017 Sep;264(9):1996-2002.*

IV- **Hooshmand B**, Polvikoski T, Kivipelto M, Tanskanen M, Myllykangas L, Mäkelä M, Oinas M, Paetau A, Solomon A. CAIDE Dementia Risk Score, Alzheimer and cerebrovascular pathology: a population-based autopsy study. *J Intern Med. 2018 Jun;283(6):597-603.*

Author contribution

Contribution to Paper I

Dr. Hooshmand had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Furthermore, he contributed to the literature search, concept and design, acquisition, statistical analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and supervision of the study.

Contribution to Paper II

Dr. Hooshmand had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. In addition, he contributed to the literature search, study concept and design, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and study supervision.

Contribution to Paper III

Dr. Hooshmand contributed to the literature search, interpretation of data, drafting of the manuscript and critical revision of the manuscript for important intellectual content.

Contribution to Paper IV

Dr. Hooshmand had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Furthermore, he conceived and designed the study, participated in acquisition and interpretation of the data and performed the statistical analysis, drafted the manuscript, critically revised the manuscript for important intellectual content, contributed to administrative, technical or material support and supervised the study.

Der Arbeitsanteil aller Ko-Autoren wird separate bestätigt und eingereicht.

Introduction

Dementia and cognitive impairment

The essential feature of dementia, a condition usually diagnosed in persons older than 65 years of age, is the development of multi-domain cognitive impairment which is severe enough to cause substantial disturbances in occupational or social status which will lead to the lack of independency, and it must present a progressive deterioration of functional status[1, 2].

Dementia has been considered as one of the most burdening conditions of the 21st century and represents a main cause of disability in elderly people[1, 3]. It has been estimated that more 50 million individuals currently suffer from dementia worldwide, a number which is expected to be tripled by 2050[1, 3, 4].

Alzheimer's disease (AD) and cerebrovascular conditions (CVD) represent the first and the second most prevalent dementia types, respectively[3], whereas Lewy body dementia (LBD) and frontotemporal lobar degeneration (FTLD) comprise less prevalent types[1, 5]. However, accumulating data from biomarker studies, especially neuroimaging neuropathological studies, imply that the majority of dementia cases are due to a combination of different pathologies in the brain, particularly vascular and neurodegenerative pathologies[6].

Advanced age and carrying one or two *APOE*ε4 alleles have been considered as the strongest dementia risk factors[1]. Dementia prevalence and incidence are very low in individuals younger than 65 years. However, from 65 to 90 years of age, it doubles every 5 years[3]. Studies on incidence of dementia after this age are mixed and yielded either a steady state or increase in incidence[3]. Although the neuropathology of dementia may begin several decades before the dementia is clinically manifest[7], the relationship between dementia and different neuropathologies has been shown to be more heterogeneous amongst the very old individuals (aged ≥ 85 years) compared with those who are younger[8-10].

Alzheimer's disease Neuropathology

The main neuropathologies of AD are extracellular plaques of amyloid and intraneuronal neurofibrillary-tangles (NFT), gradually leading to neuronal dysfunction and death[1, 11, 12].

Amyloid plaques result from processing of amyloid precursor protein (APP) and amyloid beta-40 ($A\beta_{40}$) constitutes the most abundant form of amyloid beta. About ten percent of all brain Aβ species constitute of the 42 amino-acid type of amyloid β or $A\beta_{42}$. This form of amyloid has a higher tendency to accumulate as deposits of amyloid, compared to the other forms[13]. Diffuse plaques and neuritic plaques (NP) represent the two main forms of Aβ plaques in the brains of patients with AD. The former is composed of unstructured amyloid whereas dense amyloid fibrils constitute the structure of NPs. In addition, aggregates of Aβ have been shown to be present in walls of brain vessels, leading in some individuals to another pathology, namely cerebral amyloid angiopathy (CAA)[14, 15].

Another neuropathological hallmark of AD is the formation of NFTs inside neurons. NFTs are due to pathological phosphorylation of the tau protein, which is a protein associated to microtubules. This leads to the oligomerization and destabilization of microtubules, which in turn will result in neuronal apoptosis[15, 16].

Based on the presentation and distribution of NFTs, Braak proposed a model for development of AD with a hierarchical progression of the pathologies[11, 12]. According to the Braak's model, neurofibrillary tangles first appear within the medial temporal lobe's entorhinal part. This occurs during AD's preclinical stage. During the later stages of Alzheimer's disease, the pathology spreads to the hippocampus and in the final phases to the neocortex[15]. Consequently, and as the disease proceeds, atrophy of the brain due to substantial loss of neurons will ensue, particularly in the structures of temporal lobe, observed in in-vivo imaging techniques such as magnetic resonance tomography[1, 15, 17].

This pattern changes little inter-individually and allows the classification in to 6 stages during progression of the lesions: stage I and stage II have been considered as transentorhinal stages which are usually clinically silent. Stage III and stage IV have been considered as limbic stages in which the beginning of clinical signs and symptoms may occur. Stage V and stage VI have been regarded as neocortical stages in which the full clinical signs and symptoms of Alzheimer's type dementia may be observed[18]. Interestingly, it has been suggested that via an still unknown mechanism, amyloid β may induce the progression of tau pathology[1].

Other alterations found in AD brain include reactive astrocytes' proliferations and loss of synapses particularly in structures of the temporal lobe such as hippocampus, entorhinal cortex (where the pathology of AD begins and then spreads to other regions[12, 18], see

above), and associated cortical areas. In addition, oxidative stress, neuroinflammation, aging, cerebrovascular lesions, and dysfunction of the glymphatic system seem to be important in AD[1]. However, the relevance of these alterations is not entirely understood because they can often be found in persons with MCI, or even with normal cognition as well[19]. Not every person with typical AD changes in her or his brain would develop dementia later on. Furthermore, the exact time interval between aggregation of brain pathologies and beginning of signs and symptoms of AD is still unclear.

Risk and protective factors for cognitive impairment

During recent years, a large number of studies focused on characterizing effective preventive and treatment approaches for cognitive impairment and AD. Although there is currently no disease-modifying therapy available for AD other than the debatable Aducanumab[20, 21], accumulating evidence from the latest population based studies suggest that the dementia incidence, but not necessarily prevalence, is decreasing in western societies[1]. The reason for this change could not be fully explained, but improvements in quality of life, education, and decline in the burden of vascular risk factors as well as other social and behavioral alterations such as healthier lifestyles could have favorably affected physical and mental health, and consequently, cognitive health over the life-span of the newer generations of elderly[1, 22].

This is because AD is a complex multifactorial condition involving several interrelated modifiable and non-modifiable risk factors[3, 23], and findings from epidemiological studies as well as clinical trials support targeting risk factors which are modifiable to prevent cognitive impairment. Two recent reviews suggested that about 50% of cases of Alzheimer's disease could be due to risk factors which are potentially modifiable. Accordingly, these reviews suggested that a substantial decrease in modifiable risk factors might prevent several millions of cases of cognitive impairment and AD[24, 25].

Among the non-modifiable risk factors for AD, the strongest ones are advanced age and carrying one or two Apolipoprotein-ε4 alleles. Furthermore, being female is associated with a higher probability of AD, especially in older ages (i.e. $\geq$ 80 years)[1, 3].

The 2020 report of Lancet Commission on prevention of dementia acknowledged twelve modifiable risk factors as potential targets for reducing risk of dementia. These modifiable risk factors are education, diabetes mellitus, physical activity, obesity, hypertension at mid-

life, smoking, excessive alcohol consumption, traumatic brain injury, hearing loss, air pollution, depression, and social isolation (figure 1)[25]. In comparison with the 2017 Lancet Commission report[26], the current report includes 3 additional factors. This highlights the importance of pursuing further research in order to identify additional possible candidates. Recent studies have suggested other potentially modifiable factors which, apart from their traditional characteristics, may have a role in brain health as well. In this doctoral thesis, we have examined the associations of some of these factors with the risk of cognitive impairment and structural brain changes. Pending robust evidence from clinical trials, these factors might be among potential candidates to be considered in the next version of the Lancet Commission report.

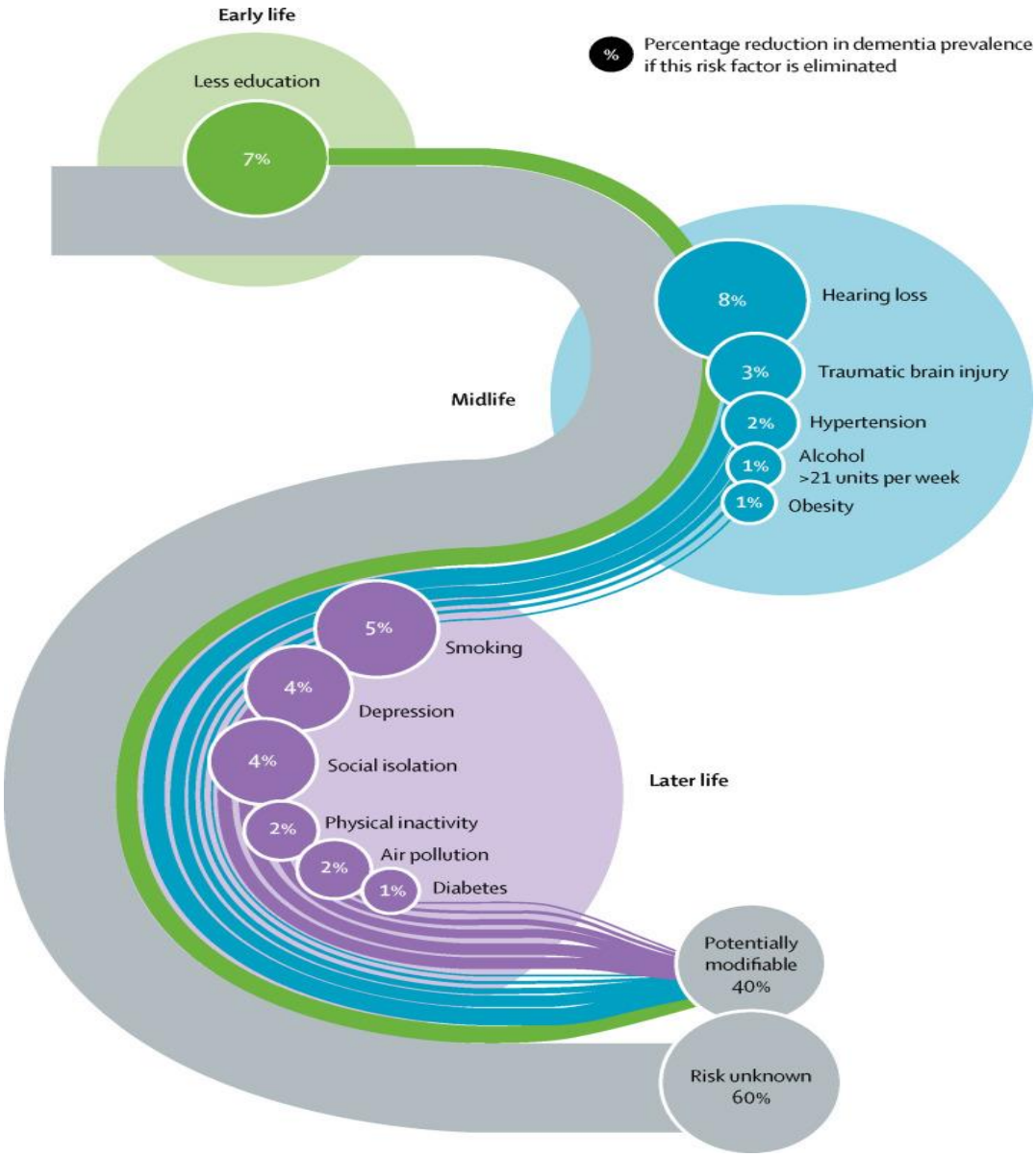

Figure 1 (reprinted with permission from[25]). Population attributable fraction of potentially modifiable risk factors for dementia[25]

Vitamin D

Low levels of Vitamin D are prevalent particularly among more vulnerable sections of the population such as elderly. This has several reasons including reduced levels of skin-7-dehydrocholesterol in older people, reduced exposure to sunlight, consuming foods which have low levels of vitamin D, and physical inactivity[27]. In addition to its traditional role in musculoskeletal system, accumulating evidence suggest that vitamin D has as an important role in well-functioning of other targets including the central nervous systems and the cardio- and cerebrovascular-system[28, 29]. Notably, receptors of vitamin D have been shown to be present in hippocampus and cerebral cortex, which are critical structures for cognition and memory[28, 30-32]. As such, vitamin D has been considered to be a possible modifiable risk-factor-candidate for cognitive impairment and dementia, although evidence has been inconclusive[27, 33-37].

Vitamin D may influence the risk of dementia through several mechanisms such as its impact on glutathione synthesis and other antioxidants or neurotrophic factors. Furthermore, vitamin D is related to well-functioning of cardiovascular and cerebrovascular systems, impairment of which has been associated with higher risk of dementia. In addition, accumulating evidence suggest a possible role of this vitamin on amyloid homeostasis in the brain or production of NFT[27, 29]. However, only a handful of studies examined the relationship between vitamin D and structural brain changes using MRI-examinations[34, 36, 38]. Furthermore, no previous research has investigated the association of vitamin D with main AD biomarkers in CSF such as $A\beta_{42}$, total-tau and phospho-tau[39].

Serum insulin and Insulin resistance

Insulin resistance is prevalent among the elderly and is closely correlated to overweight, obesity, and lack of physical activity. It has been shown to be related to several conditions, particularly different vascular disorders[40, 41].

Interestingly, accumulating evidence suggest the presence of insulin receptors in brain areas important for cognition, such as hippocampus and cerebral cortex[41]. Results from basic science studies proposed that raised values of insulin in peripheral blood due to insulin resistance may lead to its reduced transportation into the brain, resulting in lower values of insulin in the brain, causing a reduction of its effects on its receptors[41, 42].

Consequently, these changes will gradually influence brain function and its composition through numerous pathways such as the impairment of cerebrovascular system, production of Aβ or potentiation of its neuro-toxicity or enhancing the formation of neurofibrillary tangles which in turn will result in a higher risk for dementia and cognitive impairment[40-44].

Although the relationship between elevated insulin values and increased risk of dementia or cognitive impairment seems plausible, the few prospective studies which have been conducted in this context showed mixed findings[45-53]. For example, the Rotterdam study which had a follow-up duration of ten years found a relationship between higher insulin values and increased risk of AD during the first three years of follow-up. This association was no longer significant in those who were followed up longer than three years. Results of the Rotterdam study suggested that the impact of insulin on dementia may be time-dependent, before the dementia related structural brain changes are too advanced, after which insulin may no longer influence the risk[45].

Due to inconsistent findings on one hand and biologic plausibility on the other, it is important to investigate the impact of insulin resistance on dementia and cognitive impairment and identify the possible window of opportunity for its effect on dementia risk. Insulin resistance can be modified and it could be a candidate for randomized controlled trials on reducing dementia risk, pending the results of well-designed prospective studies.

Work related stress

Taking into account the large amount of time spent at work, job-strain has been frequently considered as a prevalent and significant source of stress. Several models have been proposed for conceptualizing the stress related to work such as the job-demand-control-support model (considers autonomy and gain control over job, job demands, gain support from supervisor and colleagues), job-demands-resources-model (considers not only control over job, but also imbalance between demands on the individual and the resources she/he has to deal with those demands), the effort-reward-imbalance-model (considers the balance between efforts spent and rewards received, not only in financial terms, but also in terms of job security, promotion-chances and acknowledging the work which has been done), and organizational-(in)justice-model (considers fairness and a just-climate-

communication with employees and their involvement when making important decisions)[54, 55].

Work related stress and its associated factors such as lack of social support or increased job demands have been related to various health outcomes such as dementia and cognitive impairment[54-56], whereas higher levels of job control have been correlated with a lower dementia risk, although the evidence has been inconsistent, especially regarding the specific cognitive domains affected and very few studies have examined the impact of work-related stress already at midlife on the risk of cognitive impairment later on[4, 57-60].

For instance, in the Whitehall II study, including 4146 British civil servants, a better score in verbal fluency, but not other cognitive domains, over twelve years were related to longer exposure to active jobs (a combination of high demands and high control)[14]. Furthermore, factors associated with work related stress were shown to be correlated with lower scores in episodic memory up to 20 years later among 3779 Americans from the Health and Retirement Study (mean age at baseline: 57.3 years)[61]. In addition, job strain and demands have been associated with subjective cognitive complaints and learning outcomes in other settings[62-64].

This emphasizes the importance of measuring multiple cognitive domains to identify the sensitive domains and implementing studies with a long duration of follow-up, ideally with a baseline examination already at midlife, to verify if the influence of midlife work-related stress on cognitive functioning is long-lasting, even when individuals become very old. Well-designed studies including large population-based samples with a long duration of follow-up are needed to examine this issue.

Risk scores

Based on findings on individual risk factors which were robustly associated with dementia, several integrated risk assessment tools were proposed to identify people who might profit from interventions aimed at reducing the burden of such risk factors.

Based on findings from the well-designed population-based CAIDE Study (the Cardiovascular Risk Factors, Aging and Dementia) which examined the impact of different risk factors at mid-life on risk of dementia at late-life, the main investigators proposed a risk score to estimate the risk of dementia at late-life[65]. This risk score was the first which

was validated in other settings as well and considers several well-established risk factors such as age, education, gender, blood pressure, physical activity, body mass index, hypercholesterinemia, and *APOEε4* to build a risk score of maximum 18 points with increasing the dementia risk as the CAIDE Dementia Risk Score raises.

This score was used to recruit participants for a multi-domain randomized controlled trial which aimed at reducing the risk of cognitive impairment by targeting several risk factors at the same time (the FINGER trial).[66] Since this trial was successful and its implementation in other populations seems plausible, the FINGER model is going to be adapted and tested in new World Wide FINGERS trials (http://wwfingers.com/)[67]. Therefore, it is crucial to rapidly disseminate knowledge about the different aspects of the CAIDE risk estimation tool.

Accordingly, few studies examined structural brain changes in relation to the CAIDE Risk Score. Of these, two prospective studies found associations with changes in grey and white matter volumes estimated using brain MRI[68, 69], whereas correlations with CSF total-tau and amyloid-β were found in another study which had a cross-sectional design[70]. In contrast, in a study using PIB-PET scans in 48 participants, no relationship between the CAIDE Dementia Risk Score and brain amyloid accumulation was observed[69]. Interestingly, no study has previously investigated the association of the CAIDE Risk Score with structural brain changes amongst the very old individuals (i.e. people aged 85 years or older).

This is important because several studies have shown that higher blood pressure or higher body mass index at midlife which increase the risk of dementia at late-life tend to decrease during the late-life period in those with cognitive impairment and dementia later in life[1, 3, 25]. Taken together, these facts highlight the necessity to examine midlife risk scores, such as the CAIDE Risk Score, in different contexts, especially amongst the oldest old.

Research question of the dissertation

The objective of this doctoral dissertation was to examine the impact of vitamin D, insulin, work related stress at midlife, and CAIDE Risk Score on cognitive impairment and structural brain changes in older adults.

Specific Aims

1. To investigate associations of vitamin D in plasma with cognitive functioning and structural brain changes assessed through brain MRI scans and measurement of cerebrospinal fluid biomarkers such as Aβ42, total tau and phospho-tau in a sample of patients from a memory clinic in Stockholm, Sweden *(study I)*

2. To study associations of cognitive performance assessed in several domains on two occasions seven years apart with insulin values measured in serum and insulin resistance in older Finnish adults (CAIDE study) *(study II)*

3. To investigate possible links between cognitive performance assessed in different domains at late-life and work-related stress during midlife in a large sample of Finnish older adults (CAIDE study) *(study III)*

4. To examine the association of CAIDE Risk Score and its individual components with different neuropathological findings at autopsy over ten years in a large sample of Finnish elderly aged ≥ 85 years (Vantaa 85+ study) *(study IV)*

Zusammenfassung

Es gibt momentan keine kausale Therapie für Demenz. Allerdings haben epidemiologische Studien gezeigt, dass in etwa die Hälfte der Demenzfälle die Erkrankung durch modifizierbare Risikofaktoren verursacht wird und ein gezielter Fokus auf diese Faktoren zur Prävention oder Verzögerung der Demenzen führen könnte. Das Ziel dieser Doktorarbeit ist es, den Zusammenhang von 25-hydroxyvitamin D (25(OH)D), Insulin/Insulinresistenz, arbeitsbedingter Stress im mittleren Alter und den „CAIDE Dementia Risk Score" mit kognitiven Fähigkeiten und strukturellen Veränderungen des Gehirns zu erforschen.

Studie I. Vitamin D Mangel ist sehr häufig bei älteren Personen. Es wird zunehmend angenommen, dass Vitamin D neben seiner Rolle in der Knochen Homöostase eine wichtige Rolle in anderen biologischen Systemen, wie z.B. dem Nervensystem, spielt. In Studie I wurden 75 ältere Patienten aus der Gedächtnissprechstunde des Karolinska Universitätskrankenhauses, Stockholm, Schweden, aufgenommen. Es wurden ausführliche Untersuchungen, einschließlich einer klinischen, einer neuropsychologischen und einer MRT Untersuchung durchgeführt. Zudem wurde 25(OH)D in Plasma und $A\beta_{1-42}$, t-tau und p-tau in CSF gemessen. Es wurden ordinale sowie lineare Regressionsmodelle benutzt um die Assoziation von 25(OH)D mit den verschiedenen Outcomes darzustellen. Hier zeigte sich eine Assoziation zwischen höheren 25(OH)D Spiegeln mit besserem kognitivem Profil, höheren $A\beta_{1-42}$ Spiegeln und größerem Gehirnvolumen, insbesondere Strukturen des medialen Temporallappens inklusiv Hippocampus. Die Korrektur für $A\beta_{1-42}$ verminderte den 25(OH)D Zusammenhang mit Kognition. Zusammenfassend zeigt diese Studie, dass Vitamin D mit Kognition, CSF $A\beta_{1-42}$ Spiegel und dem Gehirnvolumen korreliert ist. Der Einfluss von Vitamin D auf die Kognition könnte zum Teil durch dessen Wirkung auf CSF $A\beta_{1-42}$ erklärt werden.

Studie II. Es gibt nur wenige prospektiven Studien, die bisher den Zusammenhang zwischen Hyperinsulinämie und Insulinresistenz mit Kognition und Inzidenz der Demenz erforscht haben und die Ergebnisse sind nicht eindeutig. In Studie II wurden Serum Spiegel von Insulin und Glukose zu Studienbeginn bei 269 demenzfreien Testpersonen im Alter von 65-79 Jahren aus der Cardiovascular Risk Factors, Aging and Dementia (CAIDE)-Studie erfasst. Die Insulinresistenz wurde durch die Homeostasis Model Assessment (HOMA-IR) berechnet. Die Testpersonen wurden nach 7 Jahren erneut untersucht, um Neuerkrankungen

von Demenz zu identifizieren. Zudem wurden mehrere spezifische kognitive Fähigkeiten sowohl zu Beginn der Studie als beim Follow-Up 7 Jahre später erfasst. Lineare Regressionsmodelle wurden benutzt um die Assoziation mit der Verschlechterung der kognitiven Fähigkeiten zu erforschen. Hier wurden zuerst keine Assoziationen mit kognitiven Fähigkeiten gefunden. Nach Ausschluss von Individuen mit Neuerkrankung an Demenz zeigten sich signifikante Assoziationen von erhöhten Serum Insulinspiegeln und HOMA-IR mit schlechterer globaler Kognition und psychomotorischer Geschwindigkeit. Diese Studie deutet darauf hin, dass bei älteren demenzfreien Personen eine erhöhte Konzentration an Insulin sowie von Insulinresistenz unabhängigen Faktoren Vorboten von 7 Jahren späteren, kognitiven Fähigkeiten sein könnten. Die Ergebnisse dieser Studie zeigen zudem, dass der Insulinspiegel wahrscheinlich die Demenzentwicklung über einen begrenzten Zeitraum beeinflusst.

Studie III. Chronischer, arbeitsbedingter Stress ist mit verschiedenen somatischen und psychischen Erkrankungen wie Burnout und Depression assoziiert. Allerdings haben nur wenige prospektive Studien den Zusammenhang zwischen arbeitsbedingtem Stress und kognitiver Funktionen untersucht und die bisherigen Ergebnisse sind nicht aussagekräftig. In Studie III wurde der arbeitsbedingte Stress im mittleren Alter durch 2 Fragen bei 1273 Personen der Cardiovascular Risk Factors, Aging and Dementia (CAIDE)-Studie erfasst. Die kognitiven Funktionen wurden zu 2 Zeitpunkten (7 Jahre Abstand zwischen den 2 Untersuchungen) im späteren Leben untersucht, der durchschnittliche Zeitraum von Baseline bis zur letzten klinischen/neuropsychologischen Untersuchung betrug durchschnittlich 25 Jahre. Lineare Regressionsmodelle wurden benutzt. Hier ergab sich eine Assoziation zwischen dem höheren Niveau des arbeitsbedingten Stresses und schlechteren Funktionen in globaler Kognition und psychomotorischer Geschwindigkeit in der Nachuntersuchung. Die Ergebnisse dieser Studie weisen darauf hin, dass der Einfluss von arbeitsbedingtem Stress auf kognitive Funktionen langwierig ist und dass bestimmte kognitive Domänen für diesen Effekt anfälliger sein können.

Studie IV. Der CAIDE Dementia Risk Score ist der erste validierte Score, der auf der Grundlage von Risikofaktoren im mittleren Alter eine Abschätzung des Demenzrisikos 20 Jahre später ermöglicht. Dieser Score wurde benütz, um die Teilnehmer der FINGER Studie zu rekrutieren, eine erfolgreiche Studie zur Verhinderung des kognitiven Abbaus durch eine auf den Lebensstil abzielende, vielseitige Intervention. Allerdings ist die Langzeitassoziation

zwischen diesem Score und der Neuropathologie der Demenz in sehr hohem Alter noch nicht untersucht. In Studie IV wurde der Zusammenhang zwischen der CAIDE Dementia Risiko Score und post-mortem Neuropathologie der Demenz über 10 Jahre bei 149 demenzfreien Personen aus der Vantaa 85+ Studie (alle Teilnehmer ≥85 Jahre alt), untersucht. Logistische Regressionsmodelle wurden benutzt. Hier zeigte sich eine Assoziation zwischen dem schlechteren CAIDE Demenz Risiko Score und einem höheren Risiko von zerebralen Infarkten. Es wurden keine Korrelationen mit anderen Pathologien wie amyloid-β-Load, Neurofibrillary-Tangle, α-Synuklein oder Zerebral-Amyloid-Angiopathie gefunden. Die Ergebnisse dieser Studie suggerieren, dass zur Vorhersage des Demenzrisikos in höherem Alter der CAIDE Dementia Risk Score möglicherweise nicht so zuverlässig funktioniert, wie bei Personen, die mittleren Alters sind. Neu entwickelte Risk-Scores sind notwendig, um die Vorhersage von Demenz und deren Neuropathologie in sehr hohem Alter zu ermöglichen.

Abstract (English)

Currently, there are no causal treatments available for cognitive impairment and dementia. However, accumulating evidence support the likelihood of preventing or delaying its onset by addressing risk factors which are modifiable, as half of dementia cases has been estimated to be basically related to these factors. The main objective of this doctoral dissertation was to explore the impact of vitamin D, insulin/insulin resistance, midlife work-related stress, and the CAIDE Risk Score with cognitive performance and structural brain changes in older adults

Study I. Low levels of Vitamin D are prevalent among the elderly and accumulating evidence suggest that in addition to its traditional role in musculoskeletal system, vitamin D has as an important role in well-functioning of other systems including the nervous system as well. In Study I, we used data from 75 older patients who were examined in a memory clinic due to their memory problems. Participants underwent a comprehensive examination including a clinical assessment, cognitive assessment and brain imaging. Vitamin D was measured in plasma and total tau, phospho-tau and Aβ42 were measured in cerebrospinal fluid. Using logistic and linear regressions, raised vitamin D values were related to a better cognitive profile, higher concentration of Aβ42 in cerebrospinal fluid, and were positively correlated with brain volumetric measures of several regions such as the medial temporal lobe. Interestingly, including Aβ42 into the models attenuated the association of vitamin D with cognitive impairment, suggesting that Aβ42 in cerebrospinal fluid may partly explain the impact of vitamin D on cognitive functioning. Taken together, findings of study I imply that cognitive functioning and structural brain changes might be related to vitamin D.

Study II. Only a few prospective studies with mixed findings have examined the relationship between hyperinsulinemia/insulin resistance with dementia and cognitive performance. In Study II, insulin and glucose values were measured at baseline in serum of 269 persons from the CAIDE Study aged ≥ 65 years and homeostasis model assessment (HOMA-IR) formula was used to assess the insulin resistance. The follow-up examination was performed seven years later with similar survey methods. Incident dementia was diagnosed at follow-up and a comprehensive neuropsychological examination was performed at both baseline and follow-up to determine cognitive performance in several domains. Using regression analysis, no relationship with cognition was detected in the whole sample. In the sensitivity analysis in which the analyses were repeated after participants with incident dementia were excluded from the study (n = 19), increased insulin values and HOMA-IR were both related to lower scores on psychomotor speed

and Mini-Mental-State-Examination. This study implies that the independent impact of insulin/insulin resistance on cognitive impairment over seven years may be time-dependent, before the dementia related structural brain changes are too advanced.

Study III. Chronically elevated work-related stress has been shown to be related to several health conditions. However, prospective studies on its relationship with cognitive functioning in late-life are scare and results have been inconclusive. In Study III, work-related-stress was assessed during midlife in 1273 individuals of the CAIDE Study by taking into account questions related to work demands. Cognitive performance was evaluated using comprehensive neuropsychological examinations on two occasions at late-life which were seven years apart (mean overall follow-up from baseline: 25 years). Using linear regression models, lower scores in psychomotor speed and Mini-Mental-State-Examination at late-life were related to increased work-related-stress during midlife. This implies that especial cognitive domains may be more sensitive to the impact of work-related-stress and that this association may be observed over a long period of time.

Study IV. As a validated instrument based on several prevalent risk factors during midlife, the CAIDE Dementia Risk Score enables estimating the dementia risk at late-life. This score has been implemented to recruit participants of the FINGER trial which showed that a multi-domain intervention targeting several modifiable lifestyle factors at the same time will reduce the risk of cognitive decline. However, the relationship between the CAIDE Risk Score and structural brain changes amongst the very old people has not been examined previously. In Study IV, data from the Vantaa 85+ study was used to examine the relationship between neuropathological findings over ten years and CAIDE Risk Score in 149 subjects who were 85 years of age or older and without prevalent dementia at baseline. Using logistic regression analysis, a significant relationship between higher risk for cerebral infarcts and raised CAIDE Risk Scores was detected. No correlation was observed for other neuropathologies such as amyloid-β load or Neurofibrillary Tangles count. Findings of this study imply that the CAIDE Risk Score might not be a suitable tool to estimate the risk of dementia neuropathology in people who are very old (i.e. ≥85 years of age) and other risk scores might be needed to establish for this purpose amongst the oldest old.

Paper I

Vitamin D in relation to cognitive impairment, cerebrospinal fluid biomarkers, and brain volumes.

Hooshmand B, Lökk J, Solomon A, Mangialasche F, Miralbell J, Spulber G, Annerbo S, Andreasen N, Winblad B, Cedazo-Minguez A, Wahlund LO, Kivipelto M.

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Paper II

Serum Insulin and Cognitive Performance in Older Adults: A Longitudinal Study

Hooshmand B, Rusanen M, Ngandu T, Leiviskä J, Sindi S, von Arnim CAF, Falkai P, Soininen H, Tuomilehto J, Kivipelto M.

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Paper III

Midlife work-related stress is associated with late-life cognition

Sindi S, Kåreholt I, Solomon A, **Hooshmand B**, Soininen H, Kivipelto M.
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Paper IV

CAIDE Dementia Risk Score, Alzheimer and cerebrovascular pathology: a population-based autopsy study

Hooshmand B, Polvikoski T, Kivipelto M, Tanskanen M, Myllykangas L, Mäkelä M, Oinas M, Paetau A, Solomon A.
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Vitamin D in Relation to Cognitive Impairment, Cerebrospinal Fluid Biomarkers, and Brain Volumes

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Background. Low vitamin D status is associated with poorer cognitive function in older adults, but little is known about the potential impact on cerebrospinal fluid (CSF) biomarkers and brain volumes. The objective of this study was to examine the relations between plasma 25-hydroxyvitamin D (25(OH)D) and cognitive impairment, CSF biomarkers of Alzheimer's disease (AD), and structural brain tissue volumes.

Methods. A total of 75 patients (29 with subjective cognitive impairment, 28 with mild cognitive impairment, 18 with AD) referred to the Memory Clinic at Karolinska University Hospital, Huddinge, Sweden were recruited. Plasma 25(OH)D, CSF levels of amyloid β ($A\beta_{1–42}$), total-tau, and phosphorylated tau, and brain tissue volumes have been measured.

Results. After adjustment for several potential confounders, the odds ratios (95% confidence interval) for cognitive impairment were as follows: 0.969 (0.948–0.990) per increase of 1 nmol/L of 25(OH)D and 4.19 (1.30–13.52) for 24(OH)D values less than 50 nmol/L compared with values greater than or equal to 50 nmol/L. Adjusting for CSF $A\beta_{1–42}$ attenuated the 25(OH)D-cognition link. In a multiple linear regression analysis, higher 25(OH)D levels were related to higher concentrations of CSF $A\beta_{1–42}$ and greater brain volumes (eg, white matter, structures belonging to medial temporal lobe). The associations between 25(OH)D and tau variables were not significant.

Conclusions. This study suggests that vitamin D may be associated with cognitive status, CSF $A\beta_{1–42}$ levels, and brain tissue volumes.

Key Words: Vitamin D—Older adults—Cognition—CSF biomarkers—MRI.

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IT has been increasingly recognized that vitamin D, apart from its role in bone calcium homeostasis, plays an active role in other biologic targets such as the nervous system, the cardiovascular system, and the endocrine system (1–3). These nonclassical effects of vitamin D are not surprising because vitamin D receptors are present in many cell types including neurons (1,4). In addition, low levels of vitamin D have been linked to dementia/Alzheimer's disease (AD) and worse cognitive functioning in some but not all studies (5–9). Some biologically plausible mechanisms through which vitamin D may decrease the risk of dementia include the impact on the production of neurotrophic, antioxidative, and anti-inflammatory factors, protection against cardiovascular and cerebrovascular diseases, or the influence of vitamin D on amyloid phagocytosis and clearance (5).

Concentrations of the 42 amino-acid form of amyloid β ($A\beta_{1–42}$), total-tau (t-tau), and phosphorylated tau ($p\text{-}tau_{181}$) in cerebrospinal fluid (CSF) are considered as the core CSF biomarkers for AD (10), whereas magnetic resonance imaging (MRI)-based measures are regarded as valid biomarkers of disease state and progression (11,12). However, very few studies have so far investigated the associations of vitamin D levels in plasma with CSF biomarkers and volumetric measures of brain structures.

Vitamin D deficiency is a common condition in the elderly adults (5), and as a modifiable risk factor for dementia, it is a possible candidate for the preventive interventions. The

aim of this study was to examine the associations of plasma 25-hydroxyvitamin D (25(OH)D) levels with cognitive impairment, CSF biomarkers of AD, and brain volumes in memory clinic patients.

Methods

Study Participants

Participants included in this study were referred to the Memory Clinic at Karolinska University Hospital, Huddinge, Stockholm, Sweden, from primary care centers in the catchment area for investigation of suspected dementia. Participants were all living independently in the community (ie, they were not in need of formal care or aid from the community). A standard comprehensive protocol including clinical examination, brain imaging, electroencephalography, analyses of blood, urine, CSF, and a detailed neuropsychological evaluation was used for each individual. Diagnostic procedures have been described elsewhere (12,13). Briefly, dementia and AD were diagnosed according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, American Psychiatric Association, Fourth Edition) and NINCDS-ADRDA (the U.S. National Institute of Neurologic and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association) criteria (14). Mild cognitive impairment (MCI) patients: (i) were not demented; (ii) had self and/or informant report of cognitive decline and impairment on objective cognitive tasks; and (iii) had preserved basic activity of daily living and minimal impairment in complex instrumental functions (15). Persons categorized as subjective cognitive impairment (SCI) had subjective cognitive complaints without objective impairment on cognitive tasks, and they represented the control group for this study. Of the participants, 29 SCI, 28 MCI, and 18 AD participants had available plasma for 25(OH)D analyses. Of these, a total of 70 participants had available data on CSF biomarkers of AD. Participants with psychiatric disorders (ie, major depression, alcohol abuse) or other conditions (ie, brain tumors, normal pressure hydrocephalus) were not considered in this study. Also, 3 participants were excluded from the tau analyses because of extremely high tau (≥1200 pg/L) levels. Out of the total 75 participants, only 28 had good-quality MRI data (9 participants with SCI, 11 participants with MCI, and 8 participants with AD). The local ethics committee at Karolinska University Hospital approved the study.

Biochemical Analyses

Plasma and CSF samples were obtained during the diagnostic workup. Plasma levels of 25(OH)D were determined using the DiaSorin immunoassay method. CSF was obtained by lumbar puncture in propylene tubes, gently mixed to avoid gradient effects, and centrifuged at 2000*g* for 10 minutes. Aliquots were stored at −80°C until the biochemical analysis. Tau was determined using a sandwich enzyme-linked immunosorbent assay constructed to measure t-tau (both normal tau and hyperphosphorylated tau [p-tau$_{181}$]). P-tau$_{181}$ was determined using a sandwich enzyme-linked immunosorbent assay, with monoclonal antibody HT7 (recognizing all forms of tau) used as capturing antibody and biotinylated monoclonal antibody AT270 (specific to PThr181) used as a detection antibody. A$\beta_{1–42}$ was determined using a sandwich enzyme-linked immunosorbent assay specific for A$\beta_{1–42}$, as previously described (12).

MRI Data Acquisition and Image Processing

MRI scanning was performed with a 1.5T Siemens Magnetom Trio. T1-weighted images were collected using a three-dimensional magnetization prepared rapid acquisition gradient-echo sequence. The imaging parameters were as follows: repetition time = 11.4 ms, time to echo = 4.4 ms, flip angle = 10º, field of view = 25 cm, matrix = 512 × 144, slice thickness = 2.5 mm, 72 continuous slices, and in place voxel dimension = 0.89 × 0.89 mm. All images were checked visually for artifacts and other brain conditions (12).

Brain tissue fractions.—Brain tissue fraction volumes were calculated from the high-resolution T1-weighted images, using the cross-sectional version of the Structural Imaging Evaluation of Normalized Atrophy software, part of the FMRIB Software Library (FSL) (12). A specific value in cubic millimeter was obtained for total grey matter, white matter (WM), and CSF volumes. Total intracranial volume was calculated as the sum of grey matter, WM, and CSF values, and total brain volumes as the sum of grey matter and WM.

Regional brain volumes.—Segmentation and labeling of brain structures were performed by Freesurfer version 4.5 (http://surfer.nmr.mgh.harvard.edu/). This procedure automatically assigns a neuroanatomical label to each voxel in an MRI volume based on probabilistic information obtained from a manually labeled training set. Results were visually examined for anatomical accuracy and edits to ensure accurate surfaces and boundaries were completed where necessary. This produced measurements of several cortical and subcortical regions of interest, including the inferior temporal gyrus, entorhinal cortex, hippocampus, thalamus, and amygdala. All measures were corrected for head size by dividing each volume to total intracranial volume.

Statistical Analysis

Sociodemographic and clinical differences among diagnostic groups were compared using the χ^2 test for the proportions, analysis of variance for continuous variables with normal distributions, and nonparametric tests in case of non-normal distribution data. Results are presented as mean (standard deviation) or median (interquartile range) for continuous variables or number (%) for categorical variables.

Because of skewness, 25(OH)D values, CSF biomarkers, and brain volumes were log transformed for linear regression analyses. Additional categories for 25(OH)D status were defined according to commonly used clinical cut-offs for serum 25(OH)D: deficient (<25 nmol/L), insufficient (25–50 nmol/L), and sufficient (>50 nmol/L) (16). Because only 4 participants had vitamin D deficiency (3 participants with MCI and 1 participant with AD), re-categorization was done to create a group of 17 participants representing suboptimal vitamin D status (<50 nmol/L) and a group of 58 participants representing sufficient vitamin D status (≥50 nmol/L).

Because cognition is actually a continuum, we tried to avoid artificially sharp distinctions between the SCI, MCI, and AD categories. These three cognitive outcomes were thus considered to have an ordinal nature (irrespective of definition, MCI is a higher degree of cognitive impairment compared with SCI, and AD is a higher degree of cognitive impairment compared with MCI), and ordinal logistic regression analysis was used to examine the relation between 25(OH)D and level of cognitive functioning and results are presented as odds ratios with 95% confidence intervals (CIs). Multiple linear regression analyses were performed to investigate associations of 25(OH)D with CSF biomarkers or volumetric measures of brain structures. Analysis were adjusted for age and sex (model 1), and then additionally for other potential confounding or mediating factors, including *APOE*ε4 status (absence of ε4-allele vs presence of either 1 or 2 ε4-alleles), season of blood draw, and kidney function (model 2). Because of the small sample size, only age and sex were considered in the analysis of 25(OH)D in relation to brain structures. We analyzed the data using Stata software version 12 (StataCorp, College Station, TX).

Results

The mean (standard deviation) age of the 75 study participants was 61.6 (9.1) years, 54.7% were female, and the mean (standard deviation) 25(OH)D was 67.3 (26.5) nmol/L. Median 25(OH)D concentrations did not vary significantly by season in the total population (December–February: 62.0 nmol/L; March–May: 63.5 nmol/L; June–August: 73.0 nmol/L; September–November: 60.0 nmol/L).

The sociodemographic and clinical characteristics of the study participants were compared according to the clinical diagnoses (Table 1). As expected, AD participants were older, had lower levels of education, and had lower Mini-Mental State Examination scores compared with SCI participants. They also had decreased concentrations of $A\beta_{1–42}$ and increased t-tau and p-tau compared with both SCI and MCI patients. In addition, the SCI participants had higher 25(OH)D concentrations compared with both MCI and AD participants.

Association of 25(OH)D With Cognitive Impairment

The odds ratio for worse cognitive status for each increase of 1 nmol/L in plasma 25(OH)D was 0.980 (95% confidence

Table 1. Characteristics of the Study Population

Characteristics	SCI, *N* = 29	MCI, *N* = 28	AD, *N* = 18	*p*-Value
Age (y)*	57.7 (6.1)	61.0 (9.0)	68.6 (9.6)	SCI-AD < .001; MCI-AD = .008
Sex (% ♀)	51.7	46.4	72.2	NS
Education (y)*	13.7 (3.4)	12.5 (3.7)	10.5 (3.1)	SCI-AD = .032
25(OH)D (nmol/L)†	70 (60–96)	60.5 (41.3–74.3)	60.0 (47.3–71.8)	SCI-MCA = .014; SCI-AD = .027
Season tested (%)				
December	22.2	25.0	33.3	NS
March	22.2	35.7	33.3	
June	7.4	10.7	11.1	
September	48.1	28.6	22.2	
Mini-Mental State Examination†	29 (28–30)	28 (27.3–29)	22 (19.8–26.3)	SCI-MCI = .083; SCI-AD < .001; MCI-AD < .001
APOE (% ε4+)	42.3	65.4	66.7	NS
Elevated creatinine (%)	0	10.7	6.7	NS
$A\beta_{1–42}$ (ng/L)†	861.5 (757.5–957.0)	549.5 (448.5–627.5)	450 (357.5–555.3)	SCI-MCI < .001; SCI-AD < .001; MCI-AD = .034
T-tau (ng/L)†	275 (170–320)	270 (142–409.5)	610 (376–692.5)	SCI-AD < .001; MCI-AD = .005
P-tau (ng/L)†	49.0 (37.5–58.8)	46.0 (34.0–82.0)	91.5 (62.3–122.5)	SCI-AD < .001; MCI-AD = .017
GM (cm^3)*	408.1 (9.9)	396.8 (28.2)	386.6 (20.0)	NS
WM (cm^3)*	369.1 (7.8)	362.8 (17.3)	350.8 (20.3)	SCI-AD = .082
CSF (cm^3)†	221.0 (214.2–229.4)	242.0 (212.3–259.8)	265.4 (228–291.5)	SCI-AD = .034
TBV (cm^3)†	779 (770.6–785.5)	758 (740.2–787.7)	734.6 (708.5–772.0)	SCI-AD = .034

Notes: AD = Alzheimer's disease; CI = confidence interval; CSF = cerebrospinal fluid; GM = grey matter; MCI = mild cognitive impairment; NS = not significant; 25(OH)D = 25-hydroxyvitamin D; SCI = subjective cognitive impairment; TBV = total brain volume; WM = white matter.

*Mean (*SD*).

†Median (IQR).

interval 0.964–0.997). This association remained significant after adjusting for age and sex (model 1). Furthermore, adjusting for *APOE*ε4 status, season, and kidney function did not influence the results (Table 2). In this model, the odds ratio (95% confidence interval) for worse cognitive status was 4.19 (1.30–13.52) for individuals with 25(OH)D concentration less than 50 nmol/L compared with those with sufficient levels.

We also conducted additional analyses controlling for CSF biomarkers of AD. In the fully adjusted model, the relation between 25(OH)D and cognitive impairment was attenuated by adjusting for CSF $A\beta_{1–42}$: odds ratio (95% confidence interval) became: 0.976 (0.950–1.004) for 25(OH)D as a continuous variable and 2.13 (0.48–9.47) for those with suboptimal 25(OH)D levels compared with participants with sufficient levels. Adding t-tau or p-tau to the models did not change the association between 25(OH)D and cognitive impairment (Table 2).

25(OH)D in Relation to CSF Biomarkers

After controlling for all study covariates, increased 25(OH)D concentrations as a continuous variable were related to higher concentrations of CSF $A\beta_{1–42}$ (Table 3, model 2). This association was borderline significant when individuals with suboptimal 25(OH)D values were compared with sufficient values: β(*SE*) was −0.13 (0.07), $p = .096$. No significant associations between 25(OH)D and t-tau or p-tau were detected.

Table 2. Association of Plasma 25-Hydroxyvitamin D With a Higher Degree of Cognitive Impairment

	25-Hydroxyvitamin D
Model 1	0.972 (0.953–0.991)
Model 2	0.969 (0.948–0.990)
Model 2 and $A\beta_{1–42}$	0.976 (0.950–1.004)
Model 2 and t-tau	0.961 (0.936–0.987)
Model 2 and p-tau	0.965 (0.939–0.991)

Notes: $A\beta_{1–42}$ = amyloid β; p-tau = phosphorylated tau; t-tau = total-tau. Results are odds ratios (95% confidence intervals) from ordinal logistic regressions with cognitive status as dependent variable (subjective cognitive impairment, mild cognitive impairment, and Alzheimer's disease as categories ordered according to the severity of cognitive impairment); 25-Hydroxyvitamin D was analyzed as a continuous variable. Model 1: adjusted for age and sex. Model 2: additionally adjusted for *APOE*ε4-allele, season, and kidney function.

25(OH)D and Structural Brain Tissue Volumes

Increased 25(OH)D was related to lower CSF and greater WM and total brain volumes after taking age and sex into account (Table 4). Furthermore, 25(OH)D was associated with increased volumetric measures of the amygdala (β[*SE*]: 0.071[0.03], $p = .022$), thalamus (0.093[0.04], $p = .032$), and anterior cingulate gyrus (0.025[0.01], $p = .019$) and had a borderline significant association with hippocampus (0.391[0.217], $p = .085$) and inferior temporal gyrus (0.283[0.156], $p = .086$). No significant association between 25(OH)D and other brain volumetric measures were observed (data not shown).

Discussion

This study investigated plasma 25(OH)D in relation to cognitive impairment, CSF biomarkers of AD, and structural brain tissue volumes. Our results indicated that elevated plasma 25(OH)D may be associated with better cognitive status, irrespective of several potential confounders. In addition, higher 25(OH)D concentrations were associated with increased concentrations of CSF $A\beta_{1–42}$, greater WM volume, and greater volumetric measures of several brain structures including structures of medial temporal lobe such as amygdala and hippocampus.

These results are consistent with the findings from previous studies that reported a relationship between vitamin D and better cognitive performance (4–6,9,16–19) or decreased risk of dementia/AD (6–8,20). In contrast, no associations between 25(OH)D and cognition were found in the Tromso study, NHANES III survey, or the Osteoporotic Fractures in Men (MrOS) study (5,6). Possible explanations for the discrepancies are heterogeneity of study populations (ie, age, gender, target population), differences in 25(OH)D status, different inclusion of potential confounders, and variability in cognitive measurement methods (5,6).

The association between 25(OH)D and structural brain tissue volumes has been less investigated. One cross-sectional study reported a link between low 25(OH)D and MRI indicators of cerebrovascular diseases. However, in contrast with our findings, no significant association between 25(OH)D and volumetric measures of medial temporal lobe structures of the hippocampus and amygdala was observed (7).

Table 3. Association of Plasma 25-Hydroxyvitamin D Concentrations With Cerebrospinal Fluid Biomarkers

	Model 1		Model 2	
25-Hydroxyvitamin D	β (*SE*)	*p*-Value	β (*SE*)	*p*-Value
$A\beta_{1–42}$ ($N = 68$)	0.23 (0.12)	.051	0.26 (0.12)	.034
T-tau ($N = 70$)	0.27 (0.34)	.423	0.45 (0.36)	.213
P-tau ($N = 61$)	0.09 (0.27)	.730	0.22 (0.29)	.446

Notes: $A\beta_{1–42}$ = amyloid β; p-tau = phosphorylated tau; t-tau = total-tau. β represents the coefficient for 25-hydroxyvitamin D analyzed as a continuous variable, and *SE* represents the standard error. Model 1: adjusted for age and sex. Model 2: additionally adjusted for *APOE*ε4-allele, season, and kidney function.

Table 4. Association of Plasma 25-Hydroxyvitamin D Concentrations With Brain Tissue Volumes (n = 28)

25-Hydroxyvitamin D	β (*SE*)	*p*-Value
White matter	0.126 (0.04)	.009
Grey matter	0.059 (0.08)	.447
Total brain volume	0.437 (0.19)	.031
Cerebrospinal fluid volume	–0.437 (0.19)	.031

Notes: β represents the age- and sex-adjusted coefficient for 25-hydroxyvitamin D analyzed as a continuous variable, and *SE* represents the standard error.

Although the exact mechanisms behind the observed associations remain to be determined, certain hypotheses can be considered. Vitamin D may contribute to neuroprotection through its anti-ischemic, anti-inflammatory, and antioxidative properties (2,18). Experimental studies have suggested that 25(OH)D is related to the inhibition of nitric oxide synthase, upregulation of enzymes in glutathione and neurotrophin synthesis, regulation of neuronal calcium, protection of neuronal integrity, and the metabolism of numerous neurotransmitters in the central nervous system; including acetylcholine, dopamine, serotonin, and γ-aminobutyric acid (2,7). Furthermore, recent studies have suggested that vitamin D can stimulate amyloid phagocytosis and clearance (21), and the overexpression of vitamin D receptor or vitamin D treatment suppress amyloid precursor protein transcription (1).

The progressive deposition of Aβ peptides in the brain and the formation of neurofibrillary tangles are considered the neuropathologic hallmarks of AD (10). The CSF can reflect biochemical changes that occur in the brain because it is in direct contact with the extracellular space of the brain. Therefore, AD is characterized by increased CSF levels of t-tau (reflecting intensity of neuronal and axonal degeneration), elevated CSF p-tau (reflecting tau phosphorylation and tangle pathology), and decreased CSF $A\beta_{1–42}$ (reflecting $A\beta_{1–42}$ aggregation and amyloid plaque load in the brain parenchyma and hence, reduced availability of Aβ to diffuse into CSF) (10). Our results indicate that elevated plasma 25(OH)D is associated with increased concentration of CSF $A\beta_{1–42}$. In addition, the association between 25(OH)D and cognitive impairment was attenuated when adjusting for CSF $A\beta_{1–42}$, suggesting that the effect of vitamin D on cognition could be partly explained by its impact on $A\beta_{1–42}$. Effects of vitamin D on Aβ clearance have been reported in recent preclinical studies; positive effects on Aβ degradation by macrophages (22) and on Aβ transport across the blood–brain barrier (23) have been demonstrated.

Interestingly, one recent study did not find any significant associations between dietary intake of vitamin D and plasma Aβ levels. The authors concluded that the potential association of vitamin D with AD or cognition may involve pathways other than Aβ (24). Possible explanations for this difference are the different methods, including population characteristics, difficulties in interpreting Aβ levels in plasma compared with CSF, and vitamin D measurements (dietary intake rather than plasma concentration). Sun exposure is a larger source of vitamin D than diet, and unlike dietary intake assessment, plasma measurement is an objective measure of vitamin D, which is independent of the capacity to estimate and remember intake over a period of time. Furthermore, plasma level assessment takes into account individual variations in metabolism, giving a reliable evaluation of the micronutrient bioavailability. Nevertheless, the impact of vitamin D on CSF Aβ or CSF t-tau/p-tau has previously not been investigated, and our findings need to be confirmed in larger studies.

The main strength of this study is the accurate and comprehensive clinical assessment of the participants enrolled, which included neuroimaging and CSF analyses of biomarkers incorporated in the new diagnostic criteria for AD (25). Although an association between vitamin D and cognition has been previously reported, to the best of our knowledge, no data are available about the association between 25(OH)D and CSF biomarkers of AD. Furthermore, different levels of cognitive impairment were considered, from subjective cognitive problems to fully developed dementia syndrome.

Although our findings are of potential clinical value, some limitations should be considered. This is a cross-sectional study including patients evaluated in a memory clinic, which are not representative of community-dwelling older adults in general. The small sample size limited statistical power. Furthermore, the possibility of reverse causation cannot be excluded because cognitively impaired individuals may eat poorly or may have reduced sunlight exposure or less outdoor activity, which may lead to reduced vitamin D status (26). In addition, we could not assess the role of vitamin D determinants such as physical activity, which is considered a confounder for the association between vitamin D and cognitive outcomes (5). Although adjusting for several relevant covariates that could modify our findings did not alter the results, the possibility of residual confounding cannot be excluded. The possibility of including cognitively intact subjects was limited; however, the CSF values of the SCI participants were comparable with those identified as healthy controls in a large multicenter study of older adults (27). Finally, the proportion of poor-quality raw MRI images in the sample was relatively high. However, our restrictive image selection reduced the risk of introducing bias due to acquisition artifacts.

Taken together, our results support the hypothesis that lower 25(OH)D levels could be associated with cognitive impairment; at least partially via its association with CSF $A\beta_{1–42}$, total cerebral WM volume, and several other brain structures. Due to the potential limitations, we cautiously interpret the associations revealed herein. However, our results emphasize the need for further longitudinal studies with larger samples to confirm and refine the potential beneficial role of vitamin D in individuals who are at increased risk of dementia. The deficiency of vitamin D is

a common condition in the elderly adults because of limited sunlight exposure, decreased 7-dehydrocholesterol in the skin, inadequate dietary vitamin D intake, and limited physical activity. Hypovitaminosis D is easy to treat, however, few randomized controlled trials (RCTs) have so far investigated the usefulness of vitamin D in preventing cognitive impairment and dementia with mixed results (5,6). Limitation of statistical power, study duration, and choice of target population make such studies difficult to interpret. Supplementation might be most effective in preventing cognitive impairment during a critical time window, and larger and better planned RCTs are necessary to formulate efficient guidelines for prevention (dose, treatment start and duration, target population). Recently, several large-scale RCTs of vitamin D supplementation and cognitive function in older adults have been initiated (eg, the VITAL-Cog study, NCT01669915 (28); the DIET-D study, NCT01708005 (29); and the MERE study, NCT01315704) (30). Furthermore, a phase-III RCT investigates the impact of cholecalciferol together with memantine in participants with moderate AD and hypovitaminosis D (the AD-IDEA study, NCT01409694) (31). However, in light of the scarce evidence on the role of vitamin D in AD, and the several failures of phase-III RCTs evaluating different compounds as disease-modifying treatment (32), a more refined knowledge on the biologic effects of vitamin D in AD-related pathology is needed.

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Conflict of Interest

None declared.

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CLINICAL RESEARCH STUDY

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Serum Insulin and Cognitive Performance in Older Adults: A Longitudinal Study

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ABSTRACT

PURPOSE: The aim of this study was to examine the association of serum glucose, insulin, and insulin resistance with cognitive functioning 7 years later in a longitudinal population-based study of Finnish older adults.

METHODS: Serum glucose and insulin were measured at baseline in 269 dementia-free individuals aged 65-79 years, from the Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) study. Insulin resistance was estimated with the homeostasis model assessment (HOMA-IR). Participants were reexamined 7 years later, and global cognition, episodic memory, executive functioning, verbal expression, and psychomotor speed were assessed, both at baseline and at follow-up. Multiple linear regression was used to investigate the associations with cognitive performance at follow-up, after adjusting for several potential confounders, including common vascular risk factors.

RESULTS: In the multivariable-adjusted linear regression models, no associations of insulin resistance with cognitive functioning were observed. After excluding 19 incident dementia cases, higher baseline HOMA-IR values were related to worse performance in global cognition (β [standard error (SE)] -.050 [0.02]; $P = .043$) and psychomotor speed (β [SE] -.064 [.03]; $P = [.043]$) 7 years later. Raised serum insulin levels were associated with lower scores on global cognition (β [SE] -.054 [.03]; $P = .045$) and tended to relate to poorer performance in psychomotor speed (β [SE] -.061 [.03]; $P = .070$).

CONCLUSIONS: Serum insulin and insulin resistance may be independent predictors of cognitive performance 7 years later in elderly individuals without dementia. Randomized controlled trials are needed to determine this issue.

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INTRODUCTION

Insulin resistance and its related disorders, such as physical inactivity, overweight, and obesity, are common conditions in older adults and are associated with a variety of other disorders, including cardiovascular, cerebrovascular, and peripheral vascular disease.[1-4] Experimental studies have suggested that systemic insulin resistance or high circulating levels of insulin is accompanied by brain insulin resistance.[4,5] These metabolic derangements may directly or indirectly affect brain structure and function through several mechanisms and promote neurodegenerative disorders.[1,5-8] Population-based longitudinal studies on the association of hyperinsulinemia, insulin resistance, and blood glucose with cognitive performance or incident dementia are few and have yielded inconsistent results.[6,9-16] Therefore, it is important to determine if these metabolic parameters have an impact on cognitive functioning in longitudinal settings, particularly because they might be considered as modifiable factors, making them possible candidates for preventive interventions. The aim of the current study was to investigate serum insulin, insulin resistance, and serum glucose in relation to cognitive performance 7 years later in a subsample of the population-based Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) Study.

CLINICAL SIGNIFICANCE

- In dementia-free older adults, raised serum insulin and insulin resistance are related to worse cognitive functioning 7 years later.
- There may be a limited window for the effects of insulin on the pathology of dementia. Once dementia pathology becomes more advanced, insulin resistance may not be an important determinant of disease progression.

METHODS

The design and characteristics of the CAIDE study have been described in detail previously.[17,18] Briefly, CAIDE participants were examined at midlife within the framework of the North Karelia project and the FINMONICA study in 1972, 1977, 1982 or 1987. A random sample of 2000 individuals still alive, aged 65-79 years and living in the areas of Kuopio and Joensuu in Finland at the end of 1997 were invited to the first reexamination in 1998. Altogether, 1449 individuals participated and a total of 1409 participants completed the cognitive assessments. The second reexamination of the same cohort was conducted in 2005−2008, in which 909 individuals agreed to participate. A total of 852 completed the cognitive assessments. Both reexaminations consisted of a self-administered questionnaire on sociodemographic characteristics, health-related behaviors, and medical history, including cerebrovascular and cardiovascular conditions. Nurses trained to administer the survey checked the questionnaires to ensure that they were fully completed. Height and weight were measured, and body mass index was calculated as weight (in kilograms) divided by height squared (in meters). Blood pressure was measured from the right arm twice after participants had been seated for 5 minutes, and the mean of the 2 measurements was calculated. History of diabetes was defined by self-report or by the use of glucose lowering drugs.

This study included a subsample of 269 dementia-free people from the cohort participating in the first reexamination of the CAIDE study in 1998. Nine individuals with prevalent dementia were excluded. Participants were selected based on the availability of stored serum samples from 1998 for insulin measurements. The mean (standard deviation [SD]) duration of follow-up of the CAIDE subsample participants from the 1998 reexamination (baseline for this study) was 7.4 [0.3] years. There were no clinically significant differences in main characteristics between the CAIDE subsample and the rest of the dementia-free CAIDE cohort at the 1998 survey examination.

The CAIDE study was approved by the local ethics committee (University of Kuopio and Kuopio University Hospital, Kuopio, Finland), and written informed consent was obtained from all participants.

Measurement of Cognitive Functions

A comprehensive battery of neuropsychological tests to assess several cognitive domains was administered to CAIDE participants. Only identical tests used at both reexaminations were considered for the present study: 1) the Mini-Mental State Examination (MMSE),[19] a measure of global cognition; 2) immediate word recall test (a 1-word list that measures episodic memory); 3) the Stroop test,[20] where the time difference between the color word interference and naming tasks is used as a measure of executive functioning with higher scores representing worse performance; 4) category fluency test as a measure of verbal expression; and 5) the bimanual Purdue Pegboard Test and the Letter Digit Substitution Test, with the mean of their normalized scores used as a measure of psychomotor speed.[17,21]

Biochemical Analyses

Similar to the other settings,[5,22] venous blood samples were taken at the 1998 reexamination after a fasting period of at least 5 hours and routine analyses (including serum glucose assessment, conducted using enzymatic methods). Specimens were stored at or below −20° C for 10 years, until the determination of serum insulin using chemiluminescent microparticle immunoassay at the National Institute for Health and Welfare, Helsinki, Finland. The homeostasis model assessment index (HOMA), which was calculated by multiplying serum glucose by serum insulin divided by 22.5,[23] was

used as a measure for insulin resistance. High sensitivity C-reactive protein (hsCRP) was measured by immunoturbidimetric method. Blood leukocyte samples were analyzed to determine the APOE genotype in 1998. A standard phenol-chloroform technique was used to extract DNA; APOE genotypes were analyzed by polymerase chain reaction and HhaI digestion.[24] Participants were classified as positive for the apolipoprotein epsilon 4 (APOE ε4) allele if they had one or two ε4 alleles.

Statistical Analysis

Because of skewness of their distributions, serum insulin, HOMA, and all cognitive test scores were log transformed. Continuous variables are presented as mean (SD) or median (interquartile range [IQR]) when appropriate, and categorical variables are shown as number (percentage).

Multiple linear regression analyses were used to estimate β (standard error [SE]) for the association of serum glucose, serum insulin, and HOMA (as continuous variables) at the first reexamination in 1998 (baseline for this study) with cognitive test scores at the second reexamination 7 years later. Models were adjusted for potential confounding or mediating factors known to influence cognition, including age, sex, education level, duration of follow-up, and the corresponding cognitive measures at baseline (model 1), and then additionally for mean baseline systolic blood pressure, mean baseline diastolic blood pressure, body mass index, history of stroke, history of diabetes, smoking, and APOE ε4 allele status (model 2). As diabetes/insulin resistance are pro-inflammatory states, we additionally controlled for hsCRP.[25] All variables were entered into the models as continuous except sex, history of stroke, history of diabetes, smoking, and APOE ε4 allele status, which were dichotomized. In a sensitivity analysis, all analyses were repeated after excluding 19 people with incident dementia at follow-up (diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition criteria).[26] Interaction terms were entered in the models in order to investigate possible interactions of serum insulin and HOMA with APOE ε4 allele status in relation to the cognitive outcomes. We analyzed the data using Stata software version 15 (StataCorp, College Station, TX), and the level of significance was $< .05$ in all analyses.

RESULTS

The baseline sociodemographic and clinical characteristics of the study population are presented in Table 1. The mean (SD) age of the study population at baseline was 70.6 (3.6) years, and 165 (61.3%) participants were female. Serum insulin was negatively correlated with female gender (Spearman rho -0.118; $P = .05$) and education level (-0.120; $P = .05$) but was positively correlated with serum glucose (0.341; $P < .001$), history of diabetes (0.196; $P < .001$), body mass index (0.442; $P < .001$), and hsCRP (0.162; $P = .008$), and showed a nonsignificant trend with history of stroke (0.109; $P = .07$).

Associations with Cognitive Functioning Seven Years Later

No significant relationships between serum glucose or insulin levels or insulin resistance at baseline and any of the cognitive domains were detected in the entire study sample 7 years later (Table 2). Analyses were repeated after excluding 19 individuals with incident dementia at follow-up. These people were older at baseline (72.7 [4.3] vs 70.5 [3.5] years; $P = .006$), had lower body mass index (25.3 (3.6) vs 28.0 (4.1) kg/m^2; $P = .005$), lower systolic (138.9 (19.6) vs 153.5 (22.0) mmHg; $P = .006$) and diastolic (75.5 (9.9) vs 82.9 (10.4) mmHg; $P = .003$) blood pressures, and tended to have higher frequency of APOE ε4 allele (52.6% vs 32.7%; $P = .077$) compared with people without dementia. In addition, they had lower scores on episodic memory (4.8 (1.4) vs 5.6 (1.4); $P = .012$), executive functioning (49.8 (16.2) vs 37.2 (17.2); $P = .003$), the Letter Digit Substitution Test (16.7 (8.2) vs 20.6 (6.3); $P = .014$), and the Purdue Pegboard Test (5.3 (2.6) vs 7.6 (2.0); $P < .001$). There were no differences in serum insulin, glucose, or HOMA between participants with incident dementia and those without. Furthermore, no associations between serum insulin, glucose, or HOMA at baseline and risk of incident dementia 7 years later were detected (results not shown).

Table 1 Characteristics of the Study Population at Baseline*

Characteristic	Value	No.
Age (year)	70.6 (3.6)	269
Duration of follow-up (year)	7.4 (0.3)	269
Sex, women, n (%)	165 (61.3%)	269
Education (year)	9.2 (3.4)	269
BMI (kg/m^2)	27.8 (4.1)	269
Systolic blood pressure (mmHg)	152.4 (22.1)	269
Diastolic blood pressure (mmHg)	82.3 (10.5)	269
APOE ε4 allele, n (%)	91 (33.8%)	269
History of stroke, n (%)	18 (6.7%)	269
History of diabetes, n (%)	25 (9.3%)	269
Ever smoked, n (%)	98 (36.4%)	269
Serum insulin, μU/mL	5.7 (3.9-7.9)†	269
Serum glucose, mmol/L	5.1 (0.7)	262
HOMA	1.2 (0.8 -1.9)†	262
MMSE	27 (25-28)†	269
Immediate word recall	6 (5-6)†	269
Executive functioning	36 (27-46)†	255
Verbal fluency	20 (17-24)†	269
Purdue Pegboard Test (bimanual)	8 (7-9)†	265
Letter Digit Substitution Test	20 (16-24)†	260

APOE ε4 allele = apolipoprotein epsilon 4 allele; BMI = body mass index; HOMA-IR = homeostasis model assessment of insulin resistance; MMSE: Mini-Mental State Exam.

*Values are mean (standard deviation) or n (%), unless otherwise stated.

†Median (interquartile range).

Table 2 β (Standard Error) for the Associations of Serum Glucose, Insulin, and Homeostasis Model Assessment (HOMA) with Cognitive Functioning Seven Years Later*

	Global cognition (n = 269)	Episodic memory (n = 269)	Executive function (n = 261)	Verbal expression (n = 267)	Psychomotor speed (n = 241)
Glucose (per mmol/L)†					
Model 1	−.019 (.02); P = .424	−.029 (.03); P = .304	−.028 (.03); P = .334	−.035 (.02); P = .094	−.046 (.03); P = .130
Model 2	−.015 (.02); P = .549	−.019 (.03); P = .529	−.039 (.03); P = .217	−.026 (.02); P = .242	−.045 (.03); P = .175
Insulin (per log)					
Model 1	−.024 (.03); P = .361	.012 (.03); P = .699	.016 (.03); P = .650	.011 (.02); P = .660	−.039 (.03); P = .267
Model 2	−.034 (.03); P = .235	.014 (.04); P = .686	−.011 (.04); P = .764	.016 (.03); P = .556	−.037 (.04); P = .327
HOMA (per log)					
Model 1	−.028 (.02); P = .263	−.004 (.03); P = .900	.010 (.03); P = .742	−.004 (.02); P = .848	−.045 (.03); P = .164
Model 2	−.032 (.03); P = .227	.002 (.03); P = .940	−.017 (.04); P = .621	.002 (.02); P = .945	−.045 (.04); P = .209

*Model 1 adjusted for age, sex, education, duration of follow-up, and baseline related cognitive measure. Model 2 additionally adjusted for systolic blood pressure, diastolic blood pressure, smoking, body mass index, history of diabetes, history of stroke, apolipoprotein epsilon 4 allele, and C-reactive protein. Final cognitive scores regressed onto baseline measurements.

†Seven participants did not have glucose measurements at baseline.

After adjusting for age, sex, education, duration of follow-up, and baseline cognitive measures (model 1), raised serum insulin concentrations and insulin resistance were associated with worse performance in global cognition (β [SE] was -0.054 [0.02]; P = .031 for serum insulin and -0.051 [0.02]; P = .024 for HOMA) and psychomotor speed (β [SE] was -0.071 [0.03]; P = .020 for serum insulin and -0.070 [0.03]; P = .013 for HOMA) 7 years later. Further controlling for blood pressure, body mass index, APOE ε4, history of diabetes, stroke, smoking, and hsCRP (model 2) slightly attenuated the results (Table 3). The pattern of association remained virtually unchanged even after excluding participants with diabetes at baseline (results not shown).

No significant relationship between serum glucose and any of the cognitive domains was detected. No evidence of interaction was found between serum insulin and APOE in relation to cognitive performance.

DISCUSSION

In this longitudinal population-based study of older adults, elevated serum insulin concentrations and increase in HOMA insulin resistance estimate values were associated with worse cognitive performance 7 years later, irrespective of several potential confounders, including common socio-demographic and vascular risk factors. This association was, however, observed only after excluding individuals with incident dementia at follow-up from the analysis. This may suggest that the deleterious effects of hyperinsulinemia and insulin resistance are no longer manifest when the disease processes related to dementia become too advanced. The associations remained after adjusting for hsCRP and after excluding people with prevalent diabetes, excluding the possibility of confounding by inflammation or by diabetes or by drug treatment of diabetes.

Similar to our findings, increased values of insulin and insulin resistance in the Rotterdam study (mean age at baseline = 71.8 years) were associated with higher risk of Alzheimer's disease, but this relationship was observed only within the first 3 years of follow-up (maximum duration of follow-up = 9.7 years) This association was no longer evident with a longer follow-up period (i.e., among those who were followed between 3 and 9.7 years).The authors concluded that disturbances in insulin metabolism may only increase the risk of Alzheimer's disease for a short period of time by advancing the onset in those already on the verge of developing Alzheimer's disease.[6] Furthermore, higher risk of Alzheimer's disease and decline in memory-related cognitive scores were associated with hyperinsulinemia in a Washington Heights Northern Manhattan Study (follow-up=5.4 years).[10] In a Honolulu-Asia Aging Study, both low and high levels of insulin were related to increased risk of incident dementia in a sample of Japanese-American elderly men. However, the impact on cognition were not investigated.[9] Impaired fasting glucose and elevated HOMA index were associated with higher probability of cognitive dysfunction

Table 3 β (Standard Error) for the Associations of Serum Glucose, Insulin, and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) with Cognitive Functioning Seven Years Later, Excluding Participants with Incident Dementia*

	Global cognition (n = 250)	Episodic memory (n = 250)	Executive function (n = 244)	Verbal expression (n = 248)	Psychomotor speed (n = 225)
Glucose (per mmol/L)†					
Model 1	−.024 (.02); P = .255	−.027 (.03); P = .327	−.018 (.03); P = .507	−.035 (.02); P = .069	−.042 (.03); P = .108
Model 2	−.024 (.02); P = .293	−.019 (.03); P = .526	−.030 (.03); P = .316	−.029 (.02); P = .144	−.038 (.03); P = .185
Insulin (per log)					
Model 1	−.054 (.02); P = .031	−.007 (.03); P = .832	−.005 (.03); P = .885	−.010 (.02); P = .641	−.071 (.03); P = .020
Model 2	−.054 (.03); P = .045	.006 (.04); P = .870	−.031 (.04); P = .377	.002 (.02); P = .936	−.061 (.03); P = .070
HOMA (per log)					
Model 1	−.051 (.02); P = .024	−.020 (.03); P = .505	−.006 (.03); P = .828	−.021 (.02); P = .315	−.070 (.03); P = .013
Model 2	−.050 (.02); P = .043	−.005 (.03); P = .873	−.030 (.03); P = .258	−.010 (.02); P = .646	−.064 (.03); P = .043

*Model 1 adjusted for age, sex, education, duration of follow-up, and baseline related cognitive measure. Model 2 additionally adjusted for systolic blood pressure, diastolic blood pressure, smoking, body mass index, history of diabetes, history of stroke, apolipoprotein epsilon 4 allele, and C-reactive protein. Final cognitive scores regressed onto baseline measurements.
†Seven participants did not have glucose measurements at baseline.

assessed with 2 short cognitive screening tools in the Prospective Epidemiological Risk Factor Study, a longitudinal study of elderly women in Denmark.[11] In addition, higher serum insulin and HOMA were related to greater decline in verbal fluency during a period of 11 years in the Finnish nationwide Health Examination Survey[12] and a greater decline during a period of 6 years in delayed word recall and first letter word fluency tests in the Atherosclerosis Risk in Communities (ARIC) study,[14] both including middle aged individuals at baseline. In contrast, serum insulin or insulin sensitivity were not related to cognitive scores 20 years later or the risk of dementia in the Uppsala Longitudinal Study of Adult Men, although a low early insulin response to oral glucose challenge was associated with a higher risk of Alzheimer disease.[13,15]

Possible explanations for the discrepancies across studies are heterogeneities in study designs, populations (i.e., age, sex, target population), differences in insulin and glucose status, different inclusion of potential confounders, and variability in cognitive assessment methods. All of the studies, including ours, have been post-hoc analyses of studies that had data to be analyzed to explore the hypothesis of the role of high insulin exposure or insulin resistance in peripheral tissues. Thus, the spectrum of findings is not surprising. We are still awaiting a prospective study with the primary aim of testing this theory with an a priori design.

Although the exact mechanisms behind the observed associations have not yet been determined, certain hypotheses can be considered. Insulin resistance has been associated with endothelial dysfunction, impaired nitric oxide activity, atherosclerosis, and subsequent increase in the risk of various cardiovascular and cerebrovascular events, which may increase the risk of cognitive impairment and dementia.[4,25,27,28] Insulin receptors have been found in several brain regions, including the key regions associated with cognitive functioning.[4,7,8] Prolonged peripheral hyperinsulinemia decreases insulin transport across the blood brain barrier, leading to reduced brain insulin levels, which in turn results in reduced insulin signaling in the brain and impairment in neuronal function and synaptogenesis.[4,7,29,30] Furthermore, a close relationship between insulin and amyloid-β has been suggested in recent studies,[5,7,28,31] and insulin resistance may potentiate amyloid-β generation or its neurotoxicity or reduce its clearance.[5,7,28,31-33] Insulin may also be involved in tau phosphorylation and formation of neurofibrillary tangles through various mechanisms.[28,34-36]

The main strengths of the present study are the population-based design; follow-up period of at least 7 years; use of multiple tests to measure cognitive functioning; available data for a large number of potential confounders; and evaluation of blood glucose, insulin, and HOMA simultaneously in relation to cognitive performance. The 269 people are from a well-characterized longitudinal study (CAIDE) specifically designed to investigate risk factors for cognitive impairment and dementia. The long follow-up period, the comprehensive evaluation and diagnostic protocol at each examination, recruitment of dementia-free individuals at

baseline, and adjusting of the analyses for baseline cognitive measures make our findings less prone to the influence of reverse causality.

The main limitations of our study include the relatively small sample size and the post-hoc design, and availability of glucose and insulin measurements at only 1 timepoint, which may have underestimated their associations with cognition due to regression dilution.[37] Selective survival may also have contributed to underestimation of the associations, because insulin resistance/diabetes and their related disorders such as overweight and obesity have been related to increased mortality in previous studies.[38] Furthermore, data on glycated hemoglobin were not available in our study. Serum insulin was measured after 10 years of storage at -20˚C. Although the stability of serum insulin levels at this temperature stored long term is unknown, the stability of insulin is generally known to be sensitive to time and temperature.[39,40] Although adjusting for several relevant confounders that could modify our findings did not alter the results, the possibility of residual confounding cannot be fully excluded.

CONCLUSION

Our results suggest that serum insulin and insulin resistance may be independent predictors of cognitive performance 7 years later in elderly individuals without dementia. The sensitivity analyses of the present study, showing that insulin and insulin resistance were associated with lower cognitive test scores only in the participants who did not develop dementia during the follow-up highlight the fact that vascular risk factors tend to change close to the onset of dementia.[41] Because of the observational study design, we must caution against a causal interpretation of our findings. Further studies to investigate this question in more detail to detect possible underlying mechanisms may be interventions targeting insulin resistance in relation to cognitive impairment, before disease processes become too advanced. Once dementia becomes more advanced, insulin resistance may not be an important determinant of disease progression and the clinical profile of dementia.[25,42] Further studies are needed to identify characteristics of a high-risk profile (i.e., raised insulin concentrations or HOMA) from different age groups and whether these characteristics would be affected either by lifestyle or by pharmacological interventions

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ORIGINAL COMMUNICATION

Midlife work-related stress is associated with late-life cognition

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Abstract To investigate the associations between midlife work-related stress and late-life cognition in individuals without dementia from the general population. The Cardiovascular Risk Factors, Aging and Dementia (CAIDE) study population ($n = 2000$) was randomly selected from independent Finnish population-based surveys (baseline mean age 50 years). Participants underwent two re-examinations in late life (mean age 71 and 78 years, respectively). 1511 subjects participated in at least one re-examination (mean total follow-up 25 years). Work-related stress was measured using two questions on work demands administered in midlife. Multiple cognitive domains were assessed. Analyses were adjusted for several potential confounders. Higher levels of midlife work-related stress were associated with poorer performance on global cognition [β-coefficient, −0.02; 95% confidence interval (CI), −0.05 to −0.00], and processing speed [β −0.03, CI −0.05 to −0.01]. Results remained significant after adjusting for potential confounders. Work-related stress was not significantly associated with episodic memory, executive functioning, verbal fluency or manual dexterity. This study shows that global cognition and processing speed may be particularly susceptible to the effects of midlife work-related stress.

Keywords Work-related stress · Stress · Job demands · Job strain · Cognition · Midlife risk factors

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Introduction

Job strain is a common and important source of stress; especially considering the large proportion of time individuals spend at work throughout their lifespan. Chronically elevated work-related stress is a well-established risk factor for numerous physical and mental health outcomes, including depression, metabolic syndrome and cardiovascular diseases [5, 20, 26, 28, 33]. More recently, studies have also shown associations between work-related stress and dementia, where high job strain, low levels of job control, low social support at work, and more stress-related physical symptoms have all been associated with higher dementia risk later in life [2, 7, 25, 34]. Consistently, high levels of job control and high challenges were associated with a reduced risk for dementia [24].

Some studies have also investigated the associations between work-related stress and cognition with less than a handful of longitudinal studies simultaneously measuring multiple cognitive domains. Work-related stress in the form of low job control and high job strain was associated with

worse cognition and cognitive decline, although the findings have been mixed regarding the compromised cognitive domains. Longitudinal results showed that active jobs (characterized by high levels of both demands and control) were associated with higher performance on a phonemic test of verbal fluency, but not with other cognitive domains after adjustment for employment grade [8]. Similarly, low job control was associated with poor global cognition, whereas active jobs were associated with better global cognition [3, 20]. Low job control and more job strain were also associated with poor episodic memory at retirement, and more rapid episodic memory decline post-retirement [4]. Another study showed that high job strain and low control were associated with decline in verbal learning and memory, but not visual memory, where the tests were administered approximately 15 and 21 years after assessment of job strain [2]. Evidence also showed that job strain and demands impact subjective cognitive complaints and learning outcomes [13, 27, 29]. Taken together, these findings emphasize the importance of measuring multiple cognitive domains using a life-course approach when examining the impact of work-related stress on cognition.

The job demand-control-support model is one of the most common models for conceptualizing work-related stress [15, 30]. According to this model, high job demands, low job control and their combination are associated with various health and cognitive outcomes [19]. It has recently been suggested that more evidence is needed on the effects of self-reported job strain on cognitive outcomes [4]. In this population-based cohort study, we investigated the associations of midlife work-related stress and specifically job demands with late-life cognitive performance in several domains among individuals without dementia (average follow-up time 25 years).

Materials and methods

Study population

Participants of the Cardiovascular Risk Factors, Aging and Dementia (CAIDE) study in Finland were first examined at midlife (baseline) in the North Karelia Project and the FINMONICA study, where individuals were assessed in one of the following years for the baseline assessment: 1972, 1977, 1982 or 1987 [22]. Baseline participation rates ranged between 82 and 90%. In 1998, a random sample of 2000 survivors living in the cities of Kuopio and Joensuu, aged 65–79, were invited for a first re-examination (Fig. 1). A total of 1449 (72.5%) individuals participated and 1409 completed the cognitive assessments. The mean follow-up time was 21 years (SD = 4.9). Participants returned for a second re-examination between 2005 and 2008. In 2005, of the 2000 original sample, 1426 were still alive and were still living in the same region. When invited, 909 (63.7%) of them participated and 852 completed the cognitive assessment. A total of 1511 individuals participated in at least one re-examination, and 750 participated in both. Mean ages at each time point were: at baseline, 50 years (SD = 6.0, age range: 39-64); at the first re-examination, 71.3 years (SD = 4.0, age range 65–80); at the second re-examination, 78.6 years (SD = 3.7, age range 72–90). Local ethics committees approved the CAIDE study and participants provided written informed consent. The study complies with the Declaration of Helsinki.

Measurement of work-related stress

Perceived work-related stress was measured in midlife using two questions focusing on job demands. These questions were adapted from the questions validated by Karasek et al. [16] and have been used reliably by various research groups [3, 20, 31]. Both questions have the same 5-point likert scale. The questions were, “How often do you struggle to cope with the amount of work?” and “How often are you bothered by constant hurry at work?”. After reverse coding to facilitate the interpretation of the results, the response options were: 1 = never, 2 = rarely, 3 = sometimes, 4 = often, 5 = always. Data on work-related stress were available for 1273 participants. Both questions were highly correlated (Spearmans $\rho = 0.623$, $p < 0.001$) and were summed to produce a composite measure of work-related stress.

Cognitive assessments

At both re-examinations, a comprehensive battery of neuropsychological tests was administered to assess multiple cognitive domains. For the current study, we used the following tests that were administered at both re-examinations: (1) global cognition measured by the mini mental state examination (MMSE) [10]; (2) episodic memory assessed by an immediate word recall test (10-word list); (3) executive functioning measured by the Stroop test (time difference between the task of naming the color of the ink used to write the name of another color, and the task of naming colors of dots); (4) verbal fluency tested by category fluency test (number of correct animal names generated in 60 s); (5) psychomotor speed assessed by the letter digit substitution test; (6) manual dexterity measured by the bimanual Purdue Pegboard test. Dementia diagnosis was carried out using a three-step protocol previously described [25].

Other assessments

At baseline (midlife), assessments and survey methods were standardized and adhered to international guidelines

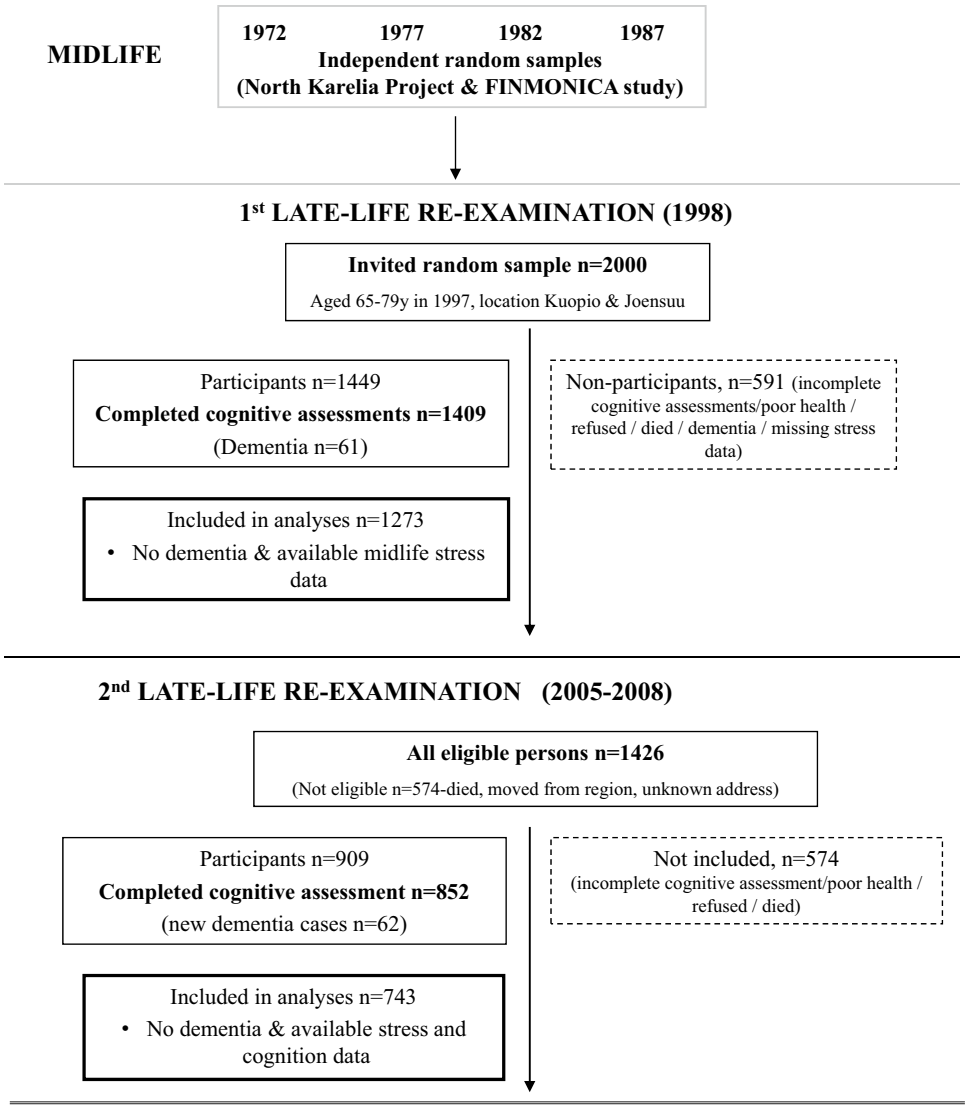

Fig. 1 Flowchart representing the study population, examinations and cognitive assessments in the CAIDE study

and the World Health Organization (WHO) (Multinational MONItoring of trends and determinants in CArdiovascular disease) MONICA protocol [21]. Re-examination surveys were similar and comparable to those at baseline. Baseline surveys involved self-administered questionnaires on medical history, sociodemographic factors, health status, health-related behaviors and psychological-related factors. We selected the following covariates previously shown to be associated with worse cognition and/or high levels of stress: age, sex, education, *APOE* ε4, respiratory, cardio/cerebrovascular and musculoskeletal conditions, and type of occupation (white collar vs. blue collar). Occupation type was measured by asking individuals to select their longest-held occupation among the following categories: office/service, farming/forestry, mining/industrial/construction work, housewives, or other. Hopelessness was measured using the following two questions described previously [11]: "I feel that it is impossible to reach the goals I would like to strive for" and "The future seems to me to be hopeless, and I cannot believe that things are changing for the better". A five-point Likert scale was used, coded as 0 = absolutely agree; 1 = somewhat agree; 2 = cannot say; 3 = somewhat disagree; or 4 = absolutely disagree. A trained nurse verified the answers and addressed participants' questions. The nurse also measured height, weight and blood pressure. A venous blood sample was obtained, and allowed for measures of biomarkers, including cholesterol and *APOE* genotype from blood leucocytes, for which HHaI digestion and polymerase chain reaction were used [32]. The Hospital Discharge Register was used for information on respiratory and cardio/cerebrovascular conditions (chronic obstructive pulmonary disease, asthma, coronary artery disease, stroke, myocardial infarction, atrial fibrillation, cardiovascular surgery, heart failure or diabetes). These conditions were combined into a dichotomous variable (yes/no) reflecting the presence of

any midlife respiratory or cardio/cerebrovascular conditions. All covariates were measured at baseline.

Statistical analyses

We conducted analyses using Stata 13.0 (Stata Corp, College Station, TX, USA). We analyzed participant baseline characteristics using Chi-square (χ^2) tests for categorical variables (data reported as percentages), and Student *t* tests for continuous variables (data reported as means (standard deviations [SD])). The significance level for all analyses was set at $p < 0.05$ (Table 1). Zero-skewness log-transformations were applied to cognitive test scores (Stata command lnskew0). Results were standardized to have SD = 1.

Participants with dementia at the first re-examination were excluded from analyses at the first re-examination. Participants with dementia at the second re-examination were excluded from analyses at the second re-examination. To maximize sample size, all subjects with cognitive assessments in at least one re-examination ($n = 1332$) were considered in analyses. This means that the analyses are based on both the first and second re-examination combined. For subjects with cognitive assessments in both re-examinations ($n = 685$), two observations were included (i.e., one for test results at the first re-examination, and one for test results at the second re-examination). Data were organized in what is often referred to as long format.

To investigate the associations between midlife work-related stress and cognition, we performed linear regression analyses for each of the cognitive domains. We reported results as β-coefficient and 95% confidence intervals (CI). All analyses were adjusted for a basic set of confounders: age, sex, years of education and follow-up time (Model 1). Model 2 additionally adjusted for the type of occupation. Model 3 additionally for *APOE* ε4 genotype, hopelessness and midlife respiratory, cardio/cerebrovascular conditions.

Results

Population characteristics

Table 1 shows sociodemographic and clinical characteristics of the participants included in the analyses. Table 2 shows the raw scores for all the cognitive tests administered at the first and second re-examinations. In this sample, 76 individuals were diagnosed with mild cognitive impairment at the first re-examination. Of them, 27 were alive at the second re-examination, and 6 of them converted to dementia (for details regarding the mild cognitive impairment diagnoses, see [25]). A total of 156 had mild cognitive impairment at the second re-examination. The dementia cases were excluded from analyses.

Associations between work-related stress and cognition

Associations between midlife work-related stress and cognition are shown in Table 3.

Higher levels of work-related stress were significantly associated with worse global cognition measured by the MMSE after the adjustment for occupation type (Model 2: β −0.02, 95% CI −0.04 to −0.00) , APOEε4, hopelessness and midlife cardio/cerebrovascular/respiratory conditions (Model 3: β −0.02, 95% CI −0.05 to −0.00).

Table 1 Sociodemographic and clinical characteristics of participants included in the analyses at the first and second re-examinations

Characteristics	First re-examination (1998)		Second re-examination (2005–2008)	
	n	Mean (SD) or *n* (%)	*n*	Mean (SD) or *n* (%)
Baseline age	1273	49.9 (5.9)	743	49.0 (5.7)
Age at follow-up	1273	71.0 (4.0)	743	78.3 (3.6)
Follow-up time	1273	21.1 (4.8)	743	29.3 (4.9)
Sex				
Women	1273	791 (62.1)	743	482 (64.9)
Education (years)	1255	8.8 (3.4)	733	9.2 (3.4)
*APOE*ε4 allele				
Carrier	1247	437 (35.0)	659	203 (30.8)
Work-related stress (range 0–8)	1273	3.4 (1.9)	743	3.4 (1.9)
Type of occupation				
White collar	1231	603 (49.0)	719	381 (53.0)
Other	1231	628 (51.0)	719	338 (47.0)
Midlife cardio/cerebrovascular/respiratory conditions				
Yes	1235	38 (3.0%)		15 (2.0%)

Column wise values are numbers (%), and χ^2 test was used. Values are means (SD)

Table 2 Descriptive statistics of cognitive test scores at the first and second re-examinations

Characteristics	First re-examination (1998)		Second re-examination (2005–2008)	
	n	Median (range)	*n*	Median (range)
Global cognition (Mini Mental State Exam)	1273	26 (20–30)	743	27 (20–30)
Episodic memory (word list recall)	1271	5 (0–10)	742	5 (1–10)
Executive functioning (Stroop)	1212	36 (1–257)	726	40 (7–250)
Verbal fluency	1268	20 (9–55)	742	20 (8–40)
Letter digit substitution	1228	19 (4–50)	655	20 (9–47)
Purdue Peg Board	1209	10 (4–18)	727	8 (1–13)

Table 3 The associations between midlife work-related and late-life cognition

	Model 1	Model 2	Model 3
Cognitive domain (test)	β-coefficient (95% CI)	β-coefficient (95% CI)	β-coefficient (95% CI)
Global cognition (Mini Mental State Exam)	−0.02 (−0.04 to 0.00)	−0.02 (−0.04 to −0.00)	−0.02 (−0.04 to −0.00)
Episodic memory (word list recall)	−0.01 (−0.03 to 0.01)	−0.01 (−0.03 to 0.01)	−0.00 (−0.02 to 0.02)
Executive functioning (Stroop)	−0.00 (−0.03 to 0.02)	−0.00 (−0.02 to 0.02)	0.00 (−0.02 to 0.03)
Verbal fluency (animal naming)	−0.02 (−0.04 to 0.00)	−0.02 (−0.04 to 0.00)	−0.01 (−0.03 to 0.01)
Processing speed (letter digit substitution)	−0.02 (−0.04 to −0.00)	−0.03 (−0.05 to −0.01)	−0.03 (−0.05 to −0.01)
Manual dexterity (Purdue Peg Board)	−0.01 (−0.03 to 0.02)	−0.01 (−0.03 to 0.02)	−0.01 (−0.03 to 0.01)

Model 1: age, sex, follow-up time, education

Model 2: Model 1 + occupation type

Model 3: Model 2 + *APOE*4, midlife hopelessness and midlife cardio/cerebrovascular/respiratory conditions

Based on data in long format, individuals with observations at both re-examinations each contribute to two observations

Higher levels of work-related stress were also associated with poorer performance on processing speed measured by the letter digit substitution test in all three models (Model 1: β −0.02, 95% CI −0.04 to −0.00; Model 2: β −0.03, 95% CI −0.05 to −0.01; Model 3: β −0.03, 95% CI −0.05 to −0.01). Work-related stress was not significantly associated with episodic memory, verbal fluency, executive functioning or manual dexterity (all $p > 0.05$).

Discussion

This study shows that midlife work-related stress characterized by high job demands and constant hurry at work is associated with poorer performance on global cognition, and lower performance on processing speed, in a large representative population without dementia. These associations remained significant after adjusting for several confounding factors. In contrast, work-related stress was not significantly associated with episodic memory, verbal fluency, executive functioning or manual dexterity.

The current study supports previous findings showing that job strain and low job control are associated with worse MMSE performance [3, 20]. Our results are inconsistent with results from the Framingham study, where job strain and low job control were associated with verbal learning and memory [2], and results from the Health and Retirement Study showing that job strain expressed as low job control was associated with poor episodic memory performance at retirement, and further decline after retirement [4]. These discrepancies may be due to different measures of work-related stress across the studies, the different follow-up durations, the different cognitive tests (immediate vs. delayed recall) and geographical differences between work-related stress levels between the USA and Nordic or other European countries.

In the current study, the associations between work-related stress and cognition were specific to a few cognitive domains, and did not extend to all measured domains. In the Framingham study, findings were also limited to verbal learning and memory, and not visual memory or abstract reasoning [2]. Similar to the interpretation of Eloviainio et al., the long duration between work-related stress and the assessment of cognitive function (assessed after retirement), may have led to a "dilution of the effects" [8]. Our study adds to the few longitudinal studies with a long follow-up duration, as it is the first to show significant associations with processing speed, in addition to the previously observed association with global cognition assessed by the MMSE.

Several mechanisms may underlie the observed associations. First the stress hormone cortisol has its receptors in brain regions involved in learning, memory and executive functioning [12]. Previous evidence has shown that higher cortisol levels are associated with worse MMSE and processing speed performance [6, 17]. Although our findings are inconsistent with studies on cortisol levels and worse episodic memory performance, these differences may be due to the different time durations between stress exposure and cognitive assessment, or the sensitivity of cognitive tasks used [17, 18]. We analyzed immediate recall. If we instead had analyzed delayed recall, we may have found an association. Another mechanism is through elevations in allostatic load, a multi-system measure of cumulative stress that has also been previously associated with poor cognition [14].

Work-related stress may be reduced through various interventions, some of which may target individuals, by providing social support, recognition, increasing the sense of control over work-related tasks, offering constructive feedback as well as professional development opportunities [9, 23]. Indeed, higher social support at work is associated with fewer cognitive complaints and reduces the risk for dementia [27]. The work environment can also be ameliorated by improving the work climate, the physical work environment and increasing cooperation/teamwork [9, 23]. As previously suggested by Andel et al., interventions may also target retired older adults who had jobs characterized by low control, and we add to that suggestion 'job strain', to prevent cognitive decline [4].

Our study has several strengths including the long follow-up duration from midlife to later life, the large population, measurement of cognitive performance using several validated tests for different cognitive domains, and adjusting for potential confounders. The study also has some limitations. First, stress in later life and non-work-related stress were not measured, so it is unclear whether the role of midlife work-related stress is independent of these other sources of stress. Second, although job control is an important dimension of work-related stress, it was not measured in the current study [24, 34].

In conclusion, our study shows that midlife work-related stress is associated with worse global cognition and processing speed performance. These findings suggest that the effects of work-related stress are long lasting, with some cognitive domains more sensitive than others.

Compliance with ethical standards

Conflicts of interest The authors have nothing to disclose.

Ethical standards The study complies with the Declaration of Helsinki. Local ethics committees approved the CAIDE study.

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Informed consent All the participants were provided the written informed consent.

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CAIDE Dementia Risk Score, Alzheimer and cerebrovascular pathology: a population-based autopsy study

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Abstract. Hooshmand B, Polvikoski T, Kivipelto M, Tanskanen M, Myllykangas L, Mäkelä M, Oinas M, Paetau A, Solomon A (Karolinska Institute, Stockholm, Sweden; Ulm University Hospital, Ulm, Germany; Newcastle University, Newcastle upon Tyne, UK; Karolinska Institute, Stockholm, Sweden; University of Eastern Finland, Kuopio, Finland; Karolinska University Hospital, Stockholm, Sweden; Imperial College London, London, UK; Helsinki University Hospital, Helsinki, Finland). CAIDE Dementia Risk Score, Alzheimer and cerebrovascular pathology: a population-based autopsy study. *J Intern Med* 2018; **283**: 597–603.

Background. CAIDE Dementia Risk Score is a tool for estimating dementia risk in the general population. Its longitudinal associations with Alzheimer or vascular neuropathology in the oldest old are not known.

Aim. To explore the relationship between CAIDE Dementia Risk Score at baseline and neuritic plaques, neurofibrillary tangles, cerebral infarcts and cerebral amyloid angiopathy (CAA) after up to 10-year follow-up in the Vantaa 85 + population.

Methods. Study population included 149 participants aged ≥85 years, without dementia at baseline, and with available clinical and autopsy data. Methenamine silver staining was used for β-amyloid and modified Bielschowsky method for neurofibrillary tangles and neuritic plaques. Macroscopic infarcts were identified from cerebral hemispheres, brainstem and cerebellum slices. Standardized methods were used to determine microscopic infarcts, CAA and α-synuclein pathologies. The CAIDE Dementia Risk Score was calculated based on scores for age, sex, BMI, total cholesterol, systolic blood pressure, physical activity and *APOE*ε4 carrier status (range 0–18 points).

Results. A CAIDE Dementia Risk Score above 11 points was associated with more cerebral infarctions up to 10 years later: OR (95% CI) was 2.10 (1.06–4.16). No associations were found with other neuropathologies.

Conclusion. In a population of elderly aged ≥85 years, higher CAIDE Dementia Risk Score was associated with increased risk of cerebral infarcts.

Keywords: Alzheimer pathology, CAIDE Dementia Risk Score, cerebrovascular pathology, dementia, elderly.

Introduction

Dementia risk scores have been developed for identifying at-risk individuals who could benefit from preventive interventions [1]. CAIDE Dementia Risk Score is the first validated tool estimating the risk of dementia 20 years later [2, 3]. It takes into account age, sex, education, systolic blood pressure, body mass index (BMI), cholesterol, physical activity and *APOE*ε4 status (maximum 18 points, Table S1). Two longitudinal studies reported associations of CAIDE Dementia Risk Score with white matter changes and grey matter atrophy on brain MRI [4, 5]. A cross-sectional study reported associations with CSF amyloid-β/tau ratio [6]. The aim of this study was to investigate links between CAIDE Dementia Risk Score and post-mortem neuropathological findings up to 10 years later in the Vantaa 85 + population including people aged ≥85 years.

Methods

Study population

The Vantaa 85 + study has been described in detail [7]. In brief, the study included 553 participants who were clinically examined at baseline and represented 92% of the 601 individuals aged ≥85 years and living in Vantaa, Finland, in 1991. A total of 149 individuals without dementia at baseline underwent consented post-mortem examination and had complete CAIDE Dementia Risk Score data. They were older at death (mean (standard deviation SD)) 92.8 (3.5) vs. 91.9 (3.4) years; $P = 0.021$), had longer follow-up (4.7 (2.5) vs 3.7 (2.3) years; $P < 0.001$) and more incident dementia over 10 years (36.9% vs. 24.2% ($P = 0.011$) compared with the rest of the study population (Table 1). The Vantaa 85 + study was approved by the Ethics Committee of the Health Centre of the city of Vantaa and by the Coordinating Ethics Committee of Helsinki University Hospital. The Finnish Health and Social Ministry approved the use of the health and social work records and death certificates. Blood samples were collected only after subjects or their relatives gave informed consent. The National Authority for Medicolegal Affairs (VALVIRA) approved the tissue sample collection at autopsy and their use for research. Written consent for autopsy was obtained from the nearest relative.

Clinical assessment

Evaluation included an interview by a trained nurse using questionnaires concerning health, health-related behaviour and clinical examination by a physician. Data on socio-demographic characteristics and medical history were collected according to a structured protocol, and dementia was diagnosed according to the DSM-III-R criteria. BMI was assessed. Blood pressure was measured from the right arm after sitting for 5 min [8]. Serum cholesterol levels were determined by enzymatic techniques [8]. *APOE*ε4 status was determined as previously described [7]. Being physically active was defined as engaging in light walking or moderate exercise several times per week. CAIDE Dementia Risk Score was calculated as specified previously [2].

Neuropathology

Paraffin-embedded brain tissue samples were assessed for neuropathology blind to clinical status. The sampling procedures and quantification of the Alzheimer's and cerebrovascular pathologies were previously described in detail [7, 9]. In brief, the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) protocol was employed for neocortical neuritic plaque score [10]. Methenamine silver staining was used for amyloid-β and modified Bielschowsky method for neurofibrillary tangles (NFT) and neuritic plaques. The average area fraction of cortex covered by methenamine silver-positive plaques and NFT per standard cortical area was determined. Gallyas silver stain was used for the Braak staging, which was carried out as originally described [10, 11].

Macroscopic infarcts were identified from cerebral hemispheres, brainstem and cerebellum slices. Microinfarcts were analysed in the haematoxylin and eosin-stained tissue sections in six brain regions (frontal, parietal, temporal and occipital lobes, hippocampus and cerebellum) [12]. Cerebral amyloid angiopathy diagnosis was based on Congo red staining within these six regions and confirmed using immunohistochemistry against amyloid-β peptide [13]. Sections of substantia nigra stained with the haematoxylin and eosin method and sections of substantia nigra and hippocampus stained with antibodies against α-synuclein were used to screen for Lewy-related pathology [14]. If any Lewy-related pathology was detected in screened areas, the immunohistochemistry for α-synuclein was performed on cortical samples [15].

Statistical analysis

Comparisons between autopsy population with available CAIDE Dementia Risk Score ($n = 149$) and the remaining study population without dementia at baseline were performed using chi-square and t-test as appropriate. Associations of CAIDE Dementia Risk Score with neuropathological variables were assessed with ordinal or logistic regressions and association with incident dementia with Cox proportional hazard regression (age as timescale). The tangle count, amyloid-β load and cerebral amyloid angiopathy were not normally distributed and were categorized into three groups: no neuropathology, and values below or above the median level of these pathologies. Dichotomous variables were created for brain infarctions (macroscopic, microscopic and all). The presence of α-synuclein pathology was categorized into three groups: none, brain stem or limbic predominant and diffuse neocortical α-synuclein. Additional analyses were performed to investigate effects of each CAIDE Dementia Risk Score component, and

Table 1 *Characteristics of the study population without dementia at baseline*

	N	Autopsy data available	*N*	Autopsy data not available	*P*
Age at baseline, mean (SD), year	149	88.1 (2.7)	190	88.0 (2.7)	0.675
Age at death, mean (SD), year	149	92.8 (3.5)	159	91.9 (3.4)	0.021
Follow-up time, mean (SD), year	149	4.7 (2.5)	159	3.7 (2.3)	<0.001
Women, *N.* (%)	149	121 (81.2%)	190	145 (76.3%)	0.277
Education, mean (SD), year	149	4.3 (2.9)	186	4.2 (2.9)	0.912
Systolic blood pressure, mean (SD), mmHg	149	154.5 (23.8)	184	156.2 (28.7)	0.563
Diastolic blood pressure, mean (SD), mmHg	149	84.5 (12.2)	184	82.6 (11.8)	0.148
Cholesterol, mean (SD), mmol L^{-1}	149	5.8 (1.3)	167	5.6 (1.3)	0.102
Low-density lipoprotein, mmol L^{-1}	137	3.8 (1.1)	157	3.7 (1.2)	0.533
High-density lipoprotein, mmol L^{-1}	149	1.0 (0.3)	166	1.0 (0.3)	0.268
Triglycerides, mmol L^{-1}	149	2.2 (1.4)	166	1.9 (1.0)	0.018
Obesity, *N.* (%)[a]	149	34 (22.8%)	189	39 (20.6%)	0.628
Physical activity (inactive), *N.* (%)	149	97 (65.1%)	187	122 (65.2%)	0.979
*APOE*ε4, *N.* (%)	149	30 (20.1%)	181	39 (21.5%)	0.753
CAIDE Dementia Risk Score, mean (SD)	149	11.9 (2.0)	158	12.0 (1.0)	0.966
CAIDE Dementia Risk Score without APOE, mean (SD)	149	9.9 (1.9)	158	9.7 (1.7)	0.558
Dementia at death, *N.* (%)	149	55 (36.9%)	190	46 (24.2%)	0.011

APOE, apolipoprotein E genotype, CAIDE, Cardiovascular Risk Factors, Aging and Dementia Study.
[a]Obesity was defined here as body mass index (BMI) ≥28 as previously described [29].

also diastolic blood pressure, triglycerides, HDL and LDL on neuropathological outcomes. As elevated homocysteine was previously related to neuropathology in the Vantaa 85 + study [7], further analyses were conducted to assess the links between CAIDE Dementia Risk Score and neuropathological outcomes according to homocysteine values above or below the cut-off of 20 μmol L^{-1} [16]. We used Stata software for the analysis.

Results

Associations between baseline CAIDE Dementia Risk Score and neuropathological measurements are shown in Table 2. Individuals with higher CAIDE Dementia Risk Score tended to have higher risk of cerebral infarcts ($P = 0.08$). This association was most evident in participants with CAIDE Dementia Risk Score above 11 points ($n = 93$) compared with below 11 points ($n = 56$): OR (95% CI) was 2.10 (1.06–4.16; $P = 0.035$). Individuals with higher CAIDE Dementia Risk Score and homocysteine >20 μmol L^{-1} tended to have higher risk of amyloid-β load: OR (95% CI) was 1.26 (0.96–1.65), $P = 0.099$. No association between CAIDE Dementia Risk Score and incident dementia was found (hazard ratio (95% confidence interval) was 1.03 (0.92–1.17)). CAIDE Dementia Risk Score without APOE was not associated with neuropathological outcomes (results not shown).

Among individual CAIDE Dementia Risk Score components, there were more amyloid-β accumulation and NFT in *APOE*ε4 carriers compared with noncarriers (Table 2). Elevated triglycerides were associated with increased NFT count and more severe Braak stage at death. Furthermore, higher HDL was related to less severe Braak stage and amyloid-β accumulation (Table 3).

Discussion

Our results indicated that a higher CAIDE Dementia Risk Score was associated with increased cerebral infarcts risk over 10 years in the oldest old. No associations with NFT burden, amyloid-β load or incident dementia were found. The CAIDE

Table 2 *Association of CAIDE Dementia Risk Score with neuropathology (OR, 95% CI)*[a]

	Continuous	Dichotomous (cut-off > 11)
Tangle count	1.03 (0.88–1.21)	0.64 (0.34–1.22)
Braak stage	*1.15 (0.98–1.34)*	0.92 (0.50–1.68)
Amyloid-β load	1.13 (0.97–1.33)	1.01 (0.54–1.87)
CERAD score	1.12 (0.95–1.33)	0.96 (0.49–1.87)
Cerebral amyloid angiopathy	1.06 (0.90–1.24)	0.77 (0.31–1.46)
Cerebral macroinfarcts	1.11 (0.94–1.32)	1.62 (0.82–3.21)
Cerebral microinfarcts	1.21 (0.96–1.53)	2.01 (0.76–5.59)
All cerebral infarcts	*1.17 (0.98–1.40)*	**2.10 (1.06–4.16)**
α-synuclein pathology	1.05 (0.87–1.27)	0.76 (0.35–1.66)

CERAD, The Consortium to Establish a Registry for Alzheimer's Disease.
[a]One hundred and fourty nine participants without dementia at baseline had available data on CAIDE Dementia Risk Score at baseline. Significant results ($P < 0.05$) are in bold, and trends ($P < 0.10$) are in italics.

Dementia Risk Score is so far the only validated dementia risk estimation tool used to select participants in a successful cognitive decline prevention trial testing a multidomain lifestyle intervention [1]. Given the increasing interest in adapting and testing this prevention model worldwide [17], it is essential to determine the full range of properties of the CAIDE Dementia Risk Score.

Because CAIDE Dementia Risk Score is mainly based on vascular risk factors, associations with cerebral infarcts may not be surprising. Furthermore, a dose–response relationship between APOE genotype and stroke has been shown [18], which may partly explain the observed stronger association with the CAIDE Dementia Risk Score including APOE. Our findings add to previous reports linking higher CAIDE Dementia Risk Score to more severe white matter lesions on MRI [4, 5].

While higher CAIDE Dementia Risk Score has been previously linked to lower grey matter and hippocampal volume, lower cortical thickness and more severe MTA on MRI [4, 5], associations with markers of amyloid accumulation seem to be context-dependent. Higher midlife CAIDE Dementia Risk Score did not predict late-life brain amyloid accumulation on PIB-PET scans in individuals from the general population [5], although a cross-sectional association with lower amyloid/tau ratio in CSF was reported in memory clinic patients without dementia [6].

CAIDE Dementia Risk Score did not predict dementia in the present study. This is in line with previous reports of differences between midlife versus late-life risk profiles for dementia [2, 19]. Risk factors such as blood pressure, BMI and cholesterol tend to decline after midlife in individuals who develop dementia later on [20]. Several studies have shown that midlife risk scores tend to perform poorly when applied to older age groups [21, 22]. CAIDE Dementia Risk Score was formulated based on midlife risk profile, while the Vantaa 85 + population was ≥85 years at baseline. A late-life risk score may perform better in this age group regarding both dementia and pathology prediction.

Our findings indicated a relationship between higher HDL and less severe Braak stage and less amyloid-β accumulation. Also, elevated triglycerides were associated with more NFT and more severe Braak stage. This is in line with previous studies showing associations of lower HDL and higher triglycerides with increased risk of neuritic plaques [23] and lower HDL levels with amyloid accumulation on PIB-PET scans [24]. Although other studies reported conflicting findings [21, 22], potentially due to differences in populations and designs, such blood markers would merit further testing as potential candidates in neuropathology prediction models. Another potential candidate would be, for example, homocysteine, which was previously associated with AD pathology in the Vantaa 85 + population [7], as well as dementia risk in several studies [25].

The major strength of this study is the prospective population-based design with comprehensive autopsy data and inclusion of participants aged

Table 3 *Associations of individual components of CAIDE Dementia Risk Score and other vascular factors at baseline with neuropathological outcomes (OR, 95% CI)*[a]

	Tangle count	Braak stage	Amyloid-β load	CERAD score	All cerebral infarcts
Age at death (n = 163)	1.01 (0.91–1.12)	1.02 (0.92–1.12)	0.95 (0.86–1.05)	0.96 (0.87–1.05)	1.04 (0.93–1.16)
Female (n = 163)	1.01 (0.49–2.11)	1.26 (0.61–2.59)	0.73 (0.35–1.55)	0.73 (0.35–1.53)	0.96 (0.43–2.15)
Education (n = 160)	1.02 (0.92–1.12)	0.97 (0.88–1.07)	1.03 (0.93–1.13)	1.05 (0.94–1.17)	0.97 (0.87–1.08)
Systolic blood pressure (n = 161)	0.99 (0.98–1.01)	1.0 (0.99–1.01)	1.00 (0.99–1.02)	1.00 (0.99–1.01)	1.00 (0.99–1.01)
Diastolic blood pressure (n = 161)	0.98 (0.96–1.01)	0.99 (0.97–1.02)	1.00 (0.98–1.02)	0.99 (0.97–1.02)	1.00 (0.98–1.03)
Obesity (n = 162)	0.87 (0.61–1.25)	1.00 (0.70–1.42)	0.76 (0.39–1.51)	0.74 (0.35–1.59)	1.83 (0.82–4.08)
Cholesterol (n = 153)	1.20 (0.92–1.55)	1.19 (0.93–1.53)	1.03 (0.80–1.32)	1.18 (0.91–1.54)	1.00 (0.77–1.30)
High-density lipoprotein (n = 153)	0.42 (0.14–1.27)	**0.23 (0.08–0.66)**	*0.40 (0.13–1.19)*	0.50 (0.16–1.61)	0.45 (0.14–1.51)
Low-density lipoprotein (n = 141)	1.19 (0.87–1.62)	1.16 (0.86–1.56)	1.04 (0.77–1.40)	1.15 (0.83–1.58)	1.01 (0.73–1.40)
Triglycerides (n = 153)	**1.31 (1.03–1.65)**	**1.33 (1.08–1.63)**	1.15 (0.92–1.44)	1.20 (0.96–1.52)	1.09 (0.86–1.38)
Physical inactivity (n = 161)	0.80 (0.43–1.46)	1.41 (0.79–2.52)	0.77 (0.42–1.40)	0.98 (0.58–1.66)	1.23 (0.64–2.35)
*APOE*ε4 (n = 160)	**3.30 (1.59–6.83)**	**2.52 (1.25–5.07)**	**9.07 (3.64–22.63)**	**4.38 (1.89–10.14)**	1.37 (0.62–3.02)

CERAD, The Consortium to Establish a Registry for Alzheimer's Disease, APOE, apolipoprotein E genotype.
[a]All analyses adjusted only for follow-up time, significant results ($P < 0.05$) are in bold, and trends ($P < 0.10$) are in italics. Only participants without dementia at baseline are included in analyses.

≥85 years. Although few studies have investigated the impact of CAIDE Dementia Risk Score on Alzheimer- and cerebrovascular-type neuropathology, none had a longitudinal design with autopsy data. However, selective survival may contribute to underestimating associations with cerebral infarcts, because individual components of CAIDE Dementia Risk Score are associated with increased mortality [26]. Although clinical dementia diagnoses were shown to correlate well with brain autopsy findings in the Vantaa 85+ study [7], association between brain pathologies and dementia is known to be more complex in older compared with younger elderly [27]. Quantitative, systematic methods were used in our study to identify neuropathological changes, but due to the use of traditional silver staining methods, there may be differences compared with studies using immunohistochemistry [28].

In conclusion, CAIDE Dementia Risk Score was related to cerebral infarcts, but not amyloid-β load or NFT count. Different risk scores will need to be developed if the aim is to predict dementia, amyloid or tangle accumulation in the oldest old.

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Author contributions

Dr Hooshmand had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Hooshmand, Polvikoski, Kivipelto and Solomon conceived and designed the study. Hooshmand, Polvikoski, Kivipelto, Tanskanen, Myllykangas, Mäkelä, Oinas, Paetau and Solomon participated in acquisition, analysed and interpreted the data. Hooshmand, Polvikoski, Kivipelto and Solomon drafted the manuscript. All authors critically revised the manuscript for important intellectual content. Hooshmand, Kivipelto and Solomon performed statistical analysis. Hooshmand, Kivipelto and Solomon obtained funding. All authors contributed to administrative, technical or material support. Hooshmand, Polvikoski, Kivipelto, Myllykangas and Solomon supervised the study.

Conflict of interest statement

No conflict of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. CAIDE Dementia Risk Score versions used in the study and number of points[1].■

