From the Aging Research Center (ARC),
Department of Neurobiology, Care Sciences and Society,
Karolinska Institutet, Stockholm, Sweden

EXPLORING THE ROLE OF VITAMIN E IN ALZHEIMER'S DISEASE

An Epidemiological and Clinical Perspective

Francesca Mangialasche

Karolinska Institutet

Stockholm 2012

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Published by Karolinska Institutet. Printed by Larserics Digital Print AB, Stockholm.

ISBN 978-91-7457-748-8

To my family

ABSTRACT English

Vitamin E, the main non-enzymatic lipophylic antioxidant in the human body, has a major role in protecting the brain from damage mediated by free radicals. The term vitamin E encompasses eight natural congeners (forms): four tocopherols and four tocotrienols, named α, β, γ, and δ. Most investigation of vitamin E in relation to dementia and Alzheimer´s disease (AD) has focused primarily only on α-tocopherol, with conflicting findings. However, increasing knowledge regarding the biological properties of vitamin E provides a strong biological rationale that other forms of vitamin E, beyond just α-tocopherol, may play a role in AD pathogenesis.
The aim of the present project is to investigate the relation of all eight natural vitamin E forms with mild cognitive impairment (MCI) and AD in older adults, by combining both an epidemiological and a clinic-based approach.

Study I. Plasma levels of all eight natural vitamin E forms, and markers of vitamin E oxidative/nitrosative damage (α-tocopherylquinone, 5-nitro-γ-tocopherol), were investigated in subjects with AD, MCI, and normal cognition (CN) in a clinical-based, multi-centre European study (AddNeuroMed Project). Compared to CN subjects, AD and MCI cases had lower plasma levels of total tocopherols, total tocotrienols and total vitamin E. Both MCI and AD cases had 85% lower odds to be in the highest tertile of plasma total tocopherols and total vitamin E, and they were, respectively, 92% and 94% less likely to be in the highest tertile of total tocotrienols than the lowest tertile. Further, both disorders were associated with increased plasma indices of vitamin E oxidative/nitrosative damage (ratios α-tocopherylquinone/α-tocopherol and 5-nitro-γ-tocopherol/γ-tocopherol).

Study II. Within the AddNeuroMed Project, analysis which integrated plasma levels of vitamin E forms with structural magnetic resonance (MRI) parameters, derived from automated regional analysis, was used to differentiate AD and MCI cases from CN individuals, and to predict MCI conversion to AD. The analysis of MRI and vitamin E data alone provided an accuracy of 83.2% and 92.8% respectively, for AD *versus* CN, and of 58.1% and 87.8% for MCI *versus* CN. The integrated analysis of plasma vitamin E and MRI data enhanced the accuracy, which were 98.2% for AD *versus* CN and 90.7% for MCI *versus* CN. This combination of data also correctly identified 85% of the MCI who converted to clinical AD at one year follow-up and 67% of the non-converters.

Study III. The association of plasma levels of eight natural vitamin E forms with the incidence of AD was examined in a Swedish population-based prospective study (Kungsholmen Project) of oldest-old individuals (age 80+), using six-year follow-up data. Subjects with higher concentrations of total tocopherols, total tocotrienols or total vitamin E had approximately a 50% reduced risk of developing AD in comparison to subjects with lower plasma levels (highest *versus* lowest tertile).

Study IV. The association of serum levels of all eight natural vitamin E forms and markers of vitamin E oxidative/nitrosative damage, with the incidence of cognitive impairment (MCI or AD) was investigated in a Finnish population-based prospective study (CAIDE) of older adults (age 65+), using eight-year follow-up data. The odds of cognitive impairment was reduced for subjects in the medium tertile of γ-tocopherol serum levels, relative to those subjects in the lowest tertile [odds ratio and 95% confidence interval: 0.27(0.10-0.78)]. Subjects with a higher serum value for the index of γ-tocopherol nitrosative damage (5-nitro-γ-tocopherol/γ-tocopherol ratio; high and middle versus lowest tertile) were about three times more likely to develop cognitive impairment.

Conclusions. α-tocopherol is the only vitamin E form currently used to define vitamin E dietary requirements, and it is the only congener tested in randomized controlled trials in subjects with AD and MCI. The results of this project provide evidence that suggests that the other natural forms of vitamin E can also be important in cognitive impairment and AD in older adults. Thus, all natural vitamin E forms should be considered when studying the association of this micronutrient with cognitive impairment and AD. These findings also suggest that some aspects of vitamin E supplementation in preventing and treating AD should be re-examined. This should include the timing of intervention, the composition of supplementation, and the assessment of plasma levels of all vitamin E forms. The latter can help identify subjects who could benefit from vitamin E supplementation, and monitor in-vivo biological response to treatment.

Key words: Alzheimer's disease, clinical-based study, mild cognitive impairment, nitrosative stress, oxidative stress, population-based study, tocopherol, tocotrienol, vitamin E, α-tocopherylquinone, 5-nitro-γ-tocopherol.

SAMMANFATTNING Svenska

Vitamin E, den främsta icke-enzymatiska lipofila antioxidanten i kroppen, har en viktig roll i att skydda hjärnan från skador orsakade av fria radikaler. Termen vitamin E omfattar åtta naturliga föreningar: fyra tokoferoler och fyra tokotrienoler, namngivna α, β, γ och δ. De flesta undersökningar av vitamin E i förhållande till Alzheimers sjukdom (Alzheimer's disease, AD) har främst fokuserat på α-tokoferol, med motstridiga resultat. Dock ger den ökade kunskapen om de biologiska egenskaperna hos vitamin E en stark logisk grund för att anta att även andra former av vitamin E kan spela en roll i AD-patogenesen.
Syftet med detta projekt är att undersöka sambandet mellan alla åtta naturliga former av vitamin E med lindrig kognitiv svikt (mild cognitive impairment, MCI) och AD hos äldre personer, genom att kombinera både epidemiologiska och kliniska metoder.

Studie I. Plasmanivåer av alla åtta naturliga former av vitamin E och markörer för vitamin E-oxidativ/nitrosativ skada (α-tokoferylquinone, 5-nitro-γ-tokoferol), undersöktes hos personer med AD, MCI och normal kognition (NK) i en klinikbaserad, europeisk multicenterstudie (AddNeuroMed Project). Jämfört med personer med NK hade personer med AD och MCI lägre plasmanivåer av totala mängder av tokoferoler, tokotrienoler och vitamin E. Både personer med MCI och AD hade 85% lägre odds att vara i den högsta tertilen av totala mängder tokoferol och vitamin E i plasma, och de hade 92% respektive 94% lägre sannolikhet att vara i den högsta tertilen av totala tokotrienoler än i den lägsta tertilen. Vidare var både MCI och AD förknippade med ökade plasmanivåer av vitamin E-oxidativa/nitrosativa skador (förhållandet mellan α-tokoferylquinone/α-tokoferol och 5-nitro-γ-tokoferol/γ-tokoferol).

Studie II. Inom AddNeuroMed-projektet användes en analys som kombinerade plasmanivåer av olika former av vitamin E med parametrar från automatiserad regional analys av strukturell magnetröntgen (MR), för att differentiera personer med AD och MCI från individer med NK och för att predicera vilka personer som övergick från MCI till AD. När bara uppgifter från MR respektive om vitamin E användes var andelen rätt differentierade personer 83,2% respektive 92,8% för AD kontra CN och 58,1% respektive 87,8% för MCI kontra CN. När både uppgifter från MR och om vitamin E användes differentierades 98,2% rätt för AD kontra CN och 90,7% för MCI kontra CN. När kombinerade data användes, blev 85% av dem med MCI rätt identifierade som att övergå till AD och 67% rätt identifierade som att inte få AD, under ett års uppföljning.

Studie III. Sambandet mellan plasmanivåer av åtta naturliga former av vitamin E och förekomst av AD undersöktes i en svenskt populationsbaserad prospektiv studie (Kungsholmsprojektet) med personer 80 år och äldre, som följdes upp efter sex år. Personer med högre koncentrationer av totala mängder tokoferoler, tokotrienoler eller vitamin E (högsta jämfört med lägsta tertilen) hade cirka 50% minskad risk att utveckla AD jämfört med personer med lägre plasmanivåer.

Studie IV. Sambandet mellan serumnivåer av alla åtta naturliga former vitamin E och markörer för vitamin E-oxidativa/nitrosativa skador, och förekomsten av kognitiv svikt (MCI eller AD) undersöktes i en finsk populationsbaserad prospektiv studie (CAIDE) hos äldre personer (65+ år), som följdes upp efter åtta år. Oddsen för kognitiv svikt var lägre för personer i den mellersta tertilen av serumnivåer av γ-tokoferol, jämfört med den lägsta [odds ratio (95% konfidensintervall): 0,27 (0,10-0,78)]. Individer med ett högre serumindex av γ-tokoferol nitrosativ skada (förhållandet mellan 5-nitro-γ-tokoferol/γ-tokoferol; högsta och mellersta jämfört med den lägsta tertilen) hade ungefär tre gånger högre risk att försämras kognitivt.

Konlusion. α-tokoferol är den enda vitamin E-form som för närvarande används för att definiera behov av vitamin E i kosten och är den enda formen som är testad i randomiserade kontrollerade studier på patienter med AD och MCI. Resultaten från detta projekt tyder på att även de andra naturliga formerna av vitamin E kan vara viktiga vid kognitiv svikt och AD hos äldre personer. Därför bör alla naturliga vitamin E-former beaktas när man studerar sambanden mellan detta mikronäringsämne, kognitiv nedsättning och AD. Dessa resultat tyder också på att vissa aspekter av vitamin E-tillskott som förebyggande och behandling av AD bör omprövas. Denna omprövning bör innefatta tidpunkt för intervention, sammansättning av tillskotten och undersökning av plasmanivåer av alla former av vitamin E. Detta för att identifiera patienter som kan dra nytta av vitamin E-tillskott och för att observera biologisk in-vivo respons på behandlingen.

Nyckelord: Alzheimers sjukdom, klinisk studie, lindrig kognitiv svikt, nitrosativ stress, oxidativ stress, populations-baserad studie, tokoferol, tokotrienol, vitamin E, α-tokoferylquinone, 5-nitro-γ-tokoferol.

RIASSUNTO Italiano

La vitamina E è il principale antiossidante lipofilo, non-enzimatico, dell'organismo, ed ha un ruolo rilevante nella protezione del cervello dal danno causato dai radicali liberi. Il termine vitamina E descrive otto composti (congeneri) naturali: quattro tocoferoli e quattro tocotrienoli, denominati α-, β-, γ- e δ. La maggior parte della ricerca sul ruolo della vitamina E nella malattia di Alzheimer (Alzheimer´s disease, AD) ha preso in considerazione solo l'α-tocoferolo, riportando risultati contradditori. Tuttavia, numerosi studi in-vitro e su modelli animali hanno dimostrato le diverse proprietà biologiche degli otto congeneri della vitamina E, che potrebbero giocare un ruolo importante nella patogenesi dell'AD.
Lo scopo del presente progetto è lo studio della relazione tra le otto forme naturali della vitamina E e le diagnosi di decadimento cognitivo lieve (mild cognitive impairment, MCI) e di AD. Il progetto integra sia studi di popolazione che studi clinici.

Studio I. I livelli plasmatici delle otto forme naturali di vitamina E e due indici di danno ossidativo e nitrosativo di tale micronutriente (α-tocoferilchinone e 5-nitro-γ-tocoferolo) sono stati analizzati in casi di AD, MCI e soggetti cognitivamente integri (CN) nell'ambito di uno studio clinico europeo multicentrico (Progetto AddNeuroMed). Rispetto ai soggetti CN ridotti livelli plasmatici di tocoferoli totali, tocotrienoli totali e vitamina E totale sono stati riscontrati nei casi di AD e MCI. Inoltre, rispetto ai soggetti CN, la diagnosi di MCI e di AD è risultata associata a elevati valori di indici plasmatici di danno ossidativo e nitrosativo della vitamina E (rapporto α-tocoferilchinone/α-tocoferolo e 5-nitro-γ-tocoferolo/γ-tocoferolo).

Studio II. Nell'ambito del Progetto AddNeuroMed, dati sui livelli plasmatici di vitamina E e dati strutturali di risonanza magnetica (MRI) ottenuti da analisi automatica sono stati utilizzati in un'analisi integrata per valutare la loro accuratezza nel distinguere casi di AD e MCI da soggetti CN e nel predire la progressione dei soggetti con MCI ad AD. L'analisi separata dei dati sulla vitamina E e dei dati di MRI ha fornito un'accuratezza dell'83.2% e del 92.8% nella distinzione di AD *versus* CN, e del 58.1% e 87.8% nella distinzione di MCI *versus* CN. L'analisi integrata di entrambi i tipi di dati (vitamina E + MRI) ha migliorato tale accuratezza: per il confronto AD *versus* CN si è ottenuta un'accuratezza del 98.2%, mentre per il confronto MCI *versus* CN l'accuratezza è risultata del 90.7%. La combinazione di tali dati ha inoltre permesso di identificare correttamente l'85% dei soggetti con MCI che sono progrediti ad AD dopo un anno di follow-up ed il 67% degli MCI che sono rimasti clinicamente stabili.

Studio III. L'incidenza di AD, in relazione ai livelli plasmatici delle otto forme naturali di vitamina E, è stata esaminata in uno studio longitudinale di popolazione condotto su ultraottantenni svedesi (Progetto Kungsholmen) durato sei anni. I soggetti con elevati livelli plasmatici di tocoferoli totali, tocotrienoli totali e vitamina E totale (terzile superiore *versus* inferiore) hanno riportato un rischio ridotto del 50% circa di sviluppare AD.

Studio IV. L'incidenza di deterioramento cognitivo (AD o MCI), in relazione ai livelli plasmatici delle otto forme naturali di vitamina E e degli indici di danno ossidativo e nitrosativo di tale micronutriente, è stata esaminata in uno studio longitudinale di popolazione condotto su ultrasessantacinquenni finlandesi (Studio CAIDE) durato otto anni. Il rischio di declino cognitivo è risultato ridotto nei soggetti nel terzile medio del γ-tocoferolo [*versus* terzile inferiore; odds ratio (intervallo di confidenza 95%): 0.27 (0.10-0.78)]. Un aumento di circa tre volte del rischio di deterioramento cognitivo è stato osservato nei soggetti con valori elevati del rapporto 5-nitro-γ-tocoferolo/γ-tocoferolo (terzili superiore e medio *versus* inferiore).

Conclusione. L'α-tocoferolo è la sola forma di vitamina E presente nelle raccomandazioni nutrizionali ed è l'unica forma che è stata testata nelle sperimentazioni cliniche controllate nei soggetti con AD e MCI. I risultati del presente progetto indicano che non solo l'α-tocoferolo, ma anche le diverse altre forme di vitamina E, possono essere importanti nello sviluppo di MCI e AD negli anziani. Alla luce di questi dati le strategie basate sulla supplementazione con vitamina E andrebbero riesaminate. In particolare, gli aspetti da rivalutare sono la "finestra-terapeutica" e la composizione dei supplementi contenenti vitamina E. Il dosaggio ematico di tutti i congeneri della vitamina E può rivelarsi utile nell'identificare i soggetti che possono beneficiare della supplementazione e nel monitoraggio in-vivo degli effetti biologici della supplementazione stessa.

Parole chiave: decadimento cognitivo lieve, malattia di Alzheimer, stress nitrosativo, stress ossidativo, studio clinico, studio di popolazione, tocoferolo, tocotrienolo, vitamina E, α-tocoferilchinone e 5-nitro-γ-tocoferolo.

LIST OF ABBREVIATIONS

AD	Alzheimer's disease
5-NO_2-γ-tocopherol	5-nitro-γ-tocopherol
ADAS-Cog	Alzheimer's Disease Assessment Scale-cognitive subscale
ADCS	Alzheimer's Disease Cooperative Study
ADL	Activities of daily living
ADNI	Alzheimer Disease Neuroimaging Initiative
AHRQ	Agency for Healthcare Research and Quality
ANOVA	Analysis of variance
APOE	Apolipoprotein E
APP	Amyloid beta precursor protein
AVED	Ataxia with vitamin E deficiency
Aβ	β-amyloid
BMI	Body mass index
CAIDE	The Finnish Cardiovascular Risk Factors, Aging, and Dementia Study
CDR	Clinical Dementia Rating scale
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CHAP	Chicago Health and Aging Project
CI	Confidence interval
CN	Cognitively normal
CNS	Central nervous system
CSBA	Conselice Study on Brain Ageing
CSF	Cerebrospinal fluid
DSM-III-R, (DSM-IV)	Diagnostic and Statistical Manual of Mental Disorders, revised third edition, (Fourth edition)
FDG-PET	Fluorodeoxyglucose-positron emission tomography
FINMONICA	Finnish part of Monitoring trends and determinants of Cardiovascular disease study
GDS	Geriatric Depression Scale
HAAS	Honolulu-Asia Aging Study
HPLC	High Performance Liquid Chromatography
HR	Hazard ratio
IU	International unit
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase
LR	Likelihood ratio
MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
NFT	Neurofibrillary tangle

NIH	National Institute of Health
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association
NS	Nitrosative stress
OPLS	Orthogonal partial least squares to latent structures
OR	Odds ratio
OS	Oxidative stress
PAQUID	Personnes Agées QUID
PCR	Polymerase chain reaction
PET	Positron emission tomography
PUFA	Polyunsaturated fatty acid
RCT	Randomized controlled trial
RDA	Recommended dietary allowance
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SD	Standard deviation
SES	Socioeconomic status
SIMCA	Soft Independent Modeling of Class Analogy
SP	Senile plaque
SPSS	Statistical Package for the Social Sciences
ULSAM	Uppsala Longitudinal Study of Adult Men
VLDL	Very low density lipoproteins
WHICAP	Washington Heights-Inwood Columbia Aging Project
α-TQ	α-tocopherylquinone
α-TTP	α-tocopherol transfer protein

LIST OF ORIGINAL PUBLICATIONS

This doctorial thesis is based on the following original papers, referred to in the text by their Roman numerals:

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CONTENTS

O frati…considerate la vostra semenza:
fatti non foste a viver come bruti,
ma per seguir virtute e conoscenza

(Dante Alighieri*, Inferno,* canto XXVI, vv.112-120)

1 INTRODUCTION

1.1 Dementia and Alzheimer's disease: epidemiology

Dementia is an acquired disabling syndrome characterized by progressive deterioration in multiple cognitive domains that is severe enough to interfere with daily functioning, including social and professional functioning.[1] Alzheimer's disease (AD) is the most common cause of dementia, but increasing evidence from population-based neuropathological and neuroimaging studies shows that mixed brain pathologies (neurodegenerative, vascular) account for a large number of dementia cases, especially in very old people.[2,3]

Increasing age is a well-established risk factor for dementia and AD. Both prevalence and incidence of dementia rise exponentially with advancing age, and 70% of all dementia cases occur in people aged 75+ years.[4] The worldwide increase in the number of older adults, which is particularly pronounced in the 80+ age group, explains the epidemic proportions assumed by dementia. Because dementia is a major cause of disability and institutionalization of elderly people, the increased prevalence of this syndrome places enormous pressure on health care systems and society. According to the World Alzheimer Report, there were 35.6 million people living with dementia worldwide in 2010, a number that will increase to 65.7 million by 2030 and 115.4 million by 2050 unless effective means of reducing disease incidence are introduced.[5] The total estimated worldwide costs of dementia were US$604 billion in 2010, including the costs of informal care (unpaid care provided by family and others), direct costs of social care (provided by community care professionals, and in residential home settings), and the direct costs of medical care (the costs of treating dementia and other conditions in primary and secondary care).[5]

The etiology of dementia has been extensively studied over the last decades with the aim of identifying efficacious prevention and treatment strategies. These efforts have indicated that dementia/AD are multifactorial disorders involving several interrelated mechanisms in which the interaction of genetic and environmental factors plays a major role. The pathways that lead from different risk factors to dementia are not fully understood, but several etiological hypotheses have been proposed: the vascular hypothesis; inflammatory hypothesis; oxidative-stress hypothesis; toxic hypothesis; and psychosocial hypothesis.[6,7] These theories highlight potential links of various risk factors to both AD and the vascular brain pathology that can cause dementia.[8,9]

A recent report commissioned by the American National institute of Health (NIH) to the Agency for Healthcare Research and Quality (AHRQ) concluded that current research evidence on many risk and protective factors for cognitive decline and AD is not of sufficient strength, thus recommendations for preventing these conditions cannot be made.[10,11] Another previous review yielded similar conclusions.[12] These negative

perspectives have been criticized, since epidemiological evidence that use of antihypertensive medications, cessation of smoking and increasing physical activity produces cognitive benefits in older adults is not inadequate,[13] and the analytical strategy used in the evidence based review did not take into account the life-course perspective.[14] In fact, observational longitudinal studies show that the risk of late-life dementia/AD is determined by exposures to multiple factors experienced over the life-span, and that the effect of specific risk/protective factors largely depends on age (Table 1).

Table 1. Proposed risk and protective factors for dementia and Alzheimer's disease

Risk factors	Protective factors	Combined effects
Age	**Psychosocial factors**	**Increased risk**
Genetic	High education and SES	*Genetic and environmental factors in midlife*
*APOE*ε4	High work complexity	*APOE*ε4 magnifies the effect of high alcohol intake, smoking, physical inactivity and high intake of saturate fat
Familial aggregation	Rich social network and social engagement	
Vascular	Mentally stimulating activity	*Vascular factors in midlife*
Cerebrovascular lesions	**Lifestyle**	Hypertension, obesity, hypercholesterolemia and physical inactivity have an additive effect when they co-occur
Cardiovascular diseases	Physical activity	
Diabetes mellitus and pre-diabetes	**Diet**	*Vascular factors/diseases in late-life*
Midlife positive association but late-life negative association	Mediterranean diet	Higher risk in individuals with brain hypoperfusion profile: chronic heart failure, low pulse pressure, low diastolic pressure
Hypertension	Polyunsaturated (PUFA) and fish-related fats	Higher risk in individuals with atherosclerosis profile: high systolic pressure, diabetes mellitus or prediabetes, stroke
High BMI (overweight and obesity)	Vitamin B_6, B_{12}, folate	
High serum cholesterol	Antioxidant vitamins (C, E)	**Decreased risk**
Lifestyle		*Genetic and environmental factors in midlife*
Smoking		High education reduces the negative effect of *APOE*ε4
High alcohol intake		Physical activity counteracts the risk due to *APOE*ε4
Diet		*Environmental factors in midlife*
Saturated fats		High work complexity modulates the increased dementia risk due to low education
Homocysteine		
Others		
Depression		

APOE: apolipoprotein E. BMI: body mass index. PUFA: polyunsaturated fatty acid. SES: socioeconomic status.

Thus, a life-course perspective is relevant for chronic disorders with a long latent period (such as dementia/AD). It allows the identification of time windows when exposures have their greatest effect on outcome and enables the assessment of whether cumulative exposures could have multiplicative or additive effects over the life course.[7] Age-dependent associations with dementia/AD have been suggested for several aging-related medical conditions. For example, elevated blood pressure, body mass index (BMI) and total cholesterol levels at a young age and in middle age (<65 years) is associated with an increased risk of dementia and AD, whereas having lower values in late-life (age >75 years) is also associated with subsequent development of dementia/AD.[15-20] Diabetes mellitus has been associated with an increased risk of dementia and AD over adult life, but the risk is stronger when diabetes occurs in mid-life than in late-life.[21] Current smoking is another major risk factor for dementia and AD, and based on the worldwide prevalence of smoking, about 14% of all AD cases are potentially attributable to this risk factor.[22] Although it is not entirely clear whether depression is a risk factor for or a preclinical symptom of dementia, studies with long-term follow-up support the risk factor hypothesis.[23]

Protective factors for dementia and AD have also been identified, including high education and socioeconomic status (SES) in early life as well as a number of factors in adult life: high work complexity, rich social network, social engagement, mentally-stimulating activity, not smoking and regular physical exercise.[6,24,25] Living with a partner during midlife has been associated with reduced risk of cognitive impairment and dementia later in life, suggesting that being in a relationship entails cognitive and social challenges that can increase cognitive reserve.[26] Even at old ages active engagement in mental, physical, and social activities may postpone the onset of dementia, possibly by increasing cognitive reserve.[27] In addition, several follow-up studies reported a decreased risk of dementia associated with healthy dietary patterns and nutritional factors, such as high adherence to a Mediterranean diet, vitamins (B_6, B_{12}, C, E, folate) and ω-3 polyunsaturated fatty acid (PUFA), which is often measured as fish consumption.[28-31]

Cumulative and combined exposure to different risk factors can lead to modified effects on dementia/AD risk (Table 1). In the Finnish Cardiovascular Risk Factors, Aging and Dementia study (CAIDE), the risk of dementia has been evaluated in relation to a score (CAIDE Dementia Risk Score) combining midlife risk factors, including low education and cardiovascular factors (i.e., hypertension, obesity, hypercholesterolemia, physical inactivity). The risk of dementia increases as the score increases in a dose-response trend, making it possible to identify individuals who can greatly benefit from preventive interventions that target vascular risk factors.[32] Similar findings have been reported for late-life exposures: in the Swedish Kungsholmen Project, the cumulative effect of vascular risk factors and vascular diseases on dementia/AD risk has been investigated in people aged 75+ years. These factors were aggregated according to two pathophysiological

hypotheses: the brain hypoperfusion profile, defined by chronic heart failure, low pulse pressure, and low diastolic pressure, and the atherosclerosis profile, which included high systolic pressure, diabetes mellitus or prediabetes, and stroke. In both profiles, dementia/AD risk increases with increasing scores in a dose-response manner, suggesting a synergy of vascular risk factors in promoting dementia/AD also in advanced age.[33] The American Cardiovascular Health Cognition Study developed a Late-life Dementia Risk Index, which groups older adults into three categories of low, moderate, and high risk of developing dementia. This index supports the cumulative effect of different factors in determining the risk of dementia after the age of 65 years, and it includes information from different domains, including demographic factors (age), genetic (presence of the Apolipoprotein E (*APOE*)-ε4 allele), lifestyle (BMI<18.5, lack of alcohol consumption), comorbid vascular conditions (internal carotid artery thickening, angina, coronary artery by-pass surgery, stroke, peripheral artery disease), evidence of brain abnormalities showed by magnetic resonance imaging (MRI) (white matter diseases or enlarged ventricles), cognitive test scores and physical performances.[34,35]

The combined effect of genetic-environmental or environmental-environmental joint exposures may also lead to the attenuation of the dementia risk. Population-based studies suggest an effect modification for the *APOE* ε4 allele, the most important genetic risk factor for sporadic AD. *APOE* ε4 carriers seem more vulnerable to risk factors like alcohol drinking, smoking, physical inactivity, and high intake of saturate fat, indicating that people with genetic susceptibility may reduce their initial AD risk by lifestyle interventions (i.e., physical activity, sufficient intake of PUFA, and avoiding excess alcohol drinking and smoking).[36] Further, it has been shown that high education may reduce dementia risk among *APOE* ε4 allele carriers.[37] Regarding the interactions among modifiable risk factors, results from the Kungsholmen Project suggested that complexity of work with data and people was related to a decreased dementia risk and that the highest level of work complexity may modulate the increased dementia risk due to low education.[24]

In conclusion, even though evidence is sparse for some risk and protective factors and their role in dementia and AD needs to be clarified, evidence from observational studies points towards a multifactorial nature of dementia/AD, with age-dependent associations for several factors. Epidemiological findings indicate the possibility to prevent or delay dementia/AD onset, by addressing modifiable risk/protective factors. It has been estimated that half of AD cases worldwide are potentially attributable to modifiable risk factors, and a 10-25% reduction in these factors could potentially prevent three million AD cases worldwide, with a reduction in all risk factors having the greatest impact on dementia prevalence.[22] However, randomized controlled trials (RCT) are needed to confirm the effect of risk reduction strategies targeting multiple risk factors. Multidomain interventional RCT, which aims to simultaneously target several risk factors for dementia in older adults, are now ongoing and will provide new insights into prevention of cognitive

impairment and dementia/AD.[38]

1.2 Alzheimer's disease diagnosis: still a challenge

The pathogenetic mechanisms leading to selective neuronal loss in AD are still unclear and effective treatments are slow to emerge.[39] AD neuropathology is characterized by regionalized neuronal death, synaptic loss, accumulation of intraneuronal neurofibrillary tangles (NFT) and extracellular senile plaques (SP), and proliferation of reactive astrocytes in the entorhinal cortex, hippocampus, amygdala and association areas of frontal, temporal, parietal, and occipital cortex. NFTs are formed by intracellular deposits of paired helical filaments composed of hyperphosphorylated tau. SP can be present as diffuse plaques, composed of amorphous extracellular deposits of β-amyloid (Aβ) that lack neuritis, and as neuritic plaques, which consist of extracellular deposits of insoluble Aβ surrounded by dystrophic neurites, reactive astrocytes, and activated microglia. Aβ is a 39-43 amino acid peptide derived from the larger amyloid beta precursor protein (APP) by proteolytic cleavage. Aβ 1-40 is the most frequent form of Aβ even if the minor species (i.e., Aβ 1-42) has a higher propensity to aggregate and is greatly enriched in amyloid deposits. Recent studies suggest that soluble Aβ oligomers are present in the AD brain and they may represent the main toxic form of Aβ.[40-42] Aβ accumulation is considered a key early event in AD pathophysiology, but its role in sporadic late-onset AD has yet to be clarified. There may be pathophysiological events that are "upstream" of Aβ accumulation, including mitochondrial, metabolic, inflammatory, oxidative/nitrosative stress (OS/NS) alterations, which may play an even earlier, or more central, role than Aβ peptides in the pathogenesis of AD.[43-47]

AD clinical onset is insidious and often characterized by memory impairment. The disease progressively evolves over a period of years, eventually leading to global cognitive impairment.[48] AD is frequently manifested by a symptomatic pre-dementia phase, which is often identified as mild cognitive impairment (MCI). Although there are different definitions of MCI, the general concept is of a condition in which memory or other cognitive abilities are slightly abnormal but they coexist with predominantly normal functions in activities of daily living (ADL) and absence of dementia.[49,50] MCI has been related to an increased risk of conversion to dementia and it may be considered, in many cases, prodromal AD. Patients in memory disorder clinics who are diagnosed with MCI progress to AD at the rate of 10-15% per year, while population-based studies show a lower rate of progression with some individuals remaining stable or even improving.[51-54]

Clinical diagnosis of AD is currently based on the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria, established in 1984.[55] These criteria essentially involve a two-step procedure, in which i) the presence of a dementia syndrome is defined, and ii) typical features of the AD phenotype (i.e., presence of memory impairment, gradual

onset and progression) are identified, and the presence of systemic or central nervous system (CNS) diseases capable of producing a dementia syndrome is excluded.

These diagnostic criteria rely on the close correspondence between clinical symptoms and the underlying brain pathology, but research on AD conducted in the last three decades has shown that AD neuropathology and the AD clinical syndrome should not be considered as synonymous of each other.[56] Indeed, extensive AD pathology, particularly diffuse SPs, can be present in individuals who are cognitively normal and in those with MCI.[57-60] Moreover, AD pathophysiology can manifest itself with clinically atypical presentations, such as prominent language and visuospatial disturbances.[61-63]

The NINCDS-ADRDA criteria create a dichotomy between dementia and non-dementia, not considering the spectrum of cognitive impairment. Indeed, several studies have shown that the pathophysiological process of AD begins years, if not decades, before the diagnosis of Alzheimer's dementia and individuals generally experience a gradual impairment of cognitive functions, which can progress to a dementia syndrome.[64,65] Recent advances in neuroimaging, cerebrospinal fluid (CSF) assays, and other techniques now provide the ability to detect evidence of the AD pathophysiological process in vivo, but the diagnostic criteria currently in use do not take into account these biomarkers.

Three international workgroups promoted by the American National Institute of Aging and the American Alzheimer's Association recently proposed new diagnostic guidelines to identify dementia due to AD, MCI due to AD, and preclinical AD.[45,66,67] These new criteria formalize the different clinical stages of AD and incorporate biomarkers (genetic, biochemical, neuroimaging) that can be detected in vivo and are believed to reflect AD pathology. These diagnostic criteria can be revised as long as new findings from research on biomarkers in AD will clarify the link between AD pathophysiology and the AD clinical syndrome.

Role of biomarkers

Biomarkers are defined as characteristics that can be objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.[68]

The new diagnostic criteria for AD, MCI due to AD and preclinical AD include two main categories of biomarkers: i) biomarkers of Aβ accumulation, which are abnormal tracer retention on amyloid positron emission tomography (PET) imaging and low CSF Aβ 1-42, and ii) biomarkers of neuronal degeneration or injury, which are elevated CSF tau (both total and phosphorylated tau); decreased fluorodeoxyglucose uptake on PET (FDG-PET) in a specific topographic pattern involving temporoparietal cortex; and atrophy on MRI - again in a specific topographic pattern - involving medial, basal, and lateral temporal lobes and medial and lateral parietal cortices.[56]

The choice of these biomarkers in the new diagnostic recommendations is based on the

assumption that SPs and NFTs define AD neuropathology. The diagnostic criteria also postulate the presence of a temporal order of biomarker abnormalities, and assign a key, early role to Aβ in AD pathophysiology. Validation and standardization of the proposed biomarkers are needed before they can be implemented in clinical practice, and the diagnostic recommendations emphasize that the clinical diagnosis is still paramount for both AD-related dementia and MCI. Further, since the diagnosis of pre-symptomatic AD is based almost entirely on biomarkers, it is intended only for research purposes.[56]

Among other candidates for biomarkers, there are compounds reflecting biochemical changes related to processes occurring in AD, such as OS/NS and inflammation. Markers of these processes may provide information about specific pathways that are altered in AD, but their utility in AD diagnosis is still unclear.[66]

The combination of different biomarkers may prove to be more useful than using single biomarkers separately. For instance, a large amount of data on different brain regions can be obtained with structural MRI, and the combined analysis of these data can increase the diagnostic accuracy for MCI and AD, compared to the examination of single brain regions.[69-71] Combined analysis could also be done by integrating biomarkers assessed with different modalities. Blood/plasma markers are less invasive to acquire than CSF and could support diagnosis and monitoring of disease progression in AD.[72,73] Combining different types of biomarkers, including neuroimaging, CSF, blood and neuropsychological data, could increase their diagnostic and prognostic value for AD and MCI, but few studies have tested the utility of a multimodal biomarkers approach.[74-76]

1.3 Alzheimer pathophysiology: oxidative and nitrosative stress

1.3.1 Definition of oxidative and nitrosative stress

OS/NS are promoted by the dysregulation of the redox balance, caused by a deficiency of antioxidants and/or overproduction of free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS).[77,78] Exogenous agents (e.g., photochemical smog, ozone, pesticides, xenobiotics and ionizing radiation) and a variety of endogenous processes (mitochondrial respiration, cytochrome P-450 detoxification reactions, phagocytic oxidative bursts, and peroxisomal leakage) can generate significant amounts of ROS and RNS in the human body.[79] Indeed, both species of free radicals are products of normal cellular metabolism, and mitochondrial oxidative phosphorylation generates the majority of free radicals in the cell.[43] ROS/RNS take part in many physiological processes, including cellular signaling, reactions to stress/noxia, induction of mitogenic and apoptotic responses.[77,78] However, ROS and RNS can be harmful as they can damage cellular components, thus inhibiting their normal function, which can compromise cell viability or induce cell death.[78]

Living systems have developed mechanisms with which to control the harmful effects of ROS/RNS. These systems are mainly based on: i) the presence of antioxidants, both

enzymatic (e.g., catalase, superoxide dismutase, glutathione peroxidase) and non-enzymatic (e.g., vitamin A, C, E, β-carotene, glutathione, uric acid); ii) the repair or removal of damaged molecules. The wide variety of antioxidant defence systems help to prevent and repair ROS and RNS-induced damage.[80] If free radicals production is not balanced by their neutralization OS/NS can occur.

Free radical attack against lipids, proteins, sugars and nucleic acids leads to the formation of respective bioproducts, which can be assessed in tissues and provide information on the oxidant/antioxidant balance of the organism. Measurements of antioxidants' levels or activities can also be used for the same purpose.

1.3.2 Oxidative and nitrosative stress in Alzheimer's disease and mild cognitive impairment

The fact that age is the main risk factor for AD has provided the basis for the involvement of oxidative and nitrosative imbalance in this disease, since products of oxidative and nitrosative damage do accumulate during aging, and senescence seems to be characterized by a shift from redox regulation to oxidative and nitrosative damage.[78,81] Also, the brain is particularly vulnerable to oxidative/nitrosative damage since: i) it contains a high proportion of PUFAs, which are highly susceptible to lipid peroxidation, and catecholamines prone to autoxidation; ii) it has a high metabolic activity that requires large amounts of oxygen (the brain uses approximately 20% of the oxygen consumed by the resting body); iii) it is relatively deficient in antioxidant systems with a lower activity of glutathione peroxidase and catalase compared to other organs; and iv) it contains redox-active metals (copper, iron) that can promote the production of free radicals.[43,82,83]

In the mid-1950's Harman was among the first to suggest that OS may promote age-related degenerative diseases causing dementia.[84] Since then, several experimental, clinical, neuropathological and population-based observational studies have shown data supporting the role of OS/NS as central processes in AD pathophysiology.[43,78] It is still unclear whether OS/NS are primary initiating events associated with neurodegeneration or a secondary effect related to other pathological pathways. It is likely that OS/NS are both early, "upstream" events which contribute to AD pathogenesis, and also secondary, "downstream" consequences of other pathogenetic mechanisms, which amplify oxidative/nitrosative damage, thus generating a vicious circle promoting neurodegeneration.[46,78,85]

Oxidative/nitrosative modification to virtually all classes of biomacromolecules has been described in brain regions susceptible to degeneration in subjects with AD and MCI, and also in the peripheral tissues (i.e., blood, urine) of these individuals.[43] In addition to their individual role, biomarkers of OS/NS in AD have been shown to be associated with altered bioenergetics and Aβ metabolism.[43,44,78,86]

It is not fully clear whether the increased oxidative/nitrosative damage in MCI and AD

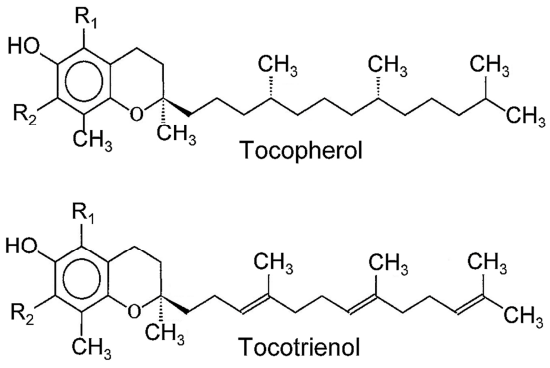
subjects constitutes an acceleration of the "normal" age-related raise in OS/NS, or whether alternative pathways of free radicals production are involved. Several sources/conditions causing ROS/RNS hyperproduction have been suggested:[87] i) loss of metal homeostasis, with a subsequent accumulation of iron and/or copper in the brain;[88,89] ii) activation of microglia/astrocytes, such as those surrounding most SPs;[90,91] iii) mitochondrial dysfunction, which seems to be a major source of ROS in AD;[87,92-94] iv) APP and Aβ, which have been directly implicated in ROS formation.[95-97] Specifically, Aβ oligomers seem to be more efficient in promoting OS, compared to the fibrilized form of Aβ.[98] Oxidative damage may, in turn, promote amyloid deposition.[78,99,100]

Not only free radical hyperproduction, but also a less efficient antioxidant system may predispose the accumulation of oxidative and nitrosative damage in AD. Particularly, reduced antioxidant defences, due to low levels of non-enzymatic antioxidants (e.g., vitamin E), have been hypothesized to increase the risk of dementia and AD.

The role of antioxidants in AD, including vitamin E, is not yet understood, but it is becoming clear that evaluation of these compounds could help in clarifying AD etiopathogenesis, and provide information which might support the monitoring of disease progression and of treatment based on antioxidant supplements.[43]

1.4 Vitamin E

Vitamin E was discovered by Evans and Bishop (1922) as a dietary factor necessary for reproduction in rats.[101] Since it supported fertility, vitamin E was scientifically named as tocopherol. This comes from the Greek word *tokos* meaning childbirth, and *phero* meaning to bring forth, and the *ol* ending was added to indicate the alcohol properties of this molecule. The term vitamin E includes eight naturally occurring, lipophilic compounds (congeners): four tocopherols and four tocotrienols, named as α-, β-, γ-, and δ (Figure 1).[102]



	R_1	R_2
α	CH_3	CH_3
β	CH_3	H
γ	H	CH_3
δ	H	H

Figure 1. Structures of tocopherols and tocotrienols.

Tocopherols consist of a chromanol ring and a saturated side chain, which serves to anchor tocopherols in biological membranes. Tocotrienols differ structurally from tocopherols by the presence of an unsaturated side chain, with three trans-double bonds, at C3, C7 and

C11. The four members of each family (*α*-, *β*-, *γ*-, *δ*-) differ by the number and placement of methyl groups on the chromanol ring (Figure 1).

Vitamin E is an essential fat soluble nutrient, which is synthesized exclusively by photosynthetic organisms.[103] So far there is no evidence that the different vitamin E forms can be interconverted in the human body.

The main dietary sources of vitamin E are vegetable oils. Tocopherols are found mainly in wheat germ oil, almonds, sunflower and olive oil, which are rich in α-tocopherol. *γ* -tocopherol is the major form of vitamin E in corn oil and soybean oil. Major food sources of tocotrienols are rice bran, barley, oats, and palm oil.[104] Palm oil is one of the most abundant natural sources of tocotrienols. It contains a mixture of tocopherols and tocotrienols, and is used by the food industry (e.g., bakery products, breakfast cereals). α-tocotrienol is the predominant form of tocotrienol in oat and barley, while β-tocotrienol is the major form of tocotrienol found in wheats. Rice bran oil, common in Asian countries, is a major natural source of γ-tocotrienol.[103,105]

Other dietary sources of vitamin E are animal fats, dairy products, fruit, and vegetables. α-tocopherol is the main form of vitamin E present in the European diet, while γ-tocopherol intake is higher than α-tocopherol in the USA diet.[106] It has been estimated that, in the Western diet, about 50% of α-tocopherol is supplied by vegetable oils, 25% by animal fats, meat, and dairy products, and 25% by vegetables and fruit. Similar data for the other vitamin E forms are not available.

1.4.1 Vitamin E metabolism

The intestinal absorption of vitamin E ingested with food or supplements follows that of lipids. Specifically, absorption of vitamin E requires the secretion of pancreatic esterases and bile acids. These secretions are needed for the micellarization of dietary fats, including vitamin E, and the hydrolysis of triglycerides that release free fatty acids. The micelles are taken up by intestinal enterocytes. Vitamin E is subsequently incorporated into chylomicrons, which are secreted into the lymphatic system, and thus enters into the bloodstream. The different vitamin E forms are equally absorbed and incorporated into the chylomicrons.[102,107,108] In the peripheral circulation, part of the newly absorbed vitamin E is released and transferred from chylomicrons to tissues and to other lipoproteins, during the lipoprotein lipase (LPL) activity and by nonspecific transfers through lipoproteins. The rest of the vitamin E remains with the chylomicron remnants and reaches the liver, where the uptake is mediated by the low-density lipoprotein (LDL) receptor.[102,108] The liver repackages the dietary fats into very low density lipoproteins (VLDL) for secretion into the plasma and uptake by peripheral tissues. The liver has a key role in regulating vitamin E distribution. In the hepatocytes the α-tocopherol transfer protein (α-TTP) enriches the nascent VLDL with vitamin E, and α-TTP has a high preference for α-tocopherol, which is the main form incorporated in the VLDL. The importance of the α-TTP for tissue delivery

of α-tocopherol is confirmed by the severe α-tocopherol deficiency detected in subjects with a genetic deficit of the α-TTP, which compromise α-tocopherol transport in the VLDL. This disease, defined as "ataxia with vitamin E deficiency" (AVED), is characterized by progressive peripheral neuropathy with ataxia, and visual and cognitive problems.[106]

The role of α-TTP in the transport of non α-tocopherol forms is still unclear. However, even if less efficiently, non α-tocopherol vitamin E forms can bind α-TTP.[109] The relative affinities of α-TTP for different vitamin E congeners have been estimated to be 100% for α-tocopherol, 38% for β-tocopherol, 9% for γ-tocopherol, 2% for δ-tocopherol, and 12% for α-tocotrienol. Furthermore, other tocopherol binding proteins have been described,[110] and tocotrienols may be carried in plasma through lipoproteins.[111] As a result, all eight natural vitamin E forms can be delivered to tissues, including the CNS. Indeed, α-, β- and γ-tocopherol have been detected in the brain,[112,113] and recent studies have shown that tocotrienols are delivered to the CNS, where they reach concentrations adequate for beneficial biological activity.[114]

The liver has a main role also in vitamin E excretion; within the hepatic cells vitamin E undergoes several steps of oxidation and enzymatic conjugation, leading to final metabolic products, which are excreted in either the bile or the urine. Different vitamin E forms are metabolized at different metabolic rates, with non α-tocopherol forms being metabolized faster than α-tocopherol, and a fraction of vitamin E is directly excreted by the liver without metabolic transformation.[108] Many aspects about hepatic vitamin E trafficking and biotransformation in humans are not yet clear. It is clear, however, that the liver has a critical role in determining plasma levels of vitamin E forms. This is due to the selective affinity of α-TTP for the different vitamin E forms, and the regulation of vitamin E metabolism and excretion.[106]

Plasma α-tocopherol concentration in humans range from 11 to 37 μmol/L, whereas γ-tocopherol concentration is generally 2 to 5 μmol/L, and tocotrienols levels are less than 1 μmol/L. When plasma lipids are taken into account, the lower limit of normal is 2.5 μmol α-tocopherol/mmol cholesterol.[106] Plasma concentrations of α-tocopherol are generally higher in European populations, compared to USA cohorts, while there is an opposite trend for γ-tocopherol levels. This difference is likely to reflect the high consumption in Europe of sunflower and olive oil (rich in α-tocopherol), and the high consumption in USA of soybean and corn oils (rich in γ-tocopherol).[106,115]

In tissues, different amount of specific vitamin E forms are found, suggesting the presence of tissue specific mechanisms for enrichment and/or storage of tocopherols and tocotrienols, but there is scarce information on this aspect in humans.[110]

1.4.2 Vitamin E biological properties

The evidence on vitamin E biological properties comes from in-vitro studies, studies in

animal models, and human studies (Table 2). The interest of researchers in tocotrienols is relatively recent, with a lower number of human studies as compared to α-tocopherol, which is the most investigated vitamin E congener.

The most well-known function of vitamin E is that of a chain-breaking antioxidant, that prevents the cyclic propagation of lipid peroxidation of PUFAs in cell membranes and lipoproteins. Peroxidation of membrane lipids is known to modify and inactivate cellular components, which can have damaging effects on crucial cellular factors leading to disease. Vitamin E functions in vivo as a potent peroxyl radical scavenger; α-tocopherol chemical reaction with oxidized lipids (peroxyl radicals) leads to the production of oxidized α-tocopherol (tocopheroxyl radical), which is regenerated to α-tocopherol by reductants compounds, such as vitamin C, ubiquinol, and glutathione.[102,106,116]

α-tocopherol is considered the major lipid-soluble, non-enzymatic antioxidant in the human body.[117] However, all tocopherols and tocotrienols have antioxidant activity, with γ-tocopherol being highly effective in scavenging RNS,[103,113] and tocotrienols being more efficient than α-tocopherol in neutralizing free radicals. This could be explained by different mechanisms, including i) the presence of the unsaturated side chain, which allows tocotrienols to distribute more efficiently into cell membranes; ii) a more efficient interaction of the chromanol ring with lipid radicals; and iii) a higher recycling efficiency from chromanoxyl radicals.[103,118]

α-tocopherylquinone (α-TQ) is among the main products of α-tocopherol oxidation, while 5-nitro-γ-tocopherol (5-NO_2-γ-tocopherol) is generated by γ-tocopherol reaction with RNS. It has been suggested that analysis of the relative levels of these compounds can provide information on the body level of OS/NS.[113,116,119,120]

Vitamin E biological activity is not limited to antioxidant properties. Vitamin E forms are involved in the regulation of membrane-bound enzymes, gene expression, inflammatory response, cellular trafficking, and proliferation.[103] Each vitamin E congener is functionally unique and exhibits specific biological functions, often not shared by the other family members (Table 2). The specificity of these biological properties is believed to rely on selective interactions of each vitamin E congener with enzymes, structural proteins, lipids, and transcription factors.[110]

Because of their biological properties, vitamin E forms can play an important role in different diseases, including atherosclerosis and cardiovascular disease, cancer, and neurodegenerative disorders (AD, Parkinson's disease, amyotrophic lateral sclerosis).[85,103,121]

Biological properties of vitamin E that could be relevant for neuroprotection include antioxidant and anti-inflammatory activity and modulation of different signaling pathways (Table 2).[103,122] As previously mentioned, OS/NS are believed to be key processes in AD onset and progression. Being the main lipid-soluble antioxidant, vitamin E has a prominent role in protecting cellular membranes. This could be crucial in mitochondria, since they are

both a main source and elective target of free radicals, and their dysfunction can promote neurodegeneration.[123]

Table 2. Tocopherols and tocotrienols biological properties.[103,110,122,124-127]

Vitamin E form	Biological properties
Tocopherols	*All forms* - antioxidant - transcriptional and/or post-translational modulation of proteins expression and activity (specific for each congener)
	α-tocopherol - anti-inflammatory activity, inhibition of platelet and monocyte adhesion, inhibition of adhesion molecules expression - modulation of cell proliferation - regulation of bone remodelling, by stimulating osteoclast fusion
	γ-tocopherol - anti-inflammatory and natriuretic functions - antineoplastic activity, due to modulation of signal transduction and apoptosis, and inhibition of cell proliferation
	δ-tocopherol - antineoplastic activity, due to modulation of signal transduction and apoptosis, and inhibition of cell proliferation
Tocotrienols	*All forms* - antioxidant - transcriptional and/or post-translational modulation of proteins expression and activity (specific for each congener)
	*All forms** - cholesterol lowering activity (total cholesterol, LDL cholesterol), due to modulation of the HMG-CoA-reductase - anti-aging effect, in cellular models of senescence and in animal models (extended mean life span) - antineoplastic activity (several cancer models), due to modulation of cell growth, proliferation and apoptotic signaling, and regulation of immunological functions - anti-inflammatory, anti-angiogenetic and anti-atherogenic activity, inhibition of adhesion molecules expression and monocyte adhesion
	α-tocotrienol - inhibition of the glutamate-induced neurotoxicity and neuronal death by modulating proteins activity, including phospholipase A2 and 12-lipoxygenase - protection against stroke related damage
	γ-tocotrienol - natriuretic properties
	δ-tocopherol - antineoplastic activity, due to modulation of signal transduction and apoptosis, inhibition of cell proliferation

*In some studies the biological activity has been tested by using tocotrienol rich fractions (TRF), which contain different proportions of all natural tocotrienols.
HMG-CoA-reductase: 3-hydroxy-3-methyl-glutaryl coenzyme A reductase.

Inflammatory pathways also seem to be involved in AD pathogenesis,[44] and some vitamin E forms (α- and γ-tocopherol, tocotrienols) exhibit anti-inflammatory properties that appear to be more potent for the non-α-tocopherol congeners.[103,122,124] Modulation of cellular signaling can also be relevant for neuroprotection. At nanomolar concentrations, α-

tocotrienol prevents neurodegeneration in mice and rat neurons, by regulating specific mediators of cell death.[103] In fact, α-tocotrienol attenuates the Arachidonic Acid cascade leading to oxidative brain injury and inhibits the glutamate-induced neurotoxicity by modulating the phospholipase A_2 activity.[128,129] Finally, in experimental studies in transgenic mice models of AD, the potential role of α-tocopherol in reducing β-amyloid deposition and β-amyloid induced damage has been shown.[85,130]

α-tocopherol is the only vitamin E form currently used to estimate vitamin E dietary requirements in humans,[131] since it has the highest bioavailability and α-tocopherol deficiency causes well-known clinical signs and symptoms, including erythrocyte hemolysis and peripheral neuropathy.[106,132]

Despite the official definition of vitamin E dietary requirement, which considers only α-tocopherol, the increasing knowledge of vitamin E family biological properties provides a strong biological rationale of a possible role of different vitamin E forms in human health and disease, including dementia and AD.

1.4.3 Vitamin E, dementia and Alzheimer's disease: clinic- and population-based observational studies

In human studies the relationship between vitamin E and dementia/AD has been investigated mainly by measuring vitamin E concentration in serum/plasma or by assessing vitamin E dietary (i.e., food sources) or supplemental intake.

Vitamin E plasma and serum levels

Several studies investigating the association between vitamin E plasma/serum levels and dementia/AD are cross-sectional analyses in small clinical samples (Table 3). In all these studies only α-tocopherol has been evaluated, and usually absolute plasma/serum values of this vitamin have been compared between subjects with dementia/AD (different disease severity among studies) and controls. However, vitamin E biokinetics is tightly related to lipoproteins, and lipid levels need to be considered when assessing the effects of vitamin E on any outcome; standardization to total cholesterol (absolute plasma/serum concentration of vitamin E divided by total cholesterol) is commonly used.[115,133,134] There is indeed some evidence that the association of vitamin E with cognitive decline/dementia is influenced by concurrent cholesterol levels, but data on this aspect are scarce.[115,135-137]

All cross-sectional clinical studies have reported reduced α-tocopherol plasma/serum levels in subjects with dementia/AD compared to controls[138-149] or no association.[148,150-155] The majority of the studies reporting reduced α-tocopherol levels in dementia/AD cases were done in non-malnourished subjects, while in some other studies the reduced value of α-tocopherol could be explained by malnourishment. Some studies showed decreased plasma α-tocopherol also in subjects with MCI,[138,147] while other studies did not confirm this finding.[115,136]

Cross-sectional analyses in population-based studies have also shown conflicting results, reporting reduced α-tocopherol plasma/serum levels in cases of dementia[156] or no association (Table 4).[115,157] Only one study, the Conselice Study on Brain Ageing (CSBA), analyzed all tocopherol forms, together with markers of vitamin E oxidative/nitrosative damage (α-TQ and 5-NO_2-γ-tocopherol). In the CSBA cohort increased prevalence of dementia was found for higher levels of δ-tocopherol and α-TQ when analyzing vitamin E values standardized by cholesterol, but there was no association when considering absolute values of vitamin E.[115] Population-based studies analyzing α-tocopherol plasma/serum concentration in relation to cognition consistently report the presence of higher α-tocopherol levels is subjects with better cognitive performance[158-161] and reduced brain atrophy.[161]

The cross-sectional design of the all above-mentioned studies does not allow the inference of any causal association between vitamin E and dementia/AD. Few prospective population-based studies have analyzed α-tocopherol plasma/serum levels in relation to the risk of cognitive decline and dementia/AD, with inconsistent results (Table 5). One study based on volunteers reported no association between α-tocopherol, measured in red blood cells, and cognition,[162] while another study in women found that higher γ-tocopherol plasma levels were associated with better cognitive performance, but only among subjects with low α-tocopherol plasma concentration, suggesting the presence of an interactive effect between the two congeners.[163]

Among studies with dementia/AD as the outcome, one (Personnes Agées QUID, PAQUID) showed an increased risk of dementia and AD in elderly, French subjects with low plasma concentration of α-tocopherol,[164] but this finding was not confirmed in another French cohort (Three-City Study),[165] in an Italian cohort (CSBA),[115] or in a Swedish study on men (Uppsala Longitudinal Study of Adult Men - ULSAM)[166] (Table 5). A lower incidence of dementia in subjects with higher plasma γ-tocopherol levels (middle but not highest tertile) was detected in the CSBA cohort.[115] The association between γ-tocopherol concentration and dementia/AD risk was not found in the ULSAM study.[166]

Inconsistency between results from the longitudinal studies could be explained by different methodological factors, including differences across investigations in the sample size, age of study participants, and duration of follow-up. These studies report different ranges of vitamin E levels, which are not always comparable, since they are reported either as absolute or as standardized values, and standardization has been done with different methods (i.e., dividing vitamin E by total cholesterol or by total cholesterol + triglyceride). Overall, studies assessing the association between vitamin E levels and incidence of dementia/AD are scarce, none of them have evaluated midlife exposure, and none examined all vitamin E family members.

Vitamin E intake

The relationship of vitamin E dietary or supplemental intake with the risk of cognitive decline and dementia/AD has been investigated in different population-based prospective studies (Table 6). Vitamin E supplements are over-the-counter products, which usually contain only α-tocopherol, and are available in doses of 100 to 1000 international units (IU) (synthetic α-tocopherol).[106]

Studies with cognitive function as the main outcome have mainly evaluated the intake of vitamin E supplements, alone or in combination with vitamin C supplements. Some of these studies reported a reduced risk of cognitive decline in supplement users,[167,168] in some cases only for the combined use of vitamin E and C supplements,[169,170] while other studies found no association.[171] In the American Chicago Health and Aging Project (CHAP), vitamin E supplement use was inversely associated with cognitive decline only among participants who had low levels of intake from food, while vitamin E supplementation was not associated with further reductions in the rate of cognitive decline among participants who had high food-intake levels of vitamin E.[172]

Population-based studies assessing vitamin E intake in relation to dementia/AD risk are difficult to compare, because of the different methodology used in each investigation (Table 6). Some studies evaluated only dietary intake, others supplements use alone, and other studies assessed total vitamin E intake (food + supplements). Almost all studies examined late-life exposure, with follow-up durations from 3 to 14 years. Only one investigation analyzed midlife exposure in men (Honolulu-Asia Aging Study - HAAS), and found no association between vitamin E dietary intake and dementia/AD risk.[173] It is worth mentioning that the results of the HAAS study are based on a single 24-hour dietary recall questionnaire, which might not be representative of usual food selection. Overall, studies investigating vitamin E intake only from supplements found no association with dementia/AD risk,[170,174-177] or a reduced incidence was found only for the combined use of vitamin E and C supplements.[169,178] On the other hand, studies examining vitamin E dietary intake consistently report a reduced risk of dementia/AD in individuals with high vitamin E intake.[179-183] Only one study found no association: the Washington Heights-Inwood Columbia Aging Project (WHICAP),[174] in which the participants had a narrow range of low vitamin E intake compared to studies reporting a positive association[180] (Table 6).

Discrepancies between results from dietary and supplement intake studies, and within both categories of investigation, can be explained by several methodological factors, including differences across investigations in the sample size, age and ethnic characteristics, and length of follow-up. In all these studies, assessment of the exposure (vitamin E intake) is based on self-report, and is thus prone to recall bias, which can occur more often in subjects having subtle cognitive problems, who could be in a very early phase of dementia.

Regarding studies on supplement use, information on vitamin E concentration was in general not available. Thus differences in vitamin E supplement dosage have been not taken into account. Further, vitamin E supplements usually contain only α-tocopherol, while dietary intake guarantees a more balanced availability of micronutrients, including all vitamin E forms. This could be more relevant for a neuroprotective effect. Indeed, in the CHAP study a high intake of vitamin E from food (all tocopherols + all tocotrienols) was associated with a reduced risk of developing AD. When considering the individual congeners, intakes of α- and γ-tocopherol and α-tocopherol equivalents (index including α-, β-, and γ-tocopherol, and α-tocotrienol) were independently associated with a reduced incidence of AD.[181] On the other side, studies evaluating dietary intake are prone to other types of bias, due to the fact that food sources of vitamin E also provide other beneficial micronutrients, which could be relevant for neuroprotection.

Vitamin E intake from food generally reflects long-term intake, whereas supplement intake is commonly of shorter duration. Information on duration of supplement use is often unavailable, and one study reported an increased positive effect on cognitive function for long duration of supplement intake.[168] Further, in some studies a time-trend in the use of vitamin E supplements was observed.[178,184]

It is also possible that diets already provide sufficient levels of nutrients for optimum functioning in supplement users. Moreover, supplement users tend to be healthy behaviour-seeking individuals and their lifestyle (diet, smoking habit, physical and mental activity, social engagement) can be significantly different compared to subjects not using supplements. Indeed, supplement users often have a higher education compared to non-users; they are also more likely to consume other antioxidants and exercise regularly.[171,177,184] Health status among supplement users and non-users can also vary, with some studies reporting better or poorer general health being associated with supplement consumption.[178,185,186] The overall impact on dementia/AD risk, due to additive and interactive effects of these factors, cannot always be fully examined. Thus residual confounding may explain the conflicting findings of studies on vitamin E intake and risk of developing dementia/AD.

1.4.4 Vitamin E and Alzheimer's disease: clinical trials

Vitamin E has been tested as a preventive/therapeutic agent in AD. α-tocopherol is the only form that has been evaluated in RCTs, with conflicting results. A review by Isaac et al., examining RCTs on α-tocopherol in patients with AD and MCI, concluded that there is no evidence of efficacy.[187] Moreover, the analysis performed by the authors on the methodological quality of the RCTs examined, highlighted the lack of high quality, robust RCTs. In fact, only two RCTs were considered as methodologically adequate. One was a multicenter RCT done by the AD Cooperative Study. The RCT enrolled 341 patients with moderate AD, who were randomly assigned to receive either α-tocopherol (2000 IU/day),

selegiline (10 mg/day), both selegiline and α-tocopherol, or placebo for two years. The primary outcome measure was the time to reach any one of the following endpoints: institutionalization, loss of basic ADL, severe dementia, or death. The risk of reaching the primary outcome was significantly reduced by vitamin E treatment, selegiline treatment, and combined treatment. However, no significant benefit on performance on cognitive tests was shown in all treatment groups.[188] The second RTC enrolled 769 subjects with amnestic MCI, who were randomly assigned to receive 2000 IU of α-tocopherol, 10 mg of donepezil daily, or placebo for three years. In this study, α-tocopherol therapy had no benefits.[189]

Different aspects should be taken into account when considering RCTs on vitamin E in AD. There are general methodological issues, which affect all RCTs in AD prevention and treatment, including the difficulties in identifying the target population and in monitoring the treatment effect with clinically relevant tools and scales.

In the vitamin E supplement RCTs in AD and MCI, baseline levels of plasma vitamin E have often not been considered, while they may support the choice of the target population (i.e., subjects with lowest levels are likely to benefit more from the supplements). Longitudinal assessment of vitamin E levels in supplemented individuals can also help in monitoring treatment adherence and biological effects of supplementation.

Other general aspects in AD prevention and treatment are related to the timing of intervention, which emphasize the importance of the "therapeutic window" (i.e., time when intervention can exert a beneficial effect), and the adequate duration.[38,39] Since OS/NS increase with age and seem to be early events in AD, which has a long preclinical phase, RCTs done in AD and MCI could miss the time-window when supplement intake would have a significant beneficial effect. So far no RCT has succeeded in reversing the disease either in AD subjects or people in the MCI phase.[39] Further, duration of supplement use has usually been short compared to the length of subjects' exposure to OS/NS, which is likely to occur during a large part of the life span. The protective effect reported by observational studies on dietary intake, which can reflect prolonged exposure, suggests that a long-term exposure is more likely to reduce the risk of AD. These studies also support the fact that a balanced combination of different vitamin E forms could be important for neuroprotection. RCTs on supplements have been done only with α-tocopherol, often in high doses, which can decrease the bioavailability of the other congeners. Indeed, α-tocopherol supplementation can decrease plasma and tissue concentration of γ-tocopherol,[122,190] and compromise tissue delivery of α-tocotrienol.[191]

In summary, the role of the vitamin E family in AD is unclear in spite of the strong biological plausibility of a possible neuroprotective activity of these micronutrients. The majority of studies investigating vitamin E in relation to cognitive decline, dementia and AD, have focused on α-tocopherol, and no study has examined all eight natural vitamin E congeners in relation to cognitive decline and dementia/AD.

Since all natural vitamin E forms are potentially relevant for neuroprotection, each of the vitamin E forms may represent an index of vitamin E status, and provide information on the association between vitamin E and AD. Further, assessment of all vitamin E forms could help develop therapeutic interventions based on vitamin E supplementation.

Table 3. Vitamin E plasma/serum levels and dementia/AD: cross-sectional clinical studies.

Study (Country)	Population	Vitamin E measure	Results
Zaman et al., 1992 (UK)[149]	20 dementia (10 AD, mean age 83 y; 10 VaD, mean age 85 y) 20 controls, mean age 80 y	Plasma α-tocopherol	Mean plasma level of α-tocopherol lower in dementia cases (both AD and VaD) compared to controls
Ahlskog et al., 1995 (USA)[150]	12 AD, mean age 74 y, 43 PD, mean age 58 y, 16 diabetes mellitus, mean age 57 y 15 controls, mean age 61y	Plasma α-tocopherol	No association
Jimènez-Jimènez et al., 1997 (Spain)[140]	44 AD, mean age 73 y, 37 controls, mean age 70 y	Serum α-tocopherol	Mean serum level of α-tocopherol lower in AD cases compared to controls
Sinclair et al., 1998 (UK)[137]	42 dementia (25 AD, 17 VaD), mean age 74 y 41 controls, mean age 73 y	Plasma α-tocopherol, standardized by cholesterol and absolute values	Absolute values of vitamin E: mean plasma level of α-tocopherol lower in AD cases compared to controls Vitamin E adjusted by cholesterol and triglyceride: no association
Rivière et al., 1998 (France)[153]	24 moderate AD, mean age 79 y, 20 severe AD, mean age 78 y, 9 AD hospitalized, mean age 80 y, 20 controls, mean age 79 y	Plasma α-tocopherol	No association
Foy et al., 1999 (UK)[135]	134 dementia (79 AD, and 37 VaD, median age 79 y; 18 PDD, median age 72 y) 58 controls, median age74 y 41 PD, median age 67 y 41 controls, median age 67 y	Plasma α-tocopherol, standardized by cholesterol and absolute values	Absolute values of vitamin E: median plasma level of α-tocopherol lower in dementia cases (both AD and VaD) compared to controls Vitamin E adjusted by cholesterol: no association
Fernandes et al., 1999 (Portugal)[151]	74 mild-severe AD, mean age 68 y, 35 controls, mean age 65 y	Plasma α-tocopherol	No association
Schippling et al., 2000 (Germany)[154]	29 mild-moderate AD, mean age 72 y, 29 controls, mean age 55 y	Plasma α-tocopherol	No association
Tabet et al., 2001 (UK)[155]	31 AD (20 mild-moderate, 11 severe) 10 VaD, 10 DLB, 30 controls, age 65+y	Plasma α-tocopherol	No association

AD: Alzheimer's disease. DLB: dementia with Lewy bodies. PD: Parkinson disease. PDD: dementia in Parkinson disease. VaD: vascular dementia. y: years.

Table 3 (continued). Vitamin E plasma/serum levels and dementia/AD: cross-sectional clinical studies.

Study (Country)	Population	Vitamin E measure	Results
McGrath et al., 2001 (UK)[152]	29 AD, mean age 74 y 46 controls, mean age 73 y	Plasma α-tocopherol standardized by cholesterol	No association
Bourdel-Marchasson et al., 2001, (France)[139]	20 mild-moderate AD, mean age 80 y, 23 controls, mean age 76 y	Plasma α-tocopherol	Mean plasma level of α-tocopherol lower in AD cases compared to controls
Ravaglia et al., 2001 (Italy)[146]	30 dementia, mean age 98 y 32 controls, mean age 96 y	Plasma α-tocopherol	Mean plasma level of α-tocopherol lower in dementia cases compared to controls, only in men
Mecocci et al., 2002 (Italy)[141]	40 mild-moderate AD, mean age 76 y, 39 controls, mean age 75 y	Plasma α-tocopherol	Mean plasma level of α-tocopherol lower in AD cases compared to controls
Polidori et al., 2002 (Italy)[143]	35 mild-moderate AD (female), mean age 86 y, 40 controls (female), mean age 85 y	Plasma α-tocopherol	Mean plasma level of α-tocopherol lower in AD cases compared to controls
Ryglewicz et al., 2002 (Poland)[148]	68 dementia (26 AD, mean age 67 y, 42 VaD, mean age 71 y) 46 controls, mean age 68 y	Plasma α-tocopherol	Mean plasma level of α-tocopherol lower in VaD cases compared to AD and controls
Praticò et al., 2000, 2004 (USA)[144,145]	25 AD, mean age 76 y 25 controls, mean age 75 y	Plasma α-tocopherol	Mean plasma level of α-tocopherol lower in AD cases compared to controls
Rinaldi et al., 2003 (Italy)[147]	63 AD, mean age 77 y 25 MCI, mean age 76 y 56 controls, mean age 76 y	Plasma α-tocopherol	Mean plasma level of α-tocopherol lower in AD and MCI cases compared to controls
Polidori et al., 2004 (Italy)[142]	86 mild-moderate dementia (63 AD, 23 VaD) mean age 77 y, 55 controls, mean age 76 y	Plasma α-tocopherol	Mean plasma level of α-tocopherol lower in dementia cases (both AD and VaD) compared to controls
Baldeiras et al., 2008 (Portugal)[138]	42 mild AD, mean age 73 y 85 MCI, mean age 71 y 37 controls, mean age 68 y	Plasma α-tocopherol Red blood cell α-tocopherol	Mean plasma level of α-tocopherol lower in AD and MCI cases compared to controls
Iuliano et al., 2010 (Italy)[136]	37 AD, mean age 76 y 53 MCI, mean age 70 y 24 controls, mean age 70 y	Plasma α-tocopherol, standardized by cholesterol and absolute values	Absolute values of vitamin E: no association Vitamin E adjusted by cholesterol: mean plasma level of α-tocopherol lower in AD cases compared to controls

AD: Alzheimer's disease. MCI: mild cognitive impairment. VaD: vascular dementia. y: years.

Table 4. Vitamin E plasma/serum levels, cognitive function and dementia/AD: cross-sectional population-based studies.

Study (Country)	Design and population	Outcomes	Vitamin E measure	Results
Perkins et al., 1999 (USA)[160]	Third National Health and Nutrition Examination Survey (NHANES III), N=4,809, 98% white, 2% non-white, age 60+ y	Cognition	Serum α-tocopherol standardized by cholesterol	Higher α-tocopherol levels were associated with better memory performance
Ortega et al., 2002 (Spain)[159]	120 community dwellers (Voluntary sample), age 65-91 y	Cognition	Plasma α-tocopherol, standardized by cholesterol and absolute values	Higher α-tocopherol levels (both absolute value and adjusted by cholesterol) were associated with better cognitive performance
Engelhart et al., 2005 (The Netherlands)[157]	Case control within the Rotterdam Study 65 AD, mean age 84 y 293 cognitive impairment, mean age 76 y 437 controls, mean age, 72 y	AD Cognitive impairment	Plasma α-tocopherol	No association
Cherubini et al., 2005 (Italy)[156]	Case-control study within the InCHIANTI cohort 58 dementia (22 degenerative, 36 VaD or mixed) mean age 85 y, 168 cognitive impairment, mean age 81 y 807 controls, mean age 74 y	Dementia Cognitive impairment	Plasma α-tocopherol	Higher α-tocopherol levels were associated with lower odds for dementia or cognitive impairment
Dunn et al., 2007 (USA)[158]	Cognitive Change in Women (CCW) study, ancillary study to the Women's Health Initiative N=526 (123 memory impairment, 61 mixed impairment), 91% white, 9% non-white, age 60+ y	Cognition	Serum α-tocopherol	Higher α-tocopherol levels were associated with lower odds for memory impairment or mixed impairment
Bowman et al., 2011 (USA)[161]	Subsample of the Oregon Brain Aging Study (OBAS), N=104, mean age 87	Cognition, MRI measures	Plasma α-tocopherol	The plasma profile of nutrients which included high α-tocopherol levels was associated with better cognition and MRI measures

AD: Alzheimer's disease. MRI: magnetic resonance imaging. VaD: vascular dementia. y: years.

Table 5. Vitamin E plasma/serum levels, cognitive decline and dementia/AD risk: prospective population-based studies.

Study (Country)	Design and population	Follow-up	Outcomes	Vitamin E measure	Results
Berr et al., 1998 (France)[162]	Etude du Vieillissement Arteriel (EVA) Study (Voluntary sample), N=1389 mean age 65 y	4 y	Cognition	Red blood cell α-tocopherol	No association
Helmer et al., 2003 (France)[164]	Nested case-control within the PAQUID cohort, N=626 (46 dementia, 39 AD) age 65+ y	9 y	Dementia AD	Plasma α-tocopherol	Higher risk of dementia among subjects in the lowest α-tocopherol tertile Similar results when examining only AD cases (results statistically significant at the 10% level)
Kang & Grodstein, 2007 (USA)[163]	Nurses' Health Study (NHS), N=858 age 43-68 y (mean 65 y)	10 y	Cognition	Plasma α- and γ-tocopherol	No association of tocopherols sum with cognition When examining individual tocopherols, higher γ-tocopherol levels were associated with better cognitive performance only among subjects with α-tocopherol levels under the median
Samieri et al., 2008 (France)[165]	Three-City (3C) Study, N=1214 (65 dementia) age 65+ y	4 y	Dementia	Plasma α-tocopherol	Higher risk of dementia in subjects with higher α-tocopherol levels (results statistically significant at the 10% level)
Ravaglia et al., 2008 (Italy)[115]	Conselice Study on Brain Ageing (CSBA), age 65+ y Baseline: N=761 (666 CN, 52 MCI, 43 dementia) 73 incident cases of dementia (45 AD, 26 VaD, 2 NOS)	4 y	Dementia MCI	Plasma α-, β-, γ- and δ-tocopherol, α-TQ, and 5-nitro-γ-tocopherol, standardized by cholesterol and absolute values	Cross-sectional analysis, vitamin E standardized by cholesterol: - increased prevalence of dementia in the highest tertile of δ-tocopherol and α-TQ levels Longitudinal analysis, vitamin E standardized by cholesterol: -reduced risk of dementia in the middle tertile of γ-tocopherol Absolute values of vitamin E: no association
Sundelöf et al., 2009 (Sweden)[166]	Uppsala Longitudinal Study of Adult Men (ULSAM), two age cohorts: - 70y, N=616 (86 dementia, 36 AD, 4 AD+VaD, 27 VaD, 19 NOS) -77y, N= 761 (80 dementia, 45 AD, 2 AD+VaD, 21 VaD, 12 NOS)	age 70: 4 y age 77: 8 y	Dementia, AD	Plasma α- and γ-tocopherol, standardized by cholesterol+triglyceride	No association

AD: Alzheimer's disease. α-TQ: , α-tocopherylquinone. CN: normal cognition. MCI: mild cognitive impairment. N: number of subjects. NOS: dementia not otherwise specified. VaD: vascular dementia. y: years.

Table 6. Vitamin E intake, cognitive decline and dementia/AD risk: prospective population-based studies.

Study (Country)	Design and population	Follow-up	Outcomes	Vitamin E source	Results
La Rue et al., 1997 (USA)[167]	New Mexico Aging Process Study, (voluntary sample) N=137 age 60-84 y	6 y	Cognition	Dietary and supplements (3-day dietary record)	Better cognitive performance in subjects taking vit. E supplements No association with vit. E dietary intake
Luchsinger et al., 2003 (USA)[174]	Washington Heights-Inwood Columbia Aging Project (WHICAP), 68% white, 32% non-white N=980 (242 AD) age 65+ y (mean 75 y)	4 y	AD	Dietary and supplements (FFQ)	No association
Grodstein et al., 2003 (USA)[168]	Nurses' Health Study (NHS) N=14968 women age 35-59 y	15 y	Cognition	Supplements	Better cognitive performance is subjects taking vit. E supplements. Evidence of increasing benefit with longer duration of supplement use
Masaki et al., 2000 Laurin et al., 2002 Laurin et al., 2004 (USA)[169,173,175]	Honolulu-Asia Aging Study (HAAS), Japanese-American men Study on prevalent cases: N=3385 (2999 CN, 47 AD, 35 VaD, 50 mixed/NOS, 254 low cognition) age 71-93 y Study on incident cases: Mid-life exposure: N=2459 (235 dementia, 102 AD, 38 AD+VaD, 44 VaD, 51 others) age 45-68 y Late-life exposure N=2369 (222 dementia, 94 AD, 38 AD+VaD, 42 VaD, 48 other dementia) age 71-92 y	5 y (10 y in a subgroup) 30 y 5 y	Cognition Dementia AD VaD Mixed dementia	Supplements of vit. E and vit. C Dietary (24h dietary recall)	Study on prevalent cases: Reduced risk of VaD and mixed dementia in subjects taking both vit. E and vit. C supplements Short term use of supplements (5y): better cognitive performance in subjects taking vit. E supplements Long term use of supplements (10 y): better cognitive performance in subjects taking both vit. E and vit. C supplements Study on incident cases: Midlife exposure: no association Late-life exposure: no association

AD: Alzheimer's disease. CN: normal cognition. FFQ: food frequency questionnaire. N: number of subjects. NOS: dementia not otherwise specified. VaD: vascular dementia. vit: vitamin. y: years.

Table 6 (continued). Vitamin E intake, cognitive decline and dementia/AD risk: prospective population-based studies.

Study (Country)	Design and population	Follow-up	Outcomes	Vitamin E source	Results
Engelhart et al., 2002 Devore et al., 2010 (Netherlands)[179,180]	Rotterdam study N=5395, age 55+ y (mean 68 y) 6 y follow-up: 197 dementia, 146 AD 9.6 y follow-up: 465 dementia, 365 AD	6 y 9.6 y	Dementia AD	Dietary and supplements (FFQ)	Reduced dementia and AD risk in subjects with higher dietary intake of vit. E
Zandi et al., 2004 Wengreen et al., 2007 (USA)[171,178]	Cache County Study on Memory, Health and Aging, age 65+ y 3 y follow up: N=4540 (185 dementia, 104 AD) 7 y follow-up: N=3632	3 y 7 y	AD Cognition	Dietary (FFQ) and supplements of vit. E and vit. C	Reduced risk of AD in subjects taking both vit. E and vit. C supplements Reduced risk of cognitive decline in subjects with higher vit. E intake from food or food+supplements
Morris et al., 2002, 2005, (USA)[172,181,184]	Chicago Health and Aging Project (CHAP)			Dietary and supplements (FFQ)	Reduced risk of cognitive decline in subjects with higher vit. E intake from food or food+supplements
	N=2889, 45% white, 55% non-white, age 65+ y (mean 74 y)	3 y	Cognition		Beneficial effect of supplement on cognition only in subjects with low vit. E dietary intake
	N=3718 age 65+ y	6 y			Reduced risk of cognitive decline in subjects with higher dietary intake of vitamin E (Total vit. E, α- and γ-tocopherol, α-TE)
	N= 815 (131 AD), 49% white, 51% non-white, age 65+ y (mean 73 y)	4 y	AD		Reduced dementia and AD risk in subjects with higher dietary intake of vitamin E, only in *APOE*ε4 non-carriers
	N=1041 (162 AD) age 65+ y				Reduced dementia and AD risk in subjects with higher dietary intake of vitamin E (Total vit. E, α- and γ-tocopherol, α-TE)

AD: Alzheimer's disease. FFQ: food frequency questionnaire. N: number of subjects. vit: vitamin. y: years. α-TE: α-tocopherol equivalents, based on conversion factors of biologic activity of the different vitamin E forms: 1.0 for α-tocopherol, 0.5 for β-tocopherol, 0.1 for γ-tocopherol, and 0.3 for α-tocotrienol.[181]

Table 6 (continued). Vitamin E intake, cognitive decline and dementia/AD risk: prospective population-based studies.

Study (Country)	Design and population	Follow-up	Outcomes	Vitamin E source	Results
Corrada et al., 2005 (USA)[182]	Baltimore Longitudinal Study of Aging (BLSA), N=579 (voluntary sample within the BLSA cohort) (57 AD) mean age 70 y	9.3 y	AD	Dietary and supplements (7-day dietary record)	Reduced AD risk in subjects with higher intake of vit. E (significant at the 10% level) No association when intake of vit. E, folate, and vit. B6 were considered simultaneously
Fillenbaum et al., 2005 (USA)[176]	Duke Established Populations for Epidemiologic Studies of the Elderly (EPESE), 38% white, 62% non-white, N=616 (141 dementia, 93 AD, 30 VaD, 18 NOS) mean age 73 y	14 y	Dementia AD	Supplements of vit. E and vit. C	No association
Maxwell et al., 2005 (Canada)[170]	Canadian Study of Health and Aging (CSHA), N=894 (279 cognitive decline), (230 dementia, 107 AD, VCI: ns) age 65+y (mean 78 y)	5 y	Cognition Dementia AD VCI	Supplements of vit. E and vit. C	Reduced risk of cognitive decline in subjects taking both vit. E and vit. C supplements. No association with dementia, AD, and VCI
Gray et al., 2008 (USA)[177]	Adult Changes in Thought study (ACT), N=2969 (405 dementia, 289 AD) age 65+y	5.5 y	Dementia AD	Supplements	No association

AD: Alzheimer's disease. N: number of subjects. NOS: dementia not otherwise specified. ns: not specified. VaD: vascular dementia. VCI: vascular cognitive impairment. vit: vitamin. y: years.

2 AIMS

2.1 GENERAL AIMS

The general aims of this thesis are to investigate and understand the relation of the vitamin E family (α-, β-, γ, and δ-tocopherol; α-, β-, γ-, and δ-tocotrienol) to cognitive impairment and AD in older adults, by combining clinic-based and population-based studies.

2.2 SPECIFIC AIMS

The specific aims addressed in four different studies are summarized below.

1. To examine the relation of plasma levels of all eight natural vitamin E forms and markers of vitamin E oxidative/nitrosative damage (α-tocopherylquinone, 5-nitro-γ-tocopherol) to MCI and AD, in a multi-centre European clinical study (AddNeuroMed Project) (*Study I*).

2. To investigate the potential use of combining plasma vitamin E forms - including all eight natural vitamin E congeners and markers of vitamin E oxidative/nitrosative damage - and structural MRI measures in differentiating AD and MCI cases from cognitively intact individuals (CN), and in predicting MCI conversion to AD (AddNeuroMed Project) (*Study II).*

3. To examine the longitudinal association between plasma levels of eight natural forms of vitamin E and the risk of AD among oldest-old individuals (age ≥ 80 years), in a Swedish population-based study (Kungsholmen Project) (*Study III)*.

4. To verify the association between serum levels of all eight natural vitamin E forms, markers of vitamin E oxidative/nitrosative damage and the incidence of cognitive impairment (MCI or AD) in older adults (age ≥ 65 years), in a Finnish population-based prospective study (CAIDE Study) *(Study IV).*

3 METHODS

The data used in this thesis are derived from three projects: The European AddNeuroMed Project, The Swedish Kungsholmen Project, and The Finnish Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) Study.

3.1 Clinic-based studies

3.1.1 AddNeuroMed Project (Studies I and II)

3.1.1.1 Study population

The AddNeuroMed project is part of Innovative Medicines in Europe (InnoMed), an Integrated Project funded by the European Union Sixth Framework programme.[72,192] AddNeuroMed is a multi-centre European longitudinal study aiming to develop and validate novel surrogate markers in AD, which can assist diagnosis, prognosis, drug discovery and treatment monitoring. The project includes a neuroimaging part utilising MRI. At baseline, 716 community-living subjects were recruited: 259 AD cases, 225 MCI and 232 CN individuals. All subjects were gathered from six different sites across Europe: University of Kuopio, Finland; University of Perugia, Italy; Aristotle University of Thessaloniki, Greece; King's College London, United Kingdom; University of Lodz, Poland; and University of Toulouse, France.[72,193] All AD and MCI cases were recruited from the local memory clinics of the six participating sites, while CN individuals were recruited from non-blood relatives of the patients' families, caregivers' relatives, and social centers for the elderly.

Study I included 521 subjects: 168 AD cases, 166 MCI, and 187 CN individuals. Sample selection was based on availability of plasma samples for vitamin E analysis. Subjects with plasma vitamin E available were similar to all AddNeuroMed participants at baseline in terms of age, gender, education, *APOE* status, Mini-Mental State Examination (MMSE)[194] score, and duration of cognitive decline. For this subsample of subjects, plasma levels of two other antioxidant micronutrients - retinol and β-carotene - were also available, and were evaluated in order to provide a broader picture of the antioxidant micronutrients status in cognitive impairment.

Study II included 253 subjects (AD: 81, MCI: 86, and CN: 86), for whom MRI and vitamin E data from baseline were available. Both MCI and AD cases included in Study II were slightly younger in comparison to MCI and AD AddNeuroMed participants without MRI (MCI: 74.6±5.2 *versus* 76.7±5.7; AD: 75.1±5.7 *versus* 78.1±6.5), while the sample was similar to all AddNeuroMed participants in terms of gender, education, *APOE* status, MMSE[194] score, and duration of cognitive decline.

3.1.1.2 Data collection

At baseline, a health interview was administrated to all participants following a standardized protocol (the full protocol is available at http://www.innomed-addneuromed.com)[195] and data on demographics, medical history, current health status, medication use, and family history were collected. For AD and MCI cases, information on the duration and severity of cognitive decline was obtained from the subjects or informants through a detailed questionnaire, which evaluated the presence of memory problems, time of their onset, subsequent course, and investigations performed for the cognitive problems.
For all participants, cognition, behaviour, functional status, and global severity were assessed and the MMSE[194] and the Clinical Dementia Rating scale (CDR)[196] were administered. Evaluation of AD included the Hachinski ischemic scale,[197] the Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog),[198] the Neuropsychiatric Inventory,[199] and the Alzheimer's Disease Cooperative Study (ADCS)-activities of daily living scale.[200] Evaluation of MCI and CN individuals comprised the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Cognitive Battery[201] and the Geriatric Depression Scale (GDS).[202] The same procedure was used for follow-up at one year.

3.1.1.3 Diagnosis of dementia and mild cognitive impairment

Dementia was diagnosed following the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV),[203] while probable AD was diagnosed according to the NINCDS-ADRDA criteria.[55] Patients with significant neurological or psychiatric illness other than AD, any significant unstable systemic illness/organ failure, alcohol/substance misuse were excluded.
The inclusion criteria for both MCI and CN subjects were: 1) absence of dementia according to the DSM-IV;[203] 2) age $\geq$ 65 years; 3) MMSE score range between 24 and 30; 4) GDS score $\leq$ 5; 5) good general health and stable medication. Subjects with significant neurological or psychiatric illness, or any significant unstable systemic disease/organ failure, or alcohol/substance misuse were excluded. The CDR total score was 0.5 for MCI cases and 0 for CN individuals.
MCI subjects were required to meet the following criteria, in line with consensus criteria for amnestic MCI:[65,204] 1) absence of dementia according to the DSM-IV;[203] 2) normal general cognitive function; 3) memory complaint: self- or informant-reported memory problem; 4) CDR[196] memory score of 0.5 or 1 (Total CDR = 0.5); and 5) normal ADL.

3.1.1.4 Vitamin E assessment

At baseline blood samples were taken from subjects after a minimum of 2 hours fasting; serum and plasma samples were aliquoted and frozen at -80 °C until analysis. Plasma tocopherols, tocotrienols, αTQ, and 5-NO_2-γ-tocopherol were measured with reverse-phase

High Performance Liquid Chromatography (HPLC) using electrochemical-coularray system (ESA-Chelmsford, MA, USA). Aliquots of 200µL were mixed and extracted three times with a 1:2 ratio of ethanol to hexane, concentrated to dryness with high-purity nitrogen gas, and reconstituted in 300µL mobile phase. β-tocopherol (Superchrome, Milan, Italy); α-, γ-, and δ-tocopherol (Sigma-Aldrich, Milan, Italy), α-, γ-, and δ-tocotrienol (LGC-Promochem, Milan, Italy), β-tocotrienol (Matreya-DBA, Pleasant-Gap, PA, USA), αTQ (Research Organics, Rome, Italy) and 5-NO_2-γ-tocopherol (gift from Prof K. Hensley, University of Toledo Health-Sciences-Center, OH, USA) were used as standards (Figure 2). After filtration, analyte separation was conducted at room temperature on a Discovery-C18-column (Sigma-Aldrich, Milan, Italy). Mobile phase [30mmol lithium acetate/L, 83% HPLC grade acetonitrile, 12% HPLC grade methanol, and 0.2% HPLC grade acetic acid (pH 6.5)] was delivered at 1mL/min.

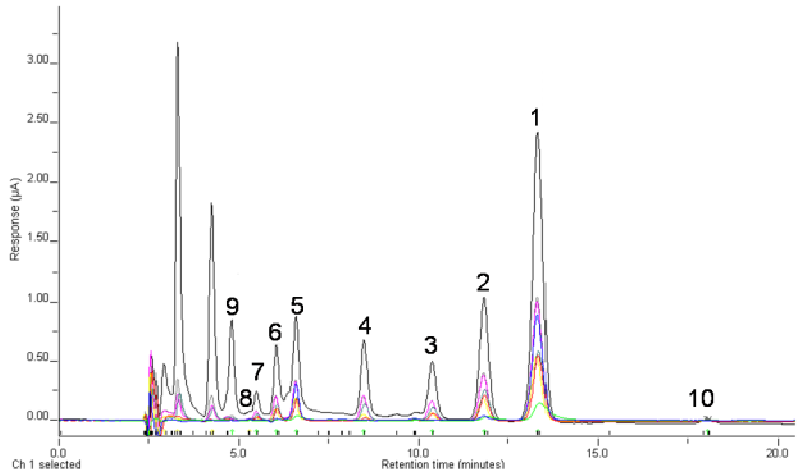


Figure 2. HPLC chromatogram of known concentration of pure standards. 1: α-tocopherol; 2: β-tocopherol; 3: γ-tocopherol; 4: δ-tocopherol; 5: α-tocotrienol; 6: β-tocotrienol; 7: γ-tocotrienol; 8: δ-tocotrienol; 9: α-TQ; 10: 5-NO2-γ-tocopherol.

3.1.1.5 Other biochemical analyses

Baseline plasma retinol and β-carotene (non-enzymatic antioxidants) were measured with reverse-phase HPLC using electrochemical-coularray system (ESA-Chelmsford, MA, USA). Pure retinol and β-carotene (Sigma-Aldrich, Milan, Italy) were used as standards, and the HPLC procedure was the same as the one used for vitamin E assessment. Baseline serum total cholesterol and albumin were measured by standardized enzymatic assay on a ILab-600-Plus Chemistry System analyzer (Instrumentation Laboratory, Milan, Italy). The *APOE* genotype was determined from blood leukocytes. DNA was extracted by a standard phenol-chloroform extraction, and *APOE* genotypes were analyzed by polymerase chain reaction (PCR), *Hha* I digestion and polyacrylamide gel electrophoresis.[205]

3.1.1.6 MRI

Data acquisition for the AddNeuroMed study took place using six different 1.5T magnetic resonance systems (four General Electric, one Siemens and one Picker), and was designed to be compatible with the Alzheimer Disease Neuroimaging Initiative (ADNI).[206] The imaging protocol for both studies included a high resolution sagittal 3D T1-weighted MPRAGE volume (voxel size 1.1x1.1x1.2 mm^3) and axial proton density/T2-weighted fast spin echo images. The MPRAGE volume was acquired using a custom pulse sequence specifically designed for the ADNI study to ensure compatibility across scanners.[206] Full brain and skull coverage was required for both of the latter datasets and detailed quality control was carried out on all MRI images according to the AddNeuroMed quality control procedure.[193,207]

Regional volume segmentation and cortical thickness parcellation

Imaging data were processed using the Freesurfer pipeline (version 4.5.0), which produces regional cortical thickness and volumetric measures (Figure 3). Cortical reconstruction and volumetric segmentation includes removal of non-brain tissue using a hybrid watershed/surface deformation procedure,[208] automated Talairach transformation, segmentation of the subcortical white matter and deep grey matter volumetric structures (including hippocampus, amygdala, caudate, putamen, ventricles),[208,209] intensity normalization,[210] tessellation of the grey matter white matter boundary, automated topology correction,[211,212] and surface deformation following intensity gradients to optimally place the grey/white and grey/CSF borders at the location where the greatest shift in intensity defines the transition to the other tissue class.[213,214] Once the cortical models are complete, registration to a spherical atlas takes place which utilizes individual cortical folding patterns to match cortical geometry across subjects.[215] This is followed by parcellation of the cerebral cortex into units based on gyral and sulcal structure.[209,216]

The pipeline generated 68 cortical thickness measures (34 from each hemisphere) and 50 regional volumes. Volumes of white matter hypointensities, optic chiasm, right and left vessel, and left and right choroid plexus were excluded from further analysis. Cortical thickness and volumetric measures from the right and left side were averaged.[69] In total 57 variables obtained from the pipeline were used as input variables for the orthogonal partial least squares to latent structures (OPLS) classification, 34 regional cortical thickness measures and 23 regional volumes (Table 7). All volumetric measures from each subject were normalized by the subject's intracranial volume. Cortical thickness measures were not normalized.

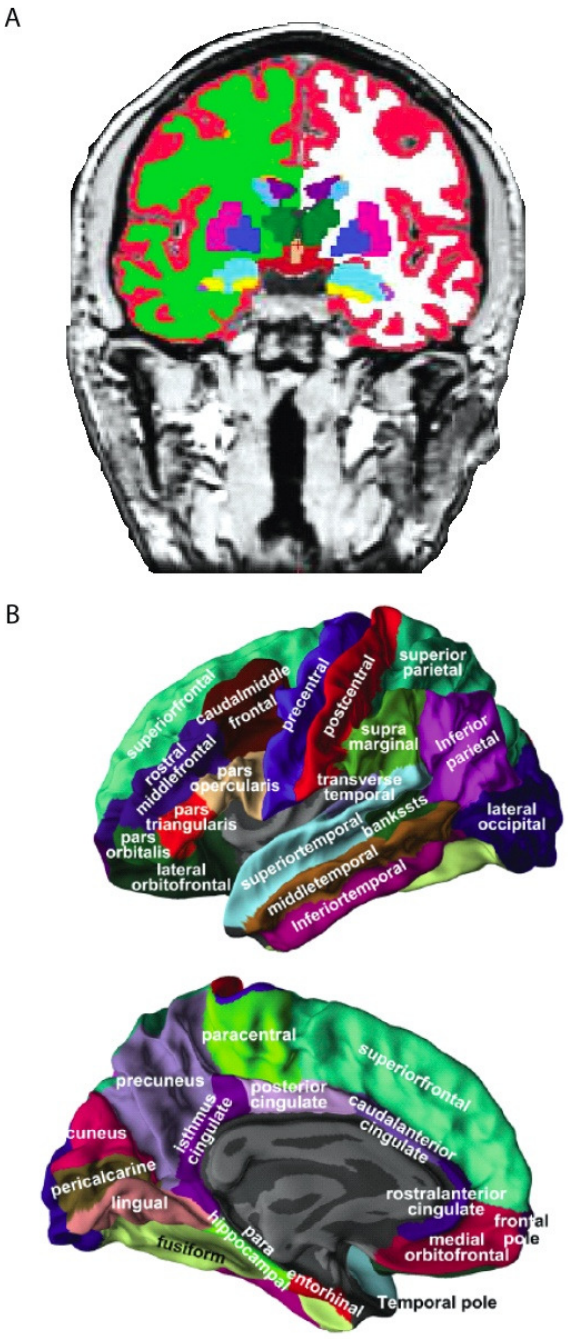


Figure 3. Representations of regions of interest (ROI) included as candidate input variables in the multivariate orthogonal partial least squares to latent structures (OPLS) model. (A) Regional volumes. (B) Regional cortical thickness measures (Figure provided by Eric Westman).

Table 7. Variable included in orthogonal partial least squares to latent structures (OPLS) analysis.

Cortical thickness measures	Volumetric measures	Vitamin E
Banks of superior temporal sulcus	Third ventricle	α-tocopherol
Caudal anterior cingulate	Fourth ventricle	β-tocopherol
Caudal middle frontal gyrus	Brainstem	γ-tocopherol
Cuneus cortex	Corpus callosum anterior	δ-tocopherol
Entorhinal cortex	Corpus callosum central	α-tocotrienol
Fusiform gyrus	Corpus callosum midanterior	β-tocotrienol
Inferior parietal cortex	Corpus callosum midposterior	γ-tocotrienol
Inferior temporal gyrus	Corpus callosum posterior	δ-tocotrienol
Isthmus of cingulate cortex	CSF	Ratio αTQ/α-tocopherol
Lateral occipital cortex	Accumbens	Ratio 5-NO_2-γ-tocopherol/ γ- tocopherol
Lateral orbitofrontal cortex	Amygdala	
Lingual gyrus	Caudate	
Medial orbitalfrontal cortex	Cerebellum Cortex	
Middle temporal gyrus	Cerebellum White Matter	
Parahippocampal gyrus	Hippocampus	
Paracentral sulcus	Inferior lateral ventricle	
Frontal operculum	Putamen	
Orbital operculum	Cerebral Cortex	
Triangular part of inferior frontal gyrus	Cerebral White Matter	
Pericalcarine cortex	Lateral Ventricle	
Postcentral gyrus	Pallidum	
Posterior cingulate cortex	Thalamus Proper	
Precentral gyrus	Ventral diencephalon (DC)	
Precuneus cortex		
Rostral anterior cingulate cortex		
Rostral middle frontal gyrus		
Superior frontal gyrus		
Superior parietal gyrus		
Superior temporal gyrus		
Supramarginal gyrus		
Frontal pole		
Temporal pole		
Transverse temporal cortex		
Insular		

In total, 67 variables were included in OPLS analysis: 34 cortical thickness measures, 23 volumetric measures and 10 vitamin E related values.

3.2 Population-based studies

3.2.1 Kungsholmen Project (Study III)

3.2.1.1 Study population

The Kungsholmen Project is a Swedish community-based prospective study on aging and dementia.[217,218] The initial population gathered all registered inhabitants (n=2,368) who were living in the Kungsholmen district of Stockholm and were aged 75+ years in 1987, and 1,810 (76.4%) individuals participated in the Phase I survey (Figure 4).

Study III included 232 subjects derived from the non-demented cohort (n=792 subjects) participating in Phase III of the Kungsholmen Project (baseline for this study) (Figure 4). Sample selection was based on availability of plasma samples for vitamin E analysis. Subjects in this sample were slightly older in comparison to the other cognitively intact participants (mean age±SD: 84.9±3.2 *versus* 83.9±4.3 years, $p<0.001$) and with a slightly lower MMSE[194] score (26.8±2 *versus* 27.1±2, $p=0.009$). The sample was representative of the entire dementia-free cohort as regard to sex, education, BMI, and *APOE* status. The 232 non-demented subjects aged 80+ years were clinically examined on two different occasions during the following six years to detect new dementia cases. Among study participants, 16 subjects refused to participate in the first follow-up examination or had moved before the assessment, and 14 refused to undertake the second follow-up examination. The other participants received a full dementia work-up, which included a structured interview by trained nurses (if the subject was not able to answer, an informant, usually a next-of-kin, was interviewed), a comprehensive clinical examination by physicians, and neuropsychological assessment by psychologists. For those subjects who had died before the first (n=53) or second (n=31) follow-up examination (Figure 4) information regarding their health status was obtained from the Stockholm Inpatient Register System, which encompasses all hospitals in the Stockholm area since 1969, and records up to six diagnoses at discharge.

3.2.1.2 Data collection

At baseline, data on age, sex, education, alcohol consumption, and smoking habit were collected from the subjects following standardized protocols.[217,218] Diagnosis of chronic diseases was made by the examining physician, based on the clinical examination, medical history, laboratory data, and current drug use. Further information on health status for all participants was derived from the computerized Stockholm Inpatient Register System. Presence of multimorbidity was defined as any co-occurrence of 2+ chronic diseases in the same individual, according to a previous study within the Kungsholmen Project.[219] Global cognitive functioning was assessed with the MMSE.[194] The Katz index of ADL was used to measure basic functional status.[220] Weight and height were measured with a standard

scale in light clothing and no shoes. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Data on lipid lowering medications and vitamin supplements use were collected from the subjects and verified by inspecting drug prescriptions and containers. Vitamin supplements were coded following the Anatomical Therapeutic and Chemical classification system.[221]

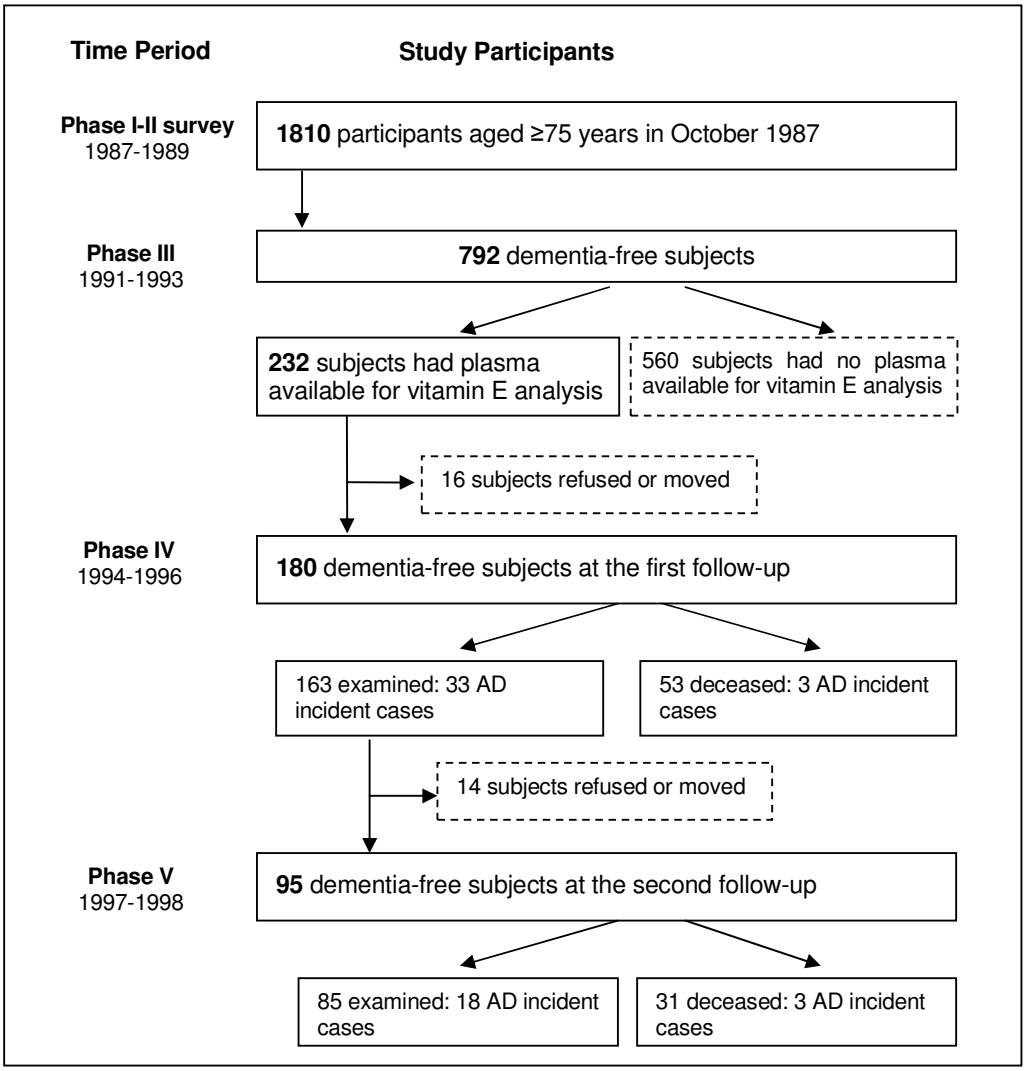


Figure 4. Flowchart of the Kungsholmen Project, 1987-1998. AD= Alzheimer's disease.

3.2.1.3 Diagnosis of dementia

At baseline and at each follow-up of the study, all participants underwent an extensive clinical and neuropsychological evaluation. Dementia was diagnosed on the basis of clinical judgment following the Diagnostic and Statistical Manual of Mental Disorders, revised third edition (DSM-III-R) criteria,[1] in which a validated three-step diagnostic procedure was used.[217,218] In brief, two examining physicians independently made a preliminary diagnosis, and in the case of disagreement, a third opinion from a specialist was sought to reach a concordant diagnosis. The diagnosis of AD required gradual onset,

progressive deterioration, and lack of any other specific causes of dementia. Diagnostic criteria for AD were equivalent to probable AD according to the NINCDS-ADRDA criteria.[55] To verify the presence of dementia and AD in subjects who died during each follow-up period specialists reviewed medical records and death certificates, and diagnoses were made using the same procedure as above. The incident cases were all subjects who developed AD over the two follow-up periods.

3.2.1.4 Vitamin E assessment

Venous blood samples were taken at baseline (fasting was not compulsory) and plasma aliquots were stored at -80 °C until analysis. Tocopherols and tocotrienols were measured on plasma with reverse-phase HPLC with electrochemical-coularray system (ESA-Chelmsford, MA, USA) as previously described (see paragraph 3.1.1.4).

3.2.1.5 Other biochemical analyses

Genomic DNA was prepared from peripheral blood samples that were taken at Phase I, and *APOE* allelic status was determined following a standard procedure.[222] Baseline serum total cholesterol was measured by standardized enzymatic assay on a ILab-300-Plus Chemistry System analyzer.

3.2.2 CAIDE (Study IV)

3.2.2.1 Study population

The CAIDE study is a prospective long-term cohort study in Finland.[223] The participants were derived from four independent, randomly selected, population-based samples originally studied within the framework of the North Karelia Project and the Finnish part of Monitoring trends and determinants of Cardiovascular disease study (FINMONICA) in 1972, 1977, 1982 or 1987 (midlife visit). The average age of the participants was then 50.6±6.0 years. These two studies were conducted to evaluate the risk factors, morbidity, and mortality from cardiovascular diseases.[224] The methods used in midlife visit were standardized and complied with international recommendations.[225]

A random sample of 2000 persons (still alive at the end of 1997, aged 65-79 years and living in the region of Kuopio and Joensuu towns, Finland) were invited for a first re-examination carried out in 1998, and 1449 persons (72.5%) participated. A second re-examination of the same cohort was conducted in 2005-2008.

Study IV included a sub-sample of 140 subjects, derived from the cohort without cognitive impairment participating in the re-examination carried out in 1998. The 140 subjects in the present study were selected based on availability of serum samples from 1998 for vitamin

E assessment (Figure 5). All subjects with cognitive impairment in 2005-2008 (MCI or AD) and available 1998 serum samples were selected, and at least one age and sex-matched control for each case. Sixty-four of the 140 subjects had cognitive impairment (24 MCI, 40 AD) at the second re-examination in 2005-2008, and 76 represented the control group. The mean duration of follow-up from 1998 examination (baseline for this study) to 2005-2008 was 8.2 years.

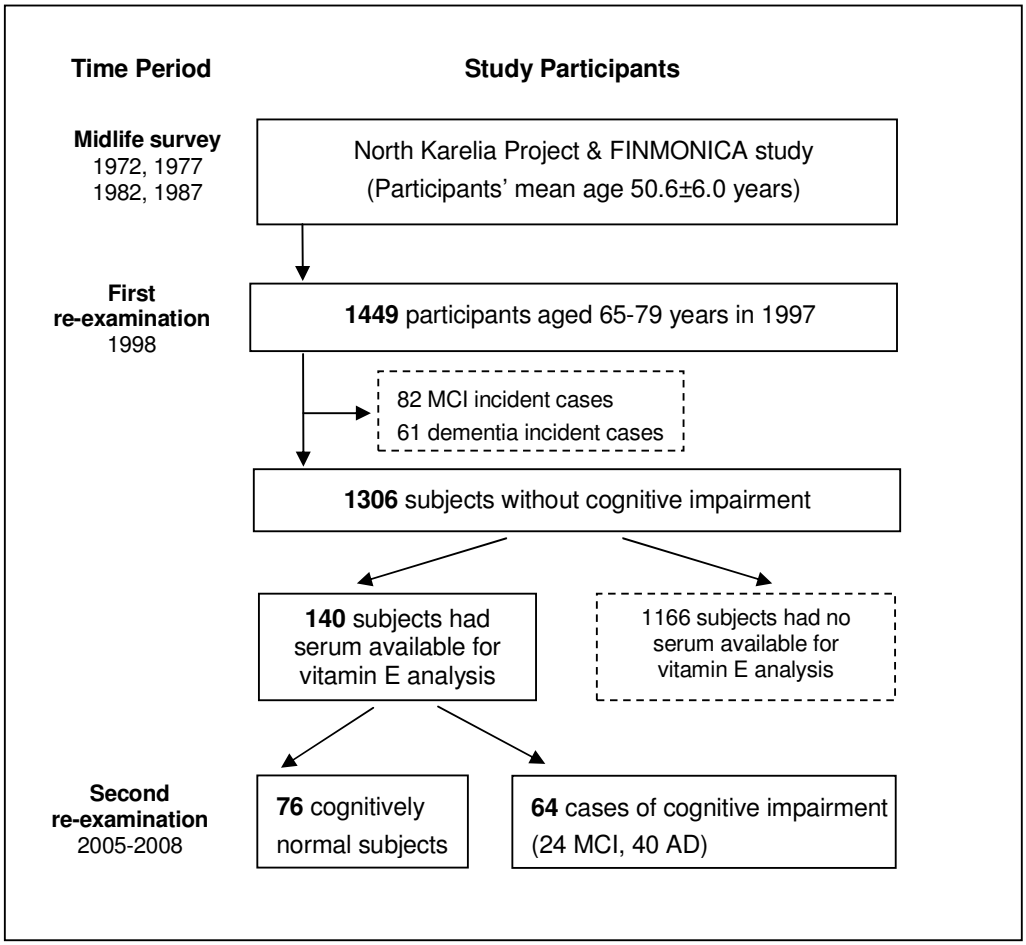


Figure 5. Flowchart of the CAIDE Study. AD= Alzheimer's disease; MCI: mild cognitive impairment

Compared to the other CAIDE participants without cognitive impairment in 1998, the sub-sample included in Study IV had a higher percentage of females (72% *versus* 61%, p<0.01), a higher education (9.5±0.3 *versus* 8.7±0.1, p<0.01), and a slightly higher MMSE[194] score (26.6±2 *versus* 26.1±2, p<0.01). There were no differences with regard to age, *APOE* status, BMI, mean serum levels of total cholesterol and history of cardio/cerebrovascular conditions (myocardial infarction or stroke or diabetes).

3.2.2.2 Data collection

The examination in 1998 comprised a self-administered questionnaire on sociodemographic characteristics, health related behaviours, including smoking habits,

alcohol consumption, medications and vitamin supplements use, medical history, including cerebrovascular and cardiovascular events, and conditions diagnosed by physicians. Nurses specifically trained for the survey checked the questionnaires to ensure that they were fully completed. Information on the use of lipid lowering medications (i.e., statins, fibrates) was also available from the Drug Reimbursement Registry. Height, weight, and blood pressure were measured. BMI was calculated as weight (in kilograms) divided by height squared (in meters). The same procedure was used for the re-examination in 2005-2008.

3.2.2.3 Diagnosis of dementia and mild cognitive impairment

Both in 1998 and 2005-2008 cognitive status of the participants was determined with a three-step protocol including a screening phase, a clinical phase and a differential diagnostic phase. In 1998 the screening phase included the following cognitive tests: MMSE, as a measure of global cognition;[194] immediate word recall tests for episodic memory;[226,227] category fluency test for semantic memory;[228] Purdue Peg Board test and letter-digit substitution test for psychomotor speed;[229,230] Stroop test for executive functioning;[231] prospective memory task;[232] subjective memory rating.[233] In 2005-2008 the screening phase included the all tests used in 1998 and the Finnish version of the CERAD neuropsychological test battery.[201] In the 1998 screening, subjects who scored 24 or less in the MMSE were referred for further investigations (clinical phase). In the 2005-2008 screening, subjects who scored 24 or less in the MMSE, had a decline of 3 points or more in MMSE since the 1998 examination, had a delayed recall in CERAD word list of <70%,[201] or serious informant concern regarding participant's cognition, were referred for further investigations. The clinical phase included neurologic, cardiovascular, and detailed neuropsychological examinations. A review board consisting of the study physician, the study neuropsychologist, and a senior neurologist ascertained the primary diagnosis based on all available information. Subjects with possible MCI or dementia were invited to the differential diagnosis phase which included including laboratory tests, brain neuroimaging (MRI), chest radiograph, electrocardiogram and CSF analysis. All data accumulated from the screening and clinical phases were carefully reanalyzed by the review board before the final diagnosis was established. Dementia was diagnosed according to the DSM-IV criteria,[203] and probable and possible AD was diagnosed according to the NINCDS-ADRDA criteria.[55] Information on the use of AD drugs (i.e., acetylcholinesterase inhibitors, memantine) was also available from the Drug Reimbursement Registry. Participants were classified as having MCI using a modified version of the Mayo Clinic AD Research Center Criteria,[234,235] which was also in line with the more recent consensus criteria.[50] These included: 1) memory complaint by patient, family or physician; 2) normal ADL; 3) normal global cognitive function as judged by physician; 4) objective impairment of memory or other areas of cognitive functioning as evidenced by scores >1.5 standard deviation (SD) below age-appropriate mean; 5) CDR score of 0.5; 6) absence of dementia.

As suggested by the Mayo Clinic AD Research Center Criteria, the cut off point 1.5 SD below the norm in the neuropsychological tests was used as a guideline in the clinical assessment of cognitive performance.[234]

3.2.2.4 Vitamin E assessment

At baseline (1998) blood samples were taken from subjects after a minimum of two hours fasting; serum samples were aliquoted and stored initially at -20 °C and then at -70 °C. Serum tocopherols, tocotrienols, αTQ and 5-NO_2-γ-tocopherol were measured with reverse-phase HPLC using electrochemical-coularray system (ESA, Chelmsford, MA, USA) as previously described (see paragraph 3.1.1.4).

3.2.2.5 Other biochemical analyses

Serum total cholesterol was assessed at baseline by standardized enzymatic essay.[236] Blood leukocyte samples were analyzed to determine *APOE* genotype. To extract DNA, a standard phenol-chloroform technique was used; *APOE* genotypes were analyzed by PCR and restriction enzyme *HhaI* digestion.[237]

3.3 Statistical analysis

All data analyses were completed with the Statistical Package for the Social Sciences (SPSS, version 17.0 for Windows; SPSS Inc., Chicago, IL, USA), the Stata version 10.0 and 12.0 for Windows (Stata-Corp, LP, Texas, USA), and the software package Soft Independent Modeling of Class Analogy (SIMCA, Umetrics AB, Umea, Sweden). Table 8 summarizes the outcome variables and the determinants for each study.

Statistical tests. The main associations examined in this thesis were between vitamin E plasma/serum levels - four tocopherols, four tocotrienols, markers of vitamin E oxidative/nitrosative damage -, MCI and AD. Throughout the four studies a range of statistical tests were employed. Chi-square test, logistic regression, Student's t-test, one-way analysis of variance (ANOVA) with Bonferroni post-hoc comparison, or linear regression were performed to assess the statistical differences of proportions and means between groups.

As vitamin E is transported by plasma lipoproteins, and lipoproteins metabolism might affect the delivery of vitamin E to tissues, the vitamin E/cholesterol ratio (absolute plasma/serum concentration of vitamin E divided by total cholesterol) has been used in the main analyses.[134] Absolute plasma/serum levels of vitamin E were considered in additional analyses in Study I, III and IV. The sum of all tocopherols (α+β+γ+δ tocopherol), all tocotrienols (α+β+γ+δ tocotrienol) and all vitamin E forms (all tocopherols+all tocotrienols) were considered, in order to have informative indices of vitamin E status in

the body; each vitamin E form was also considered separately. Further, the ratios αTQ/α-tocopherol and 5-NO_2-γ-tocopherol/γ-tocopherol were used in the analyses, as indices of α- and γ-tocopherol consumption due to oxidative and nitrosative damage, respectively. Indeed, αTQ is the primary product of α-tocopherol oxidation,[119] while 5-NO_2-γ-tocopherol is a product of reaction between γ-tocopherol and RNS.[113]

In Study I, III and IV, plasma/serum levels of each vitamin E indices were tertiled, and the lowest tertile was used as reference in the analysis. Separate models were fitted for each vitamin E index. In Study II vitamin E indices were used as continuous variables, and partial correlation was used to explore the relationship among each vitamin E index and MRI data while controlling for age and gender. Logarithmic transformation of the MRI data was done in case of non-normal distribution.

3.3.1 Specific analyses for each study

Study I: Multivariable polytomous-logistic-regression analyses were used to estimate the odds ratios (OR) and 95% confidence intervals (CI) of vitamin E plasma levels for AD and MCI, with CN subjects as the reference group. Retinol and β-carotene plasma levels were also considered. As for vitamin E indices, plasma levels of these micronutrients were tertiled, and the lowest tertile was used as reference in the analysis. Age, sex, education, *APOE* genotype, location (six dummy variables with France as reference), and disease duration were considered as potential confounders in all models. Age, education and disease duration (in years) were used as continuous variables. *APOE* genotype was used dichotomously in the analyses: absence of ε4 allele versus presence of either 1 or 2 ε4 alleles. Two main models were fitted: a basic adjusted (Model 1: adjusted by age, gender, education), and a fully adjusted model (Model 2). Albumin plasma level as a continuous variable was also adjusted for in the supplementary analysis. Statistical interactions were examined by incorporating the independent variables and cross-product terms in the same model.

Additional analyses were also performed, including i) analyses of absolute values of plasma vitamin E, using serum total cholesterol as covariate; ii) polytomous-regression excluding patients with moderate (n=39) and severe (n=2) AD, thus comprising only subjects with very mild (CDR: 0.5, n=12) or mild (CDR: 1, n=115) AD.

Study II: MRI measures and plasma vitamin E values were analyzed using OPLS, a supervised multivariate data analysis method included in the software package SIMCA (Umetrics AB, Umea, Sweden).[70,238,239] Pre-processing was performed using mean centering and unit variance scaling. Mean centering improves the interpretability of the data, by subtracting the variable average from the data. By doing so the data set is repositioned around the origin. Large variance variables are more likely to be expressed in modeling than low variance variables. Consequently, unit variance scaling was selected to

scale the data appropriately. This scaling method calculates the standard deviation of each variable. The inverse standard deviation is used as a scaling weight for each MRI and vitamin E measure.

Altogether 67 variables were used for OPLS analysis (57 MRI variables and 10 vitamin E indices, Table 7). No feature selection was performed, meaning all measured variables were included in the analysis. MRI and vitamin E variables were analyzed separately; a separate OPLS model was created for each set of variables. The two models were then combined using hierarchical modeling. This means that the scores obtained from each variable model (the MRI OPLS model and the vitamin E OPLS model) were used as new latent variables in a hierarchical model. Three OPLS models were created for AD *versus* CN and MCI *versus* CN: (1) MRI data; (2) vitamin E data; and (3) a hierarchical model containing models (1) and (2).

The AD *versus* CN OPLS model including MRI + vitamin E baseline data was used as training set to prospectively predict MCI conversion to AD at one year follow-up. This produced a discriminant score (the OPLS MRI + vitamin E based score) for each individual with MCI, reflecting the degree to which the individual's pattern (based on MRI and vitamin E) resembled the pattern of subjects with AD or the pattern of CN subjects.

A seven fold cross-validation was utilized to validate the OPLS model, which means that 1/7th of the data is omitted for each cross-validation round. The OPLS analysis generates a model with a predictive component, and it is characterized by a $Q^2(Y)$ value that describes its statistical significance for separating groups. $Q^2(Y)$ values > 0.05 are regarded as statistically significant.[240]

Variables included in the OPLS analysis were plotted according to their importance for the separation of groups. The plots describe the MRI and vitamin E measures and their corresponding jack-knifed CIs. Jack-knifing is used to estimate the bias and standard error. The cross-validation results from each model can be fed directly to jack-knifing. By doing so, the various sub-models generated by cross-validation are used to calculate the standard error of the different model parameters, which are then converted into CIs via the t-distribution.[240] Measures with CIs that include zero have low reliability.[238] A measure with high covariance is more likely to have an impact on group separation than a variable with low covariance.

Sensitivity and specificity were calculated from the cross-validated prediction values of the OPLS models. The sensitivity was calculated as the percentage of AD (or MCI) subjects classified as AD (or MCI), and the specificity as the percentage of CN subjects classified as CN subjects. Finally, the positive and negative likelihood ratios (LR) (LR+ = sensitivity/(100-specificity) and LR- =(100-sensitivity)/specificity) were calculated. A positive LR between 5-10 or a negative LR between 0.1-0.2 increases the diagnostic value in a moderate way, while a value above 10 or below 0.1 significantly increases the diagnostic value of the test.[241]

Study III: The incidence rates of AD were calculated as the number of events occurring during the entire follow-up period divided by person-years of follow-up. Cox proportional hazard models were used to estimate the hazard ratios (HRs) and 95% CI of AD in relation to vitamin E plasma levels determined at baseline. For non-demented subjects the follow-up time was calculated from the date of baseline interview to the date of the last follow-up examination or death. For demented subjects the follow-up time was estimated as the full time during which the subjects were free of dementia plus half of the follow-up time during which dementia developed. For each vitamin E index, two sets of Cox proportional hazard models were performed: i) Unadjusted analysis; ii) Multiadjusted analysis. The proportional hazard assumption was confirmed by the test based on Schoenfeld residuals.[242]

Age, sex, education, baseline MMSE score, *APOE* status, BMI, current smoking status (yes/no) and alcohol use (yes/no) were considered as potential confounders. Because ADL status (absence of disability *versus* presence of any disability) and multimorbidity (presence *versus* absence of multimorbidity) did not significantly affect the vitamin E - AD association, models without ADL and multimorbidity are presented. Age, MMSE score, and BMI were used as continuous variables; education was split in three levels: 0-7 years, 8-14 years, and ≥14 years. *APOE* genotype was used dichotomously in the analyses: absence of ε4 allele *versus* presence of either 1 or 2 ε4 alleles. As it is likely that dementia was under diagnosed in deceased individuals, and there is evidence of association between vitamin E plasma levels and mortality,[166,243] analyses were adjusted also for survival status at follow-up.

Additional analyses were performed, considering absolute values of plasma vitamin E. To take into account cholesterol levels, the population was grouped according to their median levels of vitamin E and cholesterol. This grouping was done to consider both vitamin E levels (absolute value) and the influence of lipoproteins on vitamin E biokinetics: increased lipids levels may be associated with increased plasma levels of vitamin E (absolute value) but it is not known if this promotes a linear increase in vitamin E delivery to tissues. Indeed, in impaired metabolic states, like hyperlipidaemia, vitamin E deficiency may be missed if lipid concentration is not considered. The following three groups were identified: 1) subjects with vitamin E plasma level under the median and cholesterol serum level over the median; 2) subjects with both vitamin E and cholesterol levels either under or over the median; 3) subjects with vitamin E plasma level over the median and cholesterol serum level under the median. Group 2 and 3 included subjects with vitamin E – cholesterol proportions more likely to facilitate the delivery of vitamin E to tissues. Subjects in group 1 constituted the reference category. Each vitamin E index was tested in a separate Cox proportional hazard model, while adjusting for the same confounders considered in the main analysis.

Study IV: Cognitive impairment was defined as development of MCI or AD. Logistic regression analyses were used to estimate the ORs and 95% CIs for the relation between vitamin E serum levels and cognitive impairment. Age, education, BMI (given linear representation), gender, *APOE* genotype (presence/absence of the ε4 allele), current smoking status (yes/no), alcohol use (yes/no), and history of cardio/cerebrovascular conditions (considered positive if the subjects had been diagnosed with myocardial infarction, stroke or diabetes) were considered as potential confounders. Two main models were fitted: a basic adjusted (Model 1: adjusted by age, gender, and education) and a multi-adjusted model (Model 2). Because baseline MMSE score, lipid lowering medications use, and duration of follow-up did not significantly affect the vitamin E - cognitive impairment association, they were excluded from analyses. In order to ensure representativeness of the study sample, the data were weighted for the inverse of the probability of each person from the original CAIDE population to be included in the vitamin E study population.
Absolute values of serum vitamin E were considered in additional logistic regression analyses, grouping the population according to the median levels of vitamin E and cholesterol, with the same procedure applied in Study III.

3.3.2 Pooled analyses

To further evaluate the correlation among vitamin E levels and cognition, the three populations used in the project were pooled together, which yielded a final sample of 893 subjects, including 168 AD cases, 166 MCI and 559 CN individuals.
Logistic quantile regression was used to evaluate the correlations among each vitamin E index and the MMSE total score, which was administered at the time of vitamin E assessment. This analytical method is valid for any population distribution and is appropriated for continuous outcomes variables that take on values within a defined range, such as the MMSE.[244] To describe the correlation among vitamin E and the whole range of MMSE values in our population, four percentiles of the MMSE total score were considered: 5th, 25th, 50th, and 75th. Each vitamin E form, total tocopherols, total tocotrienols, total vitamin E, the ratios α-TQ/α-tocopherol and 5-nitro-γ-tocopherol/γ-tocopherol were tested in separated models. The following variables, available for all subjects, were considered as potential confounders: age and education (given linear representation), gender, *APOE* genotype (presence/absence of the ε4 allele), and cohort of origin (three dummy variables with AddNeuroMed as reference). Two models were fitted: a basic adjusted (Model 1: adjusted by age) and a fully adjusted (Model 2). The p-values of the coefficients calculated for each percentile were based on 100 bootstrap samples.

Table 8. Statistical models used in various studies of this thesis

Clinical Studies	Model	Independent variables	Dependent variables	Covariates	Comments
Study I	Multivariable polytomous logistic regression	Vitamin E plasma levels standardized by cholesterol and absolute values Retinol, β-carotene	AD MCI CN	Age, sex, education, *APOE* genotype, location, disease duration, albumin	Model 1: age, sex, education Model 2: age, sex, education, *APOE* genotype, location, disease duration Additional model: subjects with moderate-severe AD excluded Interactions of vitamin E with the other covariates were tested by adding specific terms to the model
Study II	Orthogonal partial least squares to latent structures (OPLS)	Vitamin E plasma levels standardized by cholesterol MRI (automated regional volume and cortical thickness measures)	AD MCI CN		Model 1: 57 MRI variables Model 2: 10 vitamin E variables Model 3: 67 variables (MRI+vitamin E) AD *versus* CN (Model 3) used as training set to prospectively predict MCI conversion to AD
Population Studies	**Model**	**Exposure**	**Outcome**	**Covariates**	**Comments**
Study III	Cox proportional hazards model	Vitamin E plasma levels standardized by cholesterol and absolute values	AD	Age, sex, education, baseline MMSE score, BMI, *APOE* genotype, smoking status, alcohol use, survival status, ADL, multimorbidity	Model 1: unadjusted Model 2: age, sex, education, MMSE score, BMI, *APOE* genotype, smoking status, alcohol use, survival status
Study IV	Logistic regression	Vitamin E serum levels standardized by cholesterol and absolute values	AD+MCI	Age, sex, education, baseline MMSE score, BMI, *APOE* genotype, smoking status, alcohol use, history of cardio/cerebrovascular conditions*, lipid lowering medications use, duration of follow-up	Model 1: age, sex, education Model 2: age, sex, education, BMI, *APOE* genotype, smoking status, alcohol use, history of cardio/cerebrovascular conditions. Weighting was used to ensure representativeness of the study sample
Pooled analysis	Logistic quantile regression	Vitamin E levels standardized by cholesterol	MMSE	Age, sex, education, *APOE* genotype, cohort of origin	Model 1: age Model: all covariates included

AD: Alzheimer's disease; MCI: mild cognitive impairment; CN: normal cognition; ADL: activities of daily living; BMI: body mass index; MMSE: Mini-Mental State Examination; MRI: magnetic resonance imaging. *Cardio/cerebrovascular conditions: myocardial infarction, stroke, diabetes.

4 ETHICAL CONSIDERATIONS

4.1 The AddNeuroMed Project

For the AddNeuroMed study written informed consent was obtained directly from the study participants if they had capacity, and in those cases where dementia compromised capacity then assent from the patient and written consent from a relative, according to local law and process, was obtained. This study was approved by ethical review boards at each data acquisition site and the data coordination site (local ethical review board at University of Perugia, Italy; University of Toulouse, France; Aristotle University of Thessaloniki, Greece; Medical University of Lodz, Poland; University of Eastern Finland and University Hospital of Kuopio, Finland; King's College London, United Kingdom).

4.2 The Kungsholmen Project

For the Kungsholmen Project informed consent was obtained at baseline from the study participants, after explaining the aims of the project and clarifying that all information would be kept strictly confidential. If there was any indication that the subject had severe cognitive impairment, consent was taken from a proxy, usually a next-of-kin or close relative. However, the examination or interview was to be interrupted if the participant, in any way, expressed anguish or discomfort, regardless of whether the informed consent had been given by the subjects themselves or by a proxy. All phases of the Kungsholmen Project received approval from the Ethics Committee at the Karolinska Institutet, Stockholm, Sweden.

Study III included in this thesis used the data collected from Phase III to Phase V of the project, as well as data from medical records, death certificates, and the inpatient register database. For each phase data collection, approval from the Ethics Committee at the Karolinska Institutet was obtained:

- Phases I & II (baseline survey): Dnr. 87:148; Dnr. 87:234
- Phase III (the first follow-up examination): Dnr. 90:251
- Phase IV (the second follow-up evaluation): Dnr. 94:122
- Phase V (the third follow-up examination): Dnr. 99:308
- Death certificate and Inpatient register data: Dnr. 99:025; Dnr. 01:020

All staff working with the Kungsholmen Project database follow the guidelines of the Swedish Council for Research in the Humanities and Social Sciences: the principles of autonomy and integrity, the rule of consent, and the demand for research.[245]

4.3 The CAIDE study

For the CAIDE study written informed consent was obtained from all participants. All phases of the CAIDE Project received approval from the local ethics committee (University of Kuopio and Kuopio University Hospital).

- First examination: Dnr. 24/97
- Second examination: Dnr. 124/2004

The Ethics Committee at the Karolinska Institutet further approved the project (Dnr: 2004/2:3).

5 RESULTS

5.1 Clinic-based studies

5.1.1 Characteristics of the population

Five hundred and twenty one AddNeuroMed study participants had plasma available for vitamin E assessment at baseline: 168 cases with AD, 166 MCI, and 187 CN individuals. The characteristics of this group are reported in Table 9. Compared with MCI and CN subjects, AD patients were older and more often female. Both AD and MCI patients were less educated and more likely to be *APOE* ε4 carriers compared to CN subjects (Table 9). Mean duration of cognitive problems was 4.3±3.0 years for AD and 3.1±3.1 years for MCI ($p<0.01$) cases, respectively. Both total cholesterol and albumin mean serum levels were in the range of normality, and did not differ among the three groups. As expected, performance on the MMSE was poorest among patients with AD and best among CN, with the MCI group having scores between the AD and the CN groups (Table 9). Only 5 subjects were taking α-tocopherol supplements (CN: n=1; MCI: n=3; AD: n=1, p=0.36).

Table 9. Characteristics of the study participants (N = 521) by diagnosis of cognitive impairment.

Subjects characteristics	CN n = 187	MCI n = 166	AD n = 168
Age, y	74.7 (5.3)	75.8 (5.6)	77.4 (6.3)* §§
Gender, % female [a]	54%	58%	68%
Education, y	10.3 (4.4)	8.8 (4.3) ††	8.0 (4.1)*
Any *APOE* ε4 allele, % [a,b]	29%	41%	52%
MMSE score	28.8 (1.3)	27.1 (1.8) †	20.2 (4.7)* §
Serum albumin (g/dl)	4.3 (0.4)	4.3 (0.4)	4.3 (0.4)
Serum total cholesterol (mmol/L)	5.2 (1.1)	5.4 (1.2)	5.4 (1.2)

If not otherwise specified, data are given as mean (SD). Mini-Mental-State-Examination (MMSE) score ranged from 0 (worst) to 30 (best).

AD: Alzheimer's disease; MCI: mild cognitive impairment. CN: cognitively normal.

[a] $p<0.05$. [b] *APOE* was available for 489 subjects

AD *versus* CN: *$p<0.0001$; AD *versus* MCI: §$p<0.0001$; §§ $p<0.05$; MCI *versus* CN: †$p<0.0001$; †† $p<0.01$

5.1.2 Relation of vitamin E to Alzheimer's disease and mild cognitive impairment (Study I)

When considering plasma levels of vitamin E standardized by cholesterol, AD subjects showed reduced mean plasma levels of all vitamin E congeners, total tocopherols, total tocotrienols, and total vitamin E, as compared to CN subjects. Similar results were found for MCI cases, but for β-tocotrienol the difference with CN subject was not statistically significant (Table 10).

Table 10. Vitamin E plasma levels by diagnosis of cognitive impairment.

Vitamin E forms	CN n = 187	MCI n = 166	AD n = 168
Vitamin E adjusted by cholesterol[a]			
α-tocopherol	6.66 (1.81)	5.95 (1.83) †	5.53 (1.32)*
β-tocopherol	0.49 (0.15)	0.43 (0.13) †	0.46 (0.12)***
γ-tocopherol	0.46 (0.13)	0.38 (0.11) †	0.35 (0.09)*
δ-tocopherol	0.057 (0.015)	0.051 (0.014) †	0.053 (0.013)**
α-tocotrienol	69.85 (20.38)	54.61 (15.92) †	54.34 (13.79)*
β-tocotrienol	28.88 (7.93)	27.60 (7.70)	25.77 (6.32)*
γ-tocotrienol	16.81 (4.84)	12.92 (4.11) †	9.44 (2.51)* §
δ-tocotrienol	2.48 (1.03)	2.15 (1.06) ††	1.78 (0.81)* §§
Total tocopherols	7.67 (2.05)	6.80 (2.08) †	6.40 (1.52)*
Total tocotrienols	118.02 (30.61)	97.28 (27.44)†	91.33 (22.19)*
Total vitamin E	7.80 (2.07)	6.90 (2.10) †	6.49 (1.54)*
Ratio α-tocopherylquinone/α-tocopherol	1.55 (0.29)	1.70 (0.21) †	1.80 (0.19)* §
Ratio 5-nitro-γ-tocopherol/γ-tocopherol	9.93 (1.63)	13.22 (1.88) †	12.49 (1.74)* §
Vitamin E absolute values[b]			
α-tocopherol	33.21 (5.15)	30.24 (3.33)†	28.18 (2.42)* §
β-tocopherol	2.46 (0.44)	2.18 (0.24)†	2.33 (0.25)** §
γ-tocopherol	2.30 (0.26)	1.92 (0.19)†	1.80 (0.16)* §
δ-tocopherol	0.29 (0.02)	0.26 (0.02)†	0.27 (0.02)* §
α-tocotrienol	349.0 (68.5)	278.9 (27.6)†	276.1 (26.0)*
β-tocotrienol	143.7 (18.6)	141.0 (11.1)	131.1 (10.0)* §
γ-tocotrienol	83.63 (12.80)	65.87 (10.17)†	48.15 (7.40)* §
δ-tocotrienol	12.33 (4.27)	11.0 (4.34)††	9.00 (3.23)* §
Total tocopherols	38.26 (5.50)	34.61 (3.63)†	32.58 (2.70)* §
Total tocotrienols	588.7 (74.7)	496.8 (37.6)†	464.3 (31.2)* §
Total vitamin E	38.85 (5.55)	35.10 (3.64)†	33.04 (2.71)* §

Data are given as mean (SD). [a] Levels of tocopherols and total vitamin E are expressed as μmol per mmol cholesterol. Tocotrienols are expressed as nmol per mmol cholesterol. The ratios α-tocopherylquinone/α-tocopherol and 5-nitro-γ-tocopherol/γ-tocopherol are expressed as nmol/μmol. [b]Absolute values of plasma tocopherols and total vitamin E are expressed as μM; tocotrienols are expressed as nM.
AD *versus* CN: *p<0.0001; **p<0.01; ***p<0.05; AD *versus* MCI: §p<0.0001; §§p<0.01;
MCI *versus* CN: †p<0.0001; †† p<0.01

The mean plasma levels of the ratios αTQ/α-tocopherol and 5-NO_2-γ-tocopherol/γ-tocopherol were significantly higher in AD and MCI cases, in comparison to CN subjects (Table 10).

The evaluation of the absolute values of plasma vitamin E yielded the same pattern of differences among the three groups: mean plasma levels of each vitamin E form, total tocopherols, total tocotrienols and total vitamin E were lower in MCI and AD patients, in comparison to CN subjects, and the difference was statistically significant for all compounds, except for β-tocotrienol in MCI (Table 10).

Results of polytomous-regression are showed in Table 11.

Table 11. Association of vitamin E with diagnosis of AD and MCI.

Vitamin E plasma level	MCI		AD	
	Model 1 OR (95%CI)	Model 2 OR (95% CI)	Model 1 OR (95%CI)	Model 2 OR (95% CI)
Total tocopherols				
Lowest tertile	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Middle tertile	0.37 (0.21-0.66)	0.64 (0.21-1.92)	0.59 (0.34-1.05)	1.16 (0.39-3.51)
Highest tertile	0.21 (0.11-0.38)	**0.15 (0.05-0.44)**	0.21 (0.11-0.4)	**0.16 (0.05-0.46)**
Total tocotrienols				
Lowest tertile	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Middle tertile	0.30 (0.16-0.56)	0.32 (0.10-1.06)	0.32 (0.18-0.59)	0.38 (0.12-1.25)
Highest tertile	0.11 (0.06-0.21)	**0.08 (0.02-0.26)**	0.07 (0.04-0.14)	**0.06 (0.02-0.21)**
Total vitamin E				
Lowest tertile	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Middle tertile	0.39 (0.22-0.69)	0.65 (0.22-1.95)	0.61 (0.34-1.07)	1.15 (0.38-3.47)
Highest tertile	0.19 (0.10-0.36)	**0.15 (0.05-0.42)**	0.20 (0.11-0.39)	**0.15 (0.05-0.45)**
Ratio α-tocopherylquinone/α-tocopherol				
Lowest tertile	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Middle tertile	4.74 (2.71-8.3)	**3.97 (1.52-10.4)**	11.9 (5.8-24.2)	**10.5 (3.6-30.6)**
Highest tertile	6.53 (3.22-13.3)	**4.43 (1.27-15.5)**	32.8 (14.4-74.6)	**25.3 (6.8-93.7)**
Ratio 5-nitro-γ-tocopherol/γ-tocopherol				
Lowest tertile	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Middle tertile	14.3 (7.0-29.3)	**12.7 (4.4-36.4)**	8.2 (4.6-14.7)	**8.9 (3.3-24)**
Highest tertile	133.8 (53-338)	**48.1 (12.7-182)**	28.3 (12.3-65.5)	**11.0 (3.1-39.0)**

OR with 95% CI of vitamin E plasma levels for AD and MCI diagnosis. Multivariable polytomous logistic model, CN subjects as reference; vitamin E, as tertile, is considered as indicator variable. Model 1: adjusted by age, gender, education. Model 2: adjusted by age, gender, education, *APOE* status, location, disease duration.

In the fully adjusted model (Model 2), both diagnosis of MCI and AD were associated with reduced plasma levels of total tocopherols, total tocotrienols, and total vitamin E, and increased plasma levels of the ratios αTQ/α-tocopherol and 5-NO_2-γ-tocopherol/γ-tocopherol. Indeed, compared to CN subjects, both MCI and AD cases were less likely to be in the highest tertile of total tocopherols, total tocotrienols, and total vitamin E. Further, both AD and MCI were significantly more likely to be in the middle or in the highest tertile of the ratios αTQ/α-tocopherol and 5-NO_2-γ-tocopherol/γ-tocopherol (Table 11).

Regression analysis results for each vitamin E congener are shown in Figure 6, which illustrates the ORs with 95% CI of each vitamin E form (highest versus lowest tertile) for AD and MCI. Compared to CN subjects, both AD and MCI patients were less likely to be in the highest tertile of each vitamin E form than in the lowest; except for β- and δ-tocopherol in AD, these associations were all statistically significant (Figure 6). For each

vitamin E congener, no significant association was found with the middle tertile, for both AD and MCI (data not shown).

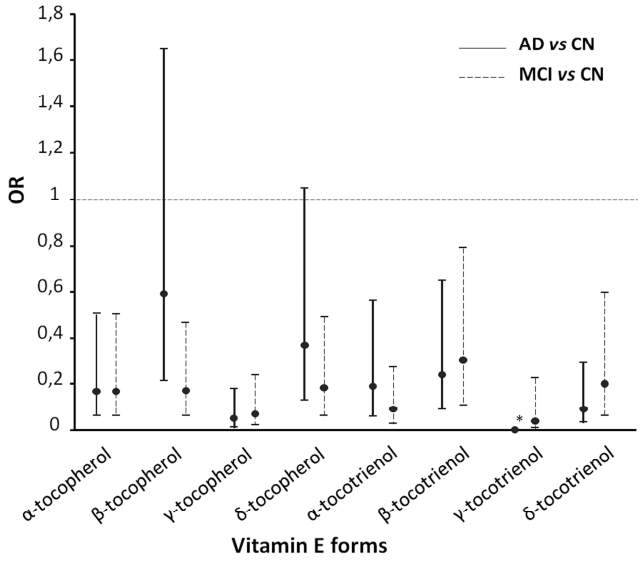


Figure 6. Association of vitamin E forms with diagnosis of AD and MCI. The x-axis shows the different vitamin E forms, and the y-axis show the adjusted OR of each form (highest versus lowest tertile) for AD or MCI diagnosis. The bars represent the 95% CI of the ORs (dots). ORs are adjusted for age, gender, education, presence of *APOE* ε4, location, disease duration. (Multivariable polytomous logistic model, CN subjects as reference).

*γ-tocotrienol: 95%CI for AD: 0.0001-0.01. There were only 4 AD subjects (of 168) in the highest tertile, and only 11 cognitively normal subjects (of 187) in the lowest tertile.

Regression analyses evaluating absolute values of plasma vitamin E were adjusted by age, sex, education, *APOE* ε4 genotype, location, disease duration and cholesterol plasma levels. The fitted regression models produced results similar to the initial analysis: with respect to CN subjects, the OR and 95% CI of total tocopherols (highest *versus* lowest tertile) was 0.20 (0.05-0.74) for MCI and 0.02 (0.01-0.09) for AD; for total tocotrienols, the OR (95% CI) was 0.06 (0.02-0.24) for MCI and 0.01 (0.002-0.03) for AD, while for total vitamin E the OR (95% CI) was 0.20 (0.05-0.74) for MCI and 0.02 (0.01-0.09) for AD. When analyzing each vitamin E congener, both MCI and AD cases were less likely to be in the higher tertile than in the lowest, in comparison to CN subjects. For MCI cases this association was statistically significant for β-, γ-, and δ-tocopherol, α and γ-tocotrienol, while for AD the correlation was statistically significant for α-, γ-, and δ-tocopherol and all tocotrienols (there were no AD cases in the highest tertile of γ-tocotrienol, and there was a significant negative correlation with the middle tertile).

In all regression models interactions between vitamin E and other covariates on the odds of AD and MCI were tested, and they were not statistically significant. Further, inclusion of albumin concentration in the models did not change the results for all plasma compounds evaluated (data not shown).

Finally, to exclude subjects at higher risk of malnutrition, polytomous-regression models were fitted excluding patients with moderate (n=39) and severe (n=2) AD, thus comprising only subjects with very mild (CDR: 0.5, n=12) or mild (CDR: 1, n=115) AD. Results were similar to those from the total population, either considering vitamin E as standardized by cholesterol or as absolute plasma values (data not shown).

Other antioxidant micronutrients.

Retinol and β-carotene plasma levels were decreased both in AD and MCI cases, compared to CN subjects. Mean (SD) values for retinol were 1.73 (0.14) μM for AD, 1.73 (0.15) μM for MCI and 1.87 (0.24) μM for CN subjects ($p<.0001$ for both AD and MCI *versus* CN subjects). β-carotene mean (SD) concentrations were 444.2 (60.8) nM for AD, 467.3 (49.7) nM for MCI and 515.0 (37.5) nM for CN subjects ($p<.0001$ for both AD and MCI *versus* CN subjects and for AD *versus* MCI). The regression analysis confirmed the negative association between plasma levels of both antioxidant micronutrients and cognitive impairment: compared to the lowest tertile, for retinol, the OR and 95%CI of the highest tertile was 0.17 (0.07-0.42) for MCI and 0.22 (0.09-0.57) for AD, while for β-carotene the correlation was statistically significant for both the middle [OR (95% CI): 0.18 (0.05-0.65)] and the highest tertile [OR (95% CI): 0.05 (0.01-0.19)] in MCI and AD [OR (95% CI), middle tertile: 0.09 (0.02-0.33); highest tertile: 0.03 (0.01-0.13)] (fully adjusted models).

5.1.3 Vitamin E and MRI in relation to Alzheimer's disease and mild cognitive impairment (Study II)

Two hundred fifty and three AddNeuroMed study participants had both MRI and vitamin E data from baseline: 81 individuals with AD, 86 MCI cases and 86 CN subjects. Among AD cases, 6 were very mild AD (CDR= 0.5), 59 individuals were mild AD (CDR= 1), and 16 were moderate AD (CDR = 2). Detailed description of the subjects is provided in the Appendix (Study II).

Classification accuracy and MCI prediction

The results from the OPLS analysis are visualized in scatter plots (Figure 7) by plotting the predictive component, which contains the information related to class separation. Figure 7A-C illustrates the separation between AD *versus* CN for the three different models (MRI, vitamin E, and the combination of the two). The separation between AD and CN increases when MRI and vitamin E measures are combined. Table 12 shows the sensitivity, specificity, accuracy, and likelihood ratios for the different models.

Similar results were found when repeating the analysis excluding the 16 individuals with moderate AD: the combination of MRI and vitamin E data had a higher predictive accuracy (97.4%) compared to the one provided by MRI (80.1%) or vitamin E (91.4%) measures alone.

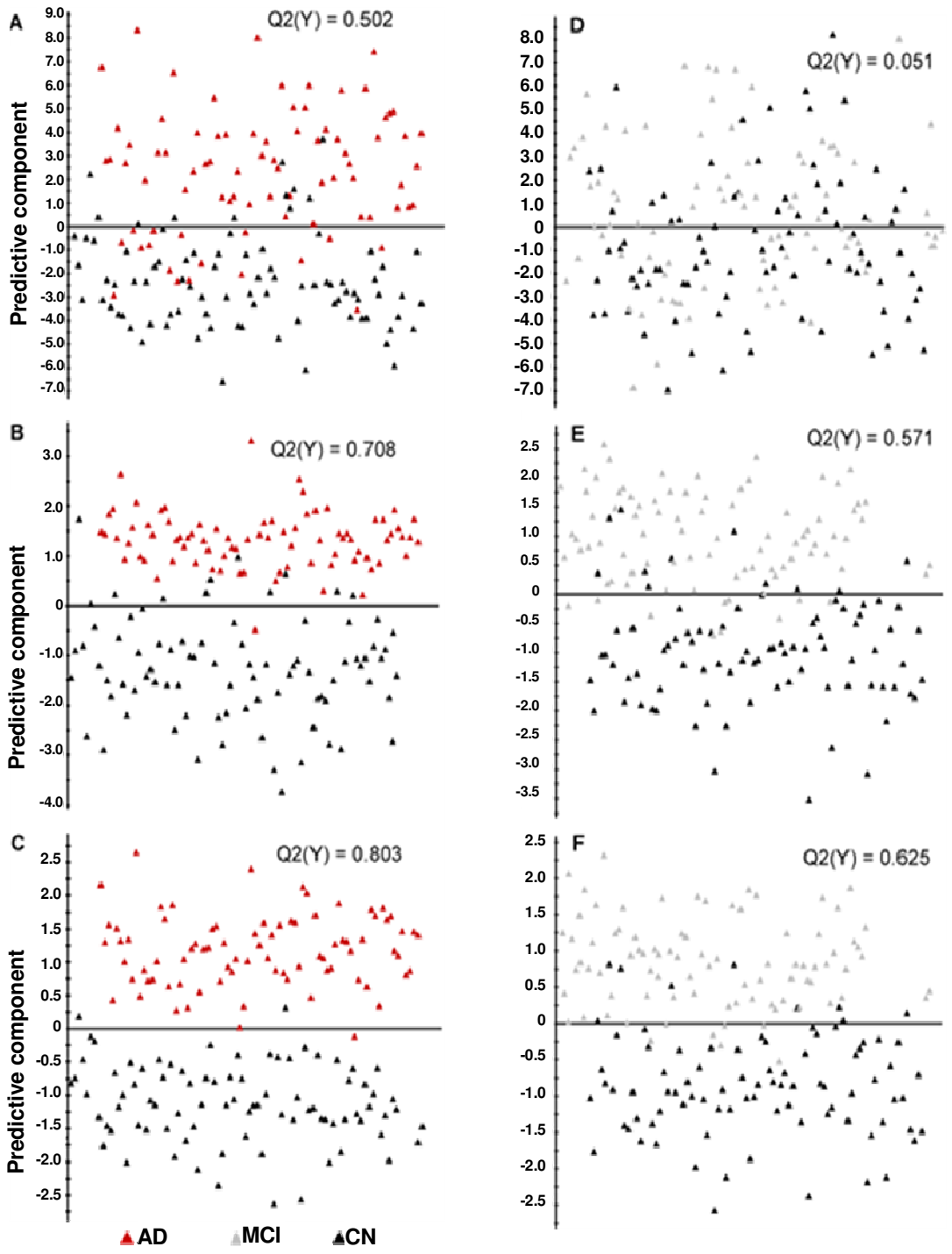


Figure 7. OPLS cross-validated score plots for AD *versus* CN (A-C) and MCI *versus* CN (D-F). (A, D) MRI; (B, E) vitamin E; (C,F) MRI + vitamin E. The scatter plots visualize group separation and the predictability of the different models. Each red triangle represents an AD subject; each grey triangle represents an MCI individual, and each black triangle a control subject. CN subjects above zero and AD or MCI subjects below zero are falsely predicted. Q2(Y) > 0.05 is a statistically significant model.

Figure 7D-F illustrates the separation for the MCI *versus* CN models (MRI, vitamin E and the combination of both). Again the combination of the two measures yielded the best results. The prediction accuracy was 90.7%, compared to 58.1% for MRI and 87.8% for vitamin E measures separately (Table 12).

Table 12. Accuracy, sensitivity, specificity, and LR for the AD *versus* CN models and the MCI *versus* CN models.

	Accuracy	Sensitivity	Specificity	LR+	LR-
AD *versus* CN					
MRI	83.2 (76.8-88.1)	79.0 (69.9-86.5)	87.2 (78.5-92.7)	6.2 (3.5-10.8)	0.24 (0.16-0.37)
Vitamin E	92.8 (87.9-95.8)	98.8 (93.3-99.8)	87.2 (78.5-92.7)	7.7 (4.4-13.4)	0.01 (0.00-0.10)
MRI + vitamin E	98.2 (94.8-99.4)	98.8 (93.3-99.8)	97.7 (91.9-99.4)	42.5 (10.8-167)	0.01 (0.00-0.09)
MCI *versus* CN					
MRI	58.1 (50.7-65.3)	53.5 (43.0-63.6)	62.8 (52.2-72.3)	1.4 (1.0-2.0)	0.74 (0.56-0.98)
Vitamin E	87.8 (82.1-91.9)	88.3 (79.9-93.6)	87.2 (78.5-92.7)	6.9 (4.0-12.1)	0.13 (0.07-0.25)
MRI + vitamin E	90.7 (85.4-94.2)	91.8 (84.1-96.0)	89.5 (81.3-94.4)	7.8 (4.7-16.3)	0.09 (0.05-0.19)

95% confidence intervals within parenthesis. AD: Alzheimer's disease; MCI: Mild Cognitive Impairment; CN: cognitively normal; LR+: positive likelihood ratio; LR-: negative likelihood ratio; MRI: magnetic resonance imaging.

Table 13 reports the analysis using the AD *versus* CN model containing MRI and vitamin E data to predict conversion from MCI to AD at one year follow-up, using baseline data. In this combined model 85% of the MCI converters were correctly classified. Even when we removed individuals with moderate AD, the model still identified 77% of the MCI converters.

Table 13. Predictions of stable and converting MCI subjects on the combined Vitamin E+MRI model for AD *versus* CN.

	Number	AD-like	CN-like
MCI Converters	13	11 (85%)	2 (15%)
MCI Non-converters	73	24 (33%)	49 (67%)

AD: Alzheimer's disease; MCI: Mild Cognitive Impairment; CN: cognitively normal

Variables of importance and correlations

The vitamin E and MRI variables used in the OPLS analysis were plotted according to their importance for the separation of groups (Figure 8 and Figure 9). The plots show the vitamin E and MRI measures and their corresponding jack-knifed CIs. Vitamin E and MRI measures below zero in the plots have lower values in AD and MCI cases compared to CN subjects, while vitamin E and MRI measures above zero are higher in AD and MCI cases compared to CN subjects in the model.

The order of importance for the vitamin E variables is very similar for the AD *versus* CN model compared to the MCI *versus* CN model (Figure 8A-B). The vitamin E measures

more relevant for discriminating both AD and MCI from CN were α- and γ-tocotrienol, γ-tocopherol, and the indices of vitamin E oxidative/nitrosative damage. The pattern of brain atrophy was not as similar for the two different group comparisons (Figure 9A-B). As expected, the most important regions for the separation were medial temporal lobe structures such as hippocampus, entorhinal cortex, and amygdala, among others, for the AD *versus* CN model (Figure 9A). The same pattern of atrophy had been observed in a larger sample of the AddNeuroMed cohort.[69] Medial temporal lobe structures were also of importance for the separation between MCI and CN; however the order of the regions was different (Figure 9B).

Age- and gender-adjusted correlations among each vitamin E index and the MRI data most relevant for group separation according to the OPLS analysis were assessed. Plasma levels of all vitamin E congeners were positively correlated with MRI regional cortical thickness and volumetric measures, but there was a negative correlation with ventricular volume (Table 14). The opposite trend was found for the indices of vitamin E oxidative/nitrosative damage, which were positively correlated with the inferior lateral ventricle measures, and negatively correlated with all other MRI measures.

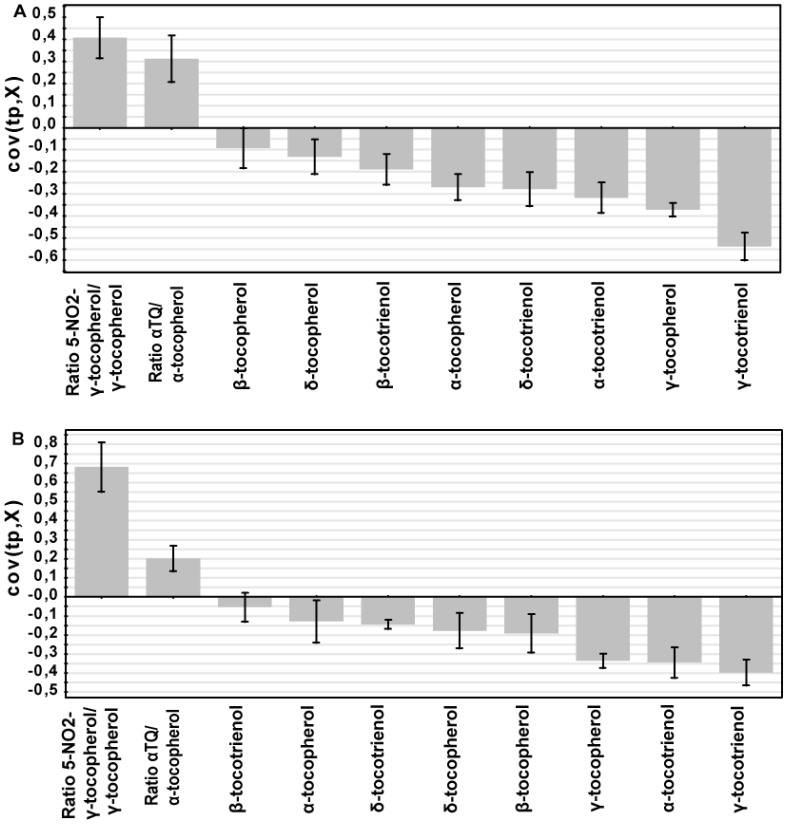


Figure 8. Vitamin E measures of importance for the separation between groups. (A) AD *versus* CN; (B) MCI *versus* CN. Measures above zero have a larger value in the patient groups compared to controls and measures below zero have a lower value the patient groups compared to controls. A measure with a high covariance is more likely to have an impact on group separation than a measure with a low covariance. Measures with jack knifed confidence intervals that include zero have low reliability.

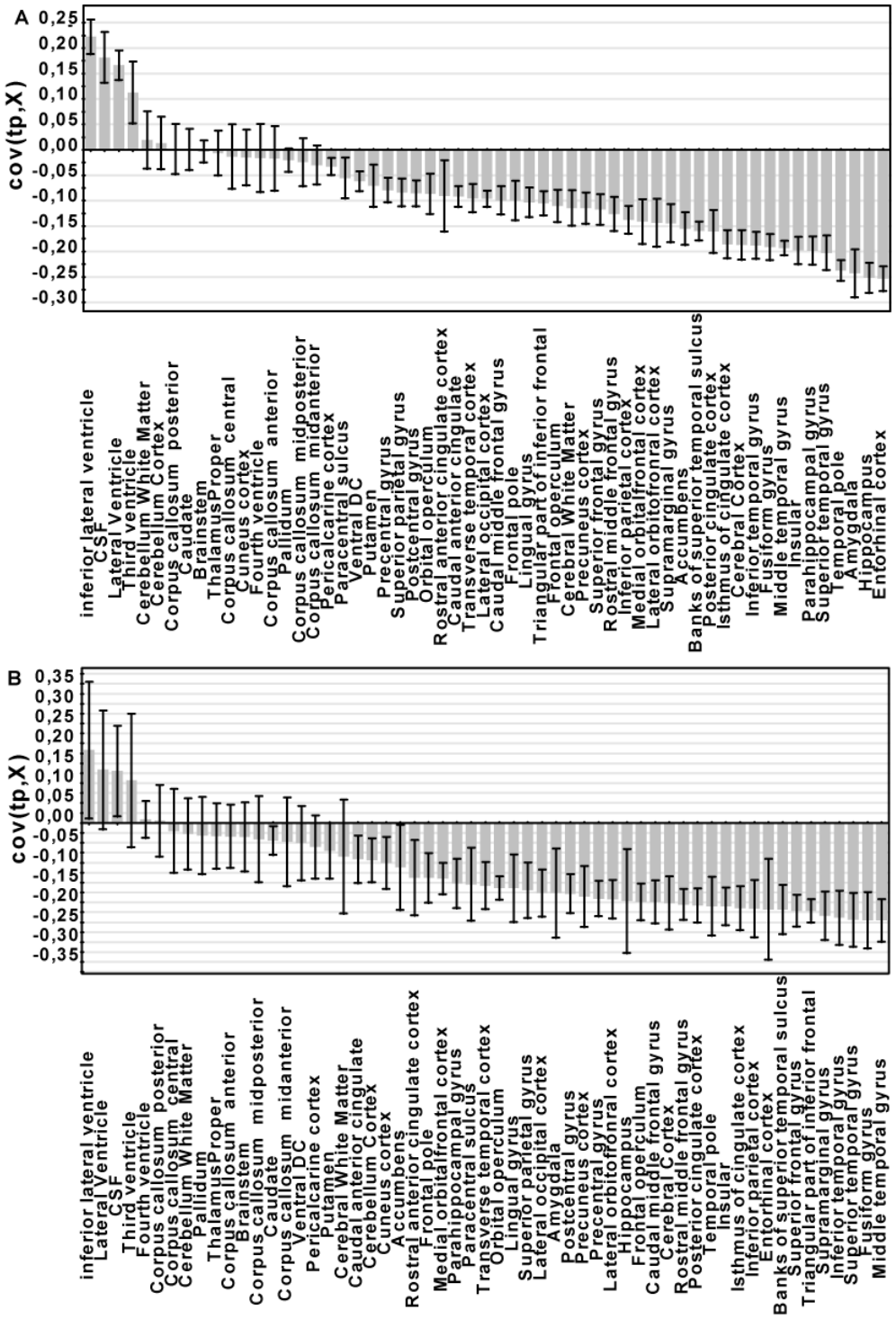


Figure 9. MRI measures of importance for the separation between groups. (A) AD *versus* CN; (B) MCI *versus* CN. Measures above zero have a larger value in the patient groups compared to controls and measures below zero have a lower value the patient groups compared to controls. A measure with a high covariance is more likely to have an impact on group separation than a measure with a low covariance. Measures with jack-knifed confidence intervals that include zero have low reliability.

Correlations were statistically significant for the vitamin E forms more relevant for discriminating AD and MCI from CN in the OPLS analyses: γ-tocopherol, α-, and γ-tocotrienol (non-significant only for α-tocotrienol and temporal pole). Some of the correlations were also significant for α-tocopherol (Table 14). Regarding the indices of vitamin E oxidative/nitrosative damage, all correlations were statistically significant, except the ones with the supramarginal gyrus (both indices) and the fusiform gyrus (only for the ratio α-TQ/α-tocopherol).

Table 14. Partial correlation among MRI data and vitamin E plasma levels.

Neuroimaging	EC	H	Am	TP	STG	MTG	ITG	FG	SMG	ILV*
Vitamin E										
α-tocopherol	0.13[c]	0.16[c]	0.12	0.09	0.11	0.12	0.11	0.14[c]	0.08	-0.12
β-tocopherol	0.09	0.06	0.05	0.03	0.01	0.05	0.04	0.07	0.004	-0.02
γ-tocopherol	0.24[a]	0.23[a]	0.17[b]	0.18[b]	0.16[c]	0.18[b]	0.17[b]	0.19[b]	0.13[c]	-0.18[b]
δ-tocopherol	0.10	0.08	0.07	0.08	0.07	0.08	0.08	0.11	0.07	-0.06
α-tocotrienol	0.17[b]	0.21[b]	0.18[b]	0.10	0.13[c]	0.15[c]	0.15[c]	0.15[c]	0.13[c]	-0.15[c]
β-tocotrienol	0.09	0.10	0.07	0.06	0.09	0.10	0.11	0.14[c]	0.10	-0.08
γ-tocotrienol	0.37[a]	0.40[a]	0.32[a]	0.26[a]	0.29[a]	0.27[a]	0.26[a]	0.27[a]	0.23[a]	-0.33[a]
δ-tocotrienol	0.13[c]	0.12	0.11	0.10	0.08	0.04	0.05	0.05	0.06	-0.15[c]
Ratio αTQ/ α-tocopherol	-0.21[b]	-0.23[a]	-0.16[c]	-0.13[c]	-0.19[b]	-0.13[c]	-0.14[c]	-0.12	-0.12	0.21[b]
Ratio 5-NO2-γ-tocopherol/ γ-tocopherol	-0.25[a]	-0.19[b]	-0.21[b]	-0.23[c]	-0.13[c]	-0.22[a]	-0.19[b]	-0.13[c]	-0.6	0.14[c]

Partial correlation (R value, adjusted by age and gender), calculated including all study participants (N=253). Am: Amygdala EC: Entorhinal cortex; FG: Fusiform gyrus; H: Hippocampus; ILV: Inferior lateral ventricle; ITG: Inferior temporal gyrus; MTG: Middle temporal gyrus; SMG: Supra marginal gyrus; STG: Superior temporal gyrus; TP: Temporal pole.

* Logarithmic transformation of this variable was done to normalize its distribution

p value: [a] <0.001, [b] <0.01, [c] <0.05

5.2 Population-based studies

5.2.1 Characteristics of the populations

The general characteristics of the Kungsholmen Project and CAIDE populations are presented in Table 15. As previously mentioned, the participants in the CAIDE cohort were younger than in the Kungsholmen Project, and the CAIDE project had a longer follow-up time (CAIDE: maximum 10 years; Kungsholmen Project: maximum 6.4 years). Sex distribution, mean education, mean baseline MMSE score and alcohol use were similar in both populations. Mean serum levels of total cholesterol were also comparable among the

two cohorts, but the use of lipid-lowering medication was present only among CAIDE participants. The presence of the *APOE* ε4 allele was higher in the Finnish population, particularly among cases of cognitive impairment. The *APOE* ε4 frequency in Finland is among the highest in the world.[246] There were more current smokers in the CAIDE cohort, and mean BMI values were higher compared to the Swedish population (Table 15). None of the CAIDE participants reported use of vitamin E supplements, which was infrequent in the Kungsholmen cohort.

Table 15. General characteristics of the Kungsholmen Project and CAIDE populations**.**

Baseline characteristics	**Kungsholmen Project**		**CAIDE**	
	Controls (n = 145)	Incident AD (n = 57)	Controls (n = 76)	Incident cognitive impairment (n = 64)
Age, y, [range]	84.6 (3.2) [80-94]	86.2 (2.8)[b] [81-94]	71.3 (4.0) [65.4-79.1]	71.5 (3.8) [65.2-79.2]
Gender, % female	78%	84%	68%	76%
Education, y	9.5 (3.1)	9.4 (3.3)	9.9 (3.5)	8.8 (3.2)[d]
MMSE score	27.1 (2.1)	26.0 (1.5)[a]	26.9 (1.9)	26.3 (1.5)
Any *APOE* ε4 allele, %	25%	26%	27%	52%[c]
BMI (Kg/m^2)	23.3 (3.4)	22.6 (2.5)	27.5 (4.0)	28.1 (4.1)
Current smokers, %	10%	7%	28%	20%
Alcohol use, % users	78%	75%	68%	79%
Serum Total cholesterol, mmol/l	5.65 (1.52)	5.93 (1.37)	5.85 (0.92)	5.83 (0.97)
Lipid lowering medication use, %	(n=1)	0	18%	11%
History of myocardial infarction/stroke/diabetes mellitus, %)	---	---	16%	28%
Multimorbidity (% with 2+ chronic diseases), %	57%	63%	---	---
Vitamin E supplements use (α-tocopherol or multivitamins), %	9%	7%	0	0

If not otherwise specified, data are given as mean (SD). AD: Alzheimer's disease; BMI: Body Mass Index; MMSE: Mini-Mental State Examination. Presence of chronic diseases is not directly comparable since assessment was different in the two cohorts.
AD or cognitive impairment versus correspondent group of controls [a] $p<0.001$ [b] $p<0.01$ [c] $p<0.05$
[d] difference significant at the 10% level

Within the Kungsholmen Project, during the six year follow-up (which covered a total of 771.98 person-years of follow-up) 57 incident cases of probable AD were detected. Among survivors, 51 subjects developed AD, while among subjects who died, 6 were diagnosed with AD. Subjects who developed AD were older than subjects who did not become demented (control group), and had a lower MMSE score at baseline. There were no significant differences among incident cases of AD and controls regarding sex, education,

presence of *APOE* ε4 allele, BMI, serum total cholesterol, presence of multimorbidity and disability at the ADL, smoking status, alcohol use, and vitamin supplements consumption (Table 15 and Appendix, Study III).

The CAIDE cohort had a mean follow-up of 8.2 years. Sixty-four of the 140 subjects had cognitive impairment (24 MCI, 40 AD) at the second re-examination in 2005-2008, while the remaining 76 were cognitively intact. Subjects who developed cognitive impairment were more likely to be *APOE* ε4 carriers and to have a lower education compared to the control group. There were no significant differences between groups on age, gender, baseline MMSE total score, BMI, smoking status, alcohol use, history of cardiovascular/cerebrovascular conditions, lipid lowering medication use, and follow-up duration (Table 15 and Appendix, Study IV).

Baseline plasma/serum concentrations of vitamin E forms for the Kungsholmen Project and CAIDE populations are presented in Table 16. Values standardized by total cholesterol and absolute plasma/serum levels are reported. Except α- and β-tocotrienol, mean levels of each tocopherol and tocotrienol form were higher in the Swedish cohort, compared to the Finnish population. This trend was present when considering both standardized and absolute values.

5.2.2 Vitamin E and risk of Alzheimer's disease in subjects aged 80+ years (Study III)

In the Kungsholmen Project, when considering vitamin E values standardized by cholesterol, mean plasma levels of each tocopherol and tocotrienol congener, total tocopherols, total tocotrienols, and total vitamin E were lower in subjects who developed AD, in comparison to subjects who remained cognitively normal, but the difference was significant (at the 10% level) only for β-tocopherol (Table 16).

The multi-adjusted Cox regression analysis showed that subjects with total tocopherols, total tocotrienols, or total vitamin E plasma levels in the highest tertile had a reduced risk of developing AD, in comparison to subjects in the lowest tertile: the risk was reduced, respectively, by 45% for total tocopherols, 54% for total tocotrienols, and 45% for total vitamin E (Table 17).

When considering each individual vitamin E form, the risk of developing AD was reduced in subjects with plasma levels of β-tocopherol in the highest tertile, with respect to the lowest tertile [HR (95% CI): 0.62 (0.39-0.99)] (Figure 10). There was also a trend for a protective effect of α-tocopherol, α- and β-tocotrienol (highest *versus* lowest tertile) but it was not statistically significant in the multi-adjusted model [HR (95% CI): α-tocopherol: 0.72 (0.48-1.09); α-tocotrienol: 0.70 (0.44-1.11); β-tocotrienol: 0.69 (0.45-1.06)] (Figure 10). For each vitamin E index considered, no significant association was found for the middle tertile (data not shown).

Table 16. Vitamin E levels in the Kungsholmen Project and CAIDE populations by final diagnosis**.**

Vitamin E levels	Kungsholmen Project		CAIDE	
	Controls (n = 145)	Incident AD (n = 57)	Controls (n = 76)	Incident cognitive impairment (n = 64)
Vitamin E forms adjusted by cholesterol#				
α-tocopherol, mean (SD)	7.34 (2.28)	6.86 (1.74)	5.13 (1.02)	5.12 (1.08)
β-tocopherol, mean (SD)	0.47 (0.17)	0.42 (0.14)[d]	0.40 (0.08)	0.41 (0.09)
γ-tocopherol, mean (SD)	0.45 (0.15)	0.41 (0.11)	0.33 (0.06)	0.32 (0.07)
δ-tocopherol, mean (SD)	0.056 (0.020)	0.054 (0.016)	0.047 (0.01)	0.047 (0.01)
α-tocotrienol, mean (SD)	29.78 (13.09)	27.81 (10.65)	48.29 (11.6)	47.81 (10.1)
β-tocotrienol, mean (SD)	16.69 (7.03)	14.99 (5.41)	23.22 (4.2)	22.72 (4.4)
γ-tocotrienol, mean (SD)	17.13 (7.34)	16.24 (7.18)	9.09 (2.28)	8.28 (2.0)[c]
δ-tocotrienol, mean (SD)	2.22 (1.24)	2.15 (1.12)	1.55 (0.51)	1.54 (0.48)
Total tocopherols, mean (SD)	8.23 (2.49)	7.62 (1.89)	5.91 (1.15)	5.90 (1.23)
Total tocotrienols, mean (SD)	65.58 (23.55)	61.04 (17.85)	82.16 (16.61)	80.36 (14.86)
Total vitamin E, mean (SD)	8.29 (2.51)	7.68 (1.90)	6.00 (1.16)	5.98 (1.24)
Ratio α-tocopherylquinone/α-tocopherol, mean (SD)	---	---	1.76 (0.16)	1.76 (0.14)
Ratio 5-nitro-γ-tocopherol/γ-tocopherol, mean (SD)	---	---	11.74 (2.11)	12.50 (1.40)[c]
Vitamin E forms absolute value ‡				
α-tocopherol, mean (SD)	38.53 (3.97)	38.67 (2.87)	29.26 (2.99)	28.91 (2.44)
β-tocopherol, mean (SD)	2.46 (0.44)	2.36 (0.43)	2.30 (0.24)	2.33 (0.20)
γ-tocopherol, mean (SD)	2.38 (0.29)	2.39 (0.32)	1.88 (0.17)	1.83 (0.15)[d]
δ-tocopherol, mean (SD)	0.29 (0.05)	0.29 (0.04)	0.27 (0.01)	0.27 (0.02)
α-tocotrienol, mean (SD)	157.02 (60.6)	155.8 (41.0)	275.7 (47.9)	272.9 (49.8)
β-tocotrienol, mean (SD)	86.51 (22.14)	84.54 (22.0)	132.5 (10.3)	128.7 (9.6)[c]
γ-tocotrienol, mean (SD)	90.04 (26.6)	94.8 (36.5)	51.75 (9.56)	46.82 (7.79)[b]
δ-tocotrienol, mean (SD)	11.57 (4.8)	11.87 (5.3)	8.86 (2.76)	8.75 (2.27)
Total tocopherols, mean (SD)	43.44 (2.8)	43.78 (2.93)	33.71 (3.16)	33.34 (2.58)
Total tocotrienols, mean (SD)	346.2 (80.2)	349.6 (47.5)	468.7 (55.5)	457.1 (53.4)
Total vitamin E, mean (SD)	43.79 (2.78)	44.14 (2.94)	34.18 (3.18)	33.79 (2.59)

Plama/Serum levels of tocopherols and total vitamin E are expressed as μmol per mmol cholesterol. Tocotrienols are expressed as nmol per mmol cholesterol. The ratios α-tocopherylquinone/α-tocopherol and 5-nitro-γ-tocopherol/γ-tocopherol are expressed as nmol/μmol.

‡ Plasma/Serum levels of tocopherols and total vitamin E are expressed as μM. Tocotrienols are expressed as nM.

AD or cognitive impairment *versus* correspondent group of controls [a] $p<0.001$ [b] $p<0.01$ [c] $p<0.05$ [d] difference significant at the 10% level.

Absolute values of plasma vitamin E were also examined in relation to AD risk by grouping the population according to their median levels of vitamin E and cholesterol. As specified in the Methods section (paragraph 3.3.1), this grouping was done to take into account the effect of cholesterol levels on vitamin E biokinetics.

Table 17. Vitamin E plasma levels and AD risk in the Kungsholmen Project.

Vitamin E plasma level	Incident AD Cases	Incidence rate (per 1,000 person-years)	Adjusted HR* (95% CI)
Total tocopherols			
lowest tertile (≤ 6.81)	18	108.9	1 (Ref.)
highest tertile (≥ 8.73)	11	65.8	0.55 (0.32-0.94)
Total tocotrienols			
lowest tertile (≤ 52.77)	15	87.4	1 (Ref.)
highest tertile (≥ 70.45)	9	52.5	0.46 (0.23-0.92)
Total vitamin E			
lowest tertile (≤ 6.87)	18	108.9	1 (Ref.)
highest tertile (≥8.81)	11	66.2	0.55 (0.32-0.94)

*Crude incidence rates and HRs adjusted for age, gender, education, MMSE score, presence of *APOE* ε4, BMI, alcohol use, smoking status, and follow-up survival status.
Plasma levels of tocopherols and total vitamin E are expressed as μmol per mmol cholesterol. Tocotrienols are expressed as nmol per mmol cholesterol.

In all fully-adjusted models we found a trend for a protective effect in subjects with vitamin E-cholesterol proportions which may facilitate vitamin E delivery to tissues (groups 2 and 3), compared to subjects with vitamin E-cholesterol proportion less favourable for vitamin E delivery to cell membranes (group 1). The positive association was significant in group 2 for α-tocopherol [HR (95% CI): 0.41 (0.20-0.85)] and α-tocotrienol [HR (95% CI): 0.40 (0.19-0.84)], and in group 3 for β-tocopherol [HR (95% CI): 0.30 (0.09-0.94)], α-tocotrienol [HR (95% CI): 0.35 (0.14-0.86)], β-tocotrienol [HR (95% CI): 0.35 (0.12-0.98)] and γ-tocotrienol [HR (95% CI): 0.26 (0.71-0.96)], and total tocotrienols [HR (95% CI): 0.12 (0.03-0.51)].

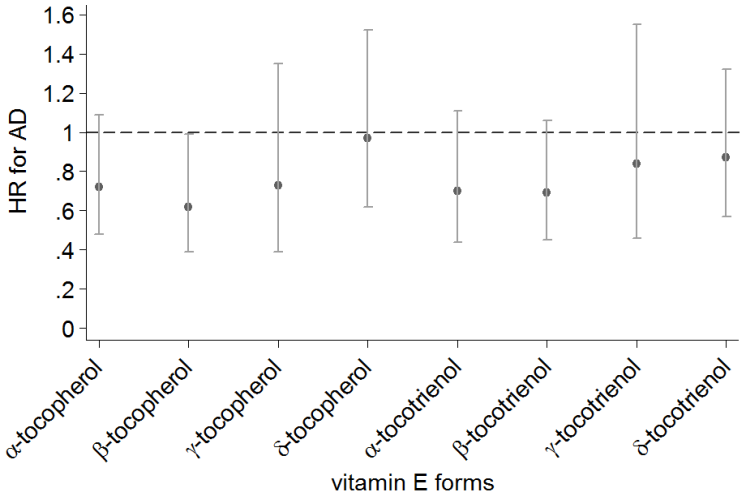


Figure 10. Vitamin E plasma levels and AD risk in the Kungsholmen Project. The x-axis shows the different vitamin E forms, and the y-axis show the adjusted hazard ratio (HR) of AD by plasma level of each form (highest versus lowest tertile). The bars represent the 95% CI of the HRs (dots).
HRs are adjusted for age, gender, education, MMSE score, presence of *APOE* ε4, BMI, alcohol use, smoking status, and follow-up survival status.

5.2.3 Vitamin E and risk of cognitive impairment in subjects aged 65+ years (Study IV)

In the CAIDE study, when considering vitamin E values standardized by cholesterol, there was a trend comparable to the Kungsholmen cohort, with lower baseline vitamin E values in subjects who developed cognitive impairment. Indeed, mean serum levels of α- and γ-tocopherol, each tocotrienol form, total tocopherols, total tocotrienols, and total vitamin E were lower in subjects who developed cognitive impairment, in comparison to controls, but the difference was statistically significant only for γ-tocotrienol (Table 16). Regarding the indices of vitamin E oxidative/nitrosative damage, development of cognitive impairment was associated with significantly higher values of 5-NO_2-γ-tocopherol/γ-tocopherol ratio, while there was no difference for αTQ/α-tocopherol ratio (Table 16).

Regression analyses results are shown in Table 18 as ORs with 95% CI for each vitamin E index (in tertiles) in relation to cognitive impairment. In the multi-adjusted model, subjects with higher γ-tocopherol/cholesterol ratio (middle compared to lowest tertile) were less likely to develop cognitive impairment [OR (95% CI): 0.27 (0.10-0.78)]. A similar association was found for the highest tertile, but it was not statistically significant [OR (95% CI): 0.85 (0.34-2.13)]. Further, subjects with a higher index of γ-tocopherol nitrosative damage (5-NO_2-γ-tocopherol/γ-tocopherol ratio, high and middle versus lowest tertile) were about three times more likely to develop cognitive impairment (Table 18). No significant associations were found for all the other vitamin E indices.

Absolute values of serum vitamin E were also analysed, by grouping the population according to the median levels of vitamin E and cholesterol, as in Study III. Similarly to the Kungsholmen Project, the analyses showed a trend for a protective effect in subjects with vitamin E-cholesterol proportions which may facilitate vitamin E delivery to tissues (groups 2 and 3), in comparison with subjects with vitamin E-cholesterol proportion less favourable for vitamin E delivery to cell membranes (group 1). In the fully adjusted model, the positive association was significant in group 2 for γ-tocopherol [OR (95% CI): 0.21 (0.08-0.56)], and in group 3 for γ-tocopherol [OR (95% CI): 0.33 (0.11-0.97)], β-tocotrienol [OR (95% CI): 0.21 (0.06-0.71)], and total tocotrienols [OR (95% CI): 0.27 (0.08-0.90)]. For γ-tocotrienol the reduced risk of cognitive impairment in group 3 was only marginally significant in the multi-adjusted model [OR (95% CI): 0.33 (0.10-1.06)].

Table 18. Vitamin E serum levels and risk of cognitive impairment in the CAIDE Study.

Vitamin E serum levels	Cases: n (%)	Model 1, OR (95% CI)	Model 2, OR (95% CI)
Total tocopherols			
L (≤5.34)	23 (36)	1.00	1.00
M (>5.34 & <6.17)	19 (30)	0.83 (0.35-1.98)	0.92 (0.35-2.43)
H (≥6.17)	22 (34)	1.10 (0.44-2.70)	1.39 (0.51-3.81)
Total tocotrienols			
L (≤73.81)	24 (38)	1.00	1.00
M (>73.81 & <86.26)	19 (30)	0.69 (0.29-1.64)	0.71 (0.27-1.87)
H (≥86.26)	21 (32)	0.84 (0.35-2.04)	0.90 (0.34-2.37)
Total vitamin E			
L (≤5.42)	24 (38)	1.00	1.00
M (>5.42 & <6.27)	18 (28)	0.69 (0.29-1.65)	0.82 (0.31-2.19)
H (≥6.27)	22 (34)	1.01 (0.41-2.49)	1.32 (0.48-3.67)
Ratio α-TQ/ α-tocopherol			
L (≤1.667)	20 (31.5)	1.00	1.00
M (>1.667 & <1.840)	20 (31.5)	0.81 (0.31-2.10)	0.58 (0.20-1.73)
H (≥1.840)	23 (37)	1.34 (0.51-3.52)	1.23 (0.44-3.58)
Ratio 5-NO-γ-tocopherol/γ-tocopherol			
L (≤ 11.408)	13 (20)	1.00	1.00
M (>11.408 & <12.85)	27 (42)	**3.50 (1.41-8.67)**	**3.41 (1.29-9.06)**
H (≥ 12.85)	24 (38)	**2.94 (1.17-7.38)**	**2.89 (1.05-7.97)**
α-tocopherol			
L (≤4.6)	23 (36)	1.00	1.00
M (>4.6 & <5.32)	20 (31)	0.93 (0.39-2.18)	1.06 (0.40-2.76)
H (≥5.32)	21 (33)	0.96 (0.40-2.33)	1.14 (0.42-3.09)
β-tocopherol			
L (≤0.368)	21 (33)	1.00	1.00
M (>0.368 & <0.435)	20 (31)	1.17 (0.48-2.81)	1.21 (0.46-3.20)
H (≥ 0.435)	23 (36)	1.54 (0.61-3.88)	1.79 (0.66-4.83)
γ-tocopherol			
L (≤ 0.295)	28 (44)	1.00	1.00
M (>0.295 & <0.342)	14 (22)	**0.29 (0.12-0.72)**	**0.27 (0.10-0.78)**
H (≥0.342)	22 (34)	0.74 (0.31-1.80)	0.85 (0.34-2.13)
δ-tocopherol			
L (≤0.043)	24 (38)	1.00	1.00
M (>0.043 & <0.05)	19 (30)	0.82 (0.34-1.96)	0.84 (0.31-2.24)
H (≥0.05)	21 (32)	0.91 (0.37-2.22)	0.89 (0.34-2.34)
α-tocotrienol			
L (≤43.0)	24 (37.5)	1.00	1.00
M (>43.0 & <51.5)	16 (25)	0.52 (0.21-1.25)	0.49 (0.18-1.33)
H (≥51.5)	24 (37.5)	1.18 (0.49-2.86)	1.03 (0.40-2.69)
β-tocotrienol			
L (≤ 21.04)	23 (36)	1.00	1.00
M (>21.04 & <24.14)	20 (31)	1.03 (0.44-2.41)	1.54 (0.58-4.07)
H (≥24.14)	21 (33)	0.90 (0.37-2.20)	1.17 (0.41-3.34)
γ-tocotrienol			
L (≤7.71)	26 (41)	1.00	1.00
M (>7.71 & <9.5)	21 (33)	0.77 (0.33-1.80)	1.04 (0.40-2.68)
H (≥9.5)	17 (26)	0.50 (0.20-1.26)	0.57 (0.21-1.57)
δ-tocotrienol			
L (≤1.30)	21 (33)	1.00	1.00
M (>1.30 & <1.66)	22 (34)	1.10 (0.45-2.67)	0.91 (0.33-2.50)
H (≥1.66)	21 (33)	0.99 (0.42-2.30)	0.95 (0.38-2.42)

Odds ratios (OR) with 95% confidence interval (CI) of vitamin E serum levels (tertiles) for cognitive impairment. L: lowest tertile (reference); M: middle tertile; H: highest tertile.

Model 1: adjusted for age, gender and education. Model 2: adjusted for age, gender, education, *APOE* status, BMI, smoking status, alcohol use, and history of cardio/cerebrovascular conditions.

5.3 Pooled analysis

The association between vitamin E and cognition has been further investigated by pooling together the three populations that were included in this project, and by examining the correlation among each vitamin E index and MMSE total score.

The characteristics of the pooled population are shown in Table 19. The MMSE total score ranged from 10 to 30, with a value of 17 for the 5th percentile, 25 for the 25th, 27 for the 50th, and 29 for the 75th percentile.

Table 19. Characteristics of the population included in the pooled analysis.

Subjects characteristics (N=893)	Value
Age, y, [range]	77.6 (6.8) [65-95]
MMSE score	26.0 (3.9)
Education	9.3 (4.0)
Gender, % female	(N=595) 67%
Any *APOE* ε4 allele*, %	(N= 309) 35%
Cholesterol, mmol/l	5.50 (1.23)
Vitamin E forms [a]	
α-tocopherol	6.20 (1.88)
β-tocopherol	0.45 (0.14)
γ-tocopherol	0.40 (0.12)
δ-tocopherol	0.053 (0.015)
α-tocotrienol	50.65 (20.59)
β-tocotrienol	24.03 (8.29)
γ-tocotrienol	13.15 (5.80)
δ-tocotrienol	2.07 (1.02)
Total tocopherols [a]	7.00 (2.05)
Total tocotrienols [a]	92.40 (30.35)
Total vitamin E [a]	7.10 (2.07)
Ratio α-tocopherylquinone/α-tocopherol [b]	1.69 (0.24)
Ratio 5-nitro-γ-tocopherol/γ-tocopherol [b]	11.86 (2.18)

If not otherwise specified, data are given as mean (SD). **APOE* was available for 861 subjects. [a] Levels of tocopherols and total vitamin E are expressed as μmol per mmol cholesterol. Tocotrienols are expressed as nmol per mmol cholesterol. [b] The ratios α-tocopherylquinone/α-tocopherol and 5-nitro-γ-tocopherol/γ-tocopherol are expressed as nmol/μmol. The ratios were available for the AddNeuroMed and CAIDE cohorts.

For each vitamin E index, the estimated regression coefficients (Beta) for the four percentiles of the MMSE are reported in Table 20, together with the 95% CI. In the fully adjusted models, a positive correlation among each vitamin E forms, total vitamin E, total

Table 20. Correlation among vitamin E and MMSE

Vitamin E forms	MMSE Percentile	Model 1 Beta (95% CI)	Model 2 Beta (95% CI)
Total vitamin E	5°	**0.16**[a]	0.03
	25°	**0.10**[b]	**0.09**[b]
	50°	**0.13**[a]	**0.09**[b]
	75°	**0.10**[b]	**0.07**[b]
Total tocopherols	5°	**0.16**[a]	0.04
	25°	**0.10**[b]	**0.09**[b]
	50°	**0.13**[a]	**0.09**[a]
	75°	**0.10**[b]	**0.07**[c]
Total tocotrienols	5°	0.01	**0.01**[c]
	25°	0.004	**0.01**[a]
	50°	**0.01**[a]	**0.01**[a]
	75°	**0.01**[b]	**0.01**[a]
α-tocopherol	5°	**0.20**[a]	0.04
	25°	**0.10**[b]	**0.07**[c]
	50°	0.11[a]	0.04
	75°	**0.08**[b]	**0.05**[c]
β-tocopherol	5°	1.16	0.55
	25°	0.40	0.01
	50°	**0.92**[c]	0.51
	75°	**0.76**[c]	0.56
γ-tocopherol	5°	**3.64**[a]	1.20
	25°	**2.18**[a]	**1.92**[a]
	50°	**2.35**[a]	**1.82**[a]
	75°	**2.39**[a]	**1.71**[a]
δ-tocopherol	5°	8.92	3.79
	25°	3.96	0.09
	50°	**7.74**[c]	2.04
	75°	**6.70**[c]	2.89
α-tocotrienol	5°	-0.02	**0.01**[b]
	25°	0.003	**0.01**[a]
	50°	**0.008**[a]	**0.01**[a]
	75°	**0.007**[b]	**0.01**[c]
β-tocotrienol	5°	-0.01	**0.01**[c]
	25°	-0.01	**0.02**[c]
	50°	0.01	**0.02**[b]
	75°	**0.01**[c]	**0.02**[b]
γ-tocotrienol	5°	**0.11**[a]	**0.05**[c]
	25°	**0.07**[a]	**0.07**[a]
	50°	**0.07**[a]	**0.07**[a]
	75°	**0.08**[a]	**0.07**[a]
δ-tocotrienol	5°	**0.41**[b]	0.07
	25°	**0.14**[a]	**0.12**[c]
	50°	**0.20**[a]	**0.11**[c]
	75°	**0.12**[c]	**0.11**[b]
Ratio α-TQ/α-tocopherol	5°	-0.51	-0.94
	25°	**-1.81**[a]	**-1.66**[a]
	50°	**-1.40**[a]	**-1.28**[a]
	75°	**-1.40**[b]	**-1.21**[b]
Ratio 5-nitro-γ-tocopherol/γ-tocopherol	5°	-0.06	-0.11
	25°	**-0.13**[a]	**-0.10**[b]
	50°	**-0.14**[a]	**-0.11**[a]
	75°	**-0.19**[a]	**-0.12**[a]

Estimated logistic quantile regression for MMSE. Model 1: adjusted by age; Model 2: adjusted by age, gender education, *APOE* genotype and cohort.
[a] $p<0.001$ [b] $p<0.01$ [c] $p<0.05$

tocopherols, total tocotrienols, and the MMSE score was shown, while there was a negative correlation between the indices of vitamin E oxidative/nitrosative damage and the MMSE score. For each vitamin E index, the strength of the correlation was similar across all MMSE percentiles. For total tocotrienols, α-, β-, and γ-tocotrienol the correlation was statistically significant for all percentiles. Figure 11 exemplifies the estimated MMSE percentiles in relation to total tocotrienols, showing that increased values of tocotrienols levels are associated with higher predicted MMSE total score within each MMSE percentile. For total vitamin E, total tocopherols, γ-tocopherol, δ-tocotrienol, and the ratios αTQ/α-tocopherol and 5-nitro-γ-tocopherol/γ-tocopherol the correlation was not significant in the fully adjusted model, only for the 5th percentile. For the other vitamin E forms the association was not statistically significant for two or all four percentiles (Table 20).

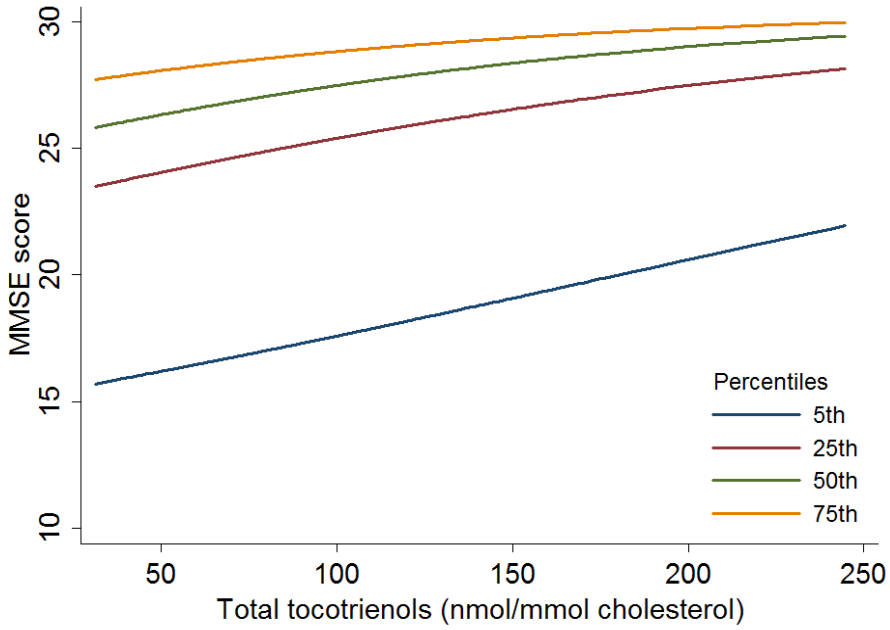


Figure 11. Age, gender, education and *APOE* status- adjusted estimated percentiles of MMSE in relation to total tocotrienols. All three cohorts included.

6 DISCUSSION

This research project has analyzed all eight natural forms of vitamin E in relation to AD and MCI, in clinic- and population-based studies. Overall, individuals from seven European countries have been included in this investigation, from North to South Europe. The results suggest the existence of a relation between different natural vitamin E forms and AD and MCI in older adults.

6.1 Vitamin E, Alzheimer's disease, and mild cognitive impairment: clinical context

Study I of this project shows that the plasma concentrations of different vitamin E forms are related to the diagnosis of AD and MCI in older adults. All previous cross-sectional clinical studies examined only α-tocopherol in subjects with dementia/AD, reporting either reduced plasma/serum levels in diseased individuals compared to controls[138-149] or no association.[148,150-155] Some studies showed decreased plasma α-tocopherol also in subjects with MCI,[138,147] while other studies did not confirm this finding.[115,136] Cross-sectional analyses in population-based studies have also shown conflicting results, and only one study (CSBA) analyzed all tocopherol forms.[115,156,157] In the CSBA cohort plasma tocopherols were assessed in a population of older adults (age 65+ years) with normal cognition, MCI, or dementia, and higher δ-tocopherol levels were found in subjects with dementia (compared to MCI and CN) when analyzing vitamin E/cholesterol ratio. No differences were found among the three groups when tocopherols levels were analyzed as absolute values.[115]

In the AddNeuroMed cohort, both cases of AD and MCI had lower plasma levels of different vitamin E forms, and reduced values for indices representing total vitamin E amounts, compared to CN subjects. This pattern was evident both when considering vitamin E values standardized by cholesterol and when evaluating absolute values of vitamin E. Further, both clinical diagnosis of AD and MCI were characterized by increased plasma indices of vitamin E oxidative/nitrosative damage.

Within the AddNeuroMed cohort, it has also been shown that the combined analysis of vitamin E plasma levels and automated MRI measures can accurately distinguish AD and MCI cases from CN individuals. Additionally, it can prospectively identify MCI cases that will progress to a clinical diagnosis of AD after one year (Study II).

The development of biomarkers for AD is an active area of research. To date, neuroimaging- and CSF-based markers have been the most widely studied. Structural MRI, PET, and CSF parameters have been included in the new diagnostic criteria for AD, MCI due to AD and preclinical AD. However, many clinical settings do not have the ability to implement some of these techniques, and further validation and standardization of the

proposed biomarkers would be advantageous.[66,67] Brain atrophy evidenced by MRI is an indicator of neuronal injury, and combined assessment of different brain regions can highlight regional patterns of abnormalities more specific for AD compared to the evaluation of single structures.[69] For example hippocampal atrophy is not specific to AD; it can also be present in hippocampal sclerosis and frontal lobe dementia.

Blood based markers can represent inexpensive and minimally-invasive tools for early identification and monitoring of AD progression. Obtaining blood samples can easily be implemented in large scale screening and prevention studies, and for repeated measures. Several studies suggest that plasma levels of different proteins and metabolites might be related to AD pathology, including indices of OS/NS and inflammation.[43,44,73,74,76,86,147,247] Such plasma markers could be a systemic metabolic hallmark of AD or reflect a change in plasma secondary to a disease-specific process in the brain. A recent study of healthy older adults concluded that pattern of nutrient biomarkers detected in plasma correlated with cognitive functioning and MRI brain volumes. The plasma profile high in α-tocopherol was associated with better global cognition and reduced total brain atrophy.[161] Plasma indices of vitamin E status could be indicators reflecting biochemical changes associated with AD pathophysiology. However, the challenge for blood-based biomarkers is still considerable. The main difficulty when studying blood is due to the variability of plasma components in response to non-AD influences (i.e., drugs, time of sampling, environmental factors). The combination of different methods, such as MRI, CSF parameters, blood measures, and neuropsychological data allow for the evaluation of different aspects of AD, increasing their diagnostic and prognostic value through a multimodality markers approach. A recent study within the ADNI project reported that combinations of neuropsychological data, CSF, and MRI measures attained an accuracy of 67.1% in predicting MCI conversion to AD after two years, outperforming the evaluation of the single modality markers.[75] In another survey, the combination of MRI, CSF, and FDG-PET separated MCI converters from non-converters with a sensitivity of 91.5% and a specificity of 73.4%.[71] However, CSF and FDG-PET are not easy to implement in large scale studies or for repeated measures. In Study II, the joint evaluation of plasma vitamin E and MRI measures increased the accuracy in separating AD and MCI from CN subjects to 98% and 91%, respectively. This combination of parameters was also able to classify 85% of the MCI converters as AD-like and 67% of the stable MCI as CN-like subjects. In a previous study using data from AddNeuroMed and ADNI cohorts, MRI data were able to identify 71% of the MCI converters and 60% of the stable MCI subjects. Thus the joint evaluation of MRI and plasma vitamin E indices seems to provide a more robust predictive tool.[69]

Other antioxidant micronutrients

Vitamin E is the main lipid-soluble, non-enzymatic antioxidant in the human body, in which other non-enzymatic antioxidants are also available. Those are a combination of hydrophilic and lipophilic compounds, which interact with each other and with enzymatic

antioxidants for a fine tuning of the redox status. In the AddNeuroMed population (Study I) β-carotene and retinol (non-enzymatic, lipophilic antioxidants) were also analysed, to provide a broader picture of the antioxidant micronutrients status in cognitive impairment. Both β-carotene and retinol (vitamin A) plasma levels were reduced in AD and MCI cases compared to CN individuals, supporting the presence of an altered antioxidant micronutrient status in these disorders.

High plasma levels or high dietary intake of carotenoids/β-carotene have been associated with better cognitive performance[160,248] and reduced risk of cognitive decline and AD in some,[249] but not all, studies.[180] Two RCTs reported protective benefits for cognition in subjects supplemented with β-carotene.[250,251] In some population-based studies plasma vitamin A was not related to dementia diagnosis.[157,164] while other studies reported reduced levels in subjects with dementia or MCI.[139,147] Carotenoids and retinol are present in the brain,[112] and evidence of their antioxidant and anti-amyloidogenic properties warrant further investigation of their role in neurodegeneration.[252]

6.2 Vitamin E, Alzheimer's disease and mild cognitive impairment: general population context

In the prospective study in Swedish octogenarians (Study III) a reduced incidence of AD was found in subjects with higher plasma levels of total tocopherols, total tocotrienols, and total vitamin E. The analyses of absolute plasma values of vitamin E, in which cholesterol levels were also accounted for, revealed a reduced AD incidence for higher plasma levels of α- and β-tocopherol, α-, β-, and γ- tocotrienol, and total tocotrienols.

In the Finnish cohort of adults aged 65+ years (Study IV) high levels of γ-tocopherol, β-tocotrienol, and total tocotrienols seemed to be protective against cognitive impairment, after different adjustments for cholesterol.

The differences in findings of the two studies could be due to different factors, including age of participants and follow-up duration. Also, the Swedish cohort had higher plasma levels of tocopherols compared to the Finnish population (the highest tertile of total tocopherols and total vitamin E in the CAIDE study fell within the lowest tertile of the Swedish cohort). The reasons for lower levels of some vitamin E forms in Finland during the 1990s are unclear, and other studies assessing tocopherols in Finnish cohorts reported values comparable to the CAIDE cohort.[253-255] Interestingly, the Italian CSBA study reported tocopherols levels similar to CAIDE participants, and found a lower incidence of dementia in subjects with plasma γ-tocopherol levels in the middle (but not highest) tertile.[115] Similar results were found in Study IV. Higher γ-tocopherol has been found to be associated with a high intake of saturated fats, another risk factor for dementia/AD,[181] which may partly explain this association.

Previous population-based studies on vitamin E levels and risk of cognitive decline or dementia/AD have evaluated mainly α-tocopherol, reporting a reduced risk in individuals

with higher α-tocopherol plasma levels[164] or no association.[162,166] Other than the CSBA, two other studies analysed γ-tocopherol; the Nurses' Health Study and the ULSAM study. The Nurses' Health Study reported better cognitive outcomes in subjects with high γ-tocopherol plasma levels and α-tocopherol concentration under the median, suggesting a possible interactive effect of different vitamin E forms in modulating the risk of cognitive decline in older adults.[163] In the ULSAM study α- and γ-tocopherol levels were evaluated in relation to the risk of dementia/AD, and no association was found.[166]

In the CAIDE population (Study IV), an elevated index of vitamin E nitrosative damage (5-NO_2-γ-tocopherol/γ-tocopherol ratio, indicating consumption of γ-tocopherol in free radical reactions), was associated with increased risk of cognitive impairment. Products of vitamin E oxidative/nitrosative damage, such as 5-NO_2-γ-tocopherol and α-TQ, were measured also in the CSBA,[115] but the ratio with the correspondent vitamer E (i.e., γ- and α-tocopherol) was not considered, and these indices have so far not been studied in relation to cognitive decline/AD in other longitudinal studies. Elevated plasma values of the ratios 5-NO_2-γ-tocopherol/γ-tocopherol and α-TQ/α-tocopherol were found in subjects with AD and MCI in the AddNeuroMed cohort (Study I), and an increased 5-NO_2-γ-tocopherol/γ-tocopherol ratio was also detected in cortical brain regions in an autopsy study of patients with AD compared to controls.[113] RNS substantially contribute to free radical-mediated damage in AD, and can be efficiently neutralized by γ-tocopherol with the generation of 5-NO_2-γ-tocopherol.[113] Thus, the increased ratio 5-NO_2-γ-tocopherol/γ-tocopherol might reflect elevated γ-tocopherol use due to increased NS.

6.3 Biological plausibility

Much evidence supports the role of OS/NS in the propagation of cellular injury leading to different types of damage observed both in brain aging and neurodegeneration.[43,78] α-tocopherol is considered the main lipophilic non-enzymatic antioxidant; it is the most abundant vitamin E form in the human body, and the most extensively investigated. However, increasing evidence is accumulating about the biological properties of the other vitamin E congeners. All vitamin E forms act as antioxidants, with γ-tocopherol being highly efficient against RNS and tocotrienols showing stronger antioxidant properties than α-tocopherol.[103,110,113] Vitamin E has a prominent role in protecting cellular membranes, as it is the main lipid-soluble antioxidant. This could be crucial in mitochondria, since they are both the main source and elective target of free radicals. Mitochondria are crucial both for cell survival and death as they produce cellular energy and are central in apoptotic signaling. Many lines of evidence suggest that mitochondria have an important role in neurodegeneration, and mitochondrial dysfunction has been shown in AD.[256,257] Different vitamin E forms may protect mitochondria through their antioxidant activity, thus supporting cell survival and synaptic functioning.[256-260]

Additionally, each vitamin E form is functionally unique, and has other biological properties that can be relevant for neuroprotection, including anti-inflammatory activity and modulation of the cellular signaling that regulates neuronal survival. Indeed, α- and γ-tocopherol, and all tocotrienols have anti-inflammatory properties.[105,113,122,124] Further, studies on mouse hippocampal and cortical neurons have shown that nanomolar concentrations of α-tocotrienol can prevent neuronal death by modulating specific cellular signaling, including phospholipase A2 and 12-lipoxygenase mediated signaling.[128]

In a neuropathological study (the Religious Order Study) CSF α-tocopherol was positively associated with perceptual speed, while no association was found for γ-tocopherol.[261] Furthermore, α-, β-, and γ-tocopherol have been detected in the brain of cognitively normal subjects, and an age-related decline of these compounds was found in the frontal lobes, which are vulnerable to AD.[112] Another neuropathological study found reduced γ-tocopherol levels in the brain of AD subjects, with concomitant increase of its reaction product with RNS (5-NO_2-γ-tocopherol).[113] A recent study has shown that tocotrienols are delivered to the CNS, where they reach concentrations adequate for beneficial biological activity.[114] Finally, population-based studies assessing vitamin E dietary intake reported a protective effect of the combination of different vitamin E forms against cognitive decline and dementia/AD.[179,181,183]

All this evidence supports the hypothesis that all vitamin E forms can have a role in the aging brain and AD. Reduced levels of vitamin E forms and increased products of their oxidative/nitrosative damage could affect neurodegeneration, both through the reductions of the antioxidant shield and the unbalance in specific cellular signaling pathways.

Results of the present project provide further evidence regarding the importance of different vitamin E forms in the development or clinical expression of AD in older adults, which warrants further investigation.

6.4 Clinical implications

α-tocopherol is the only vitamin E congener which has been tested in RCTs in subjects with AD and MCI, and the results have been inconsistent.[187] α-tocopherol is also the most commonly used vitamin E supplement, which is a widely consumed supplement in Western countries, due to the belief that vitamin E may attenuate morbidity and mortality. It has been estimated that about 35 million Americans take vitamin E supplements, which are prescription-free products.[262] Although the vitamin E recommended dietary allowance (RDA) is 15 mg [22 international units (IU)] of *RRR*-α-tocopherol (the naturally occurring form of α-tocopherol), vitamin E supplements are available in doses of 100 to 1000 IU (synthetic α-tocopherol, which includes eight different steroisomers).[131] The American Institute of Medicine's Food and Nutrition Board set the upper tolerance level for α-tocopherol at 1000 mg [1100 IU synthetic α-tocopherol; 1500 IU natural α-tocopherol] per day using data from studies in rats.[106]

Similarly to RCTs investigating α-tocopherol supplement in AD, RCTs of α-tocopherol supplementation in other disorders, including cardiovascular disorders, heart attack, and cancer risk, have resulted in conflicting outcomes: beneficial effects, limited effects, no benefit, and possible harm.[263-271] Furthermore, current data on mortality and α-tocopherol supplementation at high doses emphasize that such a micronutrient, if unbalanced, may not be as harmless as previously thought. A recent meta-analysis of RCTs testing α-tocopherol supplementation reported an increased risk of haemorrhagic stroke in users of this supplement.[272] Another recent review examining primary and secondary prevention RCTs with α-tocopherol in different disorders reported an increased mortality risk associated with α-tocopherol supplementation, confirming a previous report from the same research group.[273] The authors concluded that there is no evidence to support antioxidant supplements for primary or secondary prevention, and antioxidant supplements should be considered as medicinal products that should undergo sufficient evaluation before marketing. On the other side, in the last few years many new supplements containing vitamin E have appeared on the market. They promise to protect against different age-related disorders, including cognitive impairment and AD, and contain different combinations of tocopherols and tocotrienol, despite the fact that the scientific community has not yet defined the RDA for non-α-tocopherol forms of vitamin E. Indeed, α-tocopherol is the only vitamin E form currently used to define vitamin E dietary requirements.[131] Further, the RDA for α-tocopherol is based on studies of erythrocyte haemolysis conducted more than 60 years ago and there is general agreement that the haemolysis assays might not be the optimal test for defining α-tocopherol RDA.[121,131] The American Institute of Medicine's Food and Nutrition Board specifies that the non α-tocopherol forms of vitamin E were not used to estimate vitamin E requirement because of their failure to bind with the α-TTP. However, recent studies have shown the actual bioavailability of non α-tocopherol forms in several tissues, where tocopherols and tocotrienols can reach concentrations adequate for beneficial biological activity.[114] This, together with the results from experimental and epidemiological studies on non α-tocopherol forms, reinforces the hypothesis that the different vitamin E congeners play unique roles in human health.

A balanced intake of different vitamin E forms can be important for neuroprotection. Even if there is preferential body retention of α-tocopherol, the other congeners exhibit biological activity at very low concentrations. Additionally, high doses of α-tocopherol, such as those present in supplements, can decrease the bioavailability of the other congeners, which could be essential for normal cell functioning. In fact, α-tocopherol supplementation can decrease plasma and tissue concentration of γ-tocopherol,[122,190] and compromise tissue delivery of α-tocotrienol.[191]

Evaluating only α-tocopherol might not provide the most accurate measure of vitamin E status in humans, and assessment of all vitamin E natural congeners might be appropriate

when investigating the association of vitamin E with diseases, including age-related cognitive decline and AD. Study of the all vitamin E forms can also help refine preventive therapeutic strategies based on vitamin E supplementation.
The American Institute of Medicine's Food and Nutrition Board guidelines for dietary intake specifies that the potential role of vitamin E in the reduction of disease risk was considered in developing the Estimated Average Requirement for this micronutrient, and the acquisition of additional data relating intake to chronic disease or disability would be considered for a revision of the Estimated Average Requirement.[131]

6.5 Methodological issues

The main strength of this project is its integrative approach. Vitamin E has been evaluated both in clinic-based and population-based cohorts, and its association with AD has been investigated by taking into account all eight natural vitamin E forms, together with markers of vitamin E oxidative/nitrosative damage. Both standardized and absolute values of plasma/serum vitamin E were considered, performing different adjustments for cholesterol, and multiple potential confounders were taken into account. Although each study design, method, and population has its own inherent problems and limitations, combining them can only reinforce the results.
Results from pooled analyses confirmed the association of vitamin E levels with global cognition measured with MMSE. The main limitation of the pooled analysis is due to the combination of two population-based cohorts and a clinic-based study. However, the correlations among several vitamin E indices and MMSE were statistically significant even when adjusting for cohort of origin, and these results are consistent with the findings of independent analysis within each population.

In all studies, vitamin E congeners were measured in plasma or serum. Unlike dietary intake assessment, micronutrient plasma/serum measurement is an objective measure and is independent of the capacity to estimate/remember intake over a period of time. Moreover, plasma level assessment considers individual variations in metabolism, giving a reliable evaluation of micronutrient bioavailability.
For the longitudinal studies included in this project vitamin E measurements were available at only one time point. Due to possible intra-individual variation in vitamin E serum levels, a single measurement does not necessarily reflect the typical vitamin E bioavailability of an individual. However, some studies reported a good intra-individual stability for α- and γ-tocopherol levels, for periods up to 15 years.[243,274-276] Analogous data are not available for tocotrienols.

Both standardized and absolute values of plasma/serum vitamin E have been considered. Vitamin E biokinetics is tightly related to lipoproteins. Tissues can acquire vitamin E by several major routes: (1) during LPL mediated triglyceride-rich lipoprotein catabolism; (2)

during LDL uptake via the LDL receptor; (3) via high-density lipoprotein-mediated delivery systems; and (4) by nonspecific transfers through lipoproteins. Vitamin E rapidly transfers between various lipoproteins and also between lipoproteins and membranes, which may enrich membranes with vitamin E.[102,108] For these reasons, lipid levels need to be considered when assessing the effects of vitamin E on any outcome; standardization to total cholesterol is commonly used.[115,133,134] However, longitudinal studies have shown that the relation between serum total cholesterol and risk of cognitive decline/dementia is age-dependent. Elevated midlife total cholesterol has been associated with increased risk of dementia/AD, but cholesterol levels tend to decline after midlife in people who later develop dementia/AD.[236,277] This pattern was previously reported in the CAIDE cohort.[236] Whether the pattern of change over time in vitamin E levels is similar to that of total cholesterol remains to be determined. Data on age-related variation on vitamin E forms are scarce and inconsistent.[166,278] In a US population, an age-related increase of α-tocopherol and decrease for γ-tocopherol were described, but the increasing use of α-tocopherol supplements among older adults was suggested as a possible explanation.[278] It is unclear if adjustments for cholesterol may lead to and underestimation of the association of vitamin E with cognitive decline, and how lipid levels should be best accounted for in longitudinal vitamin E studies.

In the AddNeuroMed and CAIDE cohorts blood samples were collected after a minimum of two hours fasting, while in the Kungsholmen Project fasting was not compulsory. In the CAIDE sample fasting status was similar among cases and controls [hours, mean (SD): 9.5(4.8) for cases and 9.4(4.7) for controls, p=0.91)], and additional analyses considering fasting time as covariate provided results comparable to the main analysis. Similar data were not available for the AddNeuroMed and the Kungsholmen Project. However, transfer of vitamin E from blood to tissues occurs both during fasting and non-fasting conditions, and it is reasonable to assume that these conditions of measurements were equally distributed among all subjects, which would not affect the results. Additionally, in the Nurses' Health Study plasma levels of α- and γ-tocopherol from fasting and non-fasting samples were evaluated, and similar results were found when analyzing all samples and only fasting samples.[163]

6.5.1 Clinic-based studies

The main strengths of the clinical studies in this project are the multinational composition of the population and the sample size: the AddNeuroMed population is one of the largest cohorts of AD and MCI subjects, gathered from six European countries. The AddNeuroMed population includes subjects from the North, Center, and South of Europe, which to some extent takes into account differences in dietary habits that could influence micronutrient status. In Study I, even when considering the subject's country of origin, the differences among AD, MCI and CN subjects on vitamin E indices were still significant.

In Study II, the combined analysis of vitamin E and MRI data integrated 67 parameters, obtained by non-invasive (MRI) and minimally-invasive (venipuncture) methods. The MRI measures included a set of automated regional cortical thickness and volumetric measures. The AddNeuroMed study implemented a harmonized MRI acquisition protocol across centers, rigorous quality control, a central data analysis hub and an automated image analysis pipeline.[207,279] Automated image analysis techniques, such as the Freesurfer technique used in Study II, may have particular advantages compared to manual measures when it comes to widespread implementation in clinical or research practice. A further strength of Study II is the use of a multivariate data analysis approach, OPLS, which provides the opportunity to analyze many variables simultaneously and observe inherent patterns in the data. OPLS allows groups to be separated, highlights which measures cause the separation, and facilitates making predictive models of disease.[238]

Regarding potential study limitations, information on some potential confounders was not available for the AddNeuroMed population. This includes information on smoking status, a source of OS/NS. Nevertheless, investigations on vitamin E concentrations in smokers have yielded inconsistent evidence: smokers' plasma α- and γ-tocopherol levels were either lower or similar to non-smokers, while smoking increased 5-NO_2-γ-tocopherol levels.[280] BMI was also not available. Some studies suggest an association between BMI and γ-tocopherol levels.[281] However, data on plasma α-tocopherol and BMI are inconsistent,[243,281,282] and analogous data on tocotrienols are not available. The normal levels of serum total cholesterol and albumin in the AddNeuroMed cohort suggest that none of the study participants were malnourished. Further, after adjustment for disease duration (Study I) and even exclusion of subjects with moderate-severe AD (Study I and II), who could be at risk of malnutrition, the association between cognitive impairment and vitamin E levels was still significant.

Vitamin E status can be affected by different conditions other than lifestyle, including disorders characterized by increased OS/NS (e.g. cancer, diabetes mellitus).[78] However, in the AddNeuroMed study, individuals with unstable systemic illness/organ failure were not included. Additionally, a significant positive correlation among vitamin E plasma levels and brain volumes was found, while indices of vitamin E oxidative/nitrosative damage were positively correlated with levels of brain atrophy. The significant correlation among MRI measures and vitamin E plasma levels reduces the possibility that vitamin E differences were due only to non-specific illnesses in subjects with AD and MCI.

When considering vitamin E levels as possible indicators associated with AD pathology, it is also important to consider that different CNS disorders in which OS/NS phenomena are described (e.g., AD, vascular dementia, Parkinson's disease, amyotrophic lateral sclerosis) could influence vitamin E levels.[78,85] A similar issue also exists for β-amyloid and tau protein, which can be assessed in CSF, and directly reflect AD neuropathology. Decreased

CSF levels of β-amyloid and increased levels of tau/phospho-tau are considered AD hallmarks, but CSF changes for both proteins also occur in other neurological disorders (e.g., amyloid angiopathy, dementia with Lewy body, prion disease).[66] As in Study II, many studies on AD biomarkers are based on the comparison of diseased and disease-free subjects, and usually only AD and MCI cases are included, while cases with other diseases which can require differential diagnosis with AD are not considered.[66] There is also some evidence that β-amyloid and tau related markers are less predictive of AD development in individuals with high cognitive reserve[283] or very advanced age.[284] Other limitations related to the current state of knowledge on biomarkers for AD are due to the paucity of validation studies implementing post-mortem data, and scarcity of combined analysis of multiple biomarkers. Neither AD nor MCI can be diagnosed only by laboratory or neuroimaging tests, and clinical judgement is still paramount for the diagnosis of both disorders.[66] Vitamin E is a nutritional indicator that could reflect biochemical changes associated with AD pathophysiology, such as inflammation and OS/NS. The correct interpretation of any potential marker, including vitamin E, requires information from validation studies. The concurrent evaluation of the clinical information defining the diagnostic criteria for AD and MCI is also essential.[66]

Cognitively normal subjects gathered in the AddNeuroMed cohort were recruited from non-blood relatives of the patient's families', caregivers' relatives, and social centers for the elderly. Even though the recruitment of these CN subjects was not based on a rigorous random selection in the general population, the comprehensive examination protocols used in AddNeuroMed study excluded the presence of cognitive impairment or dementia in subjects included in the CN group.

As Study I is cross-sectional, reverse causality cannot be excluded: reduced vitamin E plasma levels in AD and MCI could mirror changes in metabolism or dietary habits promoted by these disorders. However, in the AddNeuroMed population reduced levels of α- and γ-tocopherol in AD and MCI cases were associated with increased indices of α- and γ-tocopherol consumption due to reaction with free radicals, suggesting that vitamin E depletion is due, at least in part, to increased utilization in OS/NS events.

In Study II there was a small number of MCI converters (n=13) and only one-year follow-up data to evaluate the prognostic value of the combined model based on MRI and vitamin E measures. Although a previous study in the AddNeuroMed cohort showed the validity of the OPLS approach in predicting MCI conversion on a similar sample size,[285] large studies with longer follow-up are warranted to verify this finding. Follow-up of the AddNeuroMed population is ongoing, offering the opportunity to further evaluate the relationship between vitamin E plasma levels and cognitive impairment.

6.5.2 Population-based studies

The two population-based prospective studies presented here investigated late-life vitamin E exposure in relation to cognitive decline and AD. AD has a long preclinical phase, and it is difficult to identify the time of onset of the neuropathology. This explains the age-dependent and bi-directional association with some risk factors, which can be true risk factors (i.e., increase the probability of developing the disease) or markers of risk, reflecting a preclinical phase of the disease and the risk of its clinical expression. As for some risk factors of dementia/AD, such as blood cholesterol levels, BMI and blood pressure, low vitamin E levels and increased indices on vitamin E oxidative/nitrosative damage in late-life could mirror a preclinical phase of AD, and they can be related to the risk of clinical expression of the disease. Only one study (HAAS) examined midlife vitamin E (dietary intake) in relation to dementia/AD risk, reporting no association. The HAAS cohort included only Japanese-American men, and the evaluation of vitamin E intake was based on a single 24-hour dietary recall, which might not be representative of the usual vitamin E intake.[173] Longitudinal studies considering both midlife and late-life vitamin E plasma levels are necessary to detect a possible age-dependent association of vitamin E with AD.

Kungsholmen Project

The Kungsholmen Project is a prospective survey of a community-based cohort of very old individuals, which gathered all inhabitants who lived in the Kungsholmen area, central Stockholm, Sweden (response rate 76%). Data on vitamin E were available for a subsample of the Kungsholmen Project, which was representative of the entire dementia-free cohort participating in the study phase when vitamin E was assessed. Overall, the incidence rate of AD in the Kungsholmen study participants included in Study III was 78.8 per 1,000 person-years. Similar values were reported in other studies within the whole Kungsholmen cohort.[286,287] Although the statistical power in Study III was limited due to the relatively small sample size, a significant association was found between different indices of vitamin E status and AD.

The follow-up time in Study III was up to six years but, given the long preclinical phase in AD, it is not possible to completely exclude that potential changes in dietary habits or in metabolism due to early-stage subclinical AD could have promoted a reduction in vitamin E plasma levels. However, the extensive diagnostic procedure for dementia that was implemented at baseline and at follow-ups allowed for the detection of the presence of any cognitive problem. The diagnosis of dementia was given after consensus among three independent physicians. The diagnostic procedure has been validated, with a relatively high overall agreement on diagnosis (κ=0.70).[288] Further, the analyses in Study III were adjusted for MMSE score.

The population in Study III consisted of individuals aged ≥80 years who were living in a

geographically defined central area of Stockholm. This study population was comparable to the entire population of Stockholm, in terms of age and sex composition, as well as in terms of health care system. However, the Kungsholmen population did differ from the rest of the urban area of Sweden in terms of the proportion of pensioners, women, highly educated persons, and marital status. Cautions are thus needed when generalizing the findings from the Kungsholmen population to a younger population or to rural areas. The major findings from this population may be generalized to the urban population aged over 80 in Western society. Results from the CAIDE cohort, where follow-up duration was longer and individuals from urban and suburban areas were included, further support the findings from the Kungsholmen Project.

CAIDE

The CAIDE study has been specifically designed to investigate risk factors for dementia and AD, and it is based on population-based random samples of individuals who have been investigated three times within the study. Blood samples from the second examination (1998) were used for vitamin E assessment in 140 subjects.

The long follow-up period, and comprehensive evaluation and diagnostic protocol at each examination, make the findings of Study IV less prone to the influence of reverse causality (i.e. effects of preclinical dementia on vitamin E levels).

In 1998, only participants with MMSE $\leq$ 24 at the screening phase entered the clinical phase. It would have been ideal if all persons had participated in both screening and clinical phases, but the three-phase protocol was probably high enough in sensitivity and specificity to detect cognitive impairment and dementia/AD. Nearly half of all participants with MMSE below the cut off point in the screening phase were considered as cognitively normal in the clinical phase in 1998, suggesting that the cut off score was sufficiently sensitive for detecting cognitive impairment and dementia/AD in this population. Using a higher cut off would have resulted in the inclusion of a very large proportion of the population in the clinical phase, as the median MMSE was 26.

In 2005-2008 examination, the sensitivity for detecting MCI and very mild dementia has been further improved by including in the clinical phase subjects with MMSE $\leq$ 24, decline on MMSE of 3 or more points since 1998, or delayed recall word list test < 70% in the CERAD neuropsychological test battery. Further, the Finnish Drug Reimbursement Register was also used to verify diagnosis of AD. In Finland all dementia drugs (donepezil, rivastigmine, galantamine and memantine) are available and included in the reimbursement system. To receive reimbursement, patients must be diagnosed by a specialist physician (neurologist, geriatrician) after undergoing a thorough examination (including neuropsychological and laboratory tests, and neuroimaging). The specialist's statement is submitted to the Social Insurance Institution. Compared to other countries,

reimbursement is not restricted according to dementia severity (i.e. upper or lower MMSE score limits). People living alone or in nursing homes are also entitled to reimbursed treatment. The Drug Reimbursement Register is maintained by the Social Insurance Institution and includes information on dates when patients became entitled for reimbursement, and dates of reimbursed drug purchases. Reimbursement for dementia drugs started in February 1999, and persons who are entitled for reimbursement are identified with a special code in the register.[289]
Finally, within the CAIDE study individuals diagnosed with dementia in the clinical phase underwent brain imaging in the differential diagnostic phase. Autopsy data were not available to confirm the clinical diagnoses, but a previous neuropathological study conducted in the clinic in Kuopio has shown that the accuracy of clinical AD diagnosis is good (96% for probable AD and 86% for possible AD).[290]

The main limitation of Study IV is the relatively small sample size, which may affect the statistical power. Since the CAIDE subsample included in Study IV was not randomly selected within the CAIDE cohort, data were weighted to minimize selection bias.

Vitamin E serum analyses in the CAIDE study were done using aliquots stored for twelve years, initially at -20°C and then at -70°C. Previous studies reported some loss of α-tocopherol during samples storage at -20°C,[275,291] but the reduction during ten years was uniformly distributed over the whole range of α-tocopherol values.[275] This may partly explain the lower levels of some vitamin E forms in the CAIDE cohort compared to other studies, and may also underestimate the association between vitamin E and risk of cognitive impairment.[291]

7 CONCLUSIONS

α-tocopherol is the only vitamin E form currently used to define vitamin E dietary requirements and the only congener tested in RCTs in subjects with AD and MCI. The results of this project highlight that different natural forms of vitamin E can be important in cognitive impairment and AD. In older adults, diagnoses of AD and MCI are associated with reduced plasma levels of tocopherols and tocotrienols. Further, both MCI and AD are characterized by increased values of plasma indices of vitamin E oxidative/nitrosative damage.

Biomarkers can have a relevant role in primary, secondary, and tertiary prevention of AD. The development of biomarkers for AD is an active research area, and potential candidates include indicators of biochemical events associated with AD, such as OS/NS and inflammation. In this project it is shown that combined analysis of vitamin E plasma values - tocopherols, tocotrienols, indices of vitamin E oxidative/nitrosative damage -, and structural MRI measures can support the distinction of AD and MCI cases from cognitively normal individuals, and can help identify subjects with MCI who are more at risk to progress to clinical AD.

Higher plasma or serum levels of different vitamin E forms appear to be associated with reduced risk of cognitive impairment and AD in older adults, even in very advanced age. Vitamin E biokinetics is related to lipoproteins, and it is still unclear how lipid levels should be best accounted for in longitudinal vitamin E studies. In this project, the positive association between different vitamin E forms and reduced risk of cognitive impairment and AD is evident after different adjustments for cholesterol levels.

The relationships between vitamin E and cognitive impairment as well as vitamin E and AD are controversial. The findings of this project emphasize the importance of taking into account all natural vitamin E forms when studying the association between this micronutrient and cognitive impairment and AD in older adults. Different aspects of vitamin E supplementation in preventing and treating AD should also be re-examined. This should include timing of interventions, supplements composition, plasma assessment of all vitamin E forms to identify subjects who could benefit from vitamin E supplementation, and to monitor in-vivo biological response to treatment.

8 FUTURE DIRECTIONS

Vitamin E was discovered about 90 years ago, but it has only been in the last few years that researchers have started to focus on non-α-tocopherol forms in relation to human health. Many questions related to this issue still do not have a definite answer.
Current developments in vitamin E research clearly indicate that members of the vitamin E family are functionally unique and not redundant with respect to their biological function. The functional implications of these properties are not yet fully understood, and the regulatory mechanisms that control vitamin E concentrations in plasma and tissues are largely unknown. Additional research is needed in order to clarify biokinetics and biotransformation of vitamin E family members in humans, as well as their role in physiological and pathological processes, including neurodegeneration.

In different research areas, including dementia and AD, the term vitamin E is often used as synonymous for α-tocopherol, and studies investigating all natural forms in relation to cognitive impairment and dementia/AD are lacking. This project provides the evidence of a relation between different vitamin E forms and AD, but more studies are needed to verify this association. A life-course perspective is essential in epidemiological studies of chronic disorders such as dementia. Thus, longitudinal studies considering both midlife and late-life exposures can clarify the role and the possible age-dependent association between vitamin E and AD. Repeated measures of vitamin E in the same subject over time can allow for the detection of patterns of vitamin E change, which might be more relevant than a single measure at only one point in time.
A balanced presence in the body of different vitamin E forms can be important for neuroprotection. Hence, a key point is the evaluation of all vitamin E forms, taking into account their potential cumulative effect on cognition and risk of AD. The possible influence of plasma levels of lipids (i.e., cholesterol) on the association between vitamin E forms and dementia/AD needs also to be clarified. It is also important to keep in mind that people eat foods, not nutrients, and that they eat them in combination, not in isolation. Evaluation of nutrients patterns can provide additional information on the relation among micro- and macro-nutrients and AD. Integrating the assessment of dietary intake, plasma values of vitamin E, and data on other micro- and macro-nutrients intake/levels, could help unravel the synergistic and antagonistic effects of different nutrients on cognition.

Plasma levels of vitamin E forms could be associated with biochemical changes related to AD pathophysiology. The new diagnostic criteria for AD consider cognitive impairment as a continuum and support the use of different biomarkers modalities for an accurate identification of different stages of the disease. Validation studies on the proposed biomarkers are ongoing, and the possible role of different types of plasma markers (e.g.,

inflammation, OS/NS) as direct/indirect indicators of AD pathology still requires clarification. Improvement of diagnostic accuracy might be achieved with the integration of multiple biomarker modalities (i.e., neuroimaging, blood testing) and evaluation of different conditions associated with cognitive impairment which, especially in the very early stages, can require differential diagnosis with AD (e.g., vascular cognitive impairment, Lewy body disease).

Many individuals, particularly older adults, consume dietary supplements in Western societies. α-tocopherol is among the most used supplement, and it is also the only vitamin E form tested in the currently ongoing RCTs in AD prevention and treatment. On the other side, nutriceuticals containing different proportions of all natural vitamin E congeners have become available on the market in the last few years. Since dietary supplements do not require prescriptions it can be challenging to monitor their effects on human health, including cognition. To be able to provide the public with optimal health nutritional recommendations, further studies into the effects of the different vitamin E forms are needed. This will also help better define the composition of vitamin E supplements that can be tested in the prevention/treatment of AD. Finally, since effects of supplementation are likely to depend on subjects' micronutrient levels, evaluation of plasma concentrations of all vitamin E forms may help identify people who are most likely to benefit from vitamin E supplementation. It can also help monitor treatment adherence and in-vivo biological effects of vitamin E supplementation.
In conclusion, the examination of all eight natural vitamin E forms could provide new insights into the potential role of these micronutrients in human health, including AD.

9 ACKNOWLEDGEMENTS

This doctoral thesis has been based on the joint collaboration between the *Aging Research Center (ARC)*, *Department of Neurobiology, Care Sciences and Society (NVS), Karolinska Institutet*, and the *Institute of Gerontology and Geriatrics, Department of Clinical and Experimental Medicine, University of Perugia, Italy*. In both places I found a welcoming and intellectually stimulating environment, and there are not enough words to express thanks to everyone who has encouraged me and provided me with invaluable help for the completion of this thesis. I would like to express my gratitude especially to:

Professor **Miia Kivipelto**, my main supervisor, Professor **Patrizia Mecocci**, and Professor **Laura Fratiglioni**, for making the best combination of supervisors I could have wished for. You all are an endless source of scientific ideas. Your knowledge, experience, and readily available advice have made my PhD a truly memorable experience.
Professor **Miia Kivipelto**, for your optimism and warm support, and for teaching me how to balance research projects across different institutions in various countries. Your guidance to work within a group, yet the encouragement to work independently has helped me grow as a researcher. Your energy and enthusiasm in your research and clinical activities is a constant source of inspiration.
Professor **Patrizia Mecocci**, a generous researcher and skilled geriatrician who welcomed me at the Institute of Gerontology and Geriatrics and her lab since I was a medical student, and introduced me to the field of dementia and Alzheimer's disease. Thank you for providing countless opportunities for me to learn and to grow professionally, both as a researcher and as a geriatrician, and for connecting me to Sweden. I could not wish for a better mentor.
Professor **Laura Fratiglioni**, who welcomed me at ARC. Thank you for sharing your invaluable knowledge on epidemiology and neurology, and for your constructive criticism. You are a passionate researcher, who dedicates much attention and precision in any project you are in; any meeting with you or lecture you give is an opportunity to learn. I am grateful for your dedication and insightful comments which helped me to develop as a researcher and as a person, as well as the different projects you gave me the opportunity to be part of.

Professor **Bengt Winblad**, for all support and opportunities you have given me, providing so many occasions to broaden and strengthen my work. Despite your work travels all over the world, you never missed any step of my PhD, always providing good advice.

Professor **Umberto Senin**, former Director of the Institute of Gerontology and Geriatrics and the Geriatric Unit in Perugia, for introducing me to the Geriatric discipline, being a model to follow as a medical doctor, and for the opportunity to work with you on your book on Gerontology and Geriatrics.

Laura's group at ARC: **Weili Xu**, **Debora Rizzuto**, **Sara Angleman**, **Anna-Karin Weilmer**, **Behnaz Shakersain**, **Huixin Wang**, **Almira Osmanovic Thunström**, **Anna Rita Atti**, **Alessandra Marengoni**, **Grégoria Kalpouzos**, **Anna Marseglia**, **Elisabeth Rydwik**, **Barbara Caracciolo**, **Chengxuan Qiu**, **Camilla Ferrari**, **Giola Santoni**, **Lina Keller**, **Rui Wang**, **Shervin Azadi**, for the excellent scientific discussions and practical help. Weili, you are the best office roomie I could wish for. Thank you for sharing with me your knowledge on epidemiology and statistics, as well as your chocolate, and for being a patient and supportive friend. Giola, thank you for your help with statistics. Rui, thank you for the good company and the many tasty meals during the weekends spent at ARC.

The CAIDE group, spread between Sweden and Finland, but always in touch and in sync: **Alina Solomon**, **Babak Hooshmand**, **Karin Wallin**, **Tiia Ngandu**, **Minna Rusanen**, **Marjo Eskelinen**, **Satu Ahtiluoto**, **Miika Vuorinen**, **Ulrika Akenine**, **Göran Hagman**, **Gabriela Spulber**, **Krister Håkansson**, **Jenni Lehtisalo**, and **Anna-Maija Tolppanen**, for stimulating brain-storming sessions whenever we join together, and for the endless help in times of need. Alina, your brilliant mind and provocative way to look at research and life in general is a constant source of great discussions and helps me to put many things into perspective. Babak, you are a kind and caring colleague and an even better friend, also the best conference companion.

The AddNeuroMed group, especially Professor **Simon Lovestone**, Professor **Hilkka Soininen**, Professor **Lars-Olof Wahlund**, **Andy Simmons**, and **Eric Westman**, for productive and interesting scientific discussion and for prompt help and feedback on issues related to the papers we co-authored.

Everyone at the Institute of Gerontology, Perugia University, and the Geriatric Unit, Perugia University Hospital: my senior colleagues and tutors, Dr. **Giovanni Aisa**, Dr. **Antonio Cherubini**, Dr. **Annalisa Longo**, Dr. **Dario Maggio**, and Dr. **Ivano Zampi**, for sharing your wide knowledge about medicine. Professor **Stefano Brancorsini**, from the Section of Physiopathology, for the great time spent working together in different research projects, especially the "food sharing" one.
My University companions and junior colleagues in the Geriatric Unit, **Sara Ercolani, Antonio Metastasio**, **Martina Pigliautile**, **Maria Felicita Cacioni**, **Carmelinda Ruggiero**, **Miriam Caputo**, **Angela Mariani**, **Francesca Patacchini**, **Monica Pace**, **Ilaria Montecucco**, **Eleonora Cirinei**, **Manuela Conestabile Della Staffa**, **Valentina Prenni**, **Marta Baroni**, for the wonderful times in the clinic and in more relaxed contexts. **Valeria** "**Flebsy**" **Cornacchiola**, my "twin" in the residency school, for a lovely friendship, which I deeply treasure.
Nicoletta Ricciarelli and **Massimo Roscini,** for your help in administrative issues, and for always having a good word and a smile when I come to your office. **Roberta Cecchetti**, **Mauro Baglioni** and **Patrizia Bastiani**, for taking care of the vitamin E analyses. I admire the dedication you have in your work in the lab, which produces brilliant results.

To the members of the Sector of Medicine at ARC, especially Associate Professor **Kristina Johnell**, for interesting discussion about geriatric matters and fashion; the members of the Sector of Psychology, particularly Professor **Agneta Herlitz**, for you wise advice, and **Yvonne Brehmer**, for being a good friend, sharing my ups and downs. The members of the Sector of Social Gerontology, especially Professor **Marti Parker** and Professor **Ingemar** "Pingo" **Kåreholt**. Pingo, thank you for all your help with statistics, epidemiology, and for being always there the many times I needed help and advice; thank you for the good laughs, parties and kayak excursions.
To the members working in administration, data management and scientific coordination at Karolinska, **Cecilia** "Cissi" **Annerholm**, **Hélèn von Strauss**, **Kimberly Kane**, **Maria Wahlberg, Lena Ragert Blomgren**, **Maria Yohuang** from ARC; **Gunilla Johansson** and **Anna Gustafsson** from NVS. You all provide me prompt help with countless administrative practicalities and other issues. Cissi, thanks for helping me solve many issues when I was first came to Stockholm, especially finding a place to live!

Everyone at the Äldrecentrum and the Svenks Demens Centrum, especially **Sven Erik Wånell**, **Britt-Marie Gulbrandsen**, **Inger Raune, Sara Hjulström**, **Ethel Lanesjö**, and **Zoltan Pethö**.

To the **participants of the AddNeuroMed, Kungsholmen and CAIDE projects**, who gave their time and patience to enable myself and my colleagues to conduct research on such a critical aspect of aging. Also, thanks to the many people involved in planning, data collection, and management of the three projects.

I would like to thank my friends who I met in Stockholm and made me feel at home in a new city. We shared so many wonderful times and you have been always there to help when I needed it, **Katie & Stefano**, **Debora & Nicola, Sara & Oskar, Oliver**, **Anna & Erik, Jessica**, **Kim**, **Kylie & Sebastian**, **Cynthia**, **Joakim, Scott, Vanessa**, **Sofia**, **Cajsa**, **Erika & Rui**, **Muriel**, **Alina**, **Elena & Henric**. Katie, you took me under your wings, starting with my "Inglese", when I first arrived in Stockholm, and since then you have been a great and loyal friend, always giving good advice about many life matters. Debora & Nicola, you have been my Italian family in Stockholm; thank you for your warm hospitality. Sara and Anna, I have been so lucky to have you at ARC; work is always more fun with you around, and I am grateful for all the times you dragged me away from my desk for wonderful dinners and parties. Joakim, thank you for being my advisor about practical issues related to living in Sweden, introducing me to Swedish cuisine, and the wonderful times together with Miia, Joshua and Joel, in both Stockholm and Perugia.
I am also grateful for the support and love from all my lifelong friends, who never fail to stay close no matter how far apart we are. **Paola & Riccardo**, siete amici meravigliosi, tra i pochi in grado di tener testa alla mia testardaggine; grazie per esserci sempre e comunque. **Elena**, grazie per aver condiviso i miei alti e bassi da sempre, trovando sempre il modo di sorridere e ridere di noi. **Christian**, per le lunghe e animate chiacchierate e le tue belle poesie. **Andrea, Emanuele**, **Dario & Serena, Giulia**, **Michela,** amici più recenti, sempre prodighi d'incoraggiamento e affetto.

To my big and wonderful family: papà **Mario**, mamma **Luciana**, **Lucia**, **Chiara**, **Giampaolo**, **Gianluca**, zia **Paola**, **Fausto**, and zia **Margherita**. Avete sempre creduto in me, sostenendomi e incoraggiandomi in tutti i miei progetti, incluso questo. Il vostro costante affetto e appoggio su tutti i fronti è l'ingrediente essenziale della mia felicità. Grazie per tutto l'aiuto, inclusi i passaggi in auto da e per l'aeroporto nei miei frequenti viaggi, e le animate e affettuose riunioni di famiglia ogni volta che torno a casa. Cara famiglia, senza di voi non ce l'avrei fatta, grazie di tutto!
To my grandparents, for all your love and wise advice. Nonna **Emila**, sei una fonte inesauribile di ottimismo. Grazie per le tue storie sagge e spiritose, sei il mio angelo.

To **Erik**, for your love, support and encouragement; for being a great chef, an amazing communications consultant, and for your talent in making me laugh when I am not in the mood even for a smile.

The research included in this thesis was financially supported by grants from: *Karolinska Institutet* (KID funding); *University of Perugia* (Italy); the *Swedish Research Council for Medical Research* (Vetenskapsrådet); the *Swedish Council for Working Life and Social Research*; the Swedish Foundations *Ragnhild och Einar Lundströms-Minne-Lindhés*, *Gun and Bertil Stohnes*, *Gamla-Tjänarinnor* and *C.M. Lerici*.
The AddNeuroMed-Project was funded by the *European Union* within the FP6-InnoMed (Innovative-Medicines) program (LSHB-CT-518170). I would like to thank also Dr. **Carla Mangiabene** (Lab 2000, Perugia, Italy) for cholesterol and albumin dosage (Study I, II, II), and Professor **Kenneth Hensley** (University of Toledo Health-Sciences-Center, OH, USA), for providing 5-NO_2-γ-tocopherol.

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11 APPENDIX

List of dissertations from the Aging Research Center and the Stockholm Gerontology Research Center, 1991-2011

1991

Herlitz Agneta. Remembering in Alzheimer's disease. Utilization of cognitive support. (Umeå University)

1992

Borell Lena. The activity life of persons with a dementia disease.

1993

Fratiglioni Laura. Epidemiology of Alzheimer´s disease. Issues of etiology and validity.

Almkvist Ove. Alzheimer´s disease and related dementia disorders: Neuropsychological identification, differentiation, and progression.

Basun Hans. Biological markers in Alzheimer´s disease. Diagnostic implications.

1994

Grafström Margareta. The experience of burden in care of elderly persons with dementia. (Karolinska Institutet and Umeå University)

Holmén Karin. Loneliness among elderly - Implications for those with cognitive impairment.

Josephsson Staffan. Everyday activities as meeting-places in dementia.

Stigsdotter-Neely Anna. Memory training in late adulthood: Issues of maintenance, transfer and individual differences.

Forsell Yvonne. Depression and dementia in the elderly.

1995

Mattiasson Anne-Cathrine. Autonomy in nursing home settings.

Grut Michaela. Clinical aspects of cognitive functioning in aging and dementia: Data from a population-based study of very old adults.

1996

Wahlin Åke. Episodic memory functioning in very old age: Individual differences and utilization of cognitive support.

Wills Philippa. Drug use in the elderly: Who? What? & Why? (Licentiate thesis)

Lipinska Terzis Beata. Memory and knowledge in mild Alzheimer's disease.

1997

Larsson Maria. Odor and source remembering in adulthood and aging: Influences of semantic activation and item richness.

Almberg Britt. Family caregivers experiences of strain in caring for a demented elderly person. (Licentiate thesis)

1998

Agüero-Eklund Hedda. Natural history of Alzheimer's disease and other dementias. Findings from a population survey.

Guo Zhenchao. Blood pressure and dementia in the very old. An epidemiologic study.

Björk Hassing Linda. Episodic memory functioning in nonagenarians. Effects of demographic factors, vitamin status, depression and dementia. (In collaboration with the Department of Psychology, University of Gothenburg, Sweden)

Hillerås Pernilla. Well-being among the very old. A survey on a sample aged 90 years and above. (Licentiate thesis)

1999

Almberg Britt. Family caregivers caring for relatives with dementia – Pre- and post-death experiences.

Robins Wahlin Tarja-Brita. Cognitive functioning in late senescence. Influences of age and health.

Zhu Li. Cerebrovascular disease and dementia. A population-based study.

2000

Hillerås Pernilla. Well-being among the very old. A survey on a sample aged 90 years and above. (In collaboration with H. M. Queen Sophia University College of Nursing, Stockholm, Sweden)

von Strauss Eva. Being old in our society: Health, functional status, and effects of research.

2001

Jansson Wallis. Family-based dementia care. Experiences from the perspective of spouses and adult children.

Kabir Nahar Zarina. The emerging elderly population in Bangladesh: Aspects of their health and social situation.

Wang Hui-Xin. The impact of lifestyles on the occurrence of dementia.

2002

Fahlander Kjell. Cognitive functioning in aging and dementia: The role of psychiatric and somatic factors.

Giron Maria Stella T. The rational use of drugs in a population of very old persons.

2003

Jönsson Linus. Economic evaluation of treatments for Alzheimer's disease.

2004

Berger Anna-Karin. Old age depression: Occurrence and influence on cognitive functioning in aging and Alzheimer´s disease

Cornelius Christel. Drug use in the elderly - Risk or protection? Findings from the Kungsholmen project

Qiu Chengxuan. The relation of blood pressure to dementia in the elderly: A community-based longitudinal study

Palmer Katie. Early detection of Alzheimer's disease and dementia in the general population. Results from the Kungsholmen Project.

Larsson Kristina. According to need? Predicting use of formal and informal care in a Swedish urban elderly population. (Stockholm University)

2005

Derwinger Anna. Develop your memory strategies! Self-generated versus mnemonic strategy training in old age: Maintenance, forgetting, transfer, and age differences.

De Ronchi Diana. Education and dementing disorders. The role of schooling in dementia and cognitive impairment.

Passare Galina. Drug use and side effects in the elderly. Findings from the Kungsholmen Project.

Jones Sari. Cognitive functioning in the preclinical stages of Alzheimer's disease and vascular dementia.

Karp Anita. Psychosocial factors in relation to development of dementia in late-life: a life course approach within the Kungsholmen Project.

Nilsson Jan. Understanding health-related quality of life in old age. A cross-sectional study of elderly people in rural Bangladesh.

2006

Klarin Inga. Drug use in the elderly – are quantity and quality compatible.

Nilsson Erik. Diabetes and cognitive functioning: The role of age and comorbidity.

Ngandu Tiia. Lifestyle-related risk factors in dementia and mild cognitive impairment: A population-based study.

Erika Jonsson Laukka. Cognitive functioning during the transition from normal aging to dementia.

2007

Ferdous Tamanna. Prevalence of malnutrition and determinants of nutritional status among elderly people. A population-based study of rural Bangladesh. (Licentiate thesis)

Westerbotn Margareta. Drug use among the very old living in ordinary households-Aspects on well-being, cognitive and functional ability.

Rehnman Jenny. The role of gender in face recognition. (Stockholm University)

Beckman Gyllenstrand Anna. Medication management and patient compliance in old age.

Nordberg Gunilla. Formal and informal care in an urban and a rural population. Who? When? What?

2008

Xu W. Diabetes mellitus and the risk of dementia: a population-based study.

Joachim Gavazzeni. Age differences in arousal, perception of affective pictures, and emotional memory enhancement. (Stockholm University)

Alessandra Marengoni. Prevalence and impact of chronic diseases and multimorbidity in the aging population: A clinical and epidemiological approach.

Suvi Rovio. The effect of physical activity and other lifestyle factors on dementia, Alzheimer's disease and structural brain changes.

Haider Syed Imran. Socioeconomic differences in drug use among older people: Trends, polypharmacy, quality and new drugs.

Agahi Neda. Leisure in late life: patterns of participation and relationship with health.

Meinow Bettina. Capturing health in the elderly population: Complex health problems, mortality, and the allocation of home-help services.

2009

Atti Anna Rita. The effect of somatic disorders on brain aging and dementia: Findings from population- based studies.

Livner Åsa. Prospective and retrosective memory in normal and pathological aging.

Paillard-Borg Stéphanie. Leisure activities at old age and their influence on dementia development.

Masud Rana AKM. The impact of health promotion on health in old age: results from community-based studies in rural Bangladesh.

Thilers Petra. The association between steroid hormones and cognitive performance in adulthood.

2010

Fors Stefan. Blood on the tracks. Life-course perspectives on health inequalities in later life.

Keller Lina. Genetics in dementia. Impact of sequence variations for families and populations.

2011

Rieckmann Anna. Human aging, dopamine, and cognition. Molecular and functional imaging of executive functions and implicit learning.

Caracciolo Barbara. Cognitive impairment in the nondemented elderly. Occurrence, risk factors, progression.

Schön Pär. Gender matters. Differences and change in disability and health among our oldest women and men.