Cognitive impairment - role of vascular risk factors and lipid alterations for development and progression

Anne Katrine Bergland

Avhandling for graden philosophiae doctor (ph.d.)
Universitetet i Bergen
2021

UNIVERSITETET I BERGEN

Cognitive impairment - role of vascular risk factors and lipid alterations for development and progression

Anne Katrine Bergland

Avhandling for graden philosophiae doctor (ph.d.)
ved Universitetet i Bergen

Disputasdato: 26.03.2021

År: 2021

Tittel: Cognitive impairment - role of vascular risk factors and lipid alterations for development and progression

Navn: Anne Katrine Bergland

Trykk: Skipnes Kommunikasjon / Universitetet i Bergen

Scientific environment

This thesis was conducted at the Centre for Age-Related Medicine, SESAM, Stavanger University Hospital from October 2014 to December 2020.

The supervision has been by my main supervisor Dag Aarsland at SESAM and Institute of Psychiatry, Psychology & Neuroscience, King's College London, and co-supervisors Hogne Sønnesyn at SESAM and Section of Geriatric Medicine, Stavanger University Hospital, and Alf Inge Larsen at the Department of Clinical Science, University of Bergen and Department of Cardiology, Stavanger University Hospital.

Through the entire PhD period I have been affiliated with the Department of Clinical Science at the University of Bergen. I have been part of a larger scientific environment with both local PhD students, and also national and international collaborations through SESAM, Akershus University Hospital and Institute of Clinical Medicine, Campus Ahus, University of Oslo, and Institute of Psychiatry, Psychology and Neuroscience, King's College London.

The funding has come from SESAM, and the Norwegian Health Association, grant number 7330. The Anthocyanin study was financed in part by a grant from Sandnes Sparebank, Sandnes, Norway given to Biosynth AS to support the study. Medpalett AS, Sandnes, Norway contributed to the Anthocyanin study by producing Medox® free of charge.

Acknowledgements

I would like to thank Professor Dag Aarsland who has been my main supervisor throughout the entire PhD period, and without whom this thesis never would have been written. Thank you for always providing quick responses and finding time for constructive input, good discussions and valuable advice despite your busy schedule. You have been a great support by always having an open door, showing sincere interest in my work, and supporting me through all the ups and downs in the writing process. I am very grateful for your kind interest in both my clinical work, and my daily family life.

Thanks to co-supervisor Hogne Sønnesyn who has provided guidance and support throughout the entire PhD period, especially in the process of writing the papers. You have an admirable focus on the clinical perspective in research, and on always doing everything correctly. Professor Alf Inge Larsen, co-supervisor, also deserves gratitude for his kind interest in, and important contributions to this project, especially in the planning and recruitment process of the Anthocyanin study. Additionally, I would like to thank all my co-authors. You all contributed to widening my perspective and improving the quality of the papers. A special thank you to co-author and statistician Ingvild Dalen for your support and advice during writing the papers and the thesis.

I am very grateful for all my colleagues at The Centre for age-related medicine (SESAM), led by Ingelin Testad. You all make SESAM a very friendly and inspiring environment for research. Thank you Ingelin and Martha for your support and for providing the necessary infrastructure in order to conduct the Anthocyanin study. Thanks to Martine, Lise Birgitte, Helen, and Kristin at SESAM for all your help with different practicalities and financial tasks. Thank you Ketil for both the inspiring scientific collaborations and for the invaluable and good conversations we had over the years not involving research. A great thank you to colleague and friend Ragnhild for all your support and cheering over the years. It has been a pleasure working with you and sharing research questions, late work hours, and chocolate when in need.

Thank you to the director of the Division of Medicine, Erna Harboe and the director of the Department of Emergency Medicine, Anita Lyngøy, and all my colleagues at the Department of Emergency Medicine, in particular Ole Petter, Siri and Tore for your positive and flexible attitude to my research.

Thanks to Camilla for our inspirational and fun lunches.

The thesis is based on data collected from three different studies. I therefore express my sincere gratitude towards all the patients and carers in DemVest, and the participants in the DDI and Anthocyanin study. Additionally, I would like to thank all those who have contributed to these studies, especially the research nurses Reidun, Hellen and Jorunn, without whom the Anthocyanin study could not have been done.

Lastly, I would like to thank family and friends who have been both curious and supportive about my research project. I would like to thank my parents Tove and Bjarne, who have always encouraged me to work hard and reach my goals. Thank you to my parents in law Tyra and Olaf for their invaluable support. To my brothers and siblings in law; thank you for always making me smile and laugh when we are together. Thank you Torbjørn for the last minute spell checking.

My dear friends, I am blessed to have every single one of you in my life. Thank you Helene, Silje and Linn Kathrine for brighten any rainy day.

Most importantly, I want to thank my dear husband Joar for all the support and dedication throughout the entire PhD period. Thank you for making the writing process manageable for me, even when it was at the expense of your own interests. I also want to thank our three children Tiril, Brage and Tor simply for being who you are, and putting everything into perspective. You are the loves of my life.

Abstract

Background: Dementia is a growing challenge, and there is no curative treatment available. The most common cause of dementia is Alzheimer's disease (AD), where treatment studies focusing on anti-amyloid treatment have thus far failed. Importantly, age-specific incidence of dementia has fallen in many countries, possibly due to life style changes and improved treatment of modifiable risk factors, among them vascular risk factors. However, knowledge about the effect of vascular risk factors on the progression of dementia is scarce. Of note, anthocyanins, found naturally in a number of foods, may protect against cardiovascular related cognitive impairment and dementia. Concerning AD, recent studies have investigated lipid alterations in AD pathogenesis, with the potential to complement the proteomic approaches as potential biomarkers of diagnosis and progression of AD.

Aim: To increase knowledge about the role of vascular risk factors, lipid alterations and anthocyanin supplementation with respect to development and progression of cognitive impairment in a population of people with mild dementia or at increased risk of dementia. In paper I, the objective was to examine the potential effect of vascular risk factors on the progression of AD and Lewy body dementia (LBD). Paper II explored the plasma lipid profile in mild cognitive impairment due to AD and its association with cognition. Lastly, paper III explored the potential effects of anthocyanins on mechanisms relevant for cognitive decline in people with increased risk of dementia.

Methods: Data from three different studies were analysed, including two longitudinal multicenter cohort studies; "Dementia study in Western Norway" (DemVest), and "Dementia Disease Initiation" (DDI). DemVest included newly diagnosed dementia patients from specialist clinics in old age psychiatry and geriatric medicine in Western Norway in the period of 2005-2013. DDI is a nationwide ongoing study including participants with cognitive impairment and normal controls from 2013 onwards from self-referrals from advertisements in media, newspapers and news bulletins, and referrals from general practitioners to the local memory clinics. In both studies, the baseline and follow-up assessments included a comprehensive battery of

neuropsychological tests, clinical examination, and imaging. Cerebrospinal fluid was also analysed. In addition, an open-label pilot study, the Anthocyanin study, recruited participants with increased risk of dementia from the outpatient Memory and Cardiology clinics at Stavanger University Hospital in Norway during 2015 and 2016, who received anthocyanin supplementation for 16 weeks. For comparison, normal controls not receiving anthocyanin supplementation were recruited.

Results: In paper I, smoking was the only vascular risk factor significantly associated with a more rapid cognitive decline, in patients with AD as measured by Clinical Dementia Rating Scale Sum of Boxes (CDR-SB). In contrast, being overweight was associated with a slower cognitive decline in both AD and LBD. Hypertension predicted slower decline in Mini-Mental Status Exam (MMSE) scores in all patients, and in the LBD group. Further, in the LBD group diabetes mellitus and smoking were found to be associated with a slower decline in CDR-SB scores, and in MMSE scores respectively. In paper II, a number of plasma sphingomyelin concentrations, and particularly SM(d43:2), were found to be lower in mild cognitive impairment (MCI) in cerebrospinal fluid amyloid beta positive (Aβ+) individuals compared to controls. SM(d43:2) was also nominally reduced in MCI Aβ+ individuals compared to in cerebrospinal fluid amyloid beta negative (Aβ-) participants with MCI. In addition, two phosphatidylinositols were negatively associated with visuospatial functioning at baseline. In paper III, a significant group difference was found for monocyte chemoattractant protein (MCP-1) and fasting glucose. In the anthocyanin group total cholesterol and triglycerides increased significantly, and improvements in memory and executive test scores were observed at study end.

Conclusions and implications: Smoking cessation might potentially slow down the cognitive decline in AD. Since some other vascular risk factors were associated with slower decline, further studies are needed to explore how this potentially can be translated into benefit for people with dementia. Larger studies of longer duration are warranted in order to investigate the role of lipid alterations during AD pathogenesis and progression. Randomized controlled trials are needed to explore the potential effects of anthocyanins on cognitive decline and dementia risk.

List of publications

Paper I [1]:

Bergland, A. K., et al. (2017). "Effect of vascular risk factors on the progression of mild Alzheimer's disease and Lewy body dementia." J Alzheimers Dis 56(2): 575-584. The publication is available at IOS Press through doi: 10.3233/JAD-160847. PMID: 28035932.

Paper II [2]:

Bergland, A. K., et al. (2020). "Exploration of Plasma Lipids in Mild Cognitive Impairment due to Alzheimer's Disease." J Alzheimers Dis 77(3): 1117-1127. The publication is available at IOS Press through doi: 10.3233/JAD-200441. PMID: 32804144.

Paper III [3]:

Bergland, A.K., et al. (2019). «Effects of Anthocyanin Supplementation on Serum Lipids, Glucose, Markers of Inflammation and Cognition in Adults with Increased Risk of Dementia – A Pilot Study. "Front Genet. 2019 Jun 11;10:536. doi: 10.3389/fgene.2019.00536. eCollection 2019.

Abbreviations

AD	Alzheimer's disease
Aβ	Amyloid beta
APOE	Apolipoprotein E
BMI	Body mass index
CDR	Clinical Dementia Rating Scale
CDR-SB	Clinical Dementia Rating Scale Sum of Boxes
CERAD	Consortium to Establish a Registry for Alzheimer's disease
COWAT	Controlled Oral Word Association Test
CSF	Cerebrospinal fluid
DDI	Dementia Disease Initiation
DemVest	The Dementia Study of Western Norway
DLB	Dementia with Lewy bodies
LBD	Lewy body dementia
MCI	Mild Cognitive Impairment
MRI	Magnetic resonance imaging
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PET	Positron emission tomography
VaD	Vascular dementia
VOSP	Visual Object and Space Perception
WMH	White matter hyperintensities

Contents

1. Introduction

Cognitive impairment, and particularly dementia, represents a burden for the persons diagnosed with dementia, their next of kin or carers, and the entire society [4-6]. With the increasing number of people surviving into old age, the prevalence, incidence and cost of dementia is expected to increase significantly [4]. In 2018 it was estimated to be 50 million people living with dementia worldwide, a number expected to triple by 2050 [7]. It is estimated that around 101 000 people are living with dementia in Norway [8].

Of note, there is some evidence that the incidence of dementia is decreasing in industrialized countries, possibly related to improved reduction of dementia risk factors such as hypertension, obesity, smoking, diabetes mellitus, and cerebrovascular disease and increased exposure to protective influences such as level of education, a healthy lifestyle and balanced diet [4, 9-12]. Importantly, a delay in the onset of dementia has been shown to benefit even the oldest old, possibly due to fewer years of life with dementia [13].

Nutrition has been reported to be an important modifiable risk factor for cognitive impairment [14]. The most reported diets with relevance for age-related cognitive decline, Alzheimer's disease (AD) and dementia development include the so-called "Mediterranean diet" [15], adherence to which has been reported to possibly contribute to better cognitive performance and protection against cognitive impairment and dementia [16, 17]. The Mediterranean diet contains flavonoids, a subgroup of polyphenols found in foods such as berries and fruits [15]. Further, polyphenols and anthocyanins, a subclass of the flavonoids, have been shown to possibly have a protective effect against cardiovascular related cognitive decline through the modification of a number of risk factors common to neurodegeneration due to AD, vascular dementia (VaD), and cerebrovascular disease [18]. This includes anti-inflammatory and antioxidative effects, improved lipid profile, decreased risk of diabetes mellitus type 2 and dysfunctional glucose metabolism, and improved endothelial and vascular function [19-24].

Interestingly, midlife vascular risk factors, such as hypertension, hypercholesterolemia, and obesity, have been found in large observational studies not only to be risk factors for VaD, but also AD and dementia [25-29] However, whether vascular risk factors affect the progression of dementia is not clear [30].

With respect to hypercholesterolemia, which is a risk factor for atherosclerosis, cardio- and cerebrovascular diseases, it is also associated with an accumulation of amyloid beta (Aβ) [31]. Further, cholesterol and other lipids have important structural and functional roles in the brain, and disruption of lipid homeostasis may contribute to neurodegenerative disease such as AD [32-34].

This thesis focuses on the role of vascular risk factors, lipid alterations and anthocyanin supplementation with respect to development and progression of cognitive impairment in a population of people with mild dementia or at increased risk of dementia, with a focus on AD and Lewy body dementia (LBD).

1.1 Dementia

The widely used International Classification of Diseases and Health Related Problems version 10 (ICD-10) by the World Health Organisation (WHO) defines dementia as *a syndrome due to disease of the brain, usually of a chronic or progressive nature* [35]. Impairment must be present in memory, and at least one additional cognitive domain with intact consciousness. The duration of symptoms has to be at least six months, and the impairment must represent a decline from the prior level of functioning affecting activities of daily living.

The International Classification of Diseases 11th revision recently published requires two impaired cognitive domains, however neither has to include memory [36].

Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria have also been used for diagnosing dementia, and in the latest version of DSM-5, Mild and Major Neurocognitive Disorders are introduced, the latter equated with dementia [37].

The most common cause of dementia is dementia due to AD, followed by VaD, and LBD [4]. As they share a number of clinical and pathological similarities, dementia with Lewy bodies (DLB) and Parkinson disease dementia (PDD) are often referred to as LBD [38]. Importantly, with increasing age, a combination of different pathologies, i.e. mixed dementia, is very common [4]. Other diseases include frontotemporal dementia (FTD) and the recently described limbic-predominant age-related TDP-43 encephalopathy (LATE) [39].

The most common neurodegenerative diseases causing dementia are AD and Lewy body disease. Both are pathologies of disturbed protein homeostasis involving misfolding of proteins, including amyloid, tau [40] and α-synuclein [41], leading to synaptic dysfunction and neuronal loss, and also several secondary pathologies such as inflammation, oxidative stress, and mitochondrial damage [42]. Of note, neurodegenerative conditions evolve for many years before a threshold of neuronal loss is reached that causes clinical signs [43, 44]. The ability to detect the earliest stage of these conditions is important in order to possibly intervene while neuronal viability is still present.

1.2 Mild Cognitive Impairment

Mild cognitive impairment (MCI) usually precedes dementia but the boundary between the two is not always clear. Patients with MCI are a heterogeneous group where some develop dementia, while some remain stable, and some even improve, however the risk of developing dementia is higher in persons diagnosed with MCI than in the general population [4]. According to Winblad criteria, MCI is defined as a report of cognitive decline from either the patient or an informant in addition to impairment on objective cognitive tasks, or as decline over time on cognitive tasks or tests, with relatively preserved activities of daily living [45]. Of note, ICD-11 is now using the same terminology as DSM-5; “mild neurocognitive disorder”, described as a subjective experience of cognitive decline with objective impairment in one or more cognitive domains, but not sufficiently severe to interfere with activities of daily living [36].

After fulfilling the criteria for the dementia or MCI syndrome diagnosis, relevant differential diagnosis must be considered in order to ascertain the specific underlying disease [4]. In the clinical setting this includes history taking, general clinical examination, including a brief neurological examination, as well as routine blood tests, and neuroimaging such as magnetic resonance imaging (MRI) or a computed tomography (CT). Cognitive testing must be standardised and cover relevant cognitive domains. Biomarkers such as the volume of the hippocampal region on MRI, cerebrospinal fluid (CSF) AD markers of Aβ42, phosphorylated tau (p-tau) and total-tau (t-tau), DAT-SPECT (dopamine transporter imaging), ^{18}F-fluorodeoxyglucose (FDG), and amyloid positron emission tomography (PET) are increasingly used in clinical practice [4]. A definitive diagnosis of dementia type can only be made after neuropathological analysis post mortem [46, 47].

Hence, the means used today for differentiating the underlying causes of dementias are costly procedures with potential unwanted side-effects. Thus, low-cost and safe blood-based biomarkers are of interest. Research on blood-based AD biomarkers, for example Aβ, tau, phosphorylated tau, and neurofilament light chain, is progressing and is likely to be relevant also for the differential diagnosis [48, 49]. Further, despite the focus that has been on proteomics, investigating lipid alterations during AD pathogenesis will complement the proteomic approaches channeled towards the development of early diagnosis of AD [32].

1.3 Alzheimer's Disease

1.3.1 Epidemiology

AD is the most common cause of dementia, accounting for 50-75% of all dementia cases [50]. A meta-analysis from 2017 concludes that the prevalence of AD for those above 65 years in Europe is just above 5%, the prevalence in men being 3.3 % and 7.1% in women. The incidence is increasing with age in both sexes as [51].

1.3.2 Pathology

The two core pathological hallmarks of AD are extracellular amyloid plaques and intracellular neurofibrillar tangles [40]. Amyloid plaques are deposits of Aβ peptides generated from the transmembrane amyloid precursor protein which can either be cleaved by the α- and the γ-secretases, or by the β secretase and γ-secretase where the latter has been described as the "amyloidogenic pathway" leading to formation of Aβ peptides of different lengths, including Aβ42, deposited in amyloid plaques [52]. Morphologically different subset of Aβ deposits can be found at the centre of dystrophic neurites as neuritic plagues, and in blood vessels as cerebral amyloid angiopathy [47].

The degree and distribution of the core AD hallmarks have previously been described by Braak and Braak [53]. Neurofibrillar tangles are divided into six stages where stages I-II refers to neurofibrillar tangles confined to the transenthorinal area, stages III-IV also involving the limbic area and the more severe stages V-VI having neocortical involvement [53]. Regarding amyloid deposits, three stages are described where A is characterised as low densities of amyloid deposits in the isocortex, B by medium amyloid densities in almost all isocortical association areas, and in stage C amyloid deposits can be seen in all areas of the isocortex [53].

The pathogenesis of AD is unknown. However, the amyloid cascade hypothesis proposes that deposition of Aβ causes the tau neurofibrillary tangle formation and secondary changes including glial activation, neuroinflammation, oxidative stress, autophagy and mitochondrial dysfunction, disturbance of the blood-brain barrier and microcirculation and synaptic dysfunction, triggering neuronal dysfunction and death [40, 42, 54].

1.3.3 Clinical features

AD is generally characterised by an insidious onset and slow gradual decline. The first symptoms vary from person to person, however a typical presentation of AD is the amnestic syndrome, though deficits in executive functions are also common [55]. A more atypical presentation with relatively preserved memory involves a decline in

non-amnestic aspects of cognition, such as word-finding, attention, visiospatial symptoms, impaired reasoning or judgment [55] which occur in approximately 6-14% of cases, and usually has an earlier onset [56]. The rate of decline varies but is generally irreversible, and as the disease progresses, people experience greater memory loss and other cognitive difficulties.

The first clinical stage is often labelled subjective cognitive decline, where the individual and close family members may notice some subtle changes but cognitive testing and function is still within normal range [57]. The next stage is MCI where symptoms are somewhat more pronounced, and impairment is detected on neuropsychological testing [45]. Diagnostic guidelines for MCI due to AD involving AD biomarkers have been published by the National Institute on Aging and Alzheimer's Association workgroups (NIA-AA) [58].

The dementia stage, as described previously (section 1.1) requires a cognitive decline from a prior level and impairment of activities of daily living present for at least six months [35]. The functional impairments first apparent is often problems with demanding and complex tasks such as the handling of financial issues. Then, gradually challenges evolve with different instrumental activities of daily living, such as housekeeping, cooking and shopping before progression to difficulties with basic activities such as dressing and hygiene. In the severe stage of AD the patient cannot talk, walk, or eat independently.

Of note, whereas cognitive and functional impairment typically deteriorates along the course of disease progression, behavioral and non-cognitive symptoms such as motor-, neuropsychiatric- and behavioral symptoms may occur in all phases of the disease [59], and are often found to be present at the time of dementia diagnosis [60]. Neuropsychiatric symptoms include hyperactivity (aggression, irritability, disinhibition, aberrant motor behavior and euphoria), psychosis (delusions, hallucinations and sleep disorder), affective symptoms (depression and anxiety), and apathy. The most common neuropsychiatric symptom in AD is apathy, followed by depression, aggression and anxiety [59].

1.3.4 Diagnosis

The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS-ADRA) proposed the first criteria for AD in 1984, focusing on clinical symptoms [61]. The revised 2011 criteria included not only clinical symptoms, but combined them with biomarkers of AD pathology found in CSF (Aβ42, total tau and phosphorylated tau) enabling the diagnosis of AD prior to onset of dementia [62].

The NIA-AA criteria published in 2018 describe how AD may be a biological continuum that includes formation of amyloid plaques (A), neurofibrillary tangles (T) and neurodegeneration (N), giving an ATN classification system based on CSF and imaging biomarkers [63]. In this classification biomarkers of Aβ plaques (A) are cortical amyloid PET ligand binding or low CSF Aβ42, and biomarkers of fibrillar tau (T) are elevated CSF P-tau and cortical tau PET ligand binding specific for AD. Biomarkers of neurodegeneration or neuronal injury (N) are FDG PET hypometabolism, and atrophy on MRI not specific to AD, but CSF t-tau is also classified as a neurodegeneration biomarker [63]. Of importance, the authors stress that it is premature and inappropriate to use this research framework in general medical practice [63].

1.3.5 Treatment

At the moment there are no disease-modifying treatments available for AD. Several phase III trials with anti-amyloid agents have failed [64], however with some mixed but slightly more promising results with phase-III aducanumab recently reported [65, 66]. The medications available are based on neurotransmitter manipulation involving cholinesterase inhibitors (donepezil, rivastigmine, and galantamine) increasing the acetylcholine activity, while memantine reduces the glutamatergic activity through its action on N-methyl D aspartate (NMDA) receptors. The use of these drugs alone or in combination gives, at best, a detectable, but modest response in some patients reducing the clinical symptoms of AD [67]. Of note, clinical guidelines mainly recommend non-pharmacological treatment as first line treatment, and pharmacological treatment when indicated should be used in combination with non-

pharmacological interventions [4]. Non-pharmacological interventions and behavioral strategies such as activities matched to interest, exercise programs, cognitive training, music therapy and psychosocial interventions like day-care centers are recommended as first line treatment [4, 67]. Pharmacological treatment may be necessary if patients experience severe neuropsychiatric symptoms or depression. The evidence of efficacy of antipsychotics is scarce and the side-effects are possibly severe. Regarding depression a selective serotonin-reuptake inhibitor is the preferred drug of choice [4].

1.4 Lewy body dementia

Lewy body dementia (LBD) is a common term used for Parkinson disease with dementia (PDD) and dementia with Lewy bodies (DLB). Even though they are two clinical syndromes that differ in the sequence of onset of dementia and parkinsonism, both syndromes and underlying pathological changes become similar with disease progression and can be viewed as a continuum [68].

1.4.1 Epidemiology

Dementia with lewy bodies

Numbers of DLB prevalence in the population vary, and a systematic reviews reports prevalence between 0 % - 21.9% of cases [69, 70]. Of note, DLB is reported to be underdiagnosed, especially in primary health care where the prevalence has been reported to be 4.2% while in secondary health care the prevalence was reported to be 7.5% [69, 71].

Parkinson's disease with dementia

The prevalence of PDD among all dementia cases is estimated to be 3-4%, and in the general population aged 65 years and over the prevalence estimate is 0.2 - 0.5% [72]. Further, up to 80% of patients with Parkinson's disease progress to dementia. The point-prevalence estimate of PDD in patients with Parkinson's disease is 25% with increasing estimates with age reaching 50% 10 years after diagnosis [68].

1.4.2 Pathology

The pathological hallmark of LBD are Lewy bodies in the cell soma of neurons and Lewy neurites in neuronal cell processes, mainly consisting of misfolded α-synuclein. DLB and PDD cannot be distinguished neuropathologically [68]. The lewy pathology is accompanied by neuronal loss, however whether the Lewy bodies and Lewy neurites are neurotoxic and associated with the neuronal loss is unknown as autopsies have shown severe α-synuclein pathology in some individuals who had no clinical symptoms of LBD [73, 74]

Braak et al. have proposed a caudal-to-rostral spreading of Lewy body pathology in Parkinson's disease (with or without dementia) divided into six stages. In stage 1 pathology starts in the medulla oblongata and anterior olfactory nucleus, and in stage 2 pathology spreads in the medulla ablongata and pontine structures. At stage 3 the disease has entered the substantia nigra and Lewy body lesions begin to form in the pars compacta before stage 4 were pathology involves limbic structures and temporal cortex. At stage 5 and 6 the neocortex are affected [75].

1.4.3 Diagnosis

Dementia with Lewy bodies

DLB is not defined in the ICD-10, but is described in the DSM-5 as Major and Minor Neurocognitive disorder with Lewy bodies [37] and also in the ICD-11 [36]. The DLB consortium criteria first published in 1996, and later revised in 2005 and 2017 by McKeith et al. are often being used [76-78]. Revisions were done in order to increase the sensitivity of the diagnosis.

In all the revisions, dementia as a central clinical feature is consistent. Fluctuating cognition, visual hallucinations, and parkinsonism have been constant as core clinical feature. Rapid eye movement-sleep behavior disorder was added as a supportive feature to the 2005 criteria and included as a core clinical feature in the 2017 criteria [78]. The 2005 criteria included the supportive clinical features of repeated falls, syncope, transient loss of consciousness, neuroleptic sensitivity, systematised delusions, and hallucinations [76]. However, in the latest criteria more features are

listed, such as severe autonomic dysfunction, e.g. constipation, orthostatic hypotension, urinary incontinence; hypersomnia; hyposmia; hallucinations in other modalities; systematised delusions; apathy, anxiety, and depression [78].

Further, the 2017 criteria include reduced dopamine transporter uptake in the basal ganglia by PET or SPECT (dopamine transporter imaging), abnormal metaiodobenzylguanidine (MIBG) myocardial scintigraphy and polysomnography confirming rapid eye movement sleep without atonia as indicative biomarkers.

DLB is diagnosed clinically as probable or possible, depending on symptoms and findings. According to the latest criteria, possible DLB can be diagnosed with one core clinical feature or one indicative biomarker. A probable DLB diagnosis can be made if there are two core clinical features or one core clinical feature and one indicative biomarker [78].

Dementia occurring before or concurrently with parkinsonism should be labeled DLB, while dementia which develops in patients with an established PD diagnosis should be labeled PDD [78].

Parkinson's disease with dementia

According to the ICD-10 critera, PDD is defined as "dementia developing in the course of established Parkinson disease. No particular distinguishing clinical features have yet been demonstrated" [35].

The International Parkinson and Movement Disorder Society provided in 2007 clinical diagnostic criteria for probable and possible PDD [79]. In short, the criteria require the core features of Parkinson's disease and dementia to be present in both probable and possible PDD where dementia is defined similar to the ICD-10 criteria, however without the specification that memory must be affected. Further, a probable PDD diagnosis requires that the patient has a cognitive profile with impairment of at least 2 domains attention, executive functions, visuo-spatial functions, and impaired free recall memory which usually improves with cueing. Having at least one behavioral feature such as apathy, daytime sleepiness, delusions or hallucinations,

supports the diagnosis. For a possible PDD diagnoses attention is preserved, and the associated features show atypical profile f.ex. having aphasia or if retrieval of memory is not improved when a cue or word related to the information is given. Further, behavioral symptoms may or may not be present. Possible PDD is also diagnosed if the patient has comorbidity that may explain the cognitive making the PDD diagnosis uncertain, or if the time interval from motor and cognitive symptoms is not known.

1.4.4 Clinical features

Dementia with Lewy bodies

Clinically DLB is characterised by dementia, with variable combinations of the clinical core features of parkinsonism, rapid eye movement sleep behavior disorder, fluctuating cognition/alertness, and visual hallucinations [78]. It has been reported that DLB patients present cognitive impairment characterised by impaired attention, executive- and visuospatial functions rather than impaired memory function. However, as noted in the recently proposed criteria for prodromal DLB, the symptoms and core clinical features may be mild or absent in the prodromal stages [44].

Prodromal DLB is a term used to describe a pre-dementia stage where the symptoms indicate that DLB will develop, which presents with cognitive deficits and a variable presentation of non-cognitive clinical features including motor symptoms, autonomic dysfunction, sleep disorders and neuropsychiatric disturbances [44]

Parkinson's disease with dementia

Parkinson's disease is clinically defined as a movement disorder characterised by motor symptoms such as bradykinesia, rigidity, resting tremor, and postural instability [80]. Cognitive decline, among the most common non-motor symptoms in PD develops gradually, however little is known about subjective cognitive decline in PD, and no established criteria exists for this syndrome [80].

MCI-PDD has been defined in a similar way as AD-MCI, in the context of established PD [81].

In PDD the core symptoms of both Parkinson's disease and dementia are present. Further, associated clinical features in PDD are deficits in cognitive features related to attention, visuospatial functions, executive functions, and memory. The core function and behavioral features include personality changes, excessive daytime sleepiness, apathy, delusions, and hallucinations [79]. Neuropsychiatric symptoms are frequent in PDD and the most common symptoms are reported to be depression, anxiety, apathy and hallucinations [82].

1.4.5 Treatment

As for AD, there are no available disease modifying agents for LBD treatment. A newly published review on LBD treatment concludes that rivastigmine and donepezil are the best choices of treatment for cognitive symptoms in LBD. Memantine might also have some benefits, but whether memantine should be used as a monotherapy or be combined with cholinesterase inhibitors is not clear [83].

Patients with LBD often present a variety of neuropsychiatric symptoms. Unfortunately, the evidence base for non-pharmacological interventions in patients with Lewy body dementia is weak [83]. If the symptoms are distressing pharmacological management should be given in which case cholinesterase inhibitors might help. Low dosage Quetiapine is considered to be the safest choice for patients with LBD as it has fewer side effects, while clozapine is recommended in PDD. Treatment of depression in LBD is difficult as studies are less conclusive, however a selective serotonin-reuptake inhibitor seems to be the best choice [83]. For the management of motor symptoms in patients with LBD, both acute and chronic levodopa monotherapy are found to improve motor function, although more so in patients with PDD than in those with DLB [83]. Interestingly, an epileptic drug zonisamide have been reported to improve motor function when used in combination with levodopa in patients with DLB [84].

1.5 Risk factors for cognitive impairment

Cognitive impairment, including dementia, generally results from a complicated interplay between unmodifiable and modifiable risk factors across the lifespan [4, 9].

1.5.1 Unmodifiable risk factors

Increasing age is the most important risk factor for dementia and AD, with exponential increases in incidence at age 65 and older. It has been postulated that there might be an interaction between age, neuropathology and comorbidity, and that age alone would probably be a less prominent risk factor once other risk factors and comorbidity are taken into account [4].

Genes and genetic variants are risk factors for dementia development. Generally, genetic risk in AD is by far the most studied. Genetically, AD can be subdivided into the rare (accounting for less than 1% of cases) autosomal dominant familial forms often causing early-onset AD, and the multifactorial sporadic form with several associated genes [85]. The autosomal dominant forms are caused by mutations in amyloid precursor protein, presenilin 1 or presenilin 2 genes resulting in symptom manifestation before the age of 65 years [86].

Sporadic AD has no known causative gene mutations. However, genome-wide association studies have identified multiple novel risk genes for AD pathology. Some of these genes could be linked to the amyloidogenic pathway, while others are involved in the immune system, synaptic functioning, and lipid metabolism which also show the relevance of these pathways in AD [85, 87].

Importantly, apolipoprotein E (*APOE*) ε4 gene represents the strongest genetic risk factor for sporadic AD [43]. The multifunctional APOE protein is encoded by the *APOE* gene which exists as three alleles; ε2, ε3, and ε4 producing 6 genotypes, where the resulting proteins; APOE2 APOE3 and APOE4 only differ in one or two amino acids [88]. Of the three alleles of the *APOE* gene, the ε3 is most common and neutral regarding risk for AD, while the *APOE* ε4 allele has been shown to be the major genetic risk factor of AD, whereas the *APOE* ε2 allele decreases the risk [89, 90].

However, the *APOE* ε4 cannot alone be used as a tool for diagnosing AD as it is neither sufficient nor necessary for the development of AD [91].

Genes related to LBD are not as not as thoroughly mapped as those related to AD, however studies, including recent genome-wide association studies performed in Norwegian and European cohorts reported the *APOE* ε4 allele and the glucocerebrosidase (*GBA)* gene as significantly associated with DLB [92, 93].

1.5.2 Modifiable risk factors

There is an expectation of future disease-modifying treatments for dementia. However, a need for its effective prevention still exists, as prevention is generally better and often cheaper. Currently, intervention and treatment strategies focusing on modifiable environmental and lifestyle factors are the only available approaches to reducing rates of dementia. Although not all dementia risk factors are modifiable, previous calculations have shown that up to 40 % of dementia cases may theoretically be preventable [9]. The latest Lancet Commission: "*Dementia prevention, intervention and care*" recently published a list of 12 potentially modifiable risk factors that can contribute to increased dementia risk, including hearing loss, less education, traumatic brain injury, low social contact, depression, physical inactivity alcohol consumption and air pollution in addition to vascular risk factors such as hypertension, smoking, obesity, and diabetes [9]. The recognition of all of the previously mentioned risk factors are of importance, however this thesis focuses on some vascular risk factors, which will be described in further detail in the following section.

A number of mid-life vascular risk factors such as hypertension, overweight, smoking, diabetes mellitus, and hypercholesterolemia have been identified as risk factors for late-life dementia in general, however most knowledge exists about the risk factors for AD [4, 9, 12, 94, 95]. Of note, vascular risk factors are unlikely to occur isolated but might interact (e.g. metabolic syndrome) and for most of these risk factors, the mediating pathways are not completely known, whether they are acting on the amyloid process, on the reserve capacities, or on the inflammatory pathway

[43]. Although midlife is poorly defined, midlife has been defined as 45–65 years and later life as older than 65 years in published works on dementia risk [4].

1.5.3 Vascular risk factors

Smokers are at a higher risk of dementia compared to non-smokers, possible due to the link between smoking and cardiovascular disease, and smoking cessation, even at older age reduces this risk [4, 9]. However, there are some bias and uncertainty in the association between smoking and risk of dementia as smokers also have higher risk of death before the age at which they might have developed dementia [9].

Epidemiological studies report mid-life hypertension to be a risk factor for cognitive decline, MCI and dementia, not only VaD, but also AD [25, 26, 96-98]. In the Framingham Offspring cohort comprising 1440 people, elevated systolic blood pressure (≥140 mm Hg in midlife; mean age 55 years) was associated with an increased risk of developing dementia and the risk increased further if hypertension persisted into later life [98]. Of note, among those not having hypertension, a steep decline in blood pressure during mid- to late life was associated with an increased dementia risk highlighting the potential cognitive benefits of lower blood pressures in midlife, but also suggesting that declining blood pressure in older adults with prehypertension or normotension may be a risk marker for dementia [98].

Hypertension is an established as a risk factor for cerebrovascular disease, as it affects the cerebral vasculature and is a risk factor for both extracranial and intracranial atherosclerosis and cerebral infarction. Even relatively small volumes of damage in brain regions involved in cognition, i.e. hippocampus, medial thalamus and frontal lobe can produce cognitive dysfunction [99]. Importantly, hypertension is a risk factor for small vessel disease, which is parenchymal lesions caused by abnormalities in the small vessels. Further, small vessel disease is associated with cognitive impairment [99]. The features of small vessel disease visible on conventional MRI include white matter hyperintensities (WMHs), small infarcts, dilated perivascular spaces, microbleeds and brain atrophy [100]. WMHs appear as bright areas in the more gray-appearing normal brain tissue on MRI scans, and recent

pathology studies have, interestingly, found an association between WMHs and AD pathologies [101].

Being ambitious about treating hypertension in middle aged and older people without dementia to reduce dementia incidence is recommended [9]. A meta-analysis concluded that using any antihypertensive medication in people aged 55 years and older with high blood pressure reduced the risk for developing dementia. However, there were no significant differences by use of a specific drug class [102].

Diabetes mellitus has been found to be a risk factor for future AD and dementia development [4, 103]. In a pooled meta-analysis diabetes mellitus type 2 was associated with an increased risk of any dementia and the risk of dementia increased with the severity and duration of diabetes. For VaD, the additional risk was found to be greater in women [104]. Diabetes mellitus type 2 appears to be associated with PD and neurodegenerative dementias, possibly through peripheral and cerebral insulin resistance which in turn results in altered autophagy, cell proliferation and increased inflammation [95]. Also, drugs used in diabetes treatment have shown positive effects on neurodegenerative processes and on clinical outcome, regarding memory and cognition [95]. Diabetes mellitus type 2 has been shown to be a risk factor for AD and dementia not only through increased risk of small vessel disease and increased risk of both cardio- and cerebrovascular disease, but also through insulin resistance and chronic inflammation [54, 105, 106]. Although diabetes mellitus type 2 is a risk factor for development of future dementia it is uncertain whether any particular medication ameliorates this risk [9].

Obesity in midlife has been been found to be associated with an increased risk of dementia and AD later in life [27]. A review of 19 longitudinal studies including middle aged people followed up for up to 42 years reported obesity (Body mass index (BMI) ≥30) but not being overweight (BMI = 25–30) to be associated with late-life dementia. Of note, the association between midlife underweight and dementia were inconsistent [107]. Several epidemiological studies have concurred in that there is a decline in BMI in the years prior to dementia [108, 109]. As part of metabolic

syndrome, obesity has been shown to increase peripheral inflammation, and interestingly it is suggested that excessive inflammatory mediators produced by adipose tissue during metabolic syndrome affect the brain, stimulating microglia and causing synaptic alterations, and possibly initiating the accumulation of Aβ [54, 110]. These findings reinforce the complexity of the relationship between inflammation, genetic and lifestyle risks, possibly acting cumulatively to increase the risk of developing AD.

Atherosclerosis is also a chronic inflammatory process. There is a causal relationship between plasma cholesterol levels and atherosclerosis, a common risk factor for both cardiovascular- and cerebrovascular disease, as well as AD and dementia [111-113]. Further, in peripheral tissue APOE proteins are mainly produced by the liver and regulates lipid homeostasis by mediating lipid transport between tissues. *APOE* ε4 has been shown to be associated with atherosclerosis, and to be a risk factor for coronary heart disease thus contributing to an increased risk of AD [90].

Interestingly, similar to the observations of hypertension and obesity, the relationship between serum total cholesterol and dementia seems to be bidirectional as high midlife total cholesterol is a risk factor for late-life dementia and AD [97], however high total cholesterol in late life has been shown to be associated with decreased dementia risk [114]. Decreasing serum total cholesterol after midlife may reflect ongoing disease processes and may represent a risk marker for late-life cognitive impairment [115].

Noteworthy, the brain is a highly lipid-enriched organ, requiring cholesterol, and other lipids for the long expansions of neurons and myelin construction, and the remodeling needed in relation to recovering from damages, aging-related deterioration, and disease [116]. Lipid alterations have been reported to be related to neurodegenerative processes, particularly in AD by increased Aβ production [31-33]. As lipid alterations in AD is a subject of this thesis, it will be described in greater detail below.

1.5.4 Lipid alterations in Alzheimer's Disease

Lipids are obtained through diet or synthesized and metabolised by enzymes, and are estimated to include at least 10,000 - 100,000 distinct species in the human lipidome [117]. Using biochemical approaches, lipids can be sorted according to the comprehensive classification system devised by LIPID Metabolites And Pathways Strategy (MAPS), which classify lipids into eight major categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides, isoprenols, and sterols where each category can also be further subdivided into subclasses [118] resulting in a wide diversity of lipid families where the roles of some are still poorly understood.

In the central nervous system cholesterol is produced in astrocytes, and APOE functions as a transporter of cholesterol to neurons via APOE receptors. Although the exact mechanism of APOE proteins is not known, it has been shown that APOE4 has implications for the major hallmarks of AD including deposition and clearance of Aβ peptide plaques, tau phosphorylation and neuroinflammation [90]. Interestingly, a recent study of 152 non-demented participants found *APOE* ε2 carriers to have elevated baseline phospholipids, especially phosphatidylethanolamine in plasma. Further the high baseline phosphatidylethanolamine predicted protection against cognitive decline after six years [119], suggesting a possible pathomechanism involved in AD risk by modulation of plasma lipids by *APOE* genotype.

The majority of lipids reside in cell membranes forming a lipid bilayer and carry out important functions of cell membranes such as cellular transport, energy storage, modulator for transmembrane proteins, and act as signaling molecules [120, 121]. The main lipid classes in the brain found to be disrupted in AD include cholesterol phospholipids, sphingolipids, and glycerolipids, mainly localized to myelin and neuronal membranes where they are constituents of the cellular membrane [32, 122].

The mechanisms by which lipids could be involved in AD pathology are unclear. The brain is rich in cholesterol which has important roles in the synthesis, deposition, and clearance of Aβ [123]. However, Aβ can also perturb cholesterol and lipid

metabolism [32]. Oxysterols, derivatives of cholesterol which pass the blood-brain barrier are thought to reflect cerebral cholesterol turnover, and the role of oxysterols in AD pathogenesis has been strongly supported by their involvement in modulating neuroinflammation, Aβ accumulation, and cell death [123].

Further, membrane microdomains, so-called lipid rafts, are found to be enriched with cholesterol, sphingolipids, including sphingomyelin, and glycerophospholipids, and to anchor transmembrane proteins and signalling molecules such as ion-channels [124]. The proteins amyloid precursor protein, β-secretase, and components of the γ-secretase complex taking part in the amyloidogenic pathway of AD described in section 1.4.2 are transmembrane proteins. Hence changes in the cholesterol and the sphingolipids may lead to changes in the composition and function of the lipid raft, and might thus contribute to changes in β- and γ-secretase activities and consequently affect the production of Aβ42 and disease such as AD [32, 125].

The possible implications of sphingolipids in AD and neurodegeneration have previously been explored in several studies [126-128]. The sphingolipids are associated with neuroinflammation, oxidative stress and apoptosis [126, 129]. Further, the homeostasis of membrane sphingolipids in myelin and neurons have been found to be essential to prevent the loss of synaptic plasticity, cell death and neurodegeneration [126]. Several bioactive molecules belong to the sphingolipids, including sphingomyelin, ceramide, sphingosine, and sphingosine-1-phosphate. Sphingomyelin can be hydrolysed to produce ceramide which can then be metabolised to sphingosine and further phosphorylated to produce sphingosine-1-phosphate. Ceramide can also be produced by alternative pathways and synthesised de novo [130].

It has been postulated that studying changes in brain lipid profiles could give insights not only into the pathogenesis of AD but also further the detection of potential markers for early disease diagnosis and monitoring for progression [125]. The relationship between lipid changes and cognitive impairment in AD have been studied in blood, CSF and brain tissue [32, 122, 128]. Despite only a few studies

having investigated lipid changes in CSF, sphingomyelin has been found to be elevated in CSF in AD patients, while phospholipids have mostly been unchanged or increased [122, 131-133].

Several clinical studies have reported altered blood lipid levels in sporadic AD pathology [32, 130, 134-137]. However, as previously stated by Wong et al. this field of exploration in research is still fairly new, and it is difficult to establish consensus in findings based on the number of different approaches and techniques applied across different laboratories [32]. Previous reviews have concluded that future studies should be done in well characterised longitudinal cohorts aiming to link blood-based lipidomics changes with neuropathology and integrate findings with known genomic and proteomic alterations in AD [32, 138].

1.6 Progression of cognitive impairment

Cognitive impairment, including dementia, can result from several different underlying diseases with different rates of progression. The percentage of patients diagnosed with MCI converting to dementia is a common way to report progression of cognitive impairment. Although estimates vary widely with study population and criteria used for MCI diagnosis, most studies report a rate of progression from 20 to 40%, with an annual rate of 5–17% [139, 140].

Although neurodegenerative diseases such as AD and LBD are chronic irreversible diseases where progression is expected in all patients, there is a large inter-individual variation in the rate of cognitive decline [43, 79, 80, 141, 142]. As there are no reliable predictors of neurodegenerative disease course, evidence about contributing factors associated with progression of cognitive decline are of interest as they would possibly enhance the understanding of the disease mechanisms, as well as enabling interventions to slow rate of decline. A previous study found that diabetes and prediabetes increased the risk of conversion from amnestic MCI to AD dementia. Further, diabetes, prediabetes, metabolic syndrome, and the presence of neuropsychiatric symptoms were found to increase the risk of progression from MCI

to all-cause dementia [17]. A retrospective, longitudinal, observational study using an unselected sample to test for associations between comorbidities in patients with MCI due to AD, VaD and LBD found 37.4% of the MCI patients to progress to dementia with a mean follow-up period of 27.09 ± 15.09 month, where the proportion of conversion to dementia was 39.9% in AD, 38.2% in VaD, and 27.1% in LBD [143]. Older age at onset, female sex, and a greater Clinical Dementia Rating Scale Sum of Boxes (CDR-SB) were significantly associated with a higher risk of converting from MCI to dementia both in the whole group, and also in subgroups related to dementia diagnosis [143].

The Norwegian Progression of Alzheimer's Disease and Resource use (PADR) study reported the annual proportion converting from MCI to AD to be 27% with an annual progression of AD of 1.6 points on the CDR-SB and mean annual decline in Mini Mental State Examination (MMSE) score of 1.9 [144, 145].

The role of *APOE* ε4 as a risk factor for AD is well established and it significantly lowers the age of AD onset. In addition *APOE* ε4 has been reported to be associated with increased risk of progression from MCI to AD-type dementia [90]. Controversy exists however as to whether *APOE* is associated with the rate of progression of cognitive decline in AD after its onset [91, 146].

Regarding dementia progression, little is known about this on the individual level. Dementia progression is heterogeneous both between and within persons with dementia, which can be explained by both disease and person characteristics [147, 148]. A Norwegian cohort study, with a median follow-up time of 4.3 years found DLB to decline faster (annual decline 4.4 points on MMSE) compared with AD (3.2 points on MMSE) concluding that from the mild dementia stage, patients with DLB have a more rapid cognitive decline than in AD [141]. In addition DLB has been shown to have a poorer prognosis than AD on several other important outcome measures, including higher carer stress, increased health care costs, shorter time to nursing-home admission, and shorter time to death [5, 141, 149-152].

Identification of preventable or treatable predictors likely to accelerate progression of cognitive impairment could be of importance, as this might possibly slow down cognitive decline. If so, it might reduce the costs of health care, and possibly reduce carer burden [4]. As part of this thesis focuses on progression of dementia related to the effect of vascular risk factors; this will be further addressed below.

1.6.1 Vascular risk factors and dementia progression

Studies available at the start of this doctorial project and during the writing of paper I were mainly focused on vascular risk factors associated with AD dementia progression. Findings from a systematic review suggested an association between LDL-cholesterol and the progression of dementia, while inconsistent results were found for other vascular risk factors. Of note, the review with the aim of evaluating whether vascular risk factors (hypertension, hypercholesterolemia, diabetes mellitus, overweight, and smoking) were associated with the progression of dementia, reported findings from AD dementia mainly, and to some extent VaD [30, 153], although "lewy bodies" were among the published search words [30]. Of note, in a cross-sectional analysis of late life cardiovascular factors and their relation to clinically defined neurodegenerative diseases, a specific association of AD and DLB with cardiovascular factors were found, especially with respect to BMI [154]. Further, a study from Poland examining the influence of vascular risk factors, on the survival rate of patients with DLB and AD reported diabetes mellitus, and to a lesser extent hypertension to shorten the survival time for AD cases, however no influence on DLB cases was found [155].

Although several studies have been published on the potential association of vascular risk factors and dementia progression no firm conclusion can been drawn [30]. The results of studies on various vascular risk factors, and in some cases, a sum score of these, and their association with dementia progression are summarised in Table 1. Known studies available at the time of publication of paper I are representetd in this table.

Most studies reported findings of dementia progression due to AD except one reporting on AD and VaD [153]. As evident in Table 1, the findings are conflicting. Although some studies reported an association between a single vascular risk factor, or a sum score of these, and an effect on dementia progression, most studies reported no associations. The conflicting results could have a number of explanations. Most important factors probably being different selection criteria for the study participants including age, diagnosis criteria, degree of dementia and definition of the various vascular risk factors. Further, there are differences in methodology, lengths of the different studies, and choice of outcome measure, although most studies used either MMSE, CDR or both as outcome measures. Most studies show results on a single vascular risk factor, but potentially a combination of vascular risk factors would be associated with a faster cognitive decline. However, as table 1 shows, also here the results are conflicting, probably for the same reasons as mentioned above regarding methodology.

Further studies are therefore needed in a well-characterised cohort, preferably of longer time duration. Also studies are needed to examine the possible effect of vascular risk factors on other causes of dementia than AD.

Table 1: Studies on the effect of vascular risk factors and dementia progression

Vascular risk factor	Faster progression	Slower progression	No effect on progression
Overweight	Dumont et al. 2003 [156]		Blom et al. 2014 [157], Li et al. 2010 [158], Abellan van Kan et al. 2009 [159]
Smoking			Blom et al. 2014 [157], Sona et al. 2012 [160], Li et al. 2010 [158], Van Bruchem-Visser et al. 2009 [161], Helzner et al. 2009 [162], Bhargava et al. 2006 [163], Regan et al. [164]
Hypertension	Qiao et al. 2014 [165], Blom et al. 2014 [157], Sakurai et al. 2011 [166], Ciobica et al. 2011 [167] Li et al. 2010 [158], Chaves et al. 2010 [153][1], Van Bruchem-Visser 2009 [161], Razay et al. 2009 [168], Mielke et al. 2007 [169], Bellew et al. 2004 [170]		Sona et al.2012 [160], Helzner et al. 2009 [162], Musicco et al. 2009 [171], Abellan van Kan et al. 2009 [159], Bhargava et al. [163]

Hypercholesterolemia	Ciobica et al. 2011 [167], Helzner et al. 2009 [162], Evans et al. 2000 [172]		Qiao et al. 2014 [165], Blom et al. 2014 [157], Sona et al. 2012 [160], Sakurai et al. 2011 [166], Li et al. 2010 [158], Musicco et al. 2009, [171], Abellan van Kan et al. 2009 [159], Li et al. 2008 [173]
Diabetes Mellitus	Helzner et al. 2009 [162], Roselli et al. 2009 [174], Li et al. 2010 [158]	Dominguez et al. 2012 [175], Ravona-Springer et al. 2010 [176], Musicco et al. 2009 [171], Sanz et al. 2009 [177], Mielke et al. 2007 [169]	Qiao et al. 2014 [165], Blom et al. 2014 [157], Sona et al. 2012 [160], Sakurai et al. 2011 [166], Chaves et al.2010 [153][1], Abellan van Kan et al. 2009 [159], Van Bruchem-Visser et al. 2009 [161], Bhargava et al. 2006 [163], Regan et al. 2006 [164]
Multiple vascular risk factors / increased vascular burden	Viticchi et al. 2015 [178], Kume et al. 2011 [179], Roselli et al. [174], Li et al. 2010 [158], Chaves et al. 2010 [153][1] Van Bruchem-Visser et al. 2009 [161]		Blom et al. 2014 [157], Abellan van Kan et al. [159], Mielke et al. 2007 [169], Regan et al. 2006 [164]

Unless otherwise noted the results refer to dementia due to Alzheimer's disease. 1= Dementia due to Alzheimer's disease and vascular dementia. The table is modified from Table 1 in the doctorial thesis of Eldholm R.S, 2019, p.34-35 [145].
With permission from R.S. Eldholm.

1.7 Anthocyanins

Nutritional factors may modify the risk for dementia [14]. Polyphenols and flavonoids are among the nutritional factors potentially influencing cognitive decline and dementia, possible due to a number of dementia relevant mechanisms, including on inflammation, oxidative stress, lipid metabolism, glucose metabolism and vascular function [18].

Anthocyanins constitute a subclass of flavonoids which again is a subgroup of the polyphenols, and are characterised structurally by the presence of two phenyl rings separated by a hetero-cyclic ring [18]. The anthocyanins are water soluble natural color pigments with low to no toxicity, present in most plants and foods such as berries, including blueberries, and fruits [180]. A total of about 5-600 different anthocyanin molecules have been identified, and information regarding the content of anthocyanins in food can be found in an online phenol-explorer [181].

Anthocyanins have been reported to have properties as antioxidants, which is regulated by the chemical structure involving free hydroxyl (·OH groups) around the pyrone ring, where the number of ·OH groups scattered throughout the molecule's structure determines the potency of its antioxidant activity [182]. The antioxidant mechanisms include the suppression of reactive species formation through enzyme inhibition, and the sequestration of trace elements involved in the production of free radicals, in addition to act as reducing agents in the electron-transfer reaction pathway, donating electrons to the free radicals with unpaired electrons [182, 183]. Cellular studies have shown the neuroprotective effect of anthocyanins and metabolites in human neuronal cells (SH-SY5Y) against hydrogen peroxide-induced oxidative stress [184] and modulation of the mitochondrial network [185]. Importantly, anthocyanins have been found to cross the blood-brain barrier [186] and to have anti-amyloid effect, possibly through inhibition of Aβ protein spontaneous aggregation into oligomers and preventing the fibrillogenesis of Aβ proteins, counteracting their toxicity and their triggering of neuroinflammation [187-190]. Of note, several preclinical studies have provided evidence for the potential ability of

anthocyanins to inhibit neuroinflammation through an attenuation of microglial activation and associated cytokine release, in addition to counteracting neuroinflammation related to oxidative stress [191, 192].

Studies have reported anthocyanins to have anti-inflammatory effects, possibly by suppressing pro-inflammatory mediators through the inhibition of nuclear factor (NF)-κB [182, 193]. Further, studies have suggested that anthocyanins could reduce pro-inflammatory markers such as tumor necrosis factor alpha (TNF-α) monocyte chemoattractant protein (MCP-1) and IL-6, which have been shown to play a role in the pathogenesis of obesity and increasing the risk of other associated metabolic diseases including insulin resistance, diabetes mellitus type 2, atherosclerosis and cardiovascular disease [193-195]. Of note, a meta-analysis of 32 RCTs found that anthocyanin supplementation had no relevant effects on inflammation markers, but significantly improved fasting and two hour postprandial glucose, HbA1c, total cholesterol, and LDL-cholesterol [19].

The positive effects of anthocyanins have been found to be on cardiometabolic health, and cardiovascular risk factors [19]. Anthocyanins may also protect against cardiovascular related cognitive decline by mechanisms reported to be through the modification of a number of risk factors common to neurodegeneration due to AD, VaD, and cardiovascular disease, such as improving lipid profile, decreasing risk of diabetes mellitus type 2 and dysfunctional glucose metabolism, and improving endothelial and vascular function [20-24, 196]. Anthocyanins have in several studies been reported to improve endothelial and vascular function, possibly through nitrogen oxide bioactivity [22, 23, 196-198].

There has been increased interest in- and research on the potential of flavonoids and anthocyanins to improve memory, learning, and general cognitive ability [199]. A study of the Framingham Offspring Cohort implied that higher long-term dietary intake of flavonoids is associated with lower risk of AD [200].

Over the last years several clinical studies have been done evaluating the effect of anthocyanins on cognitive function [201]. The first controlled human trials examining

neurocognitive response to dietary intervention were conducted in 2010 by Krikorian et al. reporting improved cognitive performance in elderly adults after 12 weeks of daily intake of Concord grape or blueberry juice [202, 203]. In another study of Concord grape juice relatively greater brain activation in anterior and posterior regions of the right hemisphere was observed with functional MRI in the grape juice treated subjects, which would be consistent with vascular benefit and greater hemodynamic response associated with increased neuronal activity [204]. Randomized, double-blinded, placebo-controlled studies have also reported positive effects on cognitive functions in older adults. Interestingly, relatively modest benefits were found in cognitively unimpaired older adults compared with benefits measured in participants with mild cognitive impairment [205, 206]. The cognitive functions that improved were executive function [205], and benefits in memory performance and subjective improvements in everyday function [206]. Bowtell et al. reported evidence suggesting improvement in working memory after blueberry versus placebo supplementation, and also that supplementation with an anthocyanin-rich blueberry concentrate improved brain perfusion and activation in brain areas associated with cognitive function seen on MRI in healthy older adults [207]. Another study found that regional blood oxygen level-dependent activity detected by functional MRI was enhanced in the subjects taking blueberries, but not in those taking placebo during a memory test in subjects with mild cognitive impairment [208].

Of note, the previously published studies examining the effects of anthocyanins on cognition have methodological limitations, including small sample sizes and short duration. A further limitation is the heterogeneity in sources, concentration, and dose of anthocyanin supplementation being used. Hence, it is not clear whether these promising preliminary findings can be translated into clinically meaningful effects, that is, reducing the rate of cognitive decline and risk of dementia.

1.8 Literature search

The literature searches were generally done in PubMed and in some cases Embase and Google Scholar, using the same identical search terms and combination of search terms. In a few cases searches were done in the Cochrane Library. The last comprehensive literature search for this thesis was done in March 2020. Additional references were added while reviewing the thesis before submission in December 2020.

2. Aims and hypotheses

2.1 General aim

The general aim of this thesis was to increase knowledge about the role of vascular risk factors, lipid alterations and anthocyanin supplementation with respect to development and progression of cognitive impairment in a population of people with mild dementia or at increased risk of dementia.

2.2 Specific aims

1) To examine the potential effects of vascular risk factors on disease progression in older adults with mild dementia due to AD or with mild LBD.

2) To explore plasma lipid profile in mild cognitive impairment due to AD, and its association with cognition.

3) To explore the effects of anthocyanins on mechanisms relevant for cognitive decline in adults and older adults with increased risk of dementia.

2.3 Hypotheses

a) Vascular risk factors (hypertension, overweight, smoking, diabetes mellitus, hypercholesterolemia) are associated with a faster cognitive decline in people with mild dementia due to AD or with mild LBD.

b) People with MCI due to AD have a specific plasma lipid profile which is associated with cognitive impairment.

c) Treatment with antocyanins has positive effects on dementia relevant mechanisms.

3. Materials and Methods

This thesis is based on three papers which in turn are based on data from three different studies. Table 2 provides an overview of the papers.

Table 2: Overview of the papers in the thesis.

Paper	Design	Participants		Study duration	Statistical methods
		Group	N		
I Topic: Effect of VRFs on progression of AD and LBD	Longitudinal observational study (DemVest)	Total: AD: LBD:	200 113 87	5 years	Simple comparison Spearman rho correlation Generalized Estimating Equations.
II Topic: Lipid alterations in AD-MCI	Longitudinal observational study (DDI)	Total: MCI-Aβ42pos: MCI-Aβ42neg: Normal controls:	149 50 49 50	2 years	Simple comparison Linear and Logistic Regression. Multivariate analyses
III Topic: Effect of Anthocyanins on dementia relevant mechanisms	Pilot study (Anthocyanin study)	Total: Anthocyanin: CAD: MCI: Normal controls:	47 19 8 20	16 weeks	Simple comparison

AD = Alzheimer's disease, LBD = Lewy body dementia, MCI = mild cognitive impairment, MCI-Aβ42pos = MCI with AD pathology, MCI-Aβ42neg = MCI without AD pathology, VRFs = vascular risk factors, CAD = Non-obstructive coronary artery disease, DemVest = Dementia study of Western Norway, DDI = Dementia Disease Initiation

Both the Dementia study of Western Norway (DemVest) [209] and the Dementia Disease Initiation (DDI) [210] studies are longitudinal observational studies, while the Anthocyanin study is an open label pilot study. A further description of the study participants, including inclusion and exclusion criteria will be given in the Materials section (3.1), while section 3.3 provides a description of the clinical assessments in the studies. The cognitive tests used for cognitive assessment and as primary or secondary outcome measures in the different studies are described in more detail below, in the Cognitive assessment scales section (3.2).

3.1 Materials

3.1.1 The Dementia study of Western Norway

In paper I, participants were included from the DemVest study, a prospective cohort study of older persons with a first time diagnosis of mild dementia, with a particular focus on LBD, and inclusion in the period 2005 -2013 (n=266) [209]. During the main inclusion period in DemVest from March 2005 to March 2007 all referrals to the outpatient clinics in old age psychiatry and geriatric medicine in the counties of Rogaland and Hordaland in western Norway were screened for a first time diagnosis of mild dementia. A letter was sent to all general practitioners in the area, asking them to refer new cases with mild dementia to the study. The three neurology outpatient clinics in the region were contacted, and asked to refer patients with newly diagnosed dementia to the study. In order to increase the number of LBD cases, patients with DLB and PDD were selectively recruited from April 2007.

Inclusion criteria in the DemVest study was a first-time diagnosis of mild dementia. Further, the patients had to have a MMSE score ≥ 20 [211].

Exclusion criteria were not having a dementia diagnosis or having acute delirium, terminal illness, previous bipolar disorder or psychotic disorder, or those recently having been diagnosed with a severe somatic illness which according to the clinician would significantly impact on cognition, function or study participation.

Participants included in paper I were those diagnosed with dementia due to AD or LBD, taking into account the diagnostic changes made in the 5 year follow-up reevaluation [141] and the reevaluation of the 46 brain autopsies available at the time. In addition an MMSE score of at least 20 was required for inclusion.

3.1.2 Dementia Disease Initiation

In paper II, the participants in this study were drawn from the Norwegian multicenter DDI study [210], a nationwide ongoing longitudinal cohort study for early detection of at-risk Alzheimer's disease cases, led from the Department of Neurology, Akershus University Hospital, Norway.

During the inclusion period from January 2013 to date, subjects with self-reported cognitive reduction and healthy controls have been recruited as self-referrals from advertisements in media, newspapers and news bulletins, and referral from General Practitioners to the local memory clinic. Cognitively healthy controls were also recruited among spouses of patients with cognitive disorder and from patients who had completed a lumbar puncture for orthopedic surgery. Participants in the age-group between 40 and 80 were included, and only those with a native language from one of the three Scandinavian countries. Exclusion criteria were brain trauma or brain disorder, which included dementia (CDR > 0.5 [212]), clinical stroke, severe psychiatric or somatic disorder that might influence cognitive functioning, or intellectual disability or other developmental disorders.

Out of the 658 participants available from the DDI cohort as of January 2019, 50 participants with MCI due to AD (based on CSF measurements where the CSF Aβ42 measurements were dichotomized using a cutoff of ≤ 708 ng/L (further described in 3.3.2)) were identified as MCI Aβ+. These participants were sex and age matched by manual matching with 50 Aβ- MCI participants and 50 normal controls. One participant in the MCI group was later reclassified as not having MCI and therefore excluded.

3.1.3 Anthocyanin study

In paper III, the participants were included in the Anthocyanins study, which is an open-label pilot study of people with increased risk of dementia. Recruitment of the study participants took place in the period of 2015 and 2017 at Stavanger University Hospital in Stavanger, Norway. The participants of the anthocyanin group were recruited during the period May 2015 to September 2016. The normal controls were recruited in the period December 2016 to February 2017. Participants eligible for this study were patients from the outpatient memory clinic diagnosed with MCI or mild dementia according to the ICD-10 criteria [35] and/or patients from the intervention cardiology outpatient clinic diagnosed with stable non-obstructive coronary artery disease at coronary angiography. Participants in the DDI study [210] with a diagnosis of MCI were also recruited to participate in the study. Normal controls were recruited among the staff at Stavanger University Hospital and through acquaintances.

Both the anthocyanin group and the normal control group had to have age ≥ 50 years and being on stable medication, including nutraceuticals for the past 3 months. Exclusion criteria were moderate or severe dementia (a MMSE [211] score < 24), clinically significant depression (a 15–item Geriatric Depression Scale (GDS-15) [213] score ≥7), heart failure in need of treatment, unstable coronary artery disease, having taken the anthocyanin supplement Medox® (Please see section 3.3.3 for description) during the past 3 months, inflammatory illnesses such as rheumatoid arthritis and other severe illness with < 5 years expected survival and using Warfarin, heparin or non-vitamin K antagonist oral anticoagulants.

3.2 Cognitive assessment scales

In this section the cognitive assessment scales, i.e. the ones that have been used in the MCI or dementia diagnosis in the studies and/or used as outcome measure of the papers of this thesis are described in more detail.

Mini-Mental State Examination (MMSE)

The MMSE is widely used as a screening tool for cognitive function, both in clinical practice and research where it is used to measure cognitive change over time [214, 215]. The test consists of 20 items testing cognitive function, that is, orientation, memory, attention, calculation, language and construction giving a total of 30 point where higher score indicates better cognitive functioning [211]. Due to limited response ranges, the MMSE test have floor and ceiling effects, which limits the capability of detecting both advanced dementia and early cognitive change. Further, education and age, as well as language and sensory functions i.e. hearing and vision may influence the result of the test [214]. Of note, the test is administrated directly to the patient and therefore daily fluctuations in the mood of the patient, or motivation to respond in the test situation might influence the test result.

The test can be used in settings with repeated testing as it gives specific instructions upon retesting, however there is a risk of practice-effects due to repeat exposure to the test materials [215]. As a screening tool the MMSE test has been shown to have good reliability and validity, however it is not recommended to be used alone in diagnosing dementia [214, 216].

Clinical Dementia Rating Scale

The Clinical Dementia Rating Scale (CDR) scale is a well-validated, widely used tool originally developed for measuring AD dementia severity and has shown good inter-rater reliability [217]. The CDR gives a broader assessment of both cognitive and daily functioning and is obtained through interviews of both patients and a next of kin, thus not influenced by mood or daily fluctuations [218]. The six different areas examined in this scale are memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care with global scores 0-3 where 0 denote normal functioning while 1,2 and 3 indicate mild, moderate, and severe dementia, respectively. A score of 0.5 translates into uncertain or subtle cognitive impairment. The global score is calculated based on an algorithm that weights memory as the primary domain.

For research, the CDR sum of boxes (CDR-SB) is used where the score represents the arithmetic sum of the category ratings across the six domains of functioning and serves as a means of quantifying the overall level of function. The score is obtained by summing each of the domain box scores (0-3), giving a total score of 0-18, where lower score indicates higher functioning [217]. The CDR-SB score has been reported to offer several advantages over the global score, including increased utility in tracking changes within and between stages of dementia severity, due to the increased range of values [219].

The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) wordlist memory test (WLT)

Verbal memory function was assessed using the Norwegian adaptation of the Ten Word List Learning and Recall from the the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological battery [220], a three part test where the participants are to: 1. Learn a list of 10 words presented in three trials (word list learning), giving a potential maximum of 30 points. 2. Recalling after about 10 minutes the 10 words from the word list learning task (word list delayed recall), giving a maximum of 10 points. 3. Recognition of the presented 10 words among other distracting words (word list delayed recognition), giving a maximum of 20 points [221]. The scores are interpreted based on normative data as for all tasks, and the score requirements increase with education and decreases with age. The test norms used in paper III was according to Sotanemi et al. [222] while demographically adjusted norms were used in paper II [223].

CERAD Composite

In paper II the CERAD Composite was constructed from the subtests of the CERAD word list memory test in order to construct a memory composite. Following an established method for cognitive composites [224, 225] the CERAD Composite included CERAD subtest total learning, recall and recognition. Similar CERAD memory composite scores have previously been shown accurate in detecting prodromal AD [226]. Briefly, raw scores for CERAD subtest total learning (30 items), recall (10 items) and recognition (20 items) were standardised to scores

between 0 and 1. These scores were summarized and averaged to compute a 0 – 1 standardised composite score. Further, in order to provide normative adjustment for pertinent demographics, i.e. age and education, a regression-based norming procedure [223, 227] was employed using n=146 healthy controls from the DDI cohort [210]. Standardised T-scores were then calculated for the participants in the study of paper II. (See paper II [2] for full description)

The Visual Object and Space Perception Battery

The Visual Object and Space Perception Battery (VOSP) silhouettes is a measure of visuoperceptual ability [228]. The participant are shown silhuettes of animals, 15 in total, and are to name them. After, the participant is asked to name 15 different everyday items. For both sections the test is ended if the participant makes 5 mistakes in a row. One point is given for each correct answer. Age adjusted norms are provided in the VOSP test manual [228].

The Controlled Oral Word Association Test

The Controlled Oral Word Association Test (COWAT) [229] is a measure of phonemic verbal fluency. The patient is asked to name as many words as possible on the letters F, A, and S. A 60 second limit is set for each letter. In paper II demographically adjusted norms from Heaton et al. were used [230], while in paper III unadjusted norms were used.

Trail Making Test A and B

The Trail Making Test (TMT) A and B are tests where part A assesses visual scanning and psychomotor speed while part B assesses the same abilities and in addition attention shifting [231]. Part A consists of 25 numbered circles where the participants are instructed to draw a line connecting the circles from 1 to 25 as fast as possible. Part B consists of 25 circles with numbers 1-13 and letters A-L. The participants are instructed to connect the circles, alternating between numbers and letters. For both parts the score is given as time (seconds) used to finish each part, where shorter time indicates better psychomotor speed and executive functions [231].

The results of the TMT may be influenced by age and education [232], and demographically adjusted norms were used in paper II [230]

Stroop Golden Test

The Stroop Golden test [233] is a test used to evaluate cognitive speed and inhibition [234] consisting of 3 parts, i.e. a word naming score, a colour naming score and a word-colour naming score, where the word-colour score is determined using a sheet of paper where colour words are written with incongruous ink colours. The participants are required to name the colour of ink in which the word is written rather than the word itself. The 3 scores are determined by the number of words the participants are able to read in 45 seconds in each part [235].

3.3 Clinical assessment

3.3.1 The Dementia study of Western Norway

At baseline, the patients underwent a comprehensive clinical evaluation by a study physician (old age psychiatrist, neurologist or geriatrician) with help from a research nurse. The baseline assessment included a complete physical examination and medical history by the study physician who also recorded the medication use. Other data collected according to the protocol included age, sex, weight, height, smoking status, electrocardiogram, blood tests, including *APOE* genotype analyses as previously described [236] and CSF biomarker (t-tau, p-tau and Aβ42) analyses as previously described [237].

The dementia diagnosis was made according to the Diagnostic and Statistical Manual for Mental Disorders, 4th edition (DSM-IV) [37]. The AD dementia diagnosis was made according to the criteria of the National Institute of Neurological and Communicative Disorders and the Stroke-Alzheimer's Disease and related Disorders Association [61], while a DLB diagnosis was made according to the revised consensus criteria [77] and a PDD diagnosis according to the recommendations from the Movement Disorder Society Task Force [79].

Standardised instruments including MMSE [211], Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) [238] and the CDR scale [219] were employed in the dementia diagnostic workup. Additionally a battery of neuropsychological and psychiatric tests were performed, please see reference [209].

The diagnostic criteria were independently applied by two of the researchers at baseline and the final diagnosis was made by consensus in cases of disagreement, and in the cases where the patients were fulfilling more than one diagnostic criterion.

After the baseline evaluation the participants are followed up annually, however with a less extensive protocol, until death. In order to harmonize intra- and inter-rater reliability at each centre and between centres, biannual meetings were held for the first 10 years. After five years the diagnoses were re-evaluated by three of the study clinicians based on all available data at the time [141] where the focus was on participants with "possible" AD/DLB/VaD diagnoses, or an unexpected clinical course.

A total of 46 brain autopsies with neuropathological analysis had been performed at the time of publication of paper I, supporting the clinical diagnosis of AD and LBD in approximately 85% of the patients. The accuracy of the diagnostic procedure has later been evaluated in 56 participants with neuropathological diagnosis, with a sensitivity and specificity for probable DLB of 73 % and 93 % respectively, and a sensitivity and specificity of 80 % and 92 % for AD [239].

Magnetic resonance Imaging and white matter hyperintensities

A structural MRI was performed in participants with no contraindication for this examination (otherwise a CT scan was performed) at three different sites (Stavanger University Hospital (Stavanger), Haugesund Hospital (Haugesund) and Haraldsplass Deaconess Hospital (Bergen)). In all three centres a 1.5 T scanner was used. (Philips Intera in Stavanger and Haugesund and a 1.5T GE Signa Excite scanner in Bergen). The assessment of WMH were done volumetrically as previously described [240]. In addition WMH load was rated visually according to the Scheltens scale [240, 241].

Assessment of vascular risk factors

Assessment of vascular risk factors were done based on patient history and medication use. The following vascular risk factors were included: smoking, overweight, hypertension, hypercholesterolemia, and diabetes mellitus. Participants who reported smoking at baseline were defined as smokers while those who had never smoked or had quit smoking were defined as non-smokers. Having a BMI ≥ 25kg/m^2 was defined as being overweight. Hypertension, hypercholesterolemia, and diabetes mellitus were defined as follows:

Hypertension: having a previous diagnosis of hypertension or being treated with antihypertensive drugs (i.e., beta-blockers, diuretics, Angiotensin-converting-enzyme inhibitors, calcium-antagonists or angiotensin receptor antagonists).

Hypercholesterolemia: having hypercholesterolemia reported in the medical history or using a statin.

Diabetes mellitus: having diabetes mellitus reported in the medical history, or usage of insulin or oral antidiabetic drugs (i.e., sulfonylureas, biguanides, glinides, alpha-glucosidase inhibitors, thiazolidinediones, DPP-4 inhibitors and GLP-1 analogues).

A combined total vascular risk factor summation score was created by adding the scores of the different vascular risk factors, giving a score from 0 (no vascular risk factors) to 5 (having all the vascular risk factors).

3.3.2 Dementia Disease Initiation

At baseline the participants underwent a comprehensive clinical evaluation including a full medical history from both the participant and informant, physical and neurological examinations in addition to blood tests including *APOE* genotyping, MRI scan of the brain and lumbar puncture for CSF Aβ42 analyses [210].

Blood samples were drawn by standard routine at each centre. Blood samples were immediately centrifuged and plasma and serum were aliquoted before freezing at -80 degrees Celsius, and subsequently collected and stored at the main study centre AHUS. Serum lipid analyses done locally at every centre included total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides. However LDL-cholesterol analyses were not done at 2 centres. EDTA blood was used for lipid profiling

analyses, and was centrifuged at 1200 g for 13 min before plasma were aliquoted in polypropylene tubes and stored at -80°C. Time from venepuncture until aliquoted plasma was frozen was below 2 hours. Plasma was kept at -80°C until analysis. Plasma lipid profiling was performed at King's College London, UK, using methods described previously [242, 243] and extensively described in paper II.

Lumbar puncture

The lumbar puncture procedure was performed before noon. The CSF was collected in Polypropylene tubes (Thermo Nunc) and centrifuged within 4 hours at 2000*g* for 10 minutes in room temperature. The supernatant was then transferred to new tubes and frozen at -80ºC. All CSF analyses were performed at the Department of Interdisciplinary Laboratory Medicine and Medical Biochemistry at Akershus University Hospital (AHUS). CSF from other sites was frozen at respective sites before sending to the analysing laboratory. ELISA (Innotest β-Amyloid (1–42), Innotest h-Tau Ag and Innotest Phospho-Tau (181P), Fujirebio, Ghent, Belgium) was used to determine CSF Aβ42, total tau, and phosphorylated tau. The CSF Aβ42 measurements were dichotomized using a threshold of 708 ng/mL, with values below the threshold defined as positive [244].

APOE genotyping

APOE genotyping was performed on EDTA blood samples either at AHUS (Gene Technology Division, Department of Interdisciplinary Laboratory Medicine and Medical Biochemistry) according to the laboratory's routine protocol using real-time PCR combined with a TaqMan assay (Applied Biosystems, Thermo Fisher Scientific, Waltham, USA) or at the University Hospital of Trondheim according to the protocol for the Fast Start DNA Master HybProbe Kit (Roche, Basel, Switzerland) in combination with the LightMix ApoE C112R R158C kit from TiB MolBiol (Berlin, Germany) followed by LightCycler technology (Roche, Basel, Switzerland).

Cognitive battery and MCI diagnosis

The cognitive examination included the MMSE [211], verbal learning and memory (CERAD word list test) [221], visuoperceptual ability (VOSP silhouettes) [228],

psychomotor speed (TMT-A), divided attention (TMT-B) [231] and verbal fluency (COWAT) [229]. Standardised T-scores (M=50, SD=10) were calculated for the tests based on demographically adjusted norms [223, 228, 230] except for the MMSE test. The cognitive assessment is performed approximately every 2 years. Research staff participates in bi-annual meetings, with case discussions to align procedures. A memory composite score was constructed and adjusted for age, sex and education as described in section 2.1.1

In order to diagnose MCI, the NIA-AA criteria were used, requiring reporting of subjective cognitive decline, verified objectively by lower performance on clinical cognitive tests in one or more cognitive domains [58, 62]. Originally, as described by Fladby et al. [210], the cutoff value for MCI (defined as normal versus abnormal cognition) was based on scores ≤ 1.5 SD below normative means on either CERAD word list (delayed recall) [221], VOSP silhouettes [228], TMT-B [231] or COWAT [229], or having an MMSE [211] score ≤ 27. Later, in 2019 MCI was defined as results ≤ 1.5 SD below the age, sex and education adjusted normative mean on either CERAD word list (delayed recall) [223], TMT-B, COWAT [230] or VOSP silhouettes (this test was adjusted for age only) [228]. After a thorough review of the literature, the DDI steering group found that the use of the MMSE as a MCI criterion could produce an elevated rate of false positive MCI diagnoses, and was thus excluded from the diagnostic algorithm.

More details of recruitment and diagnostic procedures have been described previously [210]. The participants of the DDI study are being examined biannually until they are diagnosed with dementia, after which they are excluded from the study. Currently the participants are mainly undergoing 2 – and 4 year follow-up assessments using same protocol as baseline.

3.3.3 Anthocyanin study

At inclusion, all participants in the anthocyanin group underwent a physical examination, including standardised blood pressure measurement, electrocardiogram (ECG), and blood tests. In addition a cognitive test battery was administered.

Cognitive functions were assessed by MMSE [211] and verbal memory function was assessed using the Norwegian adaptation of the Ten Word List Learning and Recall from the CERAD battery [220]. TMT A and B [231] and Stroop Golden test [235] were used to assess executive functioning. The GDS-15 was used to ensure that none of the participants had depression.

Following standardised procedures, participants provided blood samples in the morning after having been fasting for at least 8 hours, at baseline and after 16 weeks of anthocyanin supplementation. The blood samples were immediately centrifuged and plasma and serum were aliquoted before freezing at -80 ºC. Markers of inflammation were analysed after completion of the study by The Lipid Research Group, Department of Clinical Sciences, University of Bergen, in Bergen, Norway. After completion of the study, anthocyanin metabolites were measured in plasma at Department of Nutritional Sciences, School of Life Course Sciences, Faculty of Medicine and Life Sciences, King's College London, using a method based on microelution solid phase extraction followed by liquid chromatography and mass spectrometry, using authentic standards, as previously described, with some modifications [245]. Further details regarding the blood tests can be found in paper III [3].

The normal control group provided blood samples for analysis but was not given anthocyanin supplementation and did not complete the cognitive test battery.

Anthocyanin study design

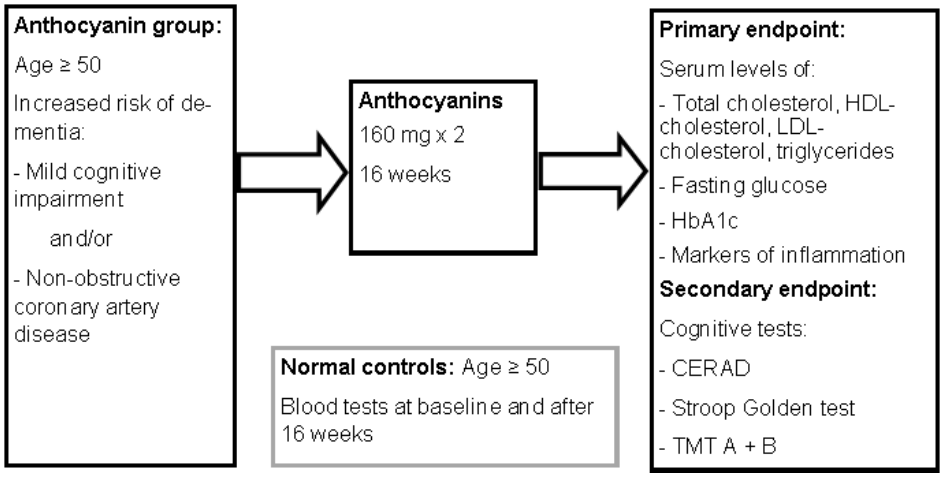


Figure 1: Overview of design of the Anthocyanin study

HbA1c = glycosylated haemoglobin. CERAD = The Consortium to Establish a Registry for Alzheimer's disease word-list learning and memory test. TMT = Trail Making Test.

In an open label design, the participants in the anthocyanin group were given anthocyanin supplementation for 16 weeks. The anthocyanin containing product used was Medox® capsules, a standardised nutraceutical product that contains naturally purified anthocyanins from bilberry (Vaccinium myrtillus) and black currant (Ribes nigrum). The content of each capsule is: Maltodextrin Glucidex IT 19, Bilberry (Vaccinium myrtillus) and Black Currant (Ribes nigrum) extract powder with 80 mg anthocyanin citrates as the 3-O-rutinosides of Cyanidin and Delphinidin and the 3-O-b-galactopyranosides, 3-O-b-glucopyranosides and 3-O-a-arabinopyranosides of Cyanidin, Peonidin, Delphinidin, Petunidin and Malvidintha [246]. The production of Medox® capsules has been described previously [247]. Each capsule contains 80 mg anthocyanins, and the participants were given the capsules at inclusion of the study, and were instructed to consume two capsules twice daily for a total daily intake of 320 mg anthocyanins for 16 weeks. The rationale for choosing this dosage was that it has previously been reported to be safe in use [248], and also to have biological effects [23, 248, 249].

The participants were asked to maintain their dietary and lifestyle habits during the study period. Further, the participants were asked explicitly during and after the study period whether they had been taking anthocyanin capsules as instructed, and were reminded of the importance of keeping the empty blister packages. Protocol adherence was assessed by collecting and counting the empty blister packages and left-over capsules at study end.

Blood tests taken for safety reasons included haemoglobin, thrombocytes, kidney function and liver function tests, which were measured at both baseline and study-end. All participants were contacted by telephone at week 8 in order to get information regarding potential side-effects and adverse events (AE).

3.4 Statistical analyses

Unless otherwise stated, the statistical data were analysed using the most recent version available of IBM® SPSS® Statistics for Windows.

In order to check the assumptions of normality of continuous variables, the Kolmogorov-Smirnov and the Shapiro-Wilk tests were used in addition to inspection of QQ-plots and histograms. When in doubt, the total number of subjects was taken into account and normality plots were discussed with a statistician.

Descriptive statistics were presented as means and standard deviations (SD) or medians and interquartile ranges (IQR) for symmetrical and non-symmetrical continuous variables respectively, and as counts and percentages for categorical variables.

For all statistical analyses $p < 0.05$ was considered statistically significant except for analysis done in paper II as described below.

In paper I, Generalised Estimating Equations (GEE) was used to explore potential associations between baseline vascular risk factors and longitudinal outcome data, while adjusting for age, sex, all other vascular risk factors, and time since baseline. GEE takes into account the correlation between multiple visits per subject through a

so-called working correlation matrix. Confidence intervals for association measures are based on robust (sandwich) estimates of standard errors. To assess whether a baseline group variable was associated with the slope of change of the outcome, the model included group and time main effects, and a group x time interaction effect.

The Spearman rho correlation was used in order to assess the associations of the different vascular risk factor scores and the Scheltens total score and CSF Aβ42.

In paper II multiple groups were compared. Between-group comparisons were done using one-way ANOVA for continuous variables with normal distributions, with Welch correction for heteroscedasticity/unequal variances. The Kruskal-Wallis tests for the continuous variables of non-normal distributions. For statistically significant ANOVA, post-hoc Bonferroni (equal variances assumed) or Tamhane's T2 (equal variances not assumed) analyses were performed as applicable by Levene's test. For Kruskal-Wallis tests Bonferroni adjusted Dunn's pairwise comparisons were applied.

The regression analyses were done using RStudio (1.2.1335). Logistic regression was applied to assess whether there was an association of plasma lipids with diagnosis at baseline. Linear regression analyses were applied to assess the association of plasma lipids with primary or secondary neuropsychological tests at baseline and the change in them between baseline and follow-up.

In order to calculate the change in cognitive outcomes between baseline and follow-up, each cognitive test at follow-up was regressed against the baseline and the residuals were used (further adjusted for months of follow-up).

For all regression analyses a stepwise adjustment model was used. Potential confounders included in the models were BMI, hypercholesterolemia, hypertension and smoking status. In addition, age, sex and education were included in the logistic models. Adjustments for age, sex and education were not done the in linear regression models as the derived T-scores were previously adjusted for these variables. Subsequently both the linear and logistic regression analyses were adjusted for *APOE* status. As 70 is the number of lipid principal components explaining >95%

of variation in the 261 identified lipids following principal component analysis, a Bonferroni threshold of $p<0.0007$ (0.05/70) was used.

As the number of variables exceeds that of the observations ($p>n$) and as lipids are highly correlated, multivariate analysis such as Partial least squares discriminant analysis (PLS-DA) and Random Forests (RA), were performed on the main outcomes. This was done in order to observe whether associations between the lipids and the tested outcomes remained when taking into account lipids' intercorrelation, and to identify which lipids and combinations of lipids are strong contributors to the outcomes.

In paper III between-groups comparisons were performed by the Independent-samples t-test, the Mann-Whitney test or Chi-square test as applicable upon comparing two independent groups. Within-group analyses were done by the Wilcoxon Signed Rank test or the paired samples t-test applying the Welch correction in situations with heteroscedasticity/unequal variances when applicable.

3.5 Ethical considerations

All the three studies were approved by the regional committee for medical and health research ethics (REK), specifically in western Norway (REK 2010/633) for the DemVest study, and in southeast for the DDI study (REK 2013/150) and the pilot study (REK 2014/1966). The Anthocyanin study was registered at Clin.Gov.Trial (NCT02409446). All the data obtained from all three studies are kept in accordance with the Norwegian recommendations on data privacy.

The participants gave their written informed consent before taking part in any of the studies, and all participants were considered to have capacity to consent on their own behalf. In addition, in the DemVest study a carer, usually the spouse or an offspring, also signed informed consent. In addition to participate in the clinical part of the study, all patients of DemVest were asked to consent for post mortem brain autopsy.

After the Anthocyanin study had started, the research group was made aware of a theoretical possibility that anthocyanins might increase the risk of bleeding [250].

Although the study group was not aware of systematic evidence supporting this claim, treatment with Warfarin, heparin or non-vitamin K antagonist oral anticoagulants was added as exclusion criteria. All participants were informed by letter about this potential issue, and given the opportunity to withdraw from the study. The REK was informed of this matter and approved the changes and let the study continue.

The Anthocynin study was financed in part by a grant from Sandnes Sparebank, Sandnes, Norway given to Biosynth AS to support the study. Medpalett AS, Sandnes, Norway contributed to the study by producing Medox® free of charge. Neither Sandnes Sparebank, Biosynth AS nor Medpalett AS had any influence on the design or conduction of the study, the analyses and interpretation of data, or regarding the decision to publish the findings.

4. Results

4.1 Paper I

A total of 200 patients with mild dementia were included (AD n=113, LBD n=87 (DLB n=69, PDD n=18)). Except for sex distribution, there were no significant differences between the AD and LBD groups when comparing the demographical data. The mean follow-up time was 3.5 years, and there was at least one follow-up assessment for 186 patients (107 AD and 79 LBD). For all patients the mean annual decline in MMSE scores was 2.6 and the annual decline was found to be 2.7 in the AD group, and 2.3 in the LBD group.

Regarding the vascular risk factors in the total sample, a total of 53% had hypertension, 9% diabetes mellitus, 25% had hypercholesterolemia, 40% were defined as being overweight (BMI ≥ 25), and 19 % were current smokers.

For both dementia groups being overweight at baseline was associated with a slower decline in MMSE scores ($p < 0.001$) and a slower increase in CDR-SB scores ($p < 0.001$). Smoking was associated with a more rapid increase in CDR-SB scores in the AD group ($p = 0.045$), and with a slower decline in MMSE in the LBD group ($p = 0.045$). In the LBD group, diabetes mellitus was found to be associated with a slower increase in CDR-SB scores ($p = 0.047$), and hypertension with a slower decline in MMSE ($p = 0.043$). Hypertension was associated with an increase in MMSE in the total group (p=0.033). In the subgroup of patients with *APOE* genotyping, adjustment for *APOE* status had no noticeable effect on the results.

The vascular risk factor summation score was significantly associated with a slower decline in cognitive function as measured by MMSE, however the results for CDR-SB were inconsistent.

WMH were associated with hypertension ($r = 0.27$, $p = 0.007$), but there were no significant associations with other vascular risk factors. Further no significant associations were found between CSF Aβ42 and any of the vascular risk factors.

4.2 Paper II

A total of 149 age and sex matched subjects took part in the study, where 3 groups were defined; MCI Aβ+ (N=50), MCI Aβ- (N=49) and normal controls (N=50). There were a few (1-5) missings on cognitive tests, but 138 participants had complete follow-up cognitive assessment after an average of 24.5 months. Regarding education, medical history, smoking status, BMI and serum lipid status the groups were equal, but as expected the groups differed in the cognitive test results. Most lipids were found to be inter-correlated and associated with many of the covariates (age, sex, education, BMI, serum lipid status, diabetes mellitus, hypercholesterolemia, hypertension, lipid lowering medication, smoking status and *APOE* status).

In this exploratory study a total of 261 plasma lipids were identified and further annotated in 8 groups as ceramides, diacylglycerols, phosphatidylcholines, Lysophosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, sphingomyelins or triglycerides.

Only one sphingomyelins (SM(d43:2)) was associated with MCI Aβ+ compared to controls after passing correction for multiple testing, being decreased in MCI Aβ+ (OR=0.29, 95% CI 0.14-0.56, $p=6.2 \times 10^{-4}$). Further, SM(d43:2) was found to be associated with MCI Aβ+ compared to MCI Aβ-, being nominally reduced in MCI Aβ+ individuals, at $p<0.05$ (OR=0.52, 95% CI 0.29-0.89, p=2.1 x10-2).

In the primary neuropsychological test analyses, no associations with CERAD composite T-score at baseline or the change in CERAD composite T-scores between baseline and follow-up passed correction for multiple testing.

In the secondary analyses, two associations passed correction for multiple testing; two phosphatidylinositols (PI(38:3) and PI(38:4)) were associated with baseline VOSP T-score, i.e. increase in two phosphatidylinositols was associated with decrease in VOSP at baseline after correction for multiple testing (beta=-3.98, 95%

CI -6.0 – -2.00 p=1.12 x10^{-4} and beta=-3.65, 95% CI -5.59 – -1.71, p p=2.89 x10^{-4} respectively).

Multivariate data analysis was also performed, finding that the lipids with the highest variable importance (VIP) in most models were the same lipids that were highlighted by univariate analysis.

4.3 Paper III

Among the 33 participants included in the study who started anthocyanin supplementation, 27 (8 MCI and 19 non-obstructive coronary artery disease) completed the study. A total of 6 participants were excluded for not being compliant with the study protocol as one participant had an exclusion criterion, two participants were not able to attend follow-up as scheduled, and three participants did not ingest the anthocyanin capsules according to protocol. In addition 20 normal controls were included in the plasma analyses, but did not receive any intervention.

Interleukin 8 (IL-8), MCP-1, CCL-5/"regulated on activation, normal T-cell expressed and secreted" (RANTES) and TNF-α, were available for statistical analyses. The other inflammation markers did not reach measurement thresholds. Regarding the between-group analyses, the only statistically significant difference were for ΔMCP-1 (difference from baseline to study end) (p=0.011) and Δfasting glucose (p=0.003).

In the within-group analyses of the anthocyanin supplementation group, statistically significant increases were found in total cholesterol (p=0.009) and triglycerides (p=0.016) from baseline to study end. In the normal control group statistically significant changes were found for MCP-1, which increased from baseline to study-end (p=0.014). Although no statistically significant changes were found for fasting glucose and HbA1c in the anthocyanin group, in the normal control group there was a statistically significant decrease in fasting glucose (p=0.009) from baseline to study end.

In the intervention group the cognitive test scores improved for CERAD learning ($p=0.016$), recall ($p < 0.001$) and recognition ($p=0.05$) and for Stroop Golden test word ($p < 0.001$) and colour ($p = 0.044$).

In plasma a total of 29 anthocyanin metabolites were quantified. Between-group analyses found a statistically significant difference for two metabolites (o-Coumaric acid ($p=0.019$) and Dihydroferulic acid-4-O-Sulfate ($p=0.010$)), which both had a larger decrease in the anthocyanin group than in the normal control group. Overall the within-group analyses of the anthocyanin metabolites were inconsistent by showing a statistically significant increase in five of the metabolites and a statistically significant decrease in five other metabolites in the anthocyanin group. Further, the within-group analyses of the normal control group showed a statistically significant decrease in four metabolites but no statistically significant increases in any of the metabolites from baseline to study end.

Regarding compliance, more than 85% of the participants returned at least 90% of the expected empty blister packages. The participants reported tolerating the anthocyanins well, and importantly, none of the participants withdrew due to adverse effects. Further, none of the participants chose to withdraw from the study after receiving the information letter regarding the theoretically increased risk of bleeding. Blood tests taken for safety reasons were all within a clinically acceptable range.

5. Discussion

In the present thesis the overall aim was to investigate the potential implications of vascular risk factors and lipid alterations for development and progression of cognitive impairment, and the possible effect of anthocyanin supplementation in a population of people with mild dementia or at increased risk of dementia. Herein the potential effects of vascular risk factors on the progression of AD and LBD were investigated in paper I. Further, paper II explored the plasma lipid profile in mild cognitive impairment due to AD and its association with cognition, while paper III explored the effect of anthocyanin supplementation in adults and older people with increased risk of dementia.

This section will be a discussion of the results in the three papers of the thesis. The findings will be compared to those of previous studies, where both similarities and inconsistencies will be discussed. In addition, a critical assessment of the methodology applied and its possible influence on the results will be provided.

5.1 Paper I

5.1.1 Vascular risk factors and progression of Alzheimer's Disease and Lewy body dementia

The potential effect of vascular risk factors on the progression of mild AD dementia and LBD was studied in paper I. Contrary to the hypothesis, with the exception of smoking, no vascular risk factors were associated with a faster cognitive decline in people with mild dementia due to AD or with mild LBD.

In the AD patients, an association of smoking at the time of dementia diagnosis with a more rapid cognitive decline was found when using CDR-SB as outcome measure. Smoking cessation is a relatively manageable modifiable risk factor found to be beneficial even in older persons [9]. Even so, the finding however, is not in line compared with both previous studies as shown in Table 1 and later research reporting no association of smoking and AD dementia progression [30, 251, 252]. In the LBD group however, smoking was associated with slower cognitive decline when MMSE

was used as outcome measure which is somewhat in line with later findings of slower cognitive decline in DLB being associated with recent or present smoking [252]. Interestingly, the latter study also found an association of smoking and slower progression of frontotemporal dementia [252].

A key finding in paper I was that at the time of dementia diagnosis, being overweight was associated with a slower progression of cognitive decline in both AD and LBD patients. This is in contradiction to previously published studies laid out in Table 1, as none of the studies reported an association of being overweight and a slower decline of cognitive impairment in patients with dementia. In paper I it was found that 40 % of the participants were overweight (defined as BMI $\geq$25). This is in line with the findings in a Norwegian study of AD patients with MCI or dementia diagnosis at baseline reporting 41.4 % of the participants to be overweight [251]. This 2-year follow-up study reported only a trend for patients with high BMI to progress less than patients of normal weight [251]. Irimata et al. analysed the effect of BMI on different types of dementia progression and did not find an association of BMI and cognitive decline in AD or DLB, but an association was found between higher BMI and slower frontotemporal dementia progression [252].

The association of being overweight at the time of dementia diagnosis with a slower progression of cognitive decline in both AD and LBD might be seen as an aspect of what has been referred to as the “obesity paradox”; while mid-life obesity is related to higher dementia risk, higher BMI after age 65 is inversely related to dementia risk [108]. Interestingly, a study has shown that weight loss negatively confounds the deleterious effect of adiposity in the brain structure in elderly and might explain the “obesity paradox” on AD risk [253]. Further, a recent study found higher late-life BMI to be associated with lower levels of CSF AD biomarkers, higher brain volumes, and slower cognitive decline [254].

The association of hypertension and hypercholesterolemia with dementia progression has in several previous studies been inconsistent, as shown in Table 1. In paper I, no association was found for hypercholesterolemia with progression of AD and LBD.

Although some studies have shown no effect on dementia progression, most studies have reported hypertension to be associated with more rapid dementia progression, which is somewhat contradictory to the findings of paper I where hypertension was found to be associated with a slower cognitive decline in the total group when MMSE was used as the outcome measure and a slower cognitive decline in the LBD when CDR-SB was used as the outcome measure. Later, Irimata et al. have reported recent or active hypertension and hypercholesterolemia to be the two most frequently co-occurring vascular risk factors at baseline and to be associated with slower cognitive decline in AD patients [252].

Of note, extensive WMH have been associated with dementia and hypertension [255, 256]. Further, in paper I a significant relationship between hypertension and WMH was found. The lack of an association between hypertension and cognitive decline might suggest that cerebrovascular pathology, as reflected in WMH load is not an important contributor to cognitive decline after onset of neurodegenerative dementia. This might also be a possible explanation as to why a relationship between diabetes mellitus and a slower increase in CDR-SB scores in LBD patients was found. That is; once a patient is diagnosed with a neurodegenerative disease, the disease will progress independently of vascular risk factors, including diabetes mellitus. However, there could be alternative explanations, as discussed below.

The vascular risk factor summation score was significantly associated with a slower decline in cognitive function as measured by MMSE, however the results regarding CDR-SB were inconsistent. Both previous studies (as shown in Table 1) and later studies have reported discrepancy regarding multiple vascular risk factors or increased vascular burden and progression of dementia. A Norwegian study with 2-year follow-up observed a trend of slower disease progression in the patients with three or more vascular risk factors, however increased vascular burden assessed by the Framingham Stroke Risk Profile had no association with AD dementia progression [252]. However, a Taiwanese study including AD dementia patients with annual follow-up for 3 years concluded that multiple vascular risk factors have summative effects leading to faster progression of AD, especially in *APOE* ε4 carriers

[257]. The discrepancies between the studies might have multiple explanations. Not only do they differ in selection criteria for their participants and methodology, they also differ in how increased vascular burden is assessed. In general, some studies use the number of vascular risk factors in an additive manner to assess vascular burden, while others use different scores allowing for differentiated weighting of individual risk factors.

The literature shows that the relationship between vascular risk factors and dementia progression is still unclear [30, 252]. Since the association of mid-life vascular risk factors and late-life dementia has been well-established in previous epidemiological studies, the discrepancy, and often lacking association of late-life vascular risk factors and dementia progression is somewhat surprising. There could be a number of reasons for these discrepancies. The comparability of the studies is limited, due to differences in outcome measurements as different cognitive tests to assess decline in cognition were used, and also different statistical analyses. Of note, for some studies it is unclear which statistical analyses they have employed, and although adjustments of the multivariate analyses were mostly similar, some studies adjusted for more factors than others. The follow-up time also varied between the studies. Furthermore, some studies only looked at one type of dementia while others analysed different dementia types. The way in which patients were recruited and selection criteria also differed. Although some studies are population based, most were based on cohort of memory or outpatient clinics.

Regarding the vascular risk factors, there is no standardised way on how they should be defined or measured in order to study their potential impact on dementia progression. Treatment of the vascular risk factors might influence the results of different studies but information regarding this is seldom available. In addition, the reporting of anti-dementia medication is also highly variable [30]. Further, as several vascular risk factors tend to decrease in the years leading up to dementia [109, 115, 258], using the evaluation of only current levels of risk factors without taking into account previous risk might influence the results. In addition, the effect of vascular risk factors or their treatment might differ between men and women but studies have

usually not been reported separate analyses per vascular risk factor by gender. Lastly, as suggested above, in individuals with dementia due to AD or LBD, the brain might already be so compromised by neuropathology that any impact of vascular risk factors does not have additional effect on cognitive functioning.

5.1.2 Study sample

The participants of paper I were drawn from the DemVest study. In line with the Norwegian health care system, most mild dementia cases are diagnosed and followed by general practitioners, however in the DemVest study all general practitioners, and all neurology clinics in the region of south-western Norway (Rogaland and Hordaland) were informed of the study, and invited to refer their patients to one of the participating outpatients clinic for evaluation and inclusion in the study. Hence, the sample in DemVest is a referral-based sample, the participants of the study may thus not be representative of the total dementia population, which might have implications for the generalizability of the results of study. Also after 2007 DLB and PPD participants were recruited selectively and are thus relatively overrepresented in the total sample which therefore may not necessarily be representative of the general dementia population. However the relative overrepresentation of the LBD would likely not influence the findings in the AD and LBD groups separately.

As the recruitment took place in outpatient clinics, one could possibly expect an overrepresentation of participants with comorbidities, which could possibly have influenced the results in paper I. Even so, the vascular comorbidity was comparable with that of another Norwegian study examining the effect of vascular risk factors on AD progression [251].

The DemVest study has been ongoing since 2005, making it a study with a long follow-up time compared to most other studies. Importantly, except for death, the study has low attrition, and from the total of 266 patients who underwent baseline assessment, only 24 (9.0%) patients have withdrawn during the study period. Further, the majority of the patients have been followed until death with repeated measurements using standardised and structured instruments at annual follow-up

times, allowing for persistent and repeated analyses in patients progressing from mild to severe dementia.

5.1.3 Dementia diagnosis

Patients screened for inclusion in the DemVest study underwent a comprehensive baseline assessment integrated into clinical practice using standardised clinical instruments and diagnostic criteria. Now after 15 years the DemVest study is still ongoing during which time there have been changes in diagnostic criteria, and increased use of supplementary diagnostic and biomarkers in clinical practice. Both 2 and 5 years into the study the diagnostic criteria were re-applied, providing a high standard of diagnostic precision [141]. Neuropathological diagnosis has also been provided, showing a good agreement between clinical and neuropathological diagnosis, although both false negative and false positive diagnoses occur. Specifically, regarding a clinical diagnosis of probable AD, the sensitivity, specificity, positive predictive value and negative predictive values were 81%, 88%, 89%, and 79%, respectively, while out of 20 patients with neuropathologically verified LBD, 16 had a clinical diagnosis of LBD (DLB=11, PDD=5), resulting in a sensitivity, specificity, positive predictive value and negative predictive value of 80%, 92%, 84%, and 89%, respectively [239]. In paper I the diagnoses from the 2 and 5 year evaluations were used, including the available neuropathological diagnoses (N=46), giving a high likelihood of correct diagnoses.

In order to increase power in the analyses of paper I, patients with DLB and PDD were combined in one LBD group. This is considered justified as they are both α-synucleinopathies and share a number of clinical and pathological similarities [38]. Further, naturalistic longitudinal data from Sweden found DLB and PDD to be similar synucleinopathies, with phenotypical variations in the order of manifestations (as expected) rather than course of progression and clinical outcome [259].

5.1.4 Clinical assessment

Study participation was integrated in the clinical practice and many clinicians took part in the multicentre design of DemVest. However, this could increase the risk of

both systematic errors, and possible random errors which could decrease the reliability of the findings.

Time of onset of risk factors is of great interest as middle age onset versus elderly onset and treatment of vascular risk factors could possibly affect the progression of AD. Per design of the DemVest study, patients were included at time of dementia diagnosis, and accordingly, information regarding previous diseases are retrospective and subject to recall bias by the patient and caregiver, and thus detailed information regarding the age at onset of diseases could not be recorded. Further, limitations to the vascular risk factors assessments of the DemVest study include the lack of some serological data, possibly introducing some bias. Most probably due to glycosylated haemoglobin (HbA1c) not being among the routine measurements at memory outpatient clinics at the time of study inclusion, only 24 patients among the 200 patients of Paper I had available measures of HbA1c, potentially resulting in underestimation of patients having diabetes mellitus as a vascular risk factor.

Hypercholesterolemia was defined as having hypercholesterolemia in the medical history and or being a statin user, without taking into account the measured level of serum cholesterol levels at baseline. This was done do to missing (n=41 missings) of this measure and the fact that the patients had not been fasting in advance of the test. Hence, patients registered as having hypercholesterolemia were all statin users which could possibly lead to misclassified data as the statin medication could have been prescribed for another reason than hypercholesterolemia, e.g. secondary prevention after cerebral and/or cardiac event. Also, patients with hypercholesterolemia might have been missed as the blood tests were not evaluated for high levels of cholesterol which could be due to hypercholesterolemia.

5.1.5 Main outcome measures

The main outcome, and also measure of AD and LBD progression were MMSE and CDR which have been previously described in section 3.2.

MMSE is well known, both in clinical and research settings [214, 215]. The limitations however, include practice effects and the relatively limited number of

cognitive domains assessed. As changes in function and behavior are not assessed, cognitive tests alone would likely not capture all changes in AD dementia and LBD. Further, the MMSE may be less sensitive in measuring change in LBD patients, possibly due to the MMSE not directly assessing psychiatric, autonomic, or executive functions often impaired in patients with DLB [260, 261].

The CDR-SB captures a broader assessment of cognitive and daily functioning and is thus potentially a more accurate and comprehensive measure of dementia severity in patients with LBD. Still, it was originally designed for use in patients diagnosed with AD dementia, and is not adequately tested in DLB [141, 217]. Further, it is more time consuming, and requires information from a next of kin.

5.1.6 Statistical considerations

In the main analyses of paper I, Generalized Estimating Equations (GEE) with unstructured working correlation was used to explore the association between vascular risk factors and cognitive decline in patients with AD and LBD.

The association analyses were adjusted for age and gender, but should preferably also have been adjusted for education, as education might influence the result on MMSE [214]. Further, the vascular risk factors were dichotomized, as in many other studies. This could potentially reduce the statistical power, thus limiting the chances of identifying associations between vascular risk factors and the progression of dementia. Further, this may also reduce the ability of making comparisons with other studies with different cut-off values for different vascular risk factors.

An unexpected finding was that AD patients were predicted to have a steeper cognitive decline than LBD patients. This is different from a previous paper [141] based on DemVest data reporting that cognitive decline is more rapid in DLB than in AD. The discrepancy in these findings are probably due to the different statistical methods applied which deals with drop-outs due to death differently. There were 20 patients (12 AD, 8 LBD) who withdrew or were lost to follow-up, and 93 (36 AD, 57 LBD) dropouts due to death. The statistical analyses have therefore later been reconsidered. Raitanen et al. [262] studies three options to deal with dropout due to

death, each of which will produce results with different interpretations. Briefly, 1) GEE (with independent working correlation) estimates the average level of functioning among those still under study at each time point, i.e. will give an estimate of how functioning develops among completers/survivors; 2) linear mixed models (LME) treat death as any other random drop-out and implicitly assumes that the subject-specific trajectories extend after death, leading to results interpretable as the expected development if death did not exist; whereas 3) joint modelling of longitudinal outcome and survival times corrects the possibly deviating development leading up to death to give estimates that can be interpreted as the expected development if no one had died.

Unpublished results using method 1) described above are similar to our published results with regard to the associations of vascular risk factors and progression of decline. Hence, the results of paper I can be interpreted as associations among survivors. Of note, some of the published findings in paper I were changed when using the other methods described by Raitanen et al. With these methods, as expected, the progression of cognitive decline was more consistent with previous reports. The findings reported in paper I should therefore be interpreted with some caution.

5.2 Paper II

5.2.1 Lipid alterations in Alzheimer's Disease

The main finding in paper II was somewhat in line with the hypothesis as lower plasma sphingomyelin concentrations, and in particular SM(d43:2), were found to be associated with AD pathology. However, no plasma lipids were associated with performance on primary neuropsychological tests at baseline or between baseline and follow-up. In the secondary outcomes, two phosphatidylinositols were found to be negatively associated with visuospatial functioning at baseline.

Previous studies have shown alterations in the sphingomyelin pathways in AD [32, 130]. A study using plasma shotgun lipidomics reported decreased sphingomyelin

and increased ceramide levels in plasma of AD patients as compared to controls [263]. This finding is supported by targeted sphingolipidomics studies that identified similar sphingolipid changes in plasma of patients with MCI [264, 265], and AD [266]. Further, a recent study using untargeted metabolomics profile analyses adjusting for *APOE* status, and ethnic/racial group reported significant differences comparing exogenous and endogenous metabolites in patients with AD and healthy controls and confirmed previous findings in sphingolipid metabolism pathways [267]. Of note, the finding of paper II is not in agreement with all previous studies as a small cross-sectional study reported lower levels of plasma sphingomyelin in AD patients compared to controls [135].

Plausible explanations to the conflicting results between different studies could possibly be, the stage of the AD may have an effect on the level on sphingomyelin measured. Mielke et al. reported that the levels of serum sphingomyelin vary according to the timing of the onset of memory impairment, a deficit observed early in AD pathogenesis [264]. Of note, age, sex and *APOE* status have been reported to have an effect on sphingomyelin metabolism, but this has not been assessed properly in all previous studies [268, 269]. Interlaboratory variability and methodology have been observed across studies. Various studies report the use of blood samples from participants who were fasting, while in other studies the participants were non-fasting at the time of blood withdrawal. Also the use of serum versus plasma and how blood products are processed varies between studies. This might affect the results, further underlining the importance of consistency across different studies and laboratories [32]. It should be noted that the provenance of SM(d43:2) is not clear. In humans, sphingomyelin levels across bio-fluids are largely regulated by multiple signaling pathways and are dependent on the contribution from diet but also de novo synthesis, recycling, and intestinal uptake. However SM(d43:2) has been detected in previous lipidomics validation studies [243].

The relationship between sphingolipid changes and cognitive impairment in AD has also been studied in brain tissue and CSF [32, 126]. Sphingomyelin has been found to be elevated in CSF in AD patients [131, 132] and studies on brain tissue have shown

alterations in lipid profiles and disturbed ceramide metabolism in AD [129]. Kosicek et al. found significantly increased sphingomyelin levels in CSF from individuals with prodromal AD compared to normal controls, however no change between mild and moderate AD groups and normal controls [131].

The research showing an association between sphingolipid metabolism and AD is intriguing. However, the exact biochemical mechanism(s) leading to the reduction of these peripheral blood lipids in people with AD is unknown. Further, the relation between systemic abnormalities and the pathogenesis of AD is poorly understood. Varma et al. designed a study in order to link alterations in metabolite signals in the brain to those in the blood, and found that higher blood concentrations of sphingolipid species were consistently associated with severity of AD pathology at autopsy and AD progression across prodromal and preclinical stages [128]. However, it is still uncertain if sphingolipid misbalance is a consequence of Aβ accumulation or one of the initiating factors of AD pathophysiology [130]. Interestingly, ceramides have not only been reported to be associated with cerebral Aβ levels, but are also intermediates linking inflammatory cytokines to insulin resistance and diabetes mellitus, and contribute to cardiovascular disease and obesity, all of which are associated with AD [270, 271].

In paper II no association between sphingomyelin, or any other lipid, and the progression of cognitive decline was found. Previous longitudinal studies that monitored the progression of cognitive decline in AD patients reported elevated plasma ceramide and low sphingomyelin to predict faster cognitive decline [136, 137]. Reason for not finding an association between plasma sphingomyelin and cognitive decline in our study could be the relatively low number of participants in and the relatively short study duration.

In the secondary outcomes of paper II, two phosphatidylinositols were found to be negatively associated with VOSP T score, suggesting that an increase in the phosphoinositols was associated with reduced performance on visuospatial functioning at baseline. Phosphatidylinositols belongs to the phospholipids which are

structurally and biologically membrane-forming lipids influencing many complex cell processes including modulation of membrane proteins and their functions [122].

Although phospholipid changes have been reported in previous studies to occur during the pathogenetic process in AD, different phospholipid species have been found to be altered [122, 133, 272].

Interestingly, Mapstone et al. [273] reported a panel of ten serum lipids to predict MCI or dementia where one of the lipids was a phosphatidylinositol [273]. These results were not be replicated in later studies possibly explained by different methodology [274, 275]. Also, a recent study examined whether phosphoinositols, and metabolites would change in erythrocytes of AD and amnestic MCI patients compared to normal controls [276]. The study found decreased levels of phosphatidylinositol in erythrocytes of amnestic MCI patients, a finding that indicates that disturbed levels of phosphatidylinositols in erythrocytes occur in the early stage of AD, possibly in the prodromal phase, but no phosphatidylinositol differences were found between amnestic MCI and AD groups, indicating that the expression of phosphatidylinositols in erythrocytes may not reflect the severity of AD [276]. These findings require verification in larger well-designed studies of longer duration.

5.2.2 Study sample

For paper II the participants were drawn from the DDI cohort, which includes participants with subjective cognitive impairment, MCI and normal controls. Altogether 150 participants were selected by manual matching. Later, one participant was excluded due to change in the diagnosis from MCI to subjective cognitive impairment after the introduction of new demographic adjusted criteria [223].

The group sample sizes are relatively small with 49 in MCIAβ- and 50 in the Aβ+, and normal control groups. The samples in the different groups were sex and age matched, and 2 year follow-up was available for most participants.

Of note, the participants in the DDI study were included from university hospitals in the four Norwegian health regions (Helse Sør-Øst, Helse Midt, Helse Nord and Helse

Vest) from general practitioners referrals. In addition recruitment was also done by self-referral following advertisements in media, newspapers or news bulletins. An important issue is that due to geographic differences in Norway, the availability of memory clinics may differ possibly leading to a biased inclusion of memory clinic referrals living in, or near city centers where the university hospitals are located. This might lead to reduced generalizability of the findings of paper II.

5.2.3 MCI-AD diagnosis

The participants were examined following the comprehensive DDI assessment protocol. By the use of published criteria, the participants were staged as either healthy controls, or having subjective cognitive decline [57] or MCI [58, 62]. An important finding by the DDI research group is that use of the Sotaniemi et al. [222] CERAD WLT normative dataset may be unfit for the DDI cohort as these norms are based on a sample that is on average 10 years older and less educated than the DDI cohort, possibly resulting in an uncertain classification of MCI and subjective cognitive decline [277]. This finding ultimately led to the development of new demographic adjusted regression-based norms for the CERAD word list (delayed recall) [223], and the use of it and other demographic adjusted tests; TMT-B, COWAT [230] or VOSP silhouettes (this test was adjusted for age only) [228].

Participants were included in the DDI in the prodromal phases and MCI stage of AD, which provides an optimal design to capture early stages of AD and follow disease development through a longitudinal study. An advantage of the sample of paper II was that the AD pathology was ensured by CSF biomarkers [244].

5.2.4 CERAD composite score

In paper II the CERAD composite score was used as a measure of cognitive decline. This score was constructed as described in section 3.2. Further standardised T-scores were then calculated and used as main outcome measure. The intention of constructing the CERAD composite was to have a more robust and reliable test of learning and memory function including being more robust against chance low performance on one measure not related to neurodegeneration or cerebral dysfunction

(e.g. low motivation or inattention during a particular test). However, the use of a CERAD composite could be a limitation, as this could possibly mask domain specific cognitive functions such as learning, recall and recognition, which are qualitatively different aspects of learning and memory. Hence, two of the subdomain measures of the CERAD WLT (learning and delayed recall) as well as other cognitive domains (psychomotor speed, executive functions, verbal fluency/language, and visual cognition) were used in the secondary analyses.

5.2.5 Statistical considerations

A possible statistical concern when analysing large numbers of variables is the risk of making type 1 errors, i.e. falsely rejecting the true null-hypothesis. However, in order reduce the risk of incorrect conclusions, post-hoc analyses as well as stringent correction for multiple testing were done in the statistical analyses of paper II. Logistic and linear regression analyses were used controlling for a number of variables associated with lipids and cognitive decline including age, sex, education, BMI, lipid lowering medication, smoking status, and history of hypercholesterolemia and hypertension.

A further strength is that a machine learning approach using Partial least squares-discriminant analysis (PLS-DA) and Random Forest (RF) was used for the main outcomes, and the results from the two approaches are in agreement.

5.3 Paper III

5.3.1 Effect of anthocyanins on dementia relevant mechanisms

In paper III, contrary to the hypothesis, the results were largely inconclusive with regard to the potential protective effects of anthocyanin supplementation.

Significant increases in serum levels of total cholesterol and triglycerides, but no significant change in LDL- and HDL-cholesterol were observed in the anthocyanin group. There were no significant changes in the lipid profile of the normal control group. The findings of the Anthocyanin study differ from several previous studies which have reported anthocyanins to improve the blood lipid profile in middle-aged

dyslipidemic participants, possibly through reverse cholesterol transport [248, 249, 278]. A systematic review and meta-analysis reported anthocyanin supplementation to reduce serum total cholesterol, triglycerides, and LDL-cholesterol but to increase HDL-cholesterol compared with controls who were given placebo. Importantly, these findings were based on studies including middle aged participants with dyslipidemia [21]. There are several limitations to the published studies as the sample sizes and the study durations are rather short. Studies are from rather few countries and involve participants in the middle-age group, many having comorbidities and treatment affecting serum lipids as exclusion criteria [21, 23, 248, 249, 278-280]. Hence, the results are often applicable to certain populations, and can not necessarily be generalized to other populations and age groups. Even so, a British study of a somewhat older population (age 63 ± 7 years) with metabolic syndrome reported an increased HDL-cholesterol concentration after six months of treatment with anthocyanins from 3 types of intervention foods (equivalent to 1 and 1/2 US cup blueberries, and placebo), especially in statin non-users, but there were no changes in total cholesterol, LDL-cholesterol or triglycerides [198].

The discrepancies in the findings between the Anthocyanin study and other studies could have a number of explanations related to differences in participants, as well as to anthocyanin supplementation dose and study duration, or other factors. The nutraceutical Medox® used for the anthocyanin supply in the Anthocyanin study has been used in previous studies showing a statistically significant increase in HDL-cholesterol [23, 247-249, 278, 279] and a decrease in LDL-cholesterol [23, 248]. Importantly, while these mentioned studies included dyslipidemic and hypercholesterolemic participants were the use of any drugs known to affect lipid metabolism were exclusion criteria [23, 248], altogether 70% of the participants in the Anthocyanin study were taking statins or other lipid lowering medication.

The Anthocyanin study did not find any significant changes in serum levels of fasting glucose and HbA1c in the anthocyanin group. However, a statistically significant lower fasting glucose was found in the normal control group. In comparison, an Australian study were a total of 55 participants in two groups of normal healthy and

metabolic syndrome (age 25-75 years) were given a daily dose of 320 mg anthocyanin supplementation 4 weeks reported significant reduction in in the serum fasting glucose in the metabolic group [281]. Meta-analysis assessing glycemic regulation in both healthy population and those with cardiometabolic disease found that anthocyanins significantly reduced fasting glucose, as well as two hour postprandial glucose, and HbA1c [19]. Further, several meta-analyses of epidemiologic research suggest anthocyanins to have a dose-dependent role in diabetes mellitus type 2 risk reduction, but the exact mechanisms explaining this potential association are not clear [20, 282]. A proposed mechanism for diabetes mellitus type 2 risk reduction is that antocyanins decrease insulin resistance [20]. However, these studies have had small sample size and short duration, and not always found decreased insulin resistance [283-286]. A recent randomized controlled trial of six months duration did not find any change in insulin resistance after treatment with anthocyanins [198]. More prospective studies in different regions and ethnic groups are warranted.

In the Anthocyanin study IL-8, MCP-1, RANTES and TNF-α, were available for statistical analyses. These are pro-inflammatory mediators involved in systemic inflammation of both metabolic syndrome and atherosclerosis, and in neuroinflammation [287-290]. Of note, the presence of systemic inflammation with increased TNF-α production is associated with increased cognitive decline, glial cells activation, and neuroinflammation [287]. RANTES decreased, however not significantly in the anthocyanin supplementation group and increased not significantly in the normal control group, however the between-group analysis showed no significant differences. Another Norwegian study of healthy adults showed that 3-week intake of purified anthocyanins decreased plasma IL-8 and RANTES. That study did not however result in significant alterations in C reactive protein (CRP), TNF-α and IL-1β levels [291]. Further, in a randomized, double-blind clinical trial, 24 weeks of anthocyanin supplementation versus placebo consumption in 150 hypercholesterolaemic individuals found significantly reduced plasma levels of RANTES as compared with the placebo [292]. Another study found however no

effect on inflammatory biomarkers, including TNF-α and RANTES following 12 weeks of consumption of anthocyanin extract in healthy postmenopausal women [293].

Possibly the efficacy of anthocyanin on inflammatory responses depends on the studied populations, the duration of the intervention and also the dose and type of anthocyanin source and how it is prepared. A recent meta-analysis indicated that dietary anthocyanins significantly decreased levels of CRP, IL-6, TNF-α, intercellular adhesion molecule-1, and vascular adhesion molecule-1 (VCAM-1) in dose dependent manner [294]. Supplementation with anthocyanins may modulate the inflammation processes and further research of anthocyanin supplementation as a potential strategy in prevention and treatment of inflammatory diseases is warranted [295].

In the secondary analyses, significant improvements in the CERAD learning, recall and recognition scores and Stroop Golden test word and color were found. The findings are in line with previous smaller studies involving participants with MCI, reporting improved cognition after ingestion of anthocyanins [202, 203]. Further, randomized, double-blinded, placebo-controlled studies have reported improved episodic memory and cognition after 3 months of anthocyanin supplementation [205, 296]. Of note, although the results of a recent review provide preliminary evidence for the potential efficacy of anthocyanins as an intervention to promote cognitive performance and mood, there is only a limited number of trials, with heterogeneous age groups, large variation in cognitive tests between trials, relatively small sample sizes, and differing anthocyanin content in the interventions. Thus, further investigations are needed, including the use of a well-defined standardised anthocyanin supplement [297].

Importantly, variations in type and dose of anthocyanins, the source of anthocyanins, preparation, and also the storage of the food containing the anthocyanins will affect the amount of anthocyanin actually being administered. As the anthocyanin composition can vary depending on the food source or whether they are fresh, frozen or dried, well-defined means of anthocyanin supplement is of importance [298].

The results of the 29 anthocyanin metabolite analyses in plasma were conflicting as various anthocyanin metabolites were found to be increased or decreased in the anthocyanin group. These results are possibly explained by high inter-individual variability and the influence of the background diet, and are in line with a previous study [245]. Interestingly, previous studies have shown anthocyanin-derived metabolites to correlate with biological effects including improvements in vascular function [22, 196]. A recent study however observed that cognitive benefits were correlated with parent anthocyanin compounds suggesting the possibility that recurrent intake of anthocyanins, rather than metabolites, was related to the cognitive benefit [299].

Currently, the evidence for anthocyanin supplementation for cognitive improvement is inconclusive, mainly due to the insufficiencies and discrepancies in the study designs and also lack of biological and physical endpoints [18]. It has previously been recommended that future studies should utilize pure anthocyanins in well-designed long-term, properly controlled and blinded trials assessing physiologic endpoints before drawing conclusions regarding their specific biological function on cardiovascular risk factors and cognitive function [18]. Importantly, although some studies of shorter duration exist [22, 300-302], longer-duration trials on dose-response effects and possible risk of long-term ingestion are needed. Therefore, based on the experience from the Anthocyanin study, our research group has been conducting a phase II randomized controlled trial examining the effect of purified anthocyanins in people at risk of dementia over 6 months including a comprehensive biomarker collection [246].

5.3.2 Study sample and design

The sample of paper III was subjects participating in the Anthocyanin study. Participants were recruited from both the outpatient Memory and Cardiology clinics at the Stavanger University Hospital, of whom a majority were recruited from the cardiologic outpatient clinic. In line with the Norwegian health care system recommendations, most patients with MCI are being diagnosed and treated in the

primary health care system. Participants of the DDI study [210] in Stavanger with a diagnosis of MCI were also invited to participate.

The Anthocyanin study has several limitations including the non-randomized open-label design, the small sample size, and the relatively short intervention period. Except for age and sex, there is no knowledge about the normal control group.

At the time when the Anthocyanin study was designed, the planning of a larger randomized controlled trial also started, and the decision was made to implement a pilot study to gain experience concerning recruitment, feasibility and safety. This was also in accordance with the decision from the the regional committee for medical and health research ethics (REK)

The Anthocyanin study was a pilot study with the aim of generating hypotheses and facilitating design and power calculations for future randomized trials rather than aiming at providing firm conclusions. A more sophisticated and robust design is required to increase the understanding of the effects of anthocyanins that may possibly benefit the aging brain and mechanisms leading to dementia. Also, valuable feedback from participants can be used in further facilitating recruitment in larger studies.

Of note, a well characterised combination of nutraceuticals was used, the contents of which has been described in section 3.3.3. This facilitates comparison with other studies. This particular nutraceutical capsule has also been used in previous clinical studies, but this is the first time it is used in a study examining cognitive function.

A limitation of the study was the lack of registration of the diet or other lifestyle measures of the participants, and thus differences in the background diet and other lifestyle factors may have influenced the findings. However, participants were asked to maintain their lifestyle during the intervention period. Reported data are prone to bias, and biological measurements of anthocyanin metabolites, which were measured, are of preference.

Since the study period was relatively short, with 16 weeks between the two test points, the cognitive tests results may have been prone to learning effects.

5.3.3 MCI diagnosis

Participants in the MCI group had been diagnosed with MCI before inclusion. Those recruited by the DDI study had been diagnosed according to the original criteria in the DDI Study [210] as described in section 3.3.2, whereas those recruited by the Memory outpatient clinic had been diagnosed by a trained physicians following standardised clinical evaluation. We cannot fully exclude that this might have influenced the findings, but this is unlikely.

The distinction between MCI and mild dementia is not always clear. The intention was to include mild dementia in the pilot study, however no participant had mild dementia. Possibly this was because of the exclusion criterion of MMSE score < 24, and most patients with mild dementia probably had a MMSE score < 24.

5.3.4 Statistical considerations

The sample of paper III was small, involving only 27 participants in the anthocyanin group and 20 normal controls. Thus there is a clear risk of both type I errors, i.e. assuming there is a difference between the groups, but there really is not, and type II errors, i.e. assuming there is no difference between the groups when there really is a difference. Non-parametric analyses were chosen as the most suitable due to the small sample size and to avoid having to rely on assumptions of symmetrically distributed data. However, descriptive statistics including variance measurements were provided, which may be helpful in planning later studies, including facilitating design and power calculations. The presented data may also be eligible for inclusion in future meta-analyses. Of note, none of the analyses were adjusted for demographic differences.

6. Conclusions

The general aim of this thesis was to increase knowledge about the role of vascular risk factors, lipid alterations and anthocyanin supplementation with respect to development and progression of cognitive impairment in a population of people with mild dementia or at increased risk of dementia.

This thesis is based on three published papers, which together provide interesting findings about vascular risk factors and mechanisms underlying cognitive impairment and dementia, and concerning the potential to intervene in order to delay or prevent the progression of cognitive decline. The main findings and conclusions from the three papers are presented below.

6.1 Paper I

The main findings in paper I were that smoking was the only vascular risk factor significantly associated with a more rapid cognitive decline in AD patients, and that being overweight at the time of dementia diagnosis was associated with a slower progression of cognitive decline in AD and LBD patients.

6.2 Paper II

The main finding in paper II was that plasma sphingomyelins, and in particular SM(d43:2), were reduced in patients with MCI due to AD pathology compared to controls, and were also nominally reduced when compared to MCI patients without AD pathology. In addition, two phosphatidylinositols were found to be negatively associated with visuospatial functioning at baseline.

6.3 Paper III

In paper III the findings are somewhat inconclusive. The only significant between-group difference was with respect to the within-group difference of MCP-1 and fasting glucose (difference from baseline to study end). In the anthocyanin group, total cholesterol and triglycerides increased, but there were no significant changes in serum levels of fasting glucose, HbA1c or markers of inflammation. Some cognitive

improvements were observed in the anthocyanin group. Anthocyanin supplementation was well tolerated, without any recorded adverse events, and the compliance was good.

7. Implications and further research

The number of people with cognitive impairment and dementia is increasing both worldwide and in Norway leading to substantial impact on the individuals, their families and societies. AD and LBD are the most common neurodegenerative causes of dementia, with AD being the most common cause of dementia overall. As there are currently no available disease-modifying treatments, measures to reduce risk of developing cognitive impairment and dementia, as well as measures to decrease disease progression are crucial.

The main aim of this thesis was to increase the knowledge about the potential effects of vascular risk factors, lipid alterations and anthocyanin supplementation on development and progression of cognitive impairment. This might have some implications as will be discussed below, and suggestions for further research will be provided.

7.1 Vascular risk factors and dementia progression

Although the association of mid-life vascular risk factors with increased risk of late-life dementia has been well established, the association of late-life vascular risk factors and dementia progression is still not clear. Vascular risk factors are potentially manageable, and a delay in the progression of dementia could benefit even the oldest old. Fewer years of life being dependent on full-time care, or in nursing home would be beneficial also with respect to carer burden and health care costs. However, based on research findings to date, it is still questionable whether intensive treatment of vascular risk factors, considering potential side-effects of medication use, and potentially polypharmacy, would benefit the older patients diagnosed with dementia. Also, little knowledge exists regarding the effect of vascular risk factors on other causes of dementia than AD. Hence, further studies are needed to explore the associations of vascular risk factors with cognitive decline, and how this could be translated into benefit for people with dementia. Future studies should be done in

well-defined longitudinal cohort studies using standardised diagnostic tools, and biological biomarkers for different dementia subtypes.

7.2 Lipid alterations in Alzheimer's Disease

The exact cause and pathomechanism of AD is not known, and research has until recently been focused on protein- and gene-centric causes. However, lipid alterations have in several studies been associated with AD pathology, and present a role not only in AD pathomechanism but possibly also as a biomarker for disease and progression, and as a treatment target. Hence, additional knowledge and understanding of the possible contribution of lipids to AD pathology is of interest. Future studies should preferably link blood based lipidomics changes with neuropathology and integrate findings with known genomic and proteomic alterations of AD pathology.

It is not known whether the observed changes in lipid levels associated with AD pathology are causally related to, or are just a marker of changes, and therefore studies that address potential causality are essential. Future analyses should use larger sample sizes to replicate already existing findings. Additionally, longer follow-up time might give a better picture of how lipids might affect changes in cognitive outcomes. Analysis consistency across laboratories is important to establish in order to be able to replicate findings.

Of importance, proteins and genes are known to be affected in AD and can function complementarily with lipids, f.ex. in lipid rafts. Hence, lipidomics experiments should not detract from investigations of proteomics and genomics. Lipidomics presents a novel and unique platform to analyse AD processes and biomarkers, but changes in lipids should preferably be evaluated in the context of gene and protein changes in order to give a more holistic picture of AD processes.

7.3 Effect of anthocyanin supplementation on dementia relevant mechanisms

At best, the effect of anthocyanin supplementation on dementia relevant mechanisms and cognitive function is encouraging. In view of the public health burden associated with neurodegeneration, in particular AD, safe, low-cost dietary interventions offer the possibility of inducing substantial benefit.

Findings from observational, preclinical, and smaller clinical studies need to be substantiated by both well designed and well defined randomized controlled trials. Importantly, studies will require the use of well defined, and hence comparable and repeatable sources of anthocyanins.

Future studies should assess cognitive function in a standardised manner, using the latest guidelines for diagnosis and should also include standardised acquisition of biomarkers, both structural and functional neuroimaging, as well as analyses of biofluids such as CSF. The intervention period needs to be long, preferably 12-24 months [18]. Genetics should be employed in order to explore whether or not there are differential responses to dietary anthocyanin supplementation in specific *APOE* genotype carriers.

In addition to further research on the potential effects of anthocyanins in terms of counteracting cardiovascular and metabolic risk factors, inflammation, oxidation and associated neurodegenerative disease, studies on intervention doses will be of importance. Although some have already been published, further confirmatory studies will be needed.

8. References

1. Bergland, A.K., et al., *Effect of vascular risk factors on the progression of mild Alzheimer's disease and Lewy body dementia.* J Alzheimers Dis, 2017. **56**(2): p. 575-584.
2. Bergland, A.K., et al., *Exploration of Plasma Lipids in Mild Cognitive Impairment due to Alzheimer's Disease.* J Alzheimers Dis, 2020. **77**(3): p. 1117-1127.
3. Bergland, A.K., et al., *Effects of Anthocyanin Supplementation on Serum Lipids, Glucose, Markers of Inflammation and Cognition in Adults With Increased Risk of Dementia - A Pilot Study.* Front Genet, 2019. **10**: p. 536.
4. Livingston, G., et al., *Dementia prevention, intervention, and care.* Lancet, 2017. **390**(10113): p. 2673-2734.
5. Svendsboe, E.J., et al., *Patterns of carer distress over time in mild dementia.* Int J Geriatr Psychiatry, 2018. **33**(7): p. 987-993.
6. Vossius, C., et al., *The use and costs of formal care in newly diagnosed dementia: a three-year prospective follow-up study.* Am J Geriatr Psychiatry, 2014. **22**(4): p. 381-8.
7. Alzheimer's Disease International, *World Alzheimer Report 2018 - The state of the art of dementia research: New frontiers*. 2018: London, UK.
8. Gjøra, L., et al., *Current and future prevalence estimates of mild cognitive impairment, dementia, and its subtypes in a population-based sample of people 70 years and older in Norway: The HUNT Study* J Alzheimers Dis, In press.
9. Livingston, G., et al., *Dementia prevention, intervention, and care: 2020 report of the Lancet Commission.* Lancet, 2020. **396**(10248): p. 413-446.
10. Wu, Y.T., et al., *The changing prevalence and incidence of dementia over time - current evidence.* Nat Rev Neurol, 2017. **13**(6): p. 327-339.
11. Rosenberg, A., et al., *Multidomain Interventions to Prevent Cognitive Impairment, Alzheimer's Disease, and Dementia: From FINGER to World-Wide FINGERS.* J Prev Alzheimers Dis, 2020. **7**(1): p. 29-36.
12. Yu, J.T., et al., *Evidence-based prevention of Alzheimer's disease: systematic review and meta-analysis of 243 observational prospective studies and 153 randomised controlled trials.* J Neurol Neurosurg Psychiatry, 2020.
13. Tom, S.E., et al., *Characterization of dementia and Alzheimer's disease in an older population: updated incidence and life expectancy with and without dementia.* Am J Public Health, 2015. **105**(2): p. 408-413.
14. Scarmeas, N., C.A. Anastasiou, and M. Yannakoulia, *Nutrition and prevention of cognitive impairment.* Lancet Neurol, 2018. **17**(11): p. 1006-1015.
15. Roman, G.C., et al., *Mediterranean diet: The role of long-chain omega-3 fatty acids in fish; polyphenols in fruits, vegetables, cereals, coffee, tea, cacao and wine; probiotics and vitamins in prevention of stroke, age-related cognitive decline, and Alzheimer disease.* Rev Neurol (Paris), 2019. **175**(10): p. 724-741.

16. Petersson, S.D. and E. Philippou, *Mediterranean Diet, Cognitive Function, and Dementia: A Systematic Review of the Evidence.* Adv Nutr, 2016. **7**(5): p. 889-904.
17. Cooper, C., et al., *Modifiable predictors of dementia in mild cognitive impairment: a systematic review and meta-analysis.* Am J Psychiatry, 2015. **172**(4): p. 323-34.
18. Del Rio, D., et al., *Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases.* Antioxid Redox Signal, 2013. **18**(14): p. 1818-92.
19. Yang, L., et al., *Effects of Anthocyanins on Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials.* Adv Nutr, 2017. **8**(5): p. 684-693.
20. Burton-Freeman, B., et al., *A Selective Role of Dietary Anthocyanins and Flavan-3-ols in Reducing the Risk of Type 2 Diabetes Mellitus: A Review of Recent Evidence.* Nutrients, 2019. **11**(4).
21. Liu, C., et al., *Effects of Anthocyanin on Serum Lipids in Dyslipidemia Patients: A Systematic Review and Meta-Analysis.* PLoS One, 2016. **11**(9): p. e0162089.
22. Rodriguez-Mateos, A., et al., *Circulating Anthocyanin Metabolites Mediate Vascular Benefits of Blueberries: Insights From Randomized Controlled Trials, Metabolomics, and Nutrigenomics.* J Gerontol A Biol Sci Med Sci, 2019. **74**(7): p. 967-976.
23. Zhu, Y., et al., *Purified anthocyanin supplementation improves endothelial function via NO-cGMP activation in hypercholesterolemic individuals.* Clin Chem, 2011. **57**(11): p. 1524-33.
24. Butterfield, D.A. and B. Halliwell, *Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease.* Nat Rev Neurosci, 2019. **20**(3): p. 148-160.
25. Kivipelto, M., et al., *Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study.* BMJ, 2001. **322**(7300): p. 1447-51.
26. Kivipelto, M., et al., *Midlife vascular risk factors and late-life mild cognitive impairment: A population-based study.* Neurology, 2001. **56**(12): p. 1683-9.
27. Kivipelto, M., et al., *Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease.* Arch Neurol, 2005. **62**(10): p. 1556-60.
28. Walker, K.A., et al., *Association of Midlife to Late-Life Blood Pressure Patterns With Incident Dementia.* Jama, 2019. **322**(6): p. 535-545.
29. Solomon, A., et al., *Midlife serum cholesterol and increased risk of Alzheimer's and vascular dementia three decades later.* Dement Geriatr Cogn Disord, 2009. **28**(1): p. 75-80.
30. Blom, K., M.H. Emmelot-Vonk, and H.L. Koek, *The influence of vascular risk factors on cognitive decline in patients with dementia: a systematic review.* Maturitas, 2013. **76**(2): p. 113-7.
31. Reed, B., et al., *Associations between serum cholesterol levels and cerebral amyloidosis.* JAMA Neurol, 2014. **71**(2): p. 195-200.

32. Wong, M.W., et al., *Dysregulation of lipids in Alzheimer's disease and their role as potential biomarkers.* Alzheimers Dement, 2017. **13**(7): p. 810-827.
33. Ricciarelli, R., et al., *Cholesterol and Alzheimer's disease: a still poorly understood correlation.* IUBMB Life, 2012. **64**(12): p. 931-5.
34. Kao, Y.C., et al., *Lipids and Alzheimer's Disease.* Int J Mol Sci, 2020. **21**(4).
35. World Health Organization, *The ICD-10 classification of mental and behavioural disorders: clinical descriptions and diagnostic guidelines*. 1992: Geneva: World Health Organization.
36. World Health Organisation. *International Classification of Diseases 11th Revision (ICD-11)*. 2018 [cited 2020 06.10]; Available from: https://www.who.int/classifications/icd/revision/en/.
37. American Psychiatric Association, *Diagnostic and statistical manual of mental disorders (DSM-5®)*. 2013: American Psychiatric Pub.
38. Aarsland, D., C. Ballard, and G. Halliday, *Are Parkinson's disease with dementia and dementia with Lewy bodies the same entity?* Journal of geriatric psychiatry and neurology, 2004. **17**(3): p. 137-145.
39. Nelson, P.T., et al., *Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report.* Brain, 2019. **142**(6): p. 1503-1527.
40. Ballard, C., et al., *Alzheimer's disease.* The Lancet, 2011. **377**(9770): p. 1019-1031.
41. Jellinger, K.A., *Dementia with Lewy bodies and Parkinson's disease-dementia: current concepts and controversies.* J Neural Transm (Vienna), 2018. **125**(4): p. 615-650.
42. Gan, L., et al., *Converging pathways in neurodegeneration, from genetics to mechanisms.* Nat Neurosci, 2018. **21**(10): p. 1300-1309.
43. Dubois, B., et al., *Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria.* Alzheimers Dement, 2016. **12**(3): p. 292-323.
44. McKeith, I.G., et al., *Research criteria for the diagnosis of prodromal dementia with Lewy bodies.* Neurology, 2020.
45. Winblad, B., et al., *Mild cognitive impairment–beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment.* Journal of internal medicine, 2004. **256**(3): p. 240-246.
46. Alafuzoff, I., et al., *Staging/typing of Lewy body related alpha-synuclein pathology: a study of the BrainNet Europe Consortium.* Acta Neuropathol, 2009. **117**(6): p. 635-52.
47. Hyman, B.T., et al., *National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease.* Alzheimers Dement, 2012. **8**(1): p. 1-13.
48. Lantero Rodriguez, J., et al., *Plasma p-tau181 accurately predicts Alzheimer's disease pathology at least 8 years prior to post-mortem and improves the clinical characterisation of cognitive decline.* Acta Neuropathol, 2020. **140**(3): p. 267-278.
49. Ashton, N.J., et al., *An update on blood-based biomarkers for non-Alzheimer neurodegenerative disorders.* Nat Rev Neurol, 2020. **16**(5): p. 265-284.
50. Prince, M., et al., *The global prevalence of dementia: a systematic review and metaanalysis.* Alzheimers Dement, 2013. **9**(1): p. 63-75 e2.

51. Niu, H., et al., *Prevalence and incidence of Alzheimer's disease in Europe: A meta-analysis.* Neurologia, 2017. **32**(8): p. 523-532.
52. Penke, B., F. Bogar, and L. Fulop, *beta-Amyloid and the Pathomechanisms of Alzheimer's Disease: A Comprehensive View.* Molecules, 2017. **22**(10).
53. Braak, H. and E. Braak, *Neuropathological stageing of Alzheimer-related changes.* Acta Neuropathol, 1991. **82**(4): p. 239-59.
54. Newcombe, E.A., et al., *Inflammation: the link between comorbidities, genetics, and Alzheimer's disease.* J Neuroinflammation, 2018. **15**(1): p. 276.
55. Scheltens, P., et al., *Alzheimer's disease.* Lancet, 2016. **388**(10043): p. 505-17.
56. Dubois, B., et al., *Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria.* Lancet Neurol, 2014. **13**(6): p. 614-29.
57. Jessen, F., et al., *A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease.* Alzheimers Dement, 2014. **10**(6): p. 844-52.
58. Albert, M.S., et al., *The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Alzheimers Dement., 2011. **7**(3): p. 270-279.
59. Zhao, Q.F., et al., *The prevalence of neuropsychiatric symptoms in Alzheimer's disease: Systematic review and meta-analysis.* J Affect Disord, 2016. **190**: p. 264-271.
60. Vik-Mo, A.O., et al., *Course of neuropsychiatric symptoms in dementia: 5-year longitudinal study.* Int J Geriatr Psychiatry, 2018. **33**(10): p. 1361-1369.
61. McKhann, G., et al., *Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease.* Neurology, 1984. **34**(7): p. 939-44.
62. McKhann, G.M., et al., *The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Alzheimer's & Dementia, 2011. **7**(3): p. 263-269.
63. Jack, C.R., Jr., et al., *NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease.* Alzheimers Dement, 2018. **14**(4): p. 535-562.
64. Doody, R.S., et al., *Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease.* N Engl J Med, 2014. **370**(4): p. 311-21.
65. Budd Haeberlein, S., et al., *Clinical Development of Aducanumab, an Anti-Aβ Human Monoclonal Antibody Being Investigated for the Treatment of Early Alzheimer's Disease.* J Prev Alzheimers Dis, 2017. **4**(4): p. 255-263.
66. Schneider, L., *A resurrection of aducanumab for Alzheimer's disease.* Lancet Neurol, 2020. **19**(2): p. 111-112.
67. Atri, A., *Current and Future Treatments in Alzheimer's Disease.* Semin Neurol, 2019. **39**(2): p. 227-240.
68. Walker, Z., et al., *Lewy Body Dementias.* Focus (Am Psychiatr Publ), 2017. **15**(1): p. 85-100.

69. Vann Jones, S.A. and J.T. O'Brien, *The prevalence and incidence of dementia with Lewy bodies: a systematic review of population and clinical studies.* Psychol Med, 2014. **44**(4): p. 673-83.
70. Hogan, D.B., et al., *The Prevalence and Incidence of Dementia with Lewy Bodies: a Systematic Review.* Can J Neurol Sci, 2016. **43 Suppl 1**: p. S83-95.
71. Kane, J.P.M., et al., *Clinical prevalence of Lewy body dementia.* Alzheimers Res Ther, 2018. **10**(1): p. 19.
72. Aarsland, D., J. Zaccai, and C. Brayne, *A systematic review of prevalence studies of dementia in Parkinson's disease.* Mov Disord, 2005. **20**(10): p. 1255-63.
73. Parkkinen, L., T. Pirttilä, and I.J.A.n. Alafuzoff, *Applicability of current staging/categorization of α-synuclein pathology and their clinical relevance.* Acta Neuropathologica, 2008. **115**(4): p. 399-407.
74. Irwin, D.J. and H.I. Hurtig, *The Contribution of Tau, Amyloid-Beta and Alpha-Synuclein Pathology to Dementia in Lewy Body Disorders.* J Alzheimers Dis Parkinsonism, 2018. **8**(4).
75. Braak, H., et al., *Staging of brain pathology related to sporadic Parkinson's disease.* Neurobiol Aging, 2003. **24**(2): p. 197-211.
76. McKeith, I.G., et al., *Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop.* Neurology, 1996. **47**(5): p. 1113-24.
77. McKeith, I.G., et al., *Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium.* Neurology, 2005. **65**(12): p. 1863-72.
78. McKeith, I.G., et al., *Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium.* Neurology, 2017. **89**(1): p. 88-100.
79. Emre, M., et al., *Clinical diagnostic criteria for dementia associated with Parkinson's disease.* Mov Disord, 2007. **22**(12): p. 1689-707; quiz 1837.
80. Aarsland, D., et al., *Cognitive decline in Parkinson disease.* Nat Rev Neurol, 2017. **13**(4): p. 217-231.
81. Litvan, I., et al., *Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines.* Mov Disord, 2012. **27**(3): p. 349-56.
82. Aarsland, D., et al., *Neuropsychiatric symptoms in patients with Parkinson's disease and dementia: frequency, profile and associated care giver stress.* J Neurol Neurosurg Psychiatry, 2007. **78**(1): p. 36-42.
83. Taylor, J.P., et al., *New evidence on the management of Lewy body dementia.* Lancet Neurol, 2020. **19**(2): p. 157-169.
84. Murata, M., et al., *Adjunct zonisamide to levodopa for DLB parkinsonism: A randomized double-blind phase 2 study.* Neurology, 2018. **90**(8): p. e664-e672.
85. Cuyvers, E. and K. Sleegers, *Genetic variations underlying Alzheimer's disease: evidence from genome-wide association studies and beyond.* Lancet Neurol, 2016. **15**(8): p. 857-868.

86. Campion, D., et al., *Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum.* Am J Hum Genet, 1999. **65**(3): p. 664-70.
87. Jansen, I.E., et al., *Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk.* Nat Genet, 2019. **51**(3): p. 404-413.
88. Martinez-Martinez, A.B., et al., *Beyond the CNS: The many peripheral roles of APOE.* Neurobiol Dis, 2020. **138**: p. 104809.
89. Corder, E.H., et al., *Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families.* Science, 1993. **261**(5123): p. 921-3.
90. Liu, C.C., et al., *Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy.* Nat Rev Neurol, 2013. **9**(2): p. 106-18.
91. Verghese, P.B., J.M. Castellano, and D.M. Holtzman, *Apolipoprotein E in Alzheimer's disease and other neurological disorders.* Lancet Neurol, 2011. **10**(3): p. 241-52.
92. Rongve, A., et al., *GBA and APOE ε4 associate with sporadic dementia with Lewy bodies in European genome wide association study.* Sci Rep, 2019. **9**(1): p. 7013.
93. Guerreiro, R., et al., *Investigating the genetic architecture of dementia with Lewy bodies: a two-stage genome-wide association study.* Lancet Neurol, 2018. **17**(1): p. 64-74.
94. Crous-Bou, M., et al., *Alzheimer's disease prevention: from risk factors to early intervention.* Alzheimers Res Ther, 2017. **9**(1): p. 71.
95. Green, H., et al., *Novel Treatment Opportunities Against Cognitive Impairment in Parkinson's Disease with an Emphasis on Diabetes-Related Pathways.* J CNS drugs, 2019. **33**(2): p. 143-160.
96. Gottesman, R.F., et al., *Midlife hypertension and 20-year cognitive change: the atherosclerosis risk in communities neurocognitive study.* JAMA Neurol, 2014. **71**(10): p. 1218-27.
97. Kivipelto, M., et al., *Apolipoprotein E epsilon4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease.* Ann Intern Med, 2002. **137**(3): p. 149-55.
98. McGrath, E.R., et al., *Blood pressure from mid- to late life and risk of incident dementia.* Neurology, 2017. **89**(24): p. 2447-2454.
99. Iadecola, C. and R.F. Gottesman, *Neurovascular and Cognitive Dysfunction in Hypertension Epidemiology, Pathobiology, and Treatment.* Circulation Research, 2019. **124**(7): p. 1025-1044.
100. Joutel, A. and H. Chabriat, *Pathogenesis of white matter changes in cerebral small vessel diseases: beyond vessel-intrinsic mechanisms.* Clin Sci (Lond), 2017. **131**(8): p. 635-651.
101. Graff-Radford, J., et al., *White matter hyperintensities: relationship to amyloid and tau burden.* Brain, 2019. **142**(8): p. 2483-2491.
102. Ding, J., et al., *Antihypertensive medications and risk for incident dementia and Alzheimer's disease: a meta-analysis of individual participant data from prospective cohort studies.* Lancet Neurol, 2020. **19**(1): p. 61-70.

103. Zhang, J., et al., *An updated meta-analysis of cohort studies: Diabetes and risk of Alzheimer's disease.* Diabetes Res Clin Pract, 2017. **124**: p. 41-47.
104. Chatterjee, S., et al., *Type 2 Diabetes as a Risk Factor for Dementia in Women Compared With Men: A Pooled Analysis of 2.3 Million People Comprising More Than 100,000 Cases of Dementia.* Diabetes Care, 2016. **39**(2): p. 300-7.
105. De Sousa, R.A.L., et al., *An update on potential links between type 2 diabetes mellitus and Alzheimer's disease.* Mol Biol Rep, 2020. **47**(8): p. 6347-6356.
106. Pantoni, L., *Potential New Horizons for the Prevention of Cerebrovascular Diseases and Dementia.* JAMA Neurol, 2019. **76**(5): p. 521-522.
107. Albanese, E., et al., *Body mass index in midlife and dementia: Systematic review and meta-regression analysis of 589,649 men and women followed in longitudinal studies.* Alzheimers Dement (Amst), 2017. **8**: p. 165-178.
108. Fitzpatrick, A.L., et al., *Midlife and late-life obesity and the risk of dementia: cardiovascular health study.* Arch Neurol, 2009. **66**(3): p. 336-42.
109. Gustafson, D.R., et al., *Adiposity indicators and dementia over 32 years in Sweden.* Neurology, 2009. **73**(19): p. 1559-66.
110. Alford, S., et al., *Obesity as a risk factor for Alzheimer's disease: weighing the evidence.* Obes Rev, 2018. **19**(2): p. 269-280.
111. Beverly, J.K. and M.J. Budoff, *Atherosclerosis: Pathophysiology of insulin resistance, hyperglycemia, hyperlipidemia, and inflammation.* J Diabetes, 2020. **12**(2): p. 102-104.
112. Nelson, R.H., *Hyperlipidemia as a risk factor for cardiovascular disease.* Prim Care, 2013. **40**(1): p. 195-211.
113. Whitmer, R.A., et al., *Midlife cardiovascular risk factors and risk of dementia in late life.* Neurology, 2005. **64**(2): p. 277-81.
114. Mielke, M.M., et al., *High total cholesterol levels in late life associated with a reduced risk of dementia.* Neurology, 2005. **64**(10): p. 1689-95.
115. Solomon, A., et al., *Serum cholesterol changes after midlife and late-life cognition: twenty-one-year follow-up study.* Neurology, 2007. **68**(10): p. 751-6.
116. Corraliza-Gomez, M., D. Sanchez, and M.D. Ganfornina, *Lipid-Binding Proteins in Brain Health and Disease.* Front Neurol, 2019. **10**: p. 1152.
117. Shevchenko, A. and K. Simons, *Lipidomics: coming to grips with lipid diversity.* Nat Rev Mol Cell Biol, 2010. **11**(8): p. 593-8.
118. Fahy, E., et al., *Update of the LIPID MAPS comprehensive classification system for lipids.* J Lipid Res, 2009. **50 Suppl**(Supplement): p. S9-14.
119. Wong, M.W.K., et al., *APOE Genotype Differentially Modulates Plasma Lipids in Healthy Older Individuals, with Relevance to Brain Health.* J Alzheimers Dis, 2019. **72**(3): p. 703-716.
120. Brugger, B., *Lipidomics: analysis of the lipid composition of cells and subcellular organelles by electrospray ionization mass spectrometry.* Annu Rev Biochem, 2014. **83**: p. 79-98.
121. Wenk, M.R., *The emerging field of lipidomics.* Nat Rev Drug Discov, 2005. **4**(7): p. 594-610.

122. Kosicek, M. and S. Hecimovic, *Phospholipids and Alzheimer's disease: alterations, mechanisms and potential biomarkers.* Int J Mol Sci, 2013. **14**(1): p. 1310-22.
123. Gamba, P., et al., *Oxidized cholesterol as the driving force behind the development of Alzheimer's disease.* Front Aging Neurosci, 2015. **7**: p. 119.
124. Mesa-Herrera, F., et al., *Lipid and Lipid Raft Alteration in Aging and Neurodegenerative Diseases: A Window for the Development of New Biomarkers.* Int J Mol Sci, 2019. **20**(15): p. 3810.
125. Xiang, Y., S.M. Lam, and G.J.B.c. Shui, *What can lipidomics tell us about the pathogenesis of Alzheimer disease?* 2015. **396**(12): p. 1281-1291.
126. Alessenko, A.V. and E. Albi, *Exploring Sphingolipid Implications in Neurodegeneration.* Front Neurol, 2020. **11**: p. 437.
127. Blasco, H., et al., *Lipidomics Reveals Cerebrospinal-Fluid Signatures of ALS.* Sci Rep, 2017. **7**(1): p. 17652.
128. Varma, V.R., et al., *Brain and blood metabolite signatures of pathology and progression in Alzheimer disease: A targeted metabolomics study.* PLoS Med, 2018. **15**(1): p. e1002482.
129. Jazvinšćak Jembrek, M., P.R. Hof, and G. Šimić, *Ceramides in Alzheimer's Disease: Key Mediators of Neuronal Apoptosis Induced by Oxidative Stress and Aβ Accumulation.* Oxid Med Cell Longev, 2015. **2015**: p. 346783.
130. Crivelli, S.M., et al., *Sphingolipids in Alzheimer's disease, how can we target them?* Adv Drug Deliv Rev, 2020.
131. Kosicek, M., et al., *Elevated cerebrospinal fluid sphingomyelin levels in prodromal Alzheimer's disease.* Neurosci Lett, 2012. **516**(2): p. 302-5.
132. Koal, T., et al., *Sphingomyelin SM(d18:1/18:0) is significantly enhanced in cerebrospinal fluid samples dichotomized by pathological amyloid-β42, tau, and phospho-tau-181 levels.* J Alzheimers Dis, 2015. **44**(4): p. 1193-201.
133. Li, D., et al., *Associations between cerebrospinal fluid total phosphatidylcholines, neurodegeneration, cognitive decline, and risk of mild cognitive impairment in the Mayo Clinic Study of Aging.* Neurobiol Aging, 2020. **93**: p. 52-54.
134. Klavins, K., et al., *The ratio of phosphatidylcholines to lysophosphatidylcholines in plasma differentiates healthy controls from patients with Alzheimer's disease and mild cognitive impairment.* Alzheimers Dement (Amst), 2015. **1**(3): p. 295-302.
135. Toledo, J.B., et al., *Metabolic network failures in Alzheimer's disease: A biochemical road map.* Alzheimers Dement, 2017. **13**(9): p. 965-984.
136. Mielke, M.M. and N.J. Haughey, *Could plasma sphingolipids be diagnostic or prognostic biomarkers for Alzheimer's disease?* Clin Lipidol, 2012. **7**(5): p. 525-536.
137. Mielke, M.M., et al., *Plasma sphingomyelins are associated with cognitive progression in Alzheimer's disease.* J Alzheimers Dis, 2011. **27**(2): p. 259-69.
138. Thambisetty, M. and S.J.B.i.m. Lovestone, *Blood-based biomarkers of Alzheimer's disease: challenging but feasible.* 2010. **4**(1): p. 65-79.

139. Zuliani, G., et al., *Clinical and demographic parameters predict the progression from mild cognitive impairment to dementia in elderly patients.* Aging Clin Exp Res, 2020.
140. Jongsiriyanyong, S. and P. Limpawattana, *Mild Cognitive Impairment in Clinical Practice: A Review Article.* American Journal of Alzheimer's Disease & Other Dementiasr, 2018. **33**(8): p. 500-507.
141. Rongve, A., et al., *Cognitive decline in dementia with Lewy bodies: a 5-year prospective cohort study.* BMJ Open, 2016. **6**(2): p. e010357.
142. Ferman, T.J., et al., *Nonamnestic mild cognitive impairment progresses to dementia with Lewy bodies.* Neurology, 2013. **81**(23): p. 2032-8.
143. Chen, P.-H., et al., *Risk Factors for the Progression of Mild Cognitive Impairment in Different Types of Neurodegenerative Disorders.* Behavioural Neurology, 2018. **2018**: p. 6929732.
144. Eldholm, R.S., et al., *Progression of Alzheimer's Disease: A Longitudinal Study in Norwegian Memory Clinics.* J Alzheimers Dis, 2018. **61**(3): p. 1221-1232.
145. Eldholm, R.S., *Alzheimer's disease - The impact of vascular comorbidity on disease progression and clinical profile*. [doctoral thesis] 2019, Norwegian University of Science and Technology. ISBN 978-82-326-3756-0.
146. Vijayaraghavan, S., et al., *Association of Butyrylcholinesterase-K Allele and Apolipoprotein E ε4 Allele with Cognitive Decline in Dementia with Lewy Bodies and Alzheimer's Disease.* Journal of Alzheimer's Disease, 2016(Preprint): p. 1-10.
147. Melis, R.J.F., M.L. Haaksma, and G. Muniz-Terrera, *Understanding and predicting the longitudinal course of dementia.* Curr Opin Psychiatry, 2019. **32**(2): p. 123-129.
148. Giil, L.M. and D. Aarsland, *Greater Variability in Cognitive Decline in Lewy Body Dementia Compared to Alzheimer's Disease.* J Alzheimers Dis, 2020. **73**(4): p. 1321-1330.
149. Mueller, C., et al., *The prognosis of dementia with Lewy bodies.* Lancet Neurol, 2017. **16**(5): p. 390-398.
150. Oesterhus, R., et al., *Risk of Hospitalization in Patients with Alzheimer's Disease and Lewy Body Dementia: Time to and Length of Stay.* J Alzheimers Dis, 2020. **74**(4): p. 1221-1230.
151. Oesterhus, R., et al., *Long-term mortality in a cohort of home-dwelling elderly with mild Alzheimer's disease and Lewy body dementia.* Dement Geriatr Cogn Disord, 2014. **38**(3-4): p. 161-9.
152. Rongve, A., et al., *Time until nursing home admission in people with mild dementia: comparison of dementia with Lewy bodies and Alzheimer's dementia.* Int J Geriatr Psychiatry, 2014. **29**(4): p. 392-8.
153. Chaves, M.L., et al., *Predictors of the progression of dementia severity in brazilian patients with Alzheimer's disease and vascular dementia.* Int J Alzheimers Dis, 2010. **2010**.
154. Dugger, B.N., et al., *A Cross-Sectional Analysis of Late-Life Cardiovascular Factors and Their Relation to Clinically Defined Neurodegenerative Diseases.* Alzheimer Disease & Associated Disorders, 2016. **30**(3): p. 223-229.

155. Magierski, R., I. Kloszewska, and T.M. Sobow, *The influence of vascular risk factors on the survival rate of patients with dementia with Lewy bodies and Alzheimer disease.* Neurol Neurochir Pol, 2010. **44**(2): p. 139-47.
156. Dumont, C., et al., *[Rapid loss of the Mini Mental State Examination: REAL.FR study].* Rev Med Interne, 2003. **24 Suppl 3**: p. 345s-350s.
157. Blom, K., et al., *The influence of vascular risk factors on cognitive decline in patients with Alzheimer's disease.* Maturitas, 2014. **79**(1): p. 96-9.
158. Li, J., et al., *Vascular risk aggravates the progression of Alzheimer's disease in a Chinese cohort.* J Alzheimers Dis, 2010. **20**(2): p. 491-500.
159. Abellan van Kan, G., et al., *Cardiovascular disease risk factors and progression of Alzheimer's disease.* Dement Geriatr Cogn Disord, 2009. **27**(3): p. 240-6.
160. Sona, A., et al., *Predictors of rapid cognitive decline in Alzheimer's disease: results from the Australian imaging, biomarkers and lifestyle (AIBL) study of ageing.* Int Psychogeriatr, 2012. **24**(2): p. 197-204.
161. van Bruchem-Visser, R.L., F.U. Mattace-Raso, and T.J. Van Der Cammen, *High systolic and pulse pressure levels are associated with better cognitive performance in patients with probable Alzheimer's disease: A cross-sectional observational study in a geriatric outpatient population.* Dementia and geriatric cognitive disorders, 2009. **28**(4): p. 320-324.
162. Helzner, E.P., et al., *Contribution of vascular risk factors to the progression in Alzheimer disease.* Arch Neurol, 2009. **66**(3): p. 343-8.
163. Bhargava, D., et al., *Vascular disease and risk factors, rate of progression, and survival in Alzheimer's disease.* Journal of geriatric psychiatry and neurology, 2006. **19**(2): p. 78-82.
164. Regan, C., et al., *Relationship of vascular risk to the progression of Alzheimer disease.* Neurology, 2006. **67**(8): p. 1357-62.
165. Qiao, J., et al., *Vascular risk factors aggravate the progression of Alzheimer's disease: a 3-year follow-up study of Chinese population.* 2014. **29**(6): p. 521-525.
166. Sakurai, H., et al., *Vascular risk factors and progression in Alzheimer's disease.* Geriatr Gerontol Int, 2011. **11**(2): p. 211-4.
167. Ciobica, A., et al., *Cardiovascular risk factors as potential markers for mild cognitive impairment and Alzheimer's disease.* Psychiatr Danub, 2011. **23**(4): p. 340-6.
168. Razay, G., et al., *Blood pressure, dementia and Alzheimer's disease: the OPTIMA longitudinal study.* Dement Geriatr Cogn Disord, 2009. **28**(1): p. 70-4.
169. Mielke, M.M., et al., *Vascular factors predict rate of progression in Alzheimer disease.* Neurology, 2007. **69**(19): p. 1850-8.
170. Bellew, K.M., et al., *Hypertension and the rate of cognitive decline in patients with dementia of the Alzheimer type.* Alzheimer Dis Assoc Disord, 2004. **18**(4): p. 208-13.
171. Musicco, M., et al., *Predictors of progression of cognitive decline in Alzheimer's disease: the role of vascular and sociodemographic factors.* J Journal of neurology, 2009. **256**(8): p. 1288.

172. Evans, R.M., et al., *Cholesterol and APOE genotype interact to influence Alzheimer disease progression.* Neurology, 2004. 62(10): p. 1869-71.
173. Li, L., et al., *Cognitive performance and plasma levels of homocysteine, vitamin B12, folate and lipids in patients with Alzheimer disease.* Dement Geriatr Cogn Disord, 2008. **26**(4): p. 384-90.
174. Roselli, F., et al., *Rate of MMSE score change in Alzheimer's disease: influence of education and vascular risk factors.* Clin Neurol Neurosurg, 2009. **111**(4): p. 327-30.
175. Domínguez, R.O., et al., *Type 2 diabetes and/or its treatment leads to less cognitive impairment in Alzheimer's disease patients.* Diabetes Res Clin Pract, 2012. **98**(1): p. 68-74.
176. Ravona-Springer, R., et al., *Diabetes is associated with increased rate of cognitive decline in questionably demented elderly.* Dement Geriatr Cogn Disord, 2010. **29**(1): p. 68-74.
177. Sanz, C., et al., *Diabetes is associated with a slower rate of cognitive decline in Alzheimer disease.* Neurology, 2009. **73**(17): p. 1359-1366.
178. Viticchi, G., et al., *Framingham Risk Score Can Predict Cognitive Decline Progression in Alzheimer's Disease.* Neurobiology of aging, 2015.
179. Kume, K., et al., *Vascular risk factors are associated with faster decline of Alzheimer disease: a longitudinal SPECT study.* J Neurol, 2011. **258**(7): p. 1295-303.
180. Khoo, H.E., et al., *Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits.* Food Nutr Res, 2017. **61**(1): p. 1361779.
181. Neveu, V., et al., *Phenol-Explorer: an online comprehensive database on polyphenol contents in foods.* Database (Oxford), 2010. **2010**: p. bap024.
182. Reis, J.F., et al., *Action mechanism and cardiovascular effect of anthocyanins: a systematic review of animal and human studies.* J Transl Med, 2016. **14**(1): p. 315.
183. Speer, H., et al., *Anthocyanins and Human Health-A Focus on Oxidative Stress, Inflammation and Disease.* Antioxidants (Basel), 2020. **9**(5).
184. Tarozzi, A., et al., *Neuroprotective effects of anthocyanins and their in vivo metabolites in SH-SY5Y cells.* Neurosci Lett, 2007. **424**(1): p. 36-40.
185. Parrado-Fernandez, C., et al., *Anthocyanins protect from complex I inhibition and APPswe mutation through modulation of the mitochondrial fission/fusion pathways.* Biochim Biophys Acta, 2016. **1862**(11): p. 2110-2118.
186. Faria, A., et al., *Flavonoid metabolites transport across a human BBB model.* Food Chem, 2014. **149**: p. 190-6.
187. Liu, F., et al., *Cyanidin-3-O-glucoside inhibits Aβ40 fibrillogenesis, disintegrates preformed fibrils, and reduces amyloid cytotoxicity.* Food Funct, 2020. **11**(3): p. 2573-2587.
188. Tarozzi, A., et al., *Neuroprotective effects of cyanidin 3-O-glucopyranoside on amyloid beta (25–35) oligomer-induced toxicity.* Neuroscience letters, 2010. **473**(2): p. 72-76.

189. Tarozzi, A., et al., *Cyanidin 3-O-glucopyranoside protects and rescues SH-SY5Y cells against amyloid-beta peptide-induced toxicity.* Neuroreport, 2008. **19**(15): p. 1483-6.
190. Thummayot, S., et al., *Cyanidin attenuates Aβ(25-35)-induced neuroinflammation by suppressing NF-κB activity downstream of TLR4/NOX4 in human neuroblastoma cells.* Acta Pharmacol Sin, 2018. **39**(9): p. 1439-1452.
191. Spencer, J.P., et al., *Neuroinflammation: modulation by flavonoids and mechanisms of action.* Mol Aspects Med, 2012. **33**(1): p. 83-97.
192. Winter, A.N. and P.C. Bickford, *Anthocyanins and Their Metabolites as Therapeutic Agents for Neurodegenerative Disease.* Antioxidants (Basel), 2019. **8**(9).
193. Xie, C., et al., *Blueberries reduce pro-inflammatory cytokine TNF-α and IL-6 production in mouse macrophages by inhibiting NF-κB activation and the MAPK pathway.* Mol Nutr Food Res, 2011. **55**(10): p. 1587-91.
194. Lee, Y.M., et al., *Dietary Anthocyanins against Obesity and Inflammation.* Nutrients, 2017. **9**(10).
195. Ellulu, M.S., et al., *Obesity and inflammation: the linking mechanism and the complications.* Arch Med Sci, 2017. **13**(4): p. 851-863.
196. Rodriguez-Mateos, A., et al., *Intake and time dependence of blueberry flavonoid–induced improvements in vascular function: a randomized, controlled, double-blind, crossover intervention study with mechanistic insights into biological activity–.* 2013. **98**(5): p. 1179-1191.
197. Rodriguez-Mateos, A., et al., *Cranberry (poly)phenol metabolites correlate with improvements in vascular function: A double-blind, randomized, controlled, dose-response, crossover study.* Mol Nutr Food Res, 2016. **60**(10): p. 2130-2140.
198. Curtis, P.J., et al., *Blueberries improve biomarkers of cardiometabolic function in participants with metabolic syndrome-results from a 6-month, double-blind, randomized controlled trial.* Am J Clin Nutr, 2019. **109**(6): p. 1535-1545.
199. Spencer, J.P., *Food for thought: the role of dietary flavonoids in enhancing human memory, learning and neuro-cognitive performance.* Proc Nutr Soc, 2008. **67**(2): p. 238-52.
200. Shishtar, E., et al., *Long-term dietary flavonoid intake and risk of Alzheimer disease and related dementias in the Framingham Offspring Cohort.* Am J Clin Nutr, 2020. **112**(2): p. 343-353.
201. Hein, S., et al., *Systematic Review of the Effects of Blueberry on Cognitive Performance as We Age.* J Gerontol A Biol Sci Med Sci, 2019. **74**(7): p. 984-995.
202. Krikorian, R., et al., *Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment.* Br J Nutr, 2010. **103**(5): p. 730-4.
203. Krikorian, R., et al., *Blueberry Supplementation Improves Memory in Older Adults†.* Journal of agricultural and food chemistry, 2010. **58**(7): p. 3996-4000.

204. Krikorian, R., et al., *Concord grape juice supplementation and neurocognitive function in human aging.* J Agric Food Chem, 2012. **60**(23): p. 5736-42.
205. Miller, M.G., et al., *Dietary blueberry improves cognition among older adults in a randomized, double-blind, placebo-controlled trial.* Eur J Nutr, 2018. **57**(3): p. 1169-1180.
206. McNamara, R.K., et al., *Cognitive response to fish oil, blueberry, and combined supplementation in older adults with subjective cognitive impairment.* Neurobiol Aging, 2018. **64**: p. 147-156.
207. Bowtell, J.L., et al., *Enhanced task-related brain activation and resting perfusion in healthy older adults after chronic blueberry supplementation.* Appl Physiol Nutr Metab, 2017. **42**(7): p. 773-779.
208. Boespflug, E.L., et al., *Enhanced neural activation with blueberry supplementation in mild cognitive impairment.* Nutr Neurosci, 2018. **21**(4): p. 297-305.
209. Aarsland, D., et al., *Frequency and case identification of dementia with Lewy bodies using the revised consensus criteria.* Dement Geriatr Cogn Disord, 2008. **26**(5): p. 445-52.
210. Fladby, T., et al., *Detecting at-risk alzheimer's disease cases.* Journal of Alzheimer's Disease, 2017. **60**(1): p. 97-105.
211. Folstein, M.F., S.E. Folstein, and P.R. McHugh, *"Mini-mental state": a practical method for grading the cognitive state of patients for the clinician.* Journal of psychiatric research, 1975. **12**(3): p. 189-198.
212. Petersen, R.C., *Mild cognitive impairment as a diagnostic entity.* Journal of Internal Medicine, 2004. **256**(3): p. 183-194.
213. Mitchell, A.J., et al., *Diagnostic validity and added value of the Geriatric Depression Scale for depression in primary care: a meta-analysis of GDS30 and GDS15.* J Affect Disord, 2010. **125**(1-3): p. 10-7.
214. Creavin, S.T., et al., *Mini-Mental State Examination (MMSE) for the detection of dementia in clinically unevaluated people aged 65 and over in community and primary care populations.* Cochrane Database Syst Rev, 2016(1): p. CD011145.
215. Hensel, A., M.C. Angermeyer, and S.G. Riedel-Heller, *Measuring cognitive change in older adults: reliable change indices for the Mini-Mental State Examination.* J Neurol Neurosurg Psychiatry, 2007. **78**(12): p. 1298-303.
216. Tombaugh, T.N. and N.J. McIntyre, *The mini-mental state examination: a comprehensive review.* J Am Geriatr Soc, 1992. **40**(9): p. 922-35.
217. Hughes, C.P., et al., *A new clinical scale for the staging of dementia.* Br J Psychiatry, 1982. **140**: p. 566-72.
218. Morris, J.C., *Clinical dementia rating: a reliable and valid diagnostic and staging measure for dementia of the Alzheimer type.* Int Psychogeriatr, 1997. **9 Suppl 1**(S1): p. 173-6; discussion 177-8.
219. O'Bryant, S.E., et al., *Staging dementia using Clinical Dementia Rating Scale Sum of Boxes scores: a Texas Alzheimer's research consortium study.* Archives of neurology, 2008. **65**(8): p. 1091-1095.

220. Morris, J.C., et al., *The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease.* Neurology, 1989. **39**(9): p. 1159-65.
221. Fillenbaum, G.G., et al., *Consortium to Establish a Registry for Alzheimer's Disease (CERAD): the first twenty years.* 2008. **4**(2): p. 96-109.
222. Sotaniemi, M., et al., *CERAD-neuropsychological battery in screening mild Alzheimer's disease.* Acta Neurologica Scandinavica, 2012. **125**(1): p. 16-23.
223. Kirsebom, B.E., et al., *Demographically adjusted CERAD wordlist test norms in a Norwegian sample from 40 to 80 years.* Clin Neuropsychol, 2019. **33**(sup1): p. 27-39.
224. Malek-Ahmadi, M., et al., *Cognitive composite score association with Alzheimer's disease plaque and tangle pathology.* Alzheimer's research & therapy, 2018. **10**(1): p. 90-90.
225. Langbaum, J.B., et al., *An empirically derived composite cognitive test score with improved power to track and evaluate treatments for preclinical Alzheimer's disease.* Alzheimers Dement, 2014. **10**(6): p. 666-74.
226. Paajanen, T., et al., *CERAD neuropsychological compound scores are accurate in detecting prodromal alzheimer's disease: a prospective AddNeuroMed study.* J Alzheimers Dis, 2014. **39**(3): p. 679-90.
227. Testa, S.M., et al., *Accounting for estimated IQ in neuropsychological test performance with regression-based techniques.* J Int Neuropsychol Soc, 2009. **15**(6): p. 1012-22.
228. Warrington, E.K. and M. James, *The Visual Object and Space Perception Battery*. 1991, Bury St Edmunds, England: Thames Valley Test Company.
229. Benton, A.L. and K.d. Hamsher, *Multilingual Aphasia Examination*. 1989, AJA Associates: Iowa City.
230. Heaton, R.K., et al., *Revised Comprehensive Norms for an Expanded Halsted-Reitan Battery: Demographically Adjusted Neuropsychological Norms for African American and Caucasian Adults.* 2004, Odessa: Psychological Assessment Resources.
231. Reitan, R. and D. Wolfson, *The Halstead-Reitan neuropsychological test battery: therapy and clinical assessment*. 1985, Tucson, AZ: Neuropsychological Press.
232. Tombaugh, T.N., *Trail Making Test A and B: normative data stratified by age and education.* Arch Clin Neuropsychol, 2004. **19**(2): p. 203-14.
233. Golden, C., *The Stroop color and Word test: a manual for clinical and experimental uses, Stoelting Company Edition.* Chicago, Illinois, 1978.
234. Scarpina, F. and S. Tagini, *The Stroop Color and Word Test.* Front Psychol, 2017. **8**: p. 557.
235. Golden, C.J., *Identification of brain disorders by the Stroop Color and Word Test.* J Clin Psychol, 1976. **32**(3): p. 654-8.
236. Skogseth, R., et al., *Neuropsychiatric correlates of cerebrospinal fluid biomarkers in Alzheimer's disease.* Dementia and geriatric cognitive disorders, 2008. **25**(6): p. 559-563.

237. Berge, G., et al., *Apolipoprotein E ε2 genotype delays onset of dementia with Lewy bodies in a Norwegian cohort.* Journal of Neurology, Neurosurgery & Psychiatry, 2014. **85**(11): p. 1227-1231.
238. Jorm, A.F. and P.A. Jacomb, *The Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE): socio-demographic correlates, reliability, validity and some norms.* Psychol Med, 1989. **19**(4): p. 1015-22.
239. Skogseth, R., et al., *Accuracy of Clinical Diagnosis of Dementia with Lewy Bodies versus Neuropathology.* J Alzheimers Dis, 2017. **59**(4): p. 1139-1152.
240. Soennesyn, H., et al., *White matter hyperintensities and the course of depressive symptoms in elderly people with mild dementia.* Dement Geriatr Cogn Dis Extra, 2012. **2**(1): p. 97-111.
241. Scheltens, P., et al., *A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging.* J Neurol Sci, 1993. **114**(1): p. 7-12.
242. O'Gorman, A., et al., *Identification of a plasma signature of psychotic disorder in children and adolescents from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort.* Transl Psychiatry, 2017. **7**(9): p. e1240.
243. Bowden, J.A., et al., *Harmonizing lipidomics: NIST interlaboratory comparison exercise for lipidomics using SRM 1950–Metabolites in Frozen Human Plasma.* J Lipid Res, 2017. **58**(12): p. 2275-2288.
244. Kalheim, L.F., et al., *[18F]-Flutemetamol Uptake in Cortex and White Matter: Comparison with Cerebrospinal Fluid Biomarkers and [18F]-Fludeoxyglucose.* J Alzheimers Dis, 2018. **62**(4): p. 1595-1607.
245. Feliciano, R.P., et al., *Plasma and Urinary Phenolic Profiles after Acute and Repetitive Intake of Wild Blueberry.* Molecules, 2016. **21**(9): p. 1120.
246. Khalifa, K., et al., *Effects of Purified Anthocyanins in People at Risk for Dementia: Study Protocol for a Phase II Randomized Controlled Trial.* Frontiers in Neurology, 2020. **11**(916).
247. Hassellund, S.S., et al., *Effects of anthocyanins on cardiovascular risk factors and inflammation in pre-hypertensive men: a double-blind randomized placebo-controlled crossover study.* J Hum Hypertens, 2013. **27**(2): p. 100-6.
248. Qin, Y., et al., *Anthocyanin supplementation improves serum LDL- and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects.* Am J Clin Nutr, 2009. **90**(3): p. 485-92.
249. Li, D., et al., *Purified anthocyanin supplementation reduces dyslipidemia, enhances antioxidant capacity, and prevents insulin resistance in diabetic patients.* J Nutr, 2015. **145**(4): p. 742-8.
250. Westergren, T. and A.K. Eek. *Antikoagulantia og samtidig bruk av Medox kosttilskudd*. 2016 08.04.2016 [cited 2018 07.02.]; Available from: https://relis.no/content/4705/Antikoagulantia-og-samtidig-bruk-av-Medox-kosttilskudd.
251. Eldholm, R.S., et al., *Association between vascular comorbidity and progression of Alzheimer's disease: a two-year observational study in Norwegian memory clinics.* BMC Geriatr, 2018. **18**(1): p. 120.

252. Irimata, K.E., B.N. Dugger, and J.R.J.C.A.R. Wilson, *Impact of the presence of select cardiovascular risk factors on cognitive changes among dementia subtypes.* J Current Alzheimer Research, 2018. **15**(11): p. 1032-1044.
253. Pegueroles, J., et al., *Obesity and Alzheimer's disease, does the obesity paradox really exist? A magnetic resonance imaging study.* Oncotarget, 2018. **9**(78): p. 34691-34698.
254. Sun, Z., et al., *Late-life obesity is a protective factor for prodromal Alzheimer's disease: a longitudinal study.* Aging (Albany NY), 2020. **12**(2): p. 2005-2017.
255. Barber, R., et al., *White matter lesions on magnetic resonance imaging in dementia with Lewy bodies, Alzheimer's disease, vascular dementia, and normal aging.* Journal of Neurology, Neurosurgery & Psychiatry, 1999. **67**(1): p. 66-72.
256. Firbank, M.J., et al., *Brain atrophy and white matter hyperintensity change in older adults and relationship to blood pressure. Brain atrophy, WMH change and blood pressure.* J Neurol, 2007. **254**(6): p. 713-21.
257. Lee, W.J., et al., *Summative Effects of Vascular Risk Factors on the Progression of Alzheimer Disease.* J Am Geriatr Soc, 2020. **68**(1): p. 129-136.
258. Walker, K.A., M.C. Power, and R.F. Gottesman, *Defining the Relationship Between Hypertension, Cognitive Decline, and Dementia: a Review.* Curr Hypertens Rep, 2017. **19**(3): p. 24.
259. Fereshtehnejad, S.M., et al., *No Significant Difference in Cognitive Decline and Mortality between Parkinson's Disease Dementia and Dementia with Lewy Bodies: Naturalistic Longitudinal Data from the Swedish Dementia Registry.* J Parkinsons Dis, 2018. **8**(4): p. 553-561.
260. Kraybill, M.L., et al., *Cognitive differences in dementia patients with autopsy-verified AD, Lewy body pathology, or both.* Neurology, 2005. **64**(12): p. 2069-73.
261. Nelson, P.T., et al., *Relative preservation of MMSE scores in autopsy-proven dementia with Lewy bodies.* Neurology, 2009. **73**(14): p. 1127-33.
262. Raitanen, J., et al., *Longitudinal change in physical functioning and dropout due to death among the oldest old: a comparison of three methods of analysis.* Eur J Ageing, 2020. **17**(2): p. 207-216.
263. Han, X., et al., *Metabolomics in early Alzheimer's disease: identification of altered plasma sphingolipidome using shotgun lipidomics.* PLoS One, 2011. **6**(7): p. e21643.
264. Mielke, M.M., et al., *Serum sphingomyelins and ceramides are early predictors of memory impairment.* Neurobiol Aging, 2010. **31**(1): p. 17-24.
265. Mielke, M.M., et al., *Plasma ceramides are altered in mild cognitive impairment and predict cognitive decline and hippocampal volume loss.* Alzheimers Dement, 2010. **6**(5): p. 378-85.
266. Savica, R., et al., *Plasma sphingolipid changes with autopsy-confirmed Lewy Body or Alzheimer's pathology.* Alzheimers Dement (Amst), 2016. **3**: p. 43-50.
267. Vardarajan, B., et al., *Differences in plasma metabolites related to Alzheimer's disease, APOE ε4 status, and ethnicity.* Alzheimers Dement (N Y), 2020. **6**(1): p. e12025.

268. Mielke, M.M., et al., *The association between plasma ceramides and sphingomyelins and risk of Alzheimer's disease differs by sex and APOE in the Baltimore Longitudinal Study of Aging.* Journal of Alzheimer's Disease, 2017. **60**(3): p. 819-828.
269. Wong, M.W.K., et al., *Plasma lipidome variation during the second half of the human lifespan is associated with age and sex but minimally with BMI.* PLoS One, 2019. **14**(3): p. e0214141.
270. Iqbal, J., et al., *Sphingolipids and Lipoproteins in Health and Metabolic Disorders.* Trends Endocrinol Metab, 2017. **28**(7): p. 506-518.
271. Mielke, M.M. and C.G. Lyketsos, *Alterations of the sphingolipid pathway in Alzheimer's disease: new biomarkers and treatment targets?* Neuromolecular Med, 2010. **12**(4): p. 331-40.
272. Gonzalez-Dominguez, R., T. Garcia-Barrera, and J.L. Gomez-Ariza, *Combination of metabolomic and phospholipid-profiling approaches for the study of Alzheimer's disease.* J Proteomics, 2014. **104**: p. 37-47.
273. Mapstone, M., et al., *Plasma phospholipids identify antecedent memory impairment in older adults.* Nat Med, 2014. **20**(4): p. 415-8.
274. Casanova, R., et al., *Blood metabolite markers of preclinical Alzheimer's disease in two longitudinally followed cohorts of older individuals.* Alzheimers Dement, 2016. **12**(7): p. 815-22.
275. Li, D., et al., *Prospective associations of plasma phospholipids and mild cognitive impairment/dementia among African Americans in the ARIC Neurocognitive Study.* Alzheimers Dement (Amst), 2017. **6**: p. 1-10.
276. Chen, Y., et al., *Altered Expression Profile of Phosphatidylinositols in Erythrocytes of Alzheimer's Disease and Amnestic Mild Cognitive Impairment Patients.* J Alzheimers Dis, 2020. **73**(2): p. 811-818.
277. Kirsebom, B.E., et al., *Screening for Alzheimer's Disease: Cognitive Impairment in Self-Referred and Memory Clinic-Referred Patients.* J Alzheimers Dis, 2017. **60**(4): p. 1621-1631.
278. Zhu, Y., et al., *Anthocyanin supplementation improves HDL-associated paraoxonase 1 activity and enhances cholesterol efflux capacity in subjects with hypercholesterolemia.* J Clin Endocrinol Metab, 2014. **99**(2): p. 561-9.
279. Zhu, Y., et al., *Anti-inflammatory effect of purified dietary anthocyanin in adults with hypercholesterolemia: a randomized controlled trial.* Nutr Metab Cardiovasc Dis, 2013. **23**(9): p. 843-9.
280. Soltani, R., et al., *Evaluation of the Effects of Vaccinium arctostaphylos L. Fruit Extract on Serum Lipids and hs-CRP Levels and Oxidative Stress in Adult Patients with Hyperlipidemia: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial.* Evid Based Complement Alternat Med, 2014. **2014**: p. 217451.
281. Aboonabi, A., et al., *Anthocyanins in berries exhibited anti-atherogenicity and antiplatelet activities in a metabolic syndrome population.* Nutr Res, 2020. **76**: p. 82-93.
282. Guo, X., et al., *Associations of dietary intakes of anthocyanins and berry fruits with risk of type 2 diabetes mellitus: a systematic review and meta-analysis of prospective cohort studies.* Eur J Clin Nutr, 2016. **70**(12): p. 1360-1367.

283. Stull, A.J., et al., *Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women.* J Nutr, 2010. **140**(10): p. 1764-8.
284. Stull, A.J., et al., *Blueberries improve endothelial function, but not blood pressure, in adults with metabolic syndrome: a randomized, double-blind, placebo-controlled clinical trial.* Nutrients, 2015. **7**(6): p. 4107-23.
285. Basu, A., et al., *Blueberries decrease cardiovascular risk factors in obese men and women with metabolic syndrome.* J Nutr, 2010. **140**(9): p. 1582-7.
286. Paquette, M., et al., *Strawberry and cranberry polyphenols improve insulin sensitivity in insulin-resistant, non-diabetic adults: a parallel, double-blind, controlled and randomised clinical trial.* Br J Nutr, 2017. **117**(4): p. 519-531.
287. Kempuraj, D., et al., *Neuroinflammation Induces Neurodegeneration.* J Neurol Neurosurg Spine, 2016. **1**(1).
288. Matter, C.M. and C. Handschin, *RANTES (regulated on activation, normal T cell expressed and secreted), inflammation, obesity, and the metabolic syndrome.* Circulation, 2007. **115**(8): p. 946-8.
289. Kwaifa, I.K., et al., *Endothelial Dysfunction in Obesity-Induced Inflammation: Molecular Mechanisms and Clinical Implications.* Biomolecules, 2020. **10**(2).
290. Zhang, Z., et al., *Chemokine Receptor 5, a Double-Edged Sword in Metabolic Syndrome and Cardiovascular Disease.* Front Pharmacol, 2020. **11**: p. 146.
291. Karlsen, A., et al., *Anthocyanins inhibit nuclear factor-κB activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults.* The Journal of nutrition, 2007. **137**(8): p. 1951-1954.
292. Song, F., et al., *Plant food anthocyanins inhibit platelet granule secretion in hypercholesterolaemia: Involving the signalling pathway of PI3K–Akt.* 2014. **111**(05): p. 981-991.
293. Curtis, P.J., et al., *Cardiovascular disease risk biomarkers and liver and kidney function are not altered in postmenopausal women after ingesting an elderberry extract rich in anthocyanins for 12 weeks.* J Nutr, 2009. **139**(12): p. 2266-71.
294. Fallah, A.A., et al., *Impact of dietary anthocyanins on systemic and vascular inflammation: Systematic review and meta-analysis on randomised clinical trials.* Food Chem Toxicol, 2020. **135**: p. 110922.
295. Karlsen, A., et al., *Bilberry juice modulates plasma concentration of NF-kappaB related inflammatory markers in subjects at increased risk of CVD.* Eur J Nutr, 2010. **49**(6): p. 345-55.
296. Whyte, A., et al., *A randomized, double-blinded, placebo-controlled study to compare the safety and efficacy of low dose enhanced wild blueberry powder and wild blueberry extract (ThinkBlue™) in maintenance of episodic and working memory in older adults.* 2018. **10**(6): p. 660.
297. Travica, N., et al., *The effect of blueberry interventions on cognitive performance and mood: A systematic review of randomized controlled trials.* Brain Behav Immun, 2020. **85**: p. 96-105.
298. Sun, Y., et al., *Analysis of the Anthocyanin Degradation in Blue Honeysuckle Berry under Microwave Assisted Foam-Mat Drying.* Foods, 2020. **9**(4).
299. Krikorian, R., et al., *Cognitive performance in relation to urinary anthocyanins and their flavonoid-based products following blueberry*

supplementation in older adults at risk for dementia. Journal of Functional Foods, 2020. **64**: p. 103667.
300. Zhang, H., et al., *Anthocyanin supplementation improves anti-oxidative and anti-inflammatory capacity in a dose-response manner in subjects with dyslipidemia.* Redox Biol, 2020. **32**: p. 101474.
301. Xu, Z., et al., *Anthocyanin supplementation at different doses improves cholesterol efflux capacity in subjects with dyslipidemia-a randomized controlled trial.* Eur J Clin Nutr, 2020.
302. Guo, Y., et al., *A dose-response evaluation of purified anthocyanins on inflammatory and oxidative biomarkers and metabolic risk factors in healthy young adults: A randomized controlled trial.* Nutrition, 2020. **74**: p. 110745.

I

II

III

frontiers in Genetics

ORIGINAL RESEARCH
published: 11 June 2019
doi: 10.3389/fgene.2019.00536

Effects of Anthocyanin Supplementation on Serum Lipids, Glucose, Markers of Inflammation and Cognition in Adults With Increased Risk of Dementia – A Pilot Study

Anne Katrine Bergland[1,2], Hogne Soennesyn[1], Ingvild Dalen[3], Ana Rodriguez-Mateos[4], Rolf Kristian Berge[5], Lasse Melvaer Giil[6], Lawrence Rajendran[7], Richard Siow[8], Michele Tassotti[4,9], Alf Inge Larsen[2,10] and Dag Aarsland[1,7]*

[1] Centre for Age-Related Medicine, Stavanger University Hospital, Stavanger, Norway, [2] Department of Clinical Science, University of Bergen, Bergen, Norway, [3] Section of Biostatistics, Department of Research, Stavanger University Hospital, Stavanger, Norway, [4] Department of Nutritional Sciences, Faculty of Life Sciences and Medicine, School of Life Course Sciences, King's College London, London, United Kingdom, [5] The Lipid Research Group, Department of Clinical Science, University of Bergen, Bergen, Norway, [6] Department of Internal Medicine, Haraldsplass Deaconess Hospital, Bergen, Norway, [7] UK Dementia Research Institute, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom, [8] School of Cardiovascular Medicine and Sciences, British Heart Foundation Centre of Research Excellence, Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom, [9] Department of Food & Drug, University of Parma, Parma, Italy, [10] Department of Cardiology, Stavanger University Hospital, Stavanger, Norway

OPEN ACCESS

Edited by:
Joseph Baur, University of Pennsylvania, United States

Reviewed by:
Indika Edirisinghe, Illinois Institute of Technology, United States
Wieslaw Wiczkowski, Institute of Animal Reproduction and Food Research (PAN), Poland

***Correspondence:**
Anne Katrine Bergland
anne.katrine.bergland@sus.no

Specialty section:
This article was submitted to Genetics of Aging, a section of the journal Frontiers in Genetics

Received: *07 January 2019*
Accepted: *16 May 2019*
Published: *11 June 2019*

Citation:
Bergland AK, Soennesyn H, Dalen I, Rodriguez-Mateos A, Berge RK, Giil LM, Rajendran L, Siow R, Tassotti M, Larsen AI and Aarsland D (2019) Effects of Anthocyanin Supplementation on Serum Lipids, Glucose, Markers of Inflammation and Cognition in Adults With Increased Risk of Dementia – A Pilot Study. Front. Genet. 10:536. doi: 10.3389/fgene.2019.00536

Background: Anthocyanins may protect against cardiovascular related cognitive decline and dementia.

Objective: Open-label study to measure changes in serum lipids, glucose, glycosylated hemoglobin (HbA1c), and markers of inflammation after anthocyanin supplementation in people with increased risk of dementia. As a secondary endpoint we examined potential changes in a battery of cognitive test in the anthocyanin group (AG). A total of 27 individuals with mild cognitive impairment (MCI) (n = 8) or stable non-obstructive coronary artery disease (CAD) (n = 19) consumed two Medox® capsules, each containing 80 mg of natural purified anthocyanins, twice daily for 16 weeks. They provided blood samples and performed a short battery of cognitive tests. Twenty healthy normal controls (NC) (n = 20) provided blood samples, but did not receive any intervention and did not perform cognitive tests.

Results: There was a significant difference between groups for CCL-5/RANTES [regulated on activation, normal T-cell expressed and secreted (RANTES)]. In addition, total cholesterol and triglycerides were significantly increased in the AG. Improvements in memory and executive test scores were observed. No adverse effects were reported.

Conclusion: The results of this pilot study were largely inconclusive with regard to the potential protective effects of anthocyanin supplementation. However,

anthocyanins were well tolerated, and compliance was high. Larger, placebo-controlled studies to explore the potential effects of anthocyanins on dementia risk are encouraged.

Clinical Trial Registration: www.ClinicalTrials.gov, identifier: NCT02409446.

Keywords: mild cognitive impairment, MCI, anthocyanins, lipids, inflammation markers

INTRODUCTION

Anthocyanins, a subclass of the flavonoids, are found in foods such as berries and fruits and information regarding their content in food can be found in an online phenol-explorer (Neveu et al., 2010). Anthocyanins have been shown in previous studies to have a number of positive health effects, such as improving the blood lipid profile (Qin et al., 2009; Li et al., 2015), and also fasting serum glucose and glycosylated hemoglobin (HbA1c) in diabetic patients (Li et al., 2015; Yang et al., 2017), and have anti-inflammatory effects (Xia et al., 2007; Spencer et al., 2012; Spagnuolo et al., 2017). Anthocyanins can improve endothelial and vascular function (Rodriguez-Mateos et al., 2013, 2016, 2019), and can cross the blood–brain barrier (Faria et al., 2014) and thus may reduce neurodegenerative and cerebrovascular changes and possibly protect against cognitive decline and dementia. Interestingly, some studies have found that food-based anthocyanins can improve memory functioning in older adults with mild cognitive impairment (MCI; Krikorian et al., 2010a,b; Hein et al., 2019). However, these studies were based on relatively small samples, and had a short duration. In addition, food-based anthocyanin supplementation leads to heterogeneity, i.e., variations in types of food sources, concentration, and dose of anthocyanins.

In this exploratory open-label pilot study, we aimed as a primary endpoint to examine potential changes in dementia-relevant mechanisms after 16 weeks of treatment with purified anthocyanin containing capsules, in people with increased risk of dementia. As a secondary endpoint we also explored the potential change in a battery of cognitive tests.

MATERIALS AND METHODS

Material

Participants were recruited from the outpatient Memory and Cardiology clinics at Stavanger University Hospital in Norway during 2015 and 2016. Eligible for this study were patients with MCI or mild dementia and/or stable non-obstructive coronary artery disease (CAD). Potential participants identified at the respective outpatient clinics were contacted for a telephone interview by a study doctor, regarding inclusion and exclusion criteria. Participants were also recruited from the dementia disease initiation (DDI) study (Fladby et al., 2017). Inclusion criteria were age $\geq$50 years and being on stable medication, including nutraceuticals for the past 3 months, and either (a) confirmed CAD without physiologically significant stenosis evaluated by coronary angiography, or (b) having MCI or mild dementia according to ICD 10 (World Health Organization [WHO], 1992).

Exclusion criteria were moderate to severe dementia [operationalized as a mini-mental status exam (MMSE) (Folstein et al., 1975) score < 24], clinically significant depression [15–item Geriatric Depression Scale (GDS-15) (Mitchell et al., 2010) score ≥ 7], unstable CAD, heart failure in need of treatment, having taken Medox® during the past 3 months, using Warfarin, heparin or non-vitamin K antagonist oral anticoagulants (NOAC), inflammatory illnesses such as rheumatoid arthritis and other severe illness with <5 years expected survival. Any treatment with vitamins, minerals or nutraceuticals had to have remained stable for the last 3 months prior to inclusion and during the study. Healthy normal controls (NC) ($n = 20$) recruited from the staff at Stavanger University Hospital and through acquaintances, were $\geq$50 years, had stable medication, and had not been taking Medox® for the last 3 months. This group provided blood samples at inclusion and 16 weeks later, but did not take Medox® or other interventions, and did not perform the cognitive test battery. The rationale for including this comparison group was to have a reference group with respect to any observed changes in the blood analyses in the intervention group.

Ethics Statement

All participants provided written informed consent, and the study has been approved by the Regional Ethics Committee (Approval 2014/1966). The study has been registered at ClinicalTrials.gov (NCT02409446).

Intervention, Design, and Assessment

Intervention

The participants were given open-label Medox® capsules, provided free of charge by the manufacturer Medpalett AS, Sandnes, Norway. Medox® capsules, which contain specific quantities of natural purified anthocyanins from bilberry (*Vaccinium myrtillus*) and blackcurrant (*Ribes nigrum*), have been used previously in human studies (Karlsen et al., 2007). The production of the Medox® capsule (Hassellund et al., 2013), and its anthocyanin content (Qin et al., 2009) have been described previously.

The capsules were dispensed at inclusion in the study, and the participants were instructed to consume two 80 mg anthocyanin capsules twice daily for a total daily intake of 320 mg anthocyanins for 16 weeks. This dosage was chosen because it has previously been shown to have biological effects (Qin et al., 2009; Zhu et al., 2011; Li et al., 2015) and found to be safe in use (Qin

et al., 2009). A review found that doses up to 640 mg/day showed no adverse events (Wallace et al., 2016).

Participants were instructed to maintain their dietary and lifestyle habits in order to avoid interferences in the study results.

Design and Assessment

At inclusion, all participants underwent a physical examination, including standardized blood pressure measurement, electrocardiogram (ECG), and blood tests. In addition a cognitive test battery (see below) was administered, including the MMSE and GDS-15. Following standardized procedures, participants provided blood samples in the morning after having been fasting for at least 8 h, before and after 16 weeks of treatment. They were contacted by telephone after 8 weeks regarding safety and compliance.

Blood Sampling and Analyses

Blood was collected, centrifugated, and stored at −80 °C until analysis according to standardized procedures. The serum samples were analyzed for lipids (total cholesterol, triglycerides, HDL- and LDL cholesterol) and fasting glucose using Architect c16000 TM (Abbott Diagnostics, Chicago, IL, United States) and HbA1c using Variant II turbo (BioRad, Hercules, CA, United States) at Stavanger University Hospital.

Markers of inflammation were analyzed after completion of the study by The Lipid Research Group, Department of Clinical Sciences, University of Bergen, Bergen, Norway. Concentrations of cytokines were measured in serum using the Bio-Plex Pro™ Human Cytokine 8-plex assay (Cat.: M50000007A) which included GM-CSF, IFN-γ, IL-2, IL-4, IL-6, IL-8, IL-10, and TNF-α, in addition to five Bio-Plex Pro Human Cytokine single-plexes: MCP-1 (Cat.: 171B5021M), RANTES (Cat.: 171B5025M), G-CSF (Cat.: 171B5017M), IL-17 (Cat.: 171B5014M), and IL-Iβ (Cat.:171B5001M). All plexes were manufactured by Bio-Rad (Hercules, CA, United States). The cytokines were detected by the Bio-Plex™ 200 System and determined with the Bio-Plex Manager Software 6.1. The samples were prepared as described in the protocol (Cat.: 10014905) with a dilution factor of three.

Anthocyanin metabolites were measured in plasma after completion of the study at Department of Nutritional Sciences, School of Life Course Sciences, Faculty of Medicine and Life Sciences, King's College London, using a method based on microelution solid phase extraction followed by liquid chromatography and mass spectrometry, using authentic standards, as previously described with some modifications (Feliciano et al., 2016). The detection of plasma (poly)phenol metabolites was performed on a Exactive™ Orbitrap Mass Spectrometer (Thermo Scientific, Waltham, CA, United States) after separation on an Accela 1250 pump UHPLC system (Thermo Scientific, Waltham, CA, United States). The autosampler injected 5 μL of each sample in a Zorbax Eclipse Plus RRHD column 2.1 mm × 50 mm, 1.8 m with a compatible Eclipse Plus guardcolumn 2.1 mm × 5 mm, 1.8 m (Agilent, Waldbronn, Germany). The mobile phase consisted of 0.1% HCOOH (solvent A) and acetonitrile with 0.1% HCOOH (solvent B) in a 10 min gradient program. Quantification analysis of the plasma (poly)phenols was done using Xcalibur 2.2 (Thermo Scientific, Waltham, CA, United States).

Cognitive Tests

Verbal memory function was assessed using the Norwegian adaptation of the Ten Word List Learning and Recall from the CERAD battery (Morris et al., 1989), a three part test; word list learning, word list delayed recall, and word list delayed recognition.

Executive functioning was assessed by the Trail Making Test (TMT) A and B (Reitan and Wolfson, 1985) and Stroop Golden (Golden, 1976). The Trail Making Tests A and B are tests of psychomotor speed and attention shifting (Ashendorf et al., 2008), while Stroop Golden is a test used to evaluate cognitive speed and inhibition (Scarpina and Tagini, 2017).

Safety

Participants were contacted by phone at week 8 to ask about potential side-effects and adverse events (AE).

The safety blood tests included hemoglobin, thrombocytes, kidney function and liver function tests, which were measured at both baseline and study-end.

Compliance

Participants were contacted by phone at week 8 and asked about adherence to the protocol. Specifically, they were asked whether they had been taking Medox® capsules as instructed, and they were reminded about keeping the empty blister packages. Protocol adherence was also assessed by collecting and counting the empty blister packages and left-over capsules at study-end.

Statistical Analyses

Descriptive statistics are presented as medians and interquartile ranges (IQR), and illustrated using Box plots. Most data were not normally distributed and thus the main analyses were non-parametric. The Mann–Whitney *U* test and the Chi-square test were used for between-group comparisons. Changes from baseline to follow-up at 24 weeks within groups were analyzed with the Wilcoxon Signed Rank test. For all tests $p \leq 0.05$ was considered statistically significant.

Supplementary parametric analyses were performed, from which we present means, standard deviations (SD), and *p*-values from paired and independent samples *t*-tests.

The IBM SPSS statistical package version 24 was used for all statistical analyses.

RESULTS

During the period May 2015 to September 2016, 33 participants started anthocyanin supplementation, of whom 27 (8 MCI and 19 CAD) completed the study (6 were excluded for administrative and logistical reasons) (**Figure 1**). The NC were included in the period December 2016 to February 2017. No subjects with mild dementia were included.

Baseline characteristics are shown in **Table 1**.

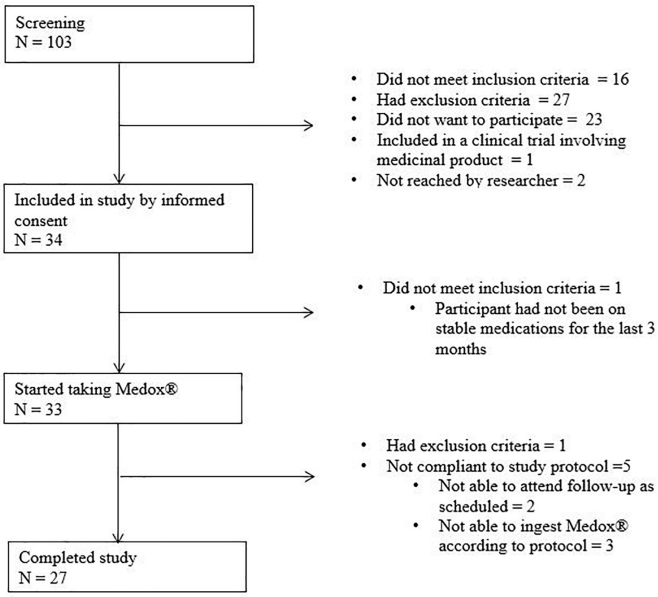


FIGURE 1 | Enrolment of participants. N, number.

TABLE 1 | Baseline characteristics.

	Active (*n* = 27)	Controls (*n* = 20)
	Median (IQR)	**Median (IQR)**
Women, count (%)	9 (33)	11 (55)
Age (years)	61 (55 – 70)	58 (55 – 62)
CAD, count (%)	19 (70)	
Education (years)	11.5 (10 – 14)	
BMI	27.7 (26.0 – 30.3)	
Lipid lowering therapy, count (%)	19 (70)	
Acetylsalicylic acid, count (%)	17 (63)	
Oral antidiabetic treatment count (%)	3 (11)	
Dietary supplement, count (%)	18 (67)	

CAD, coronary artery disease; BMI, body mass index; IQR, interquartile range.

Only IL-8, MCP-1, CCL-5/RANTES [regulated on activation, normal T-cell expressed and secreted (RANTES)] and TNF were available for statistical analyses, as the other inflammation markers did not reach measurement thresholds. The findings are summarized in **Table 2** and **Supplementary Figures S1**–**S3**.

The only significant between-group difference was for ΔRANTES (difference from baseline to study end) which decreased in the supplementation group and increased in the NC group (**Table 2**).

When analyzing the groups separately, significant increases were found in total cholesterol and triglycerides in the anthocyanin supplementation group (AG) from baseline to study end (**Table 2**), and MCP-1 which increased in the NC group (**Table 2**).

No significant changes were found for fasting glucose and HbA1c in the AG group.

A total of 29 plasma anthocyanin metabolites were quantified (**Table 3** and **Supplementary Figure S4**). When comparing the two groups, a statistically significant difference was found for two metabolites (*o*-Coumaric acid and Dihydroferulic acid-4-*O*-Sulfate), which both had a larger decrease in the AG than in the NC group (**Table 3**).

In the AG, there was a statistically significant increase in five of the metabolites (Pyrogallol-2-*O*-sulfate, Protocatechuic acid-3-*O*-sulfate, Pyrogallol-1-*O*-sulfate, Ferulic acid-4-*O*-β-*D*-glucuronide, Isoferulic_acid-3-*O*-β-*D*-glucuronide) and a statistically significant decrease in five other metabolites (3-Hydroxyhippuric acid, 4-Hydroxybenzaldehyde, Dihydroferulic acid-4-*O*-Sulfate, *m*-Coumaric acid, *o*-Coumaric acid) after 16 weeks of anthocyanin consumption in comparison with baseline.

In the NC group, there was a statistically significant decrease in four metabolites (Protocatechuic acid, 3,4-Dihydroxyphenylacetic acid, 4-Hydroxybenzaldehyde and *o*-Coumaric acid), whereas there were no statistically significant increases in any of the metabolites.

The cognitive test scores improved in the intervention group, with improvements for CERAD learning ($p = 0.016$), recall ($p < 0.001$) and recognition ($p = 0.047$) and for STROOP

TABLE 2 | Changes from baseline to 16 weeks follow-up in serum variables, for participants with supplementation (active) and for control participants.

	Active (n = 27)		Control (n = 20)		Active vs. control
	Median (IQR)	p^*	**Median (IQR)**	p^*	$p^\#$
Cholesterol (mmol/L)			Cholesterol		
Pre	4.0 (3.1 to 5.5)		5.1 (4.5 to 5.5)		
Post	4.6 (3.3 to 6.0)		5.1 (4.6 to 5.6)		
Diff	0.2 (0.1 to 0.7)	0.009	0.1 (− 0.2 to 0.5)	0.29	0.34
HDL (mmol/L)			HDL		
Pre	1.2 (1.0 to 1.4)		1.5 (1.1 to 1.7)		
Post	1.2 (1.1 to 1.5)		1.4 (1.2 to 1.8)		
Diff	0.0 (− 0.1 to 0.1)	0.81	0.1 (− 0.1 to 0.1)	0.21	0.23
LDL (mmol/L)			LDL$^{n = 19}$		
Pre	2.4 (1.8 to 3.9)		3.3 (2.9 to 3.9)		
Post	3.0 (1.8 to 4.3)		3.3 (2.8 to 4.0)		
Diff	0.1 (− 0.1 to 0.3)	0.21	0.0 (− 0.1 to 0.4)	0.62	0.72
Triglycerides (mmol/L)			Triglycerides		
Pre	1.0 (0.7 to 1.4)		0.9 (0.6 to 1.3)		
Post	1.0 (0.7 to 1.7)		0.9 (0.6 to 1.8)		
Diff	0.1 (0.7 to 1.7)	0.016	0.0 (− 0.1 to 0.4)	0.072	0.84
Fasting glucose (mmol/L)			Fasting glucose		
Pre	5.4 (4.9 to 5.6)		5.3 (5.0 to 5.6)		
Post	5.5 (5.3 to 6.3)		5.0 (4.8 to 5.7)		
Diff	0.2 (− 0.1 to 0.4)	0.058	−0.2 (− 0.4 to − 0.03)	0.009	0.71
HbA1c (%)			HbA1c		
Pre	5.8 (5.6 to 6.1)		5.6 (5.4 to 5.8)		
Post	5.8 (5.6 to 6.1)		5.4 (5.2 to 5.6)		
Diff	0.0 (− 0.1 to 0.1)	0.87	−0.05 (− 0.2 to 0.0)	0.057	0.11
IL-8 (mmol/L)			IL-8		
Pre	9.0 (7.7 to 10.3)		7.5 (7.2 to 8.4)		
Post	9.2 (6.9 to 11.1)		7.8 (7.2 to 8.9)		
Diff	0.0 (− 1.5 to 1.2)	0.80	0.2 (− 1.0 to 1.5)	0.79	0.41
MCP-1 (pg/mL)			MCP-1		
Pre	42.2 (10.3 to 59.4)		51.7 (40.9 to 70.2)		
Post	41.3 (11.1 to 60.2)		52.8 (45.1 to 93.7)		
Diff	0.0 (− 5.4 to 1.7)	0.55	1.9 (0.2 to 17.5)	0.014	0.95
RANTES (pg/mL)			RANTES		
Pre	9206 (8172 to 9833)		8800 (8370 to 9761)		
Post	8918 (8046 to 9942)		9164 (8651 to 10027)		
Diff	−161 (− 730 to 677)	0.81	19.09 (− 633 to 1105)	0.41	0.003
TNFa (pg/mL)			TNFa$^{n = 19}$		
Pre	10.1 (7.8 to 13.3)		6.5 (6.1 to 10.9)		
Post	9.9 (6.5 to 13.9)		8.0 (5.8 to 11.4)		
Diff	0.9 (− 2.8 to 2.9)	0.74	−0.4 (− 1.5 to 3.9)	0.66	0.26

*RANTES; CCL-5/RANTES (regulated on activation, normal T-cell expressed and secreted); Diff, median difference between baseline and follow up serum measurements; IQR, interquartile range; mmol/L, millimol/liter; pg/mL, picomol/liter. *The within group difference from baseline to study end. #The between group differences for Δ (difference from baseline to study end).*

test word ($p < 0.001$) and color ($p = 0.044$) (**Table 4** and **Supplementary Figures S5–S7**).

Overall, findings using parametric analyses differed only marginally from the non-parametric findings reported above (**Supplementary Tables S1**, **S2**).

The compliance was good. More than 85% of the participants returned at least 90% of the empty blister packages. The anthocyanins were well tolerated, and none of the participants withdrew due to adverse effects. Blood tests taken for safety reasons were all within a clinically acceptable range. Increased bleeding tendency was not observed.

DISCUSSION

In this pilot study anthocyanin supplementation was well tolerated, without any AE, and the compliance was good.

TABLE 3 | Changes from baseline to 16 weeks follow-up in plasma anthocyanin metabolites, for participants with supplementation (active) and for control participants.

	Active (n = 27)		Control (n = 20)		Active vs. control
nmol/L	**Median (IQR)**	p^*	**Median (IQR)**	p^*	$p^\#$
Methylpyrogallol-*O*-sulfate					
Pre	33.1 (17.5 to 52.5)		26.2 (10.2 to 67.6)		
Post	45.5 (24.1 to 104.2)		20.6 (7 to 65.3)		
Diff	15.6 (− 3.3 to 70.7)	0.068	−1.7 (− 23.8 to 13.0)	0.85	0.14
Pyrogallol-2-*O*-sulfate					
Pre	36.4 (17.2 to 110.5)		58.4 (20.4 to 102.4)		
Post	63.8 (41.5 to 199.1)		35.2 (21.2 to 93.1)		
Diff	17.5 (− 8.3 to 106.4)	0.001	−12.3 (− 82.4 to 32.1)	0.41	0.89
Protocatechuic acid-3-*O*-sulfate					
Pre	3.5 (0.3 to 11.1)		5.4 (1.6 to 10.1)		
Post	11.0 (5.5 to 26.5)		6.5 (0.3 to 16.4)		
Diff	6.2 (0.0 to 21.6)	0.007	0.8 (− 6.3 to 10.1)	0.81	0.071
1-Methylpyrogallol-*O*-sulfate					
Pre	44.7 (29.2 to 77.3)		60.8 (34.8 to 123)		
Post	52.7 (33.6 to 97.2)		71.8 (30.3 to 106)		
Diff	12.8 (− 19.6 to 32.7)	0.14	10.4 (− 27.8 to 65.5)	0.60	0.70
4-Methylgallic-3-*O*-sulfate					
Pre	14.0 (5.6 to 23.8)		13.1 (8.5 to 28.7)		
Post	12.8 (7.1 to 24.6)		12.3 (7.3 to 21.1)		
Diff	1.6 (− 7.8 to 14.3)	0.65	−5.5 (− 15.6 to 12.0)	0.35	0.25
4-Hydroxybenzoic acid-*O*-sulfate					
Pre	2027 (1211 to 4284)		2664 (1455 to 3764)		
Post	1704 (772 to 3597)		1723 (420 to 4354)		
Diff	−97 (− 1039 to 234)	0.14	−924 (− 2691 to 1046)	0.26	0.67
4-Hydroxyhippuric acid					
Pre	164 (93 to 272)		145 (117 to 275)		
Post	123 (94 to 283)		111 (91 to 163)		
Diff	6 (− 84 to 44)	0.84	−33 (− 236 to 33)	0.079	0.21
Protocatechuic acid					
Pre	57.5 (22.0 to 106.8)		66.6 (35.5 to 127)		
Post	51.3 (29.4 to 114.2)		48.7 (5.0 to 76.9)		
Diff	1.6 (− 49.7 to 51.3)	0.75	−22.0 (− 85.2 to 17.2)	0.049	0.057
Pyrogallol-1-*O*-sulfate					
Pre	25.6 (15.3 to 61.2)		55.1 (25.3 to 106.5)		
Post	52.9 (25.5 to 97.6)		63.8 (41.4 to 94.4)		
Diff	18.8 (1.5 to 68.4)	0.006	−2.9 (− 36.3 to 45.7)	0.85	0.093
3,4-Dihydroxyphenylacetic acid					
Pre	174 (100 to 237)		122 (85 to 197)		
Post	106 (84 to 213)		98 (75 to 163)		
Diff	−26 (− 135 to 69)	0.20	−33 (− 67 to 2)	0.044	0.97
Catechol-*O*-sulfate					
Pre	5518 (2718 to 8056)		5986 (4284 to 7527)		
Post	4640 (3183 to 7843)		4690 (3576 to 5627)		
Diff	367 (− 2569 to 1608)	0.61	−937 (− 3502 to 2010)	0.25	0.67
Vanillic acid-4-*O*-sulfate					
Pre	30.6 (9.5 to 47.8)		24.6 (9.6 to 39.6)		
Post	26.2 (9.8 to 42.3)		22.6 (7.9 to 34.7)		
Diff	−6.5 (− 27.6 to 10.8)	0.20	−5.2 (− 15.0 to 23.8)	1.0	0.41
3-Hydroxyhippuric acid					
Pre	709 (191 to 3060)		451 (269 to 1343)		

(Continued)

TABLE 3 | Continued

	Active (n = 27)		Control (n = 20)		Active vs. control
nmol/L	**Median (IQR)**	p^*	**Median (IQR)**	p^*	$p^{\#}$
Post	339 (63 to 1233)		162 (35 to 599)		
Diff	−503 (− 1246 to − 55)	0.002	−183 (− 1117 to 196)	0.10	0.28
***p*-Coumaric acid-4-*O*-β-*D*-glucuronide**					
Pre	0.29 (0.00 to 0.52)		0.23 (0.00 to 0.46)		
Post	0.21 (0.11 to 0.41)		0.17 (0.00 to 0.30)		
Diff	−0.07 (− 0.26 to 0.24)	0.92	0.01 (− 0.20 to 0.20)	0.94	0.84
Isovanillic acid-3-*O*-sulfate					
Pre	2.3 (0.0 to 8.3)		5.5 (0.0 to 19.1)		
Post	2.8 (0.0 to 11.3)		5.7 (0.3 to 14.4)		
Diff	0.0 (− 3.5 to 11.3)	0.90	−1.1 (− 10.6 to 3.6)	0.50	0.48
Catechol-*O*-1-glucuronide					
Pre	1.7 (0.0 to 9.2)		2.2 (0.1 to 8.0?)		
Post	4.6 (1.6 to 14.2)		1.1 (0.1 to 6.6)		
Diff	1.5 (− 1.5 to 8.8)	0.075	0.1 (− 2.2 to 1.7)	0.97	0.14
Ferulic acid-4-*O*-β-*D*-glucuronide					
Pre	4.3 (0.4 to 33.4)		7.7 (1.0 to 19.4)		
Post	19.4 (2.6 to 59.3)		5.8 (1.1 to 21.5)		
Diff	5.5 (0.2 to 31.4)	0.013	−0.3 (− 5.9 to 15.4)	0.98	0.064
Hippuric acid					
Pre	17680 (9444 to 55008)		24075 (11958 to 39646)		
Post	15225 (8654 to 40673)		12211 (11086 to 28936)		
Diff	−3572 (− 18398 to 1041)	0.068	−2788 (− 15907 to 4975)	0.33	0.68
4-Methylcatechol-*O*-sulfate					
Pre	1630 (815 to 3580)		1209 (753 to 1889)		
Post	1228 (691 to 2722)		997 (647 to 1899)		
Diff	−182 (− 1087 to 733)	0.47	−221 (− 617 to 255)	0.28	0.95
4-Hydroxybenzaldehyde					
Pre	72.6 (40.3 to 130)		81.2 (58.4 to 105)		
Post	53.5 (48.9 to 93.3)		48.7 (39.5 to 75.5)		
Diff	−24.8 (− 56.1 to 6.5)	0.029	−34.3 (− 52.8 to − 6.8)	0.019	0.78
Ferulic acid-4-*O*-sulfate					
Pre	2.3 (0.5 to 7.0)		4.0 (0.0 to 12.3)		
Post	1.9 (0.8 to 23.8)		6.1 (1.6 to 9.7)		
Diff	0.5 (− 2.9 to 21.1)	0.20	1.1 (− 7.7 to 9.1)	0.55	0.69
Dihydroisoferulic acid-3-*O*-sulfate					
Pre	7.2 (0.0 to 17.5)		4.9 (0.0 to 14.1)		
Post	9.2 (2.1 to 23.7)		7.6 (1.0 to 19.4)		
Diff	1.0 (− 7.5 to 8.6)	0.47	0.9 (− 6.9 to 13.0)	0.69	0.94
Isoferulic acid-3-*O*-sulfate					
Pre	1.1 (0.3 to 2.3)		0.8 (0.4 to 5.7)		
Post	4.5 (0.4 to 13.7)		1.5 (0.3 to 8.6)		
Diff	0.6 (− 1.7 to 10.2)	0.25	0.4 (− 1.6 to 2.4)	0.55	0.67
Dihydroisoferulic acid-3-*O*-β-*D*-glucuronide					
Pre	19.1 (7.2 to 45.7)		25.8 (2.5 to 82.4)		
Post	20.7 (8.1 to 60.8)		16.1 (5.4 to 43.4)		
Diff	1.2 (− 15.4 to 34.5)	0.47	−4.9 (− 70.9 to 27.7)	0.55	0.31
Isoferulic acid-3-*O*-β-*D*-glucuronide					
Pre	22.3 (5.2 to 67.5)		21.2 (1.8 to 54.6)		
Post	27.3 (8.5 to 136.4)		21.3 (5.7 to 58.6)		
Diff	24.4 (− 18.0 to 96.2)	0.044	2.7 (− 10.1 to 36.8)	0.55	0.21

(Continued)

TABLE 3 | Continued

	Active (n = 27)		Control (n = 20)		Active vs. control
nmol/L	**Median (IQR)**	p^*	**Median (IQR)**	p^*	$p^{\#}$
Dihydroferulic acid-4-*O*-sulfate					
Pre	3.7 (1.2 to 8.3)		1.9 (0.2 to 3.6)		
Post	1.6 (0.9 to 5.1)		2.7 (1.4 to 5.2)		
Diff	−1.5 (− 3.1 to − 0.3)	0.006	0.8 (− 0.9 to 2.9)	0.31	0.010
3-(3-hydroxyphenyl)propanoic acid					
Pre	805 (44 to 2377)		350 (14 to 1479)		
Post	470 (72 to 4306)		414 (112 to 1624)		
Diff	29 (− 495 to 831)	0.43	36 (− 917 to 345)	0.74	0.76
***m*-Coumaric acid**					
Pre	111 (35 to 146)		84 (63 to 110)		
Post	55 (38 to 78)		58 (53 to 85)		
Diff	−62 (− 82 to 16)	0.014	−28 (− 58 to 2)	0.093	0.33
***o*-Coumaric acid**					
Pre	218 (81 to 356)		125 (81 to 234)		
Post	87 (47 to 158)		81 (50 to 162)		
Diff	−116 (− 230 to − 35)	<0.001	−36 (− 111 to − 1)	0.006	0.019

*Diff, median difference between baseline and follow up plasma measurements; IQR, interquartile range; nmol/L = nanomol/liter. *The within group difference from baseline to study end. #The between group differences for Δ (difference from baseline to study end).*

This indicates that larger RCTs might be feasible, to confirm exploratory results in the current pilot study.

Our findings are somewhat inconclusive. While some cognitive improvements were observed in the AG, there were no significant changes in serum levels of some risk factors for dementia; i.e., fasting glucose, HbA1c or pro-inflammatory cytokines. There was a non-significant decrease in serum levels of RANTES in the AG and a non-significant increase in the NC during the study period. However, the between-group difference in Δ serum levels of RANTES was statistically significant.

Furthermore, we observed a significant increase in serum levels of total cholesterol and triglycerides in AG. The lipid profile of the NC group did not change significantly, and since we have no information about statin use or use of other lipid lowering medications in the NC group, the observed difference should be interpreted cautiously.

Previous studies using Medox® have shown a statistically significant increase in HDL-cholesterol (Qin et al., 2009; Zhu et al., 2011; Hassellund et al., 2013) and a decrease in LDL-cholesterol (Qin et al., 2009; Zhu et al., 2011). This is of clinical interest, as higher HDL-cholesterol is associated with lower cardiovascular risk (Barter et al., 2007), while high levels of total cholesterol, triglycerides and LDL are associated with higher cardiovascular risk (Stone et al., 2014).

The differences in the findings between our study and these previous studies might be due to differences in participants, as well as in anthocyanin supplementation dose and duration, or other factors. Furthermore, other studies included dyslipidemic and hypercholesterolemic participants not using statins or any other lipid lowering treatment (Qin et al., 2009; Zhu et al., 2011), whereas in our study, the median cholesterol at baseline in the intervention group was 4.0 mmol/l, in addition 70% were taking statins or other lipid lowering medication.

Regarding the inflammation markers, RANTES promotes activation and migration of leukocytes and mediates neuroinflammation and brain microvascular dysfunction (Appay and Rowland-Jones, 2001; Dénes et al., 2010; Yilmaz and Granger, 2010). There was a significant between-group difference for ΔRANTES, although anthocyanin supplementation did not significantly reduce RANTES in the AG. Still, our results are consistent with similar findings in a randomized, double-blind trial in hypercholesterolemic individuals consuming purified anthocyanins for 24 weeks (Song et al., 2014), and in a parallel-designed, placebo-controlled trial (Karlsen et al., 2007). Other studies did not report a reduction of pro-inflammatory mediators after anthocyanin supplementation (Hassellund et al., 2013; Kent et al., 2015). Therefore, the anti-inflammatory effect of anthocyanins and the potential to reduce neuroinflammation and brain microvascular dysfunction associated with cognitive decline in adults at risk of dementia (Grammas, 2011) should be studied in larger randomized studies.

The beneficial effect of anthocyanins might possibly be due to their degradation products and metabolites (Feliciano et al., 2016) as absorption of intact anthocyanins is reported to be low (Zhong et al., 2017). Rodriguez-Mateos et al. quantified metabolites in plasma after blueberry consumption, and showed that anthocyanin-derived metabolites correlated with *in vivo* effects (Rodriguez-Mateos et al., 2013, 2019). Furthermore, circulating anthocyanin metabolites were shown to improve vascular function when injected in an *in vivo* model of vascular function, and cardiovascular benefits after consumption of anthocyanins were linked with anthocyanin

TABLE 4 | Changes from baseline to 16 weeks follow-up in cognitive variables, for participants with supplementation (active).

	Active (n = 27)	
	Median (IQR)	p*
CERAD (points) Learning		
Pre	20 (16 to 22)	
Post	21 (17 to 25)	
Diff	2 (− 1 to 3)	0.016
Recall		
Pre	6 (4 to 8)	
Post	7 (5 to 9)	
Diff	1 (0 to 2)	<0.001
Recognition		
Pre	20 (16 to 20)	
Post	20 (19 to 20)	
Diff	0 (0 to 1)	0.047
TMT A (sec)		
Pre	32 (21 to 53)	
Post	34 (23 to 39)	
Diff	−2 (− 6 to 2)	0.081
TMT B$^{n\ =\ 19}$ (sec)		
Pre	85.50 (62.25 to 118)	
Post	69.50 (56 to 100.75)	
Diff	−2.0 (− 19.75 to 2.25)	0.16
STROOP (score) Word		
Pre	87 (72 to 67)	
Post	87 (80 to 103)	
Diff	6 (1 to 10)	<0.001
Color		
Pre	61 (52 to 67)	
Post	62 (54 to 69)	
Diff	2 (− 1 to 6)	0.044
Word-color		
Pre	34 (29 to 41)	
Post	34 (28 to 41)	
Diff	0 (− 3 to 5)	0.67

*TMT, Trail Making Test; Diff, median difference between baseline and follow up results in cognitive tests; IQR, interquartile range. *The within group difference from baseline to study end.*

metabolites as mediators of change in cellular gene programs (Rodriguez-Mateos et al., 2019).

In our study, we were able to measure a total of 29 anthocyanin metabolites. The results were conflicting, as we found various anthocyanin metabolites to be both significantly increasing and decreasing in the AG. In the NC group as well, we found significant changes. This is in line with findings in another study reporting an increase of some metabolites, and a decrease in others after ingestion of anthocyanins over time (Feliciano et al., 2016). A possible explanation for our results could be high inter-individual variability and the influence of the background diet on the concentration of these compounds in blood, as some of these metabolites could arise from the consumption of other anthocyanin-rich foods such as berries and red wine or other food components in the diet. It is also possible that the results could be related to the handling and analysis of the blood samples.

As far as we know, the presence of anthocyanin metabolites in plasma after Medox® use is reported in only one other clinical study, where 8 out of 17 anthocyanin metabolites were found to be significantly increased. However, this was analyzed in blood samples collected 1–3 h after ingestion of the morning dose of anthocyanins (320 mg), and not after daily consumption and an overnight fast (Hassellund et al., 2013).

The improvement on several cognitive tests should be interpreted cautiously due to potential learning effects related to the relatively short test–retest interval, and the lack of a comparison group. Nonetheless, our results are in line with previous smaller studies involving participants with MCI, reporting improved cognition after ingestion of anthocyanins (Krikorian et al., 2010a,b). Our results are also in line with the results of a randomized, double-blinded, placebo-controlled study reporting improved episodic memory after 3 months of anthocyanin supplementation (Whyte et al., 2018).

Intake of anthocyanin capsules in the dosage of 320 mg/day appears to be well tolerated and safe. None of the safety blood tests were found to be out of a clinically acceptable range or necessitating medical follow-up after study-end. This is consistent with previous studies (Karlsen et al., 2007; Qin et al., 2009).

The major limitations of this pilot study are the non-randomized open-label design, the small sample size, and the relatively short intervention period. Although all the participants were told not to change their lifestyle during the intervention period, we have no data on this. Further we had no detailed dietary assessment, and thus we cannot exclude the possibility of differences in the background diet between the groups before or during the study. However, the participants were instructed to maintain their dietary and lifestyle habits during the study period, and to take the capsules 30 min before or 120 min after meals, as concomitant ingestion of certain types of food may counteract the effect of flavonoids (Lorenz et al., 2007).

The NC were recruited separately and differed from the participants by being a healthy group that did not receive any intervention, and did not perform cognitive testing. Thus power calculations for further studies might be compromised. However, we provide descriptive statistics including variance measurements which may be helpful in planning sample size in later studies.

An important strength of our study is the well characterized combination of nutraceuticals used, which facilitates comparison with other studies regarding both source of anthocyanins and dosage. Of note, this is, to our knowledge, the first study on cognitive function in adults with increased risk of dementia where Medox® is being used as the source of anthocyanin. Thus our study might also facilitate investigation of the effect of different proprietary blueberry formulatons, shown by Whyte et al. to be of importance (Whyte et al., 2018).

All things considered, adequately powered, randomized studies are warranted to better understand how anthocyanins and their metabolites may affect relevant mechanisms, including their possible protective role in epigenetic modifications that potentially benefit the aging brain and reduce the risk for dementia.

ETHICS STATEMENT

All participants provided written informed consent, and the study has been approved by the Regional Ethics Committee (Approval 2014/1966). The study has been registered at ClinicalTrials.gov (NCT02409446).

AUTHOR CONTRIBUTIONS

DA, HS, AB, and AL planned and designed the study. AB conducted the study and collected the data. RB performed the serum analyses. MT and AR-M performed the plasma analyses. AB and ID conducted the statistical analyses. All authors wrote the manuscript and critically reviewed the manuscript.

FUNDING

This study was financed by a grant from Sandnes Sparebank, Sandnes, Norway and the Norwegian Health Association, grant number 7330. Medpalett, Sandnes, Norway contributed to the study by producing Medox® free of charge. Medpalett had no influence on the design or conduction of the study, the analyses and interpretation of data, or regarding the decision to publish the findings. This paper represents independent research partly funded by the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed are those of the author and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care.

ACKNOWLEDGMENTS

We would like to thank the research nurses Reidun Sikveland Meling and Jorunn Margrete Nilsen for their contribution in the conduction of the study. We also thank Hellen Svalestad for her contribution to participant recruitment from the cardiology department, and Bjarne Hervik for his contribution in recruiting healthy normal controls.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2019.00536/full#supplementary-material

FIGURE S1 | Changes from baseline to 16 weeks follow-up in serum lipids, for participants with anthocyanin supplementation. mmol/L, millimole/liter.

FIGURE S2 | Changes from baseline to 16 weeks follow-up in serum fasting glucose and HbA1c, for participants with anthocyanin supplementation. HbA1a, glycosylated hemoglobin; mmol/L, millimole/liter.

FIGURE S3 | Changes from baseline to 16 weeks follow-up in serum cytokines, for participants with anthocyanin supplementation. RANTES;CCL-5/RANTES (regulated on activation, normal T-cell expressed and secreted). pg/mL, picomolar/milliliter.

FIGURE S4 | Changes from baseline to 16 weeks follow-up in plasma anthocyanin metabolites, for participants with and without anthocyanin supplementation. Measurement unit: nmol/L, nanomolar/liter, except for hippuric acid (nmol/10L) and coumaric acid-4-*O*-β-*D*-glucuronide (nmol/0.033L).

FIGURE S5 | Changes from baseline to 16 weeks follow-up in CERAD.

FIGURE S6 | Changes from baseline to 16 weeks follow-up in Trail Making Test (TMT). Sec, seconds.

FIGURE S7 | Changes from baseline to 16 weeks follow-up in Stroop test.

TABLE S1 | Changes from baseline to 16 weeks follow-up in serum variables, for participants with supplementation (active) and for control participants. $\bar{x}$, mean; SD, standard deviation; *The within group difference from baseline to study end tested with paired-samples *t*-test. $^{\$}$independent samples *t*-test for the between group differences for Δ (difference from baseline to study end). $^{\#}$With Welch correction for heteroscedasticity. RANTES;CCL-5/RANTES (regulated on activation, normal T-cell expressed and secreted). Diff, mean difference between baseline and follow up results in serum measurements.

TABLE S2 | Changes from baseline to 16 weeks follow-up in cognitive variables, for participants with supplementation (active). $\bar{x}$, mean; SD, standard deviation; TMT, Trail Making Test; Diff, mean difference between baseline and follow up results in cognitive tests. *The within group difference from baseline to study end tested with paired-samples *t*-test.

REFERENCES

Appay, V., and Rowland-Jones, S. L. (2001). RANTES: a versatile and controversial chemokine. *Trends Immunol.* 22, 83–87. doi: 10.1016/s1471-4906(00)01812-3

Ashendorf, L., Jefferson, A. L., O'connor, M. K., Chaisson, C., Green, R. C., and Stern, R. A. (2008). Trail making test errors in normal aging, mild cognitive impairment, and dementia. *Arch. Clin. Neuropsychol.* 23, 129–137. doi: 10.1016/j.acn.2007.11.005

Barter, P., Gotto, A. M., Larosa, J. C., Maroni, J., Szarek, M., Grundy, S. M., et al. (2007). HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N. Engl. J. Med.* 357, 1301–1310. doi: 10.1056/nejmoa064278

Dénes, Á, Humphreys, N., Lane, T. E., Grencis, R., and Rothwell, N. (2010). Chronic systemic infection exacerbates ischemic brain damage via a CCL5 (regulated on activation, normal T-cell expressed and secreted)-mediated proinflammatory response in mice. *J. Neurosci.* 30, 10086–10095. doi: 10.1523/JNEUROSCI.1227-10.2010

Faria, A., Meireles, M., Fernandes, I., Santos-Buelga, C., Gonzalez-Manzano, S., Dueñas, M., et al. (2014). Flavonoid metabolites transport across a human BBB model. *Food Chem.* 149, 190–196. doi: 10.1016/j.foodchem.2013.10.095

Feliciano, R. P., Istas, G., Heiss, C., and Rodriguez-Mateos, A. J. M. (2016). Plasma and urinary phenolic profiles after acute and repetitive intake of wild blueberry. *Molecules* 21, 1120. doi: 10.3390/molecules21091120

Fladby, T., Pålhaugen, L., Selnes, P., Waterloo, K., Bråthen, G., Hessen, E., et al. (2017). Detecting at-risk alzheimer's disease cases. *J. Alzheimer's Dis.* 60, 97–105.

Folstein, M. F., Folstein, S. E., and Mchugh, P. R. (1975). "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198.

Golden, C. J. (1976). Identification of brain disorders by the stroop color and word test. *J. Clin. Psychol.* 32, 654–658. doi: 10.1002/1097-4679(197607)32:3<654::aid-jclp2270320336>3.0.co;2-z

Grammas, P. (2011). Neurovascular dysfunction, inflammation and endothelial activation: implications for the pathogenesis of Alzheimer's disease. *J. Neuroinflamm.* 8:26. doi: 10.1186/1742-2094-8-26

Hassellund, S., Flaa, A., Kjeldsen, S., Seljeflot, I., Karlsen, A., Erlund, I., et al. (2013). Effects of anthocyanins on cardiovascular risk factors and inflammation in pre-hypertensive men: a double-blind randomized placebo-controlled crossover study. *J. Hum. Hypertens.* 27, 100–106. doi: 10.1038/jhh.2012.4

Hein, S., Whyte, A. R., Wood, E., Rodriguez-Mateos, A., and Williams, C. M. (2019). Systematic review of the effects of blueberry on cognitive performance as we age. *J. Gerontol. A Biol. Sci. Med. Sci.* [Epub ahead of print]

Karlsen, A., Retterstøl, L., Laake, P., Paur, I., Kjølsrud-Bøhn, S., Sandvik, L., et al. (2007). Anthocyanins inhibit nuclear factor-κB activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults. *J. Nutr.* 137, 1951–1954. doi: 10.1093/jn/137.8.1951

Kent, K., Charlton, K., Roodenrys, S., Batterham, M., Potter, J., Traynor, V., et al. (2015). Consumption of anthocyanin-rich cherry juice for 12 weeks improves memory and cognition in older adults with mild-to-moderate dementia. *Eur. J. Nutr.* 56, 333–341. doi: 10.1007/s00394-015-1083-y

Krikorian, R., Nash, T. A., Shidler, M. D., Shukitt-Hale, B., and Joseph, J. A. (2010a). Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment. *Br. J. Nutr.* 103, 730–734. doi: 10.1017/S0007114509992364

Krikorian, R., Shidler, M. D., Nash, T. A., Kalt, W., Vinqvist-Tymchuk, M. R., Shukitt-Hale, B., et al. (2010b). Blueberry supplementation improves memory in older adults†. *J. Agric. Food Chem.* 58, 3996–4000. doi: 10.1021/jf9029332

Li, D., Zhang, Y., Liu, Y., Sun, R., and Xia, M. (2015). Purified anthocyanin supplementation reduces dyslipidemia, enhances antioxidant capacity, and prevents insulin resistance in diabetic patients. *J. Nutr.* 145, 742–748. doi: 10.3945/jn.114.205674

Lorenz, M., Jochmann, N., Von Krosigk, A., Martus, P., Baumann, G., Stangl, K., et al. (2007). Addition of milk prevents vascular protective effects of tea. *Eur. Heart J.* 28, 219–223. doi: 10.1093/eurheartj/ehl442

Mitchell, A. J., Bird, V., Rizzo, M., and Meader, N. (2010). Diagnostic validity and added value of the geriatric depression scale for depression in primary care: a meta-analysis of GDS30 and GDS15. *J. Affect. Disord.* 125, 10–17. doi: 10.1016/j.jad.2009.08.019

Morris, J. C., Heyman, A., Mohs, R. C., Hughes, J. P., Van Belle, G., Fillenbaum, G., et al. (1989). The consortium to establish a registry for Alzheimer's disease (CERAD). Part I. clinical and neuropsychological assessment of Alzheimer's disease. *Neurology* 39, 1159–1165.

Neveu, V., Perez-Jiménez, J., Vos, F., Crespy, V., Du Chaffaut, L., Mennen, L., et al. (2010). Phenol-explorer: an online comprehensive database on polyphenol contents in foods. *Database* 2010:ba024. doi: 10.1093/database/bap024

Qin, Y., Xia, M., Ma, J., Hao, Y., Liu, J., Mou, H., et al. (2009). Anthocyanin supplementation improves serum LDL-and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. *Am. J. Clin. Nutr.* 90, 485–492. doi: 10.3945/ajcn.2009.27814

Reitan, R., and Wolfson, D. (1985). *The Halstead-Reitan Neuropsychological Test Battery: Therapy and Clinical Assessment*. Tucson, AZ: Neuropsychological Press.

Rodriguez-Mateos, A., Istas, G., Boschek, L., Feliciano, R. P., Mills, C. E., Boby, C., et al. (2019). Circulating anthocyanin metabolites mediate vascular benefits of blueberries: insights from randomized controlled trials, metabolomics, and nutrigenomics. *J. Gerontol. A Biol. Sci. Med. Sci.* [Epub ahead of print]

Rodriguez-Mateos, A., Rendeiro, C., Bergillos-Meca, T., Tabatabaee, S., George, T. W., Heiss, C., et al. (2013). Intake and time dependence of blueberry flavonoid–induced improvements in vascular function: a randomized, controlled, double-blind, crossover intervention study with mechanistic insights into biological activity. *Am. J. Clin. Nutr.* 98, 1179–1191. doi: 10.3945/ajcn.113.066639

Rodriguez-Mateos, A., Feliciano, R. P., Boeres, A., Weber, T., Dos Santos, C. N., Ventura, M. R., et al. (2016). Cranberry (poly) phenol metabolites correlate with improvements in vascular function: a double-blind, randomized, controlled, dose-response, crossover study. *Mol. Nutr. Food Res.* 60, 2130–2140. doi: 10.1002/mnfr.201600250

Scarpina, F., and Tagini, S. (2017). The stroop color and word test. *Front. Psychol.* 8:557. doi: 10.3389/fpsyg.2017.00557

Song, F., Zhu, Y., Shi, Z., Tian, J., Deng, X., Ren, J., et al. (2014). Plant food anthocyanins inhibit platelet granule secretion in hypercholesterolaemia: involving the signalling pathway of PI3K–Akt. *Thromb. Haemost.* 112, 981–991. doi: 10.1160/th13-12-1002

Spagnuolo, C., Moccia, S., and Russo, G. L. (2017). Anti-inflammatory effects of flavonoids in neurodegenerative disorders. *Eur. J. Med. Chem.* 153, 105–115. doi: 10.1016/j.ejmech.2017.09.001

Spencer, J. P., Vafeiadou, K., Williams, R. J., and Vauzour, D. (2012). Neuroinflammation: modulation by flavonoids and mechanisms of action. *Mol. Aspects Med.* 33, 83–97. doi: 10.1016/j.mam.2011.10.016

Stone, N. J., Robinson, J. G., Lichtenstein, A. H., Merz, C. N. B., Blum, C. B., Eckel, R. H., et al. (2014). 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the american college of cardiology/american heart association task force on practice guidelines. *J. Am. Coll. Cardiol.* 63, 2889–2934.

Wallace, T. C., Slavin, M., and Frankenfeld, C. L. (2016). Systematic review of anthocyanins and markers of cardiovascular disease. *Nutrients* 8:32. doi: 10.3390/nu8010032

Whyte, A., Cheng, N., Fromentin, E., and Williams, C. J. N. (2018). A randomized, double-blinded, placebo-controlled study to compare the safety and efficacy of low dose enhanced wild blueberry powder and wild blueberry extract (ThinkBlue) in maintenance of episodic and working memory in older adults. *Nutrients* 10:E660. doi: 10.3390/nu10060660

World Health Organization [WHO] (1992). *The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Descriptions and Diagnostic Guidelines*. Geneva: World Health Organization.

Xia, M., Ling, W., Zhu, H., Wang, Q., Ma, J., Hou, M., et al. (2007). Anthocyanin prevents CD40-activated proinflammatory signaling in endothelial cells by regulating cholesterol distribution. *Arterioscler. Thromb. Vasc. Biol.* 27, 519–524. doi: 10.1161/01.atv.0000254672.04573.2d

Yang, L., Ling, W., Du, Z., Chen, Y., Li, D., Deng, S., et al. (2017). Effects of anthocyanins on cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials. *Adv. Nutr.* 8, 684–693. doi: 10.3945/an.116.014852

Yilmaz, G., and Granger, D. N. (2010). Leukocyte recruitment and ischemic brain injury. *Neuromol. Med.* 12, 193–204. doi: 10.1007/s12017-009-8074-1

Zhong, S., Sandhu, A., Edirisinghe, I., Burton-Freeman, I., and Research, F. (2017). Characterization of wild blueberry polyphenols bioavailability and kinetic profile in plasma over 24-h period in human subjects. *Mol. Nutr. Food Res.* 61:1700405. doi: 10.1002/mnfr.201700405

Zhu, Y., Xia, M., Yang, Y., Liu, F., Li, Z., Hao, Y., et al. (2011). Purified anthocyanin supplementation improves endothelial function via NO-cGMP activation in hypercholesterolemic individuals. *Clin. Chem.* 57, 1524–1533. doi: 10.1373/clinchem.2011.167361

Conflict of Interest Statement: AB has received support for conference participation from Evonik. DA has received research support and/or honoraria from Astra-Zeneca, H. Lundbeck, Novartis Pharmaceuticals, and GE Health, and serves as paid consultant for H. Lundbeck and Axovant.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Material

Figure S1

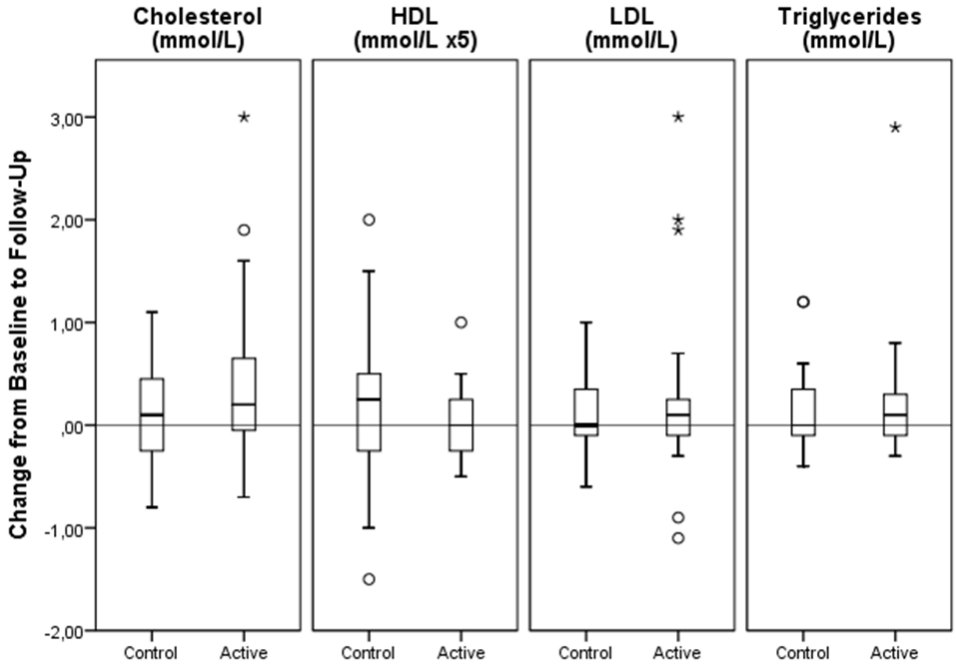


Figure S2

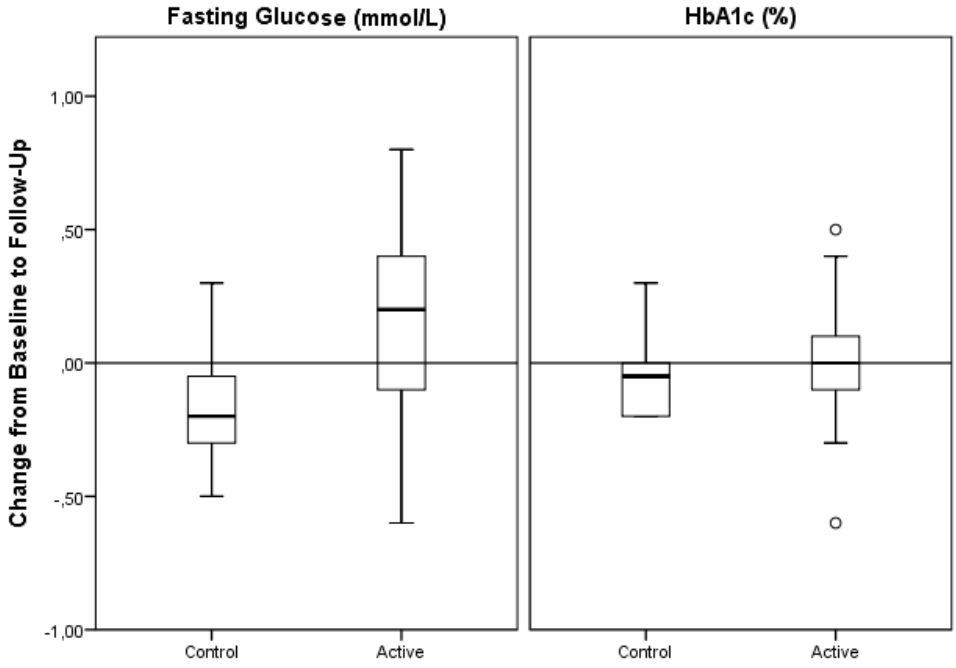

Fasting Glucose (mmol/L)
HbA1c (%)
Change from Baseline to Follow-Up
1,00
,50
,00
-,50
-1,00
Control
Active
Control
Active

Figure S3

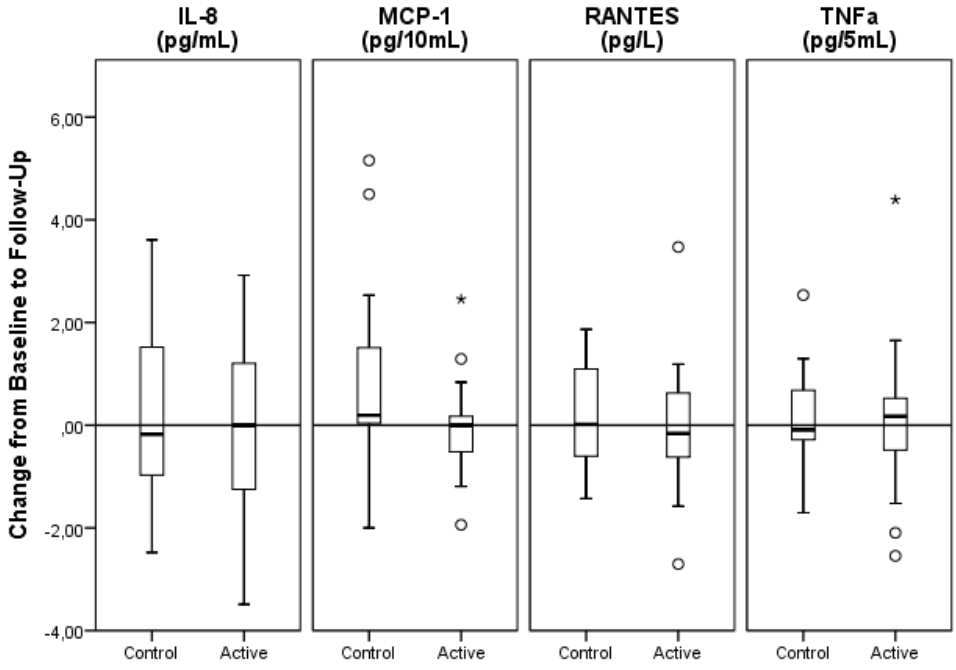
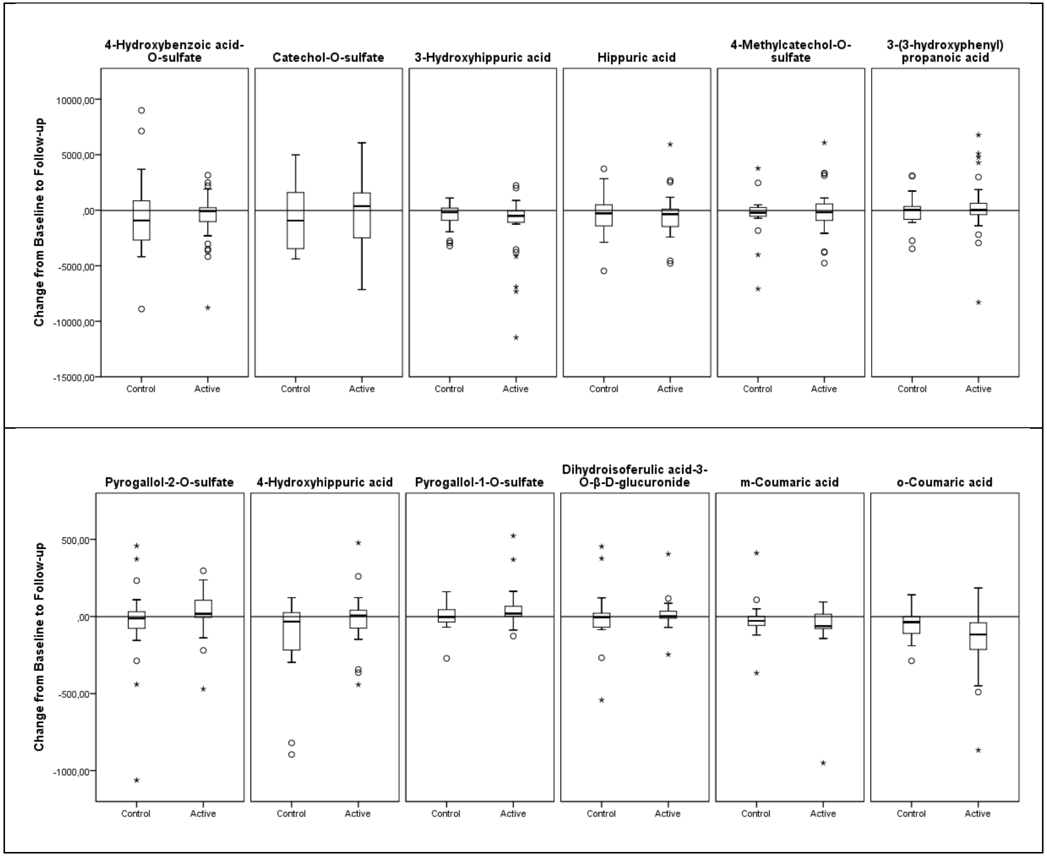
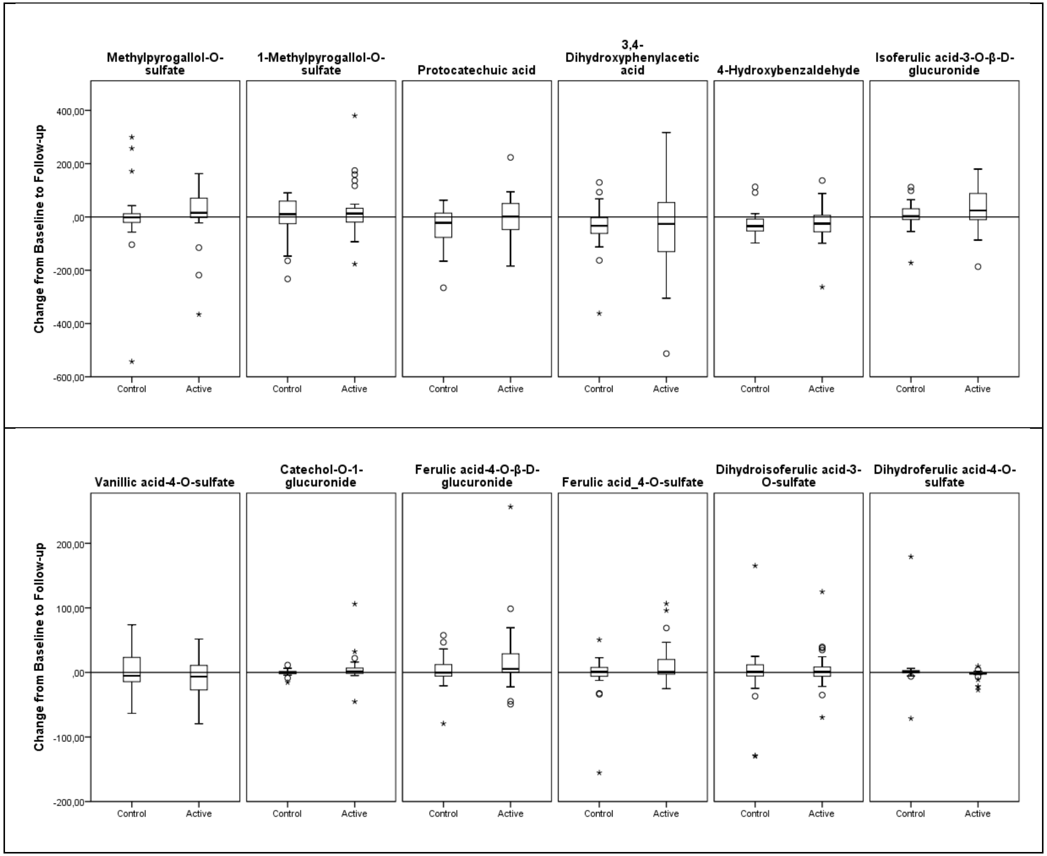

IL-8
(pg/mL)
MCP-1
(pg/10mL)
RANTES
(pg/L)
TNFa
(pg/5mL)
Change from Baseline to Follow-Up
6,00
4,00
2,00
,00
-2,00
-4,00
Control
Active
Control
Active
Control
Active
Control
Active

Figure S4


4-Hydroxybenzoic acid-O-sulfate
Catechol-O-sulfate
3-Hydroxyhippuric acid
Hippuric acid
4-Methylcatechol-O-sulfate
3-(3-hydroxyphenyl) propanoic acid
Change from Baseline to Follow-up
10000,00
5000,00
,00
-5000,00
-10000,00
-15000,00
Control
Active
Pyrogallol-2-O-sulfate
4-Hydroxyhippuric acid
Pyrogallol-1-O-sulfate
Dihydroisoferulic acid-3-O-β-D-glucuronide
m-Coumaric acid
o-Coumaric acid
500,00
-500,00
-1000,00


Methylpyrogallol-O-sulfate
1-Methylpyrogallol-O-sulfate
Protocatechuic acid
3,4-Dihydroxyphenylacetic acid
4-Hydroxybenzaldehyde
Isoferulic acid-3-O-β-D-glucuronide
Vanillic acid-4-O-sulfate
Catechol-O-1-glucuronide
Ferulic acid-4-O-β-D-glucuronide
Ferulic acid_4-O-sulfate
Dihydroisoferulic acid-3-O-sulfate
Dihydroferulic acid-4-O-sulfate
Change from Baseline to Follow-up
400,00
200,00
,00
-200,00
-400,00
-600,00
100,00
-100,00
Control
Active

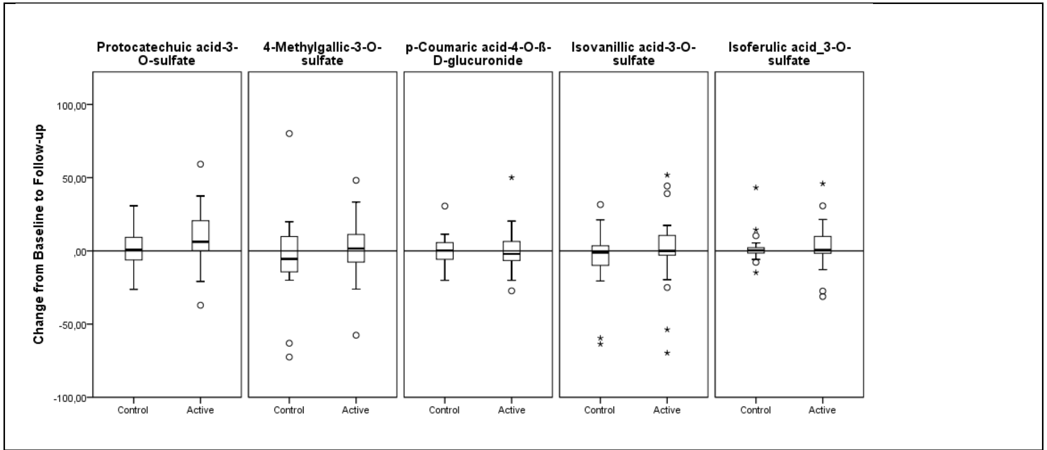

Protocatechuic acid-3-O-sulfate
4-Methylgallic-3-O-sulfate
p-Coumaric acid-4-O-ß-D-glucuronide
Isovanillic acid-3-O-sulfate
Isoferulic acid_3-O-sulfate
Change from Baseline to Follow-up
100,00
50,00
,00
-50,00
-100,00
Control
Active

Figure S5

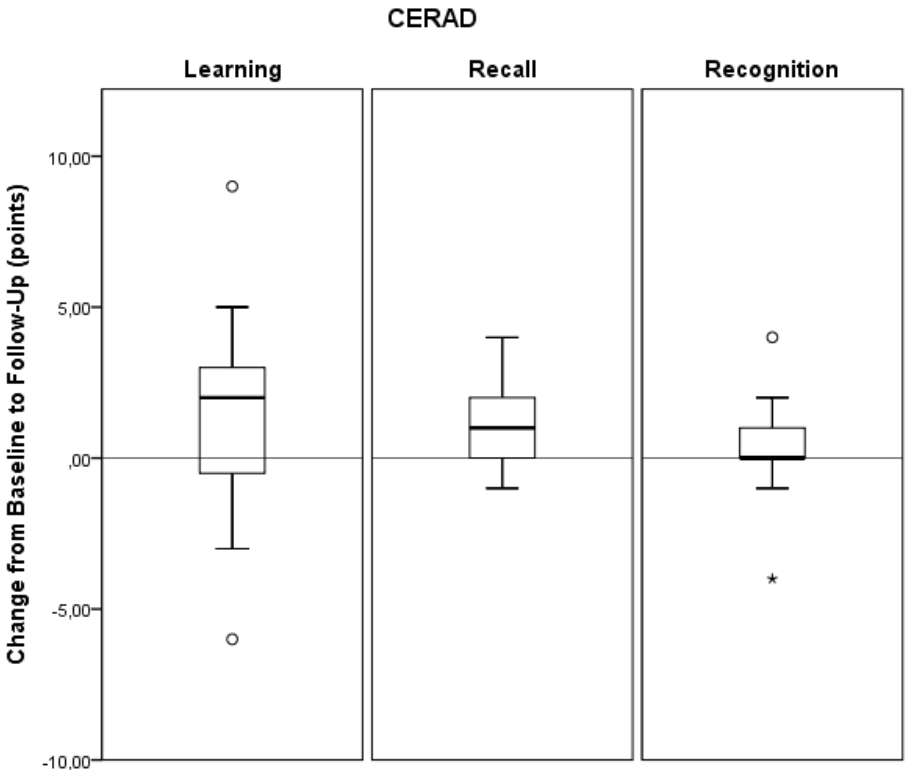

CERAD
Learning
Recall
Recognition
Change from Baseline to Follow-Up (points)
10,00
5,00
,00
-5,00
-10,00

Figure S6

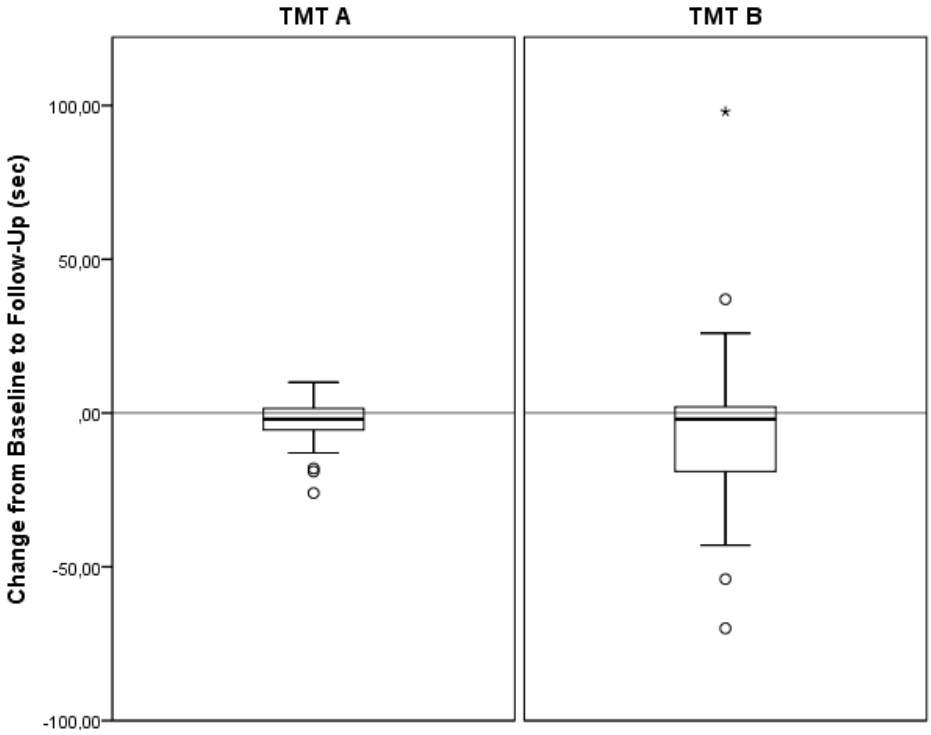

TMT A
TMT B
Change from Baseline to Follow-Up (sec)
100,00
50,00
,00
-50,00
-100,00

Figure S7

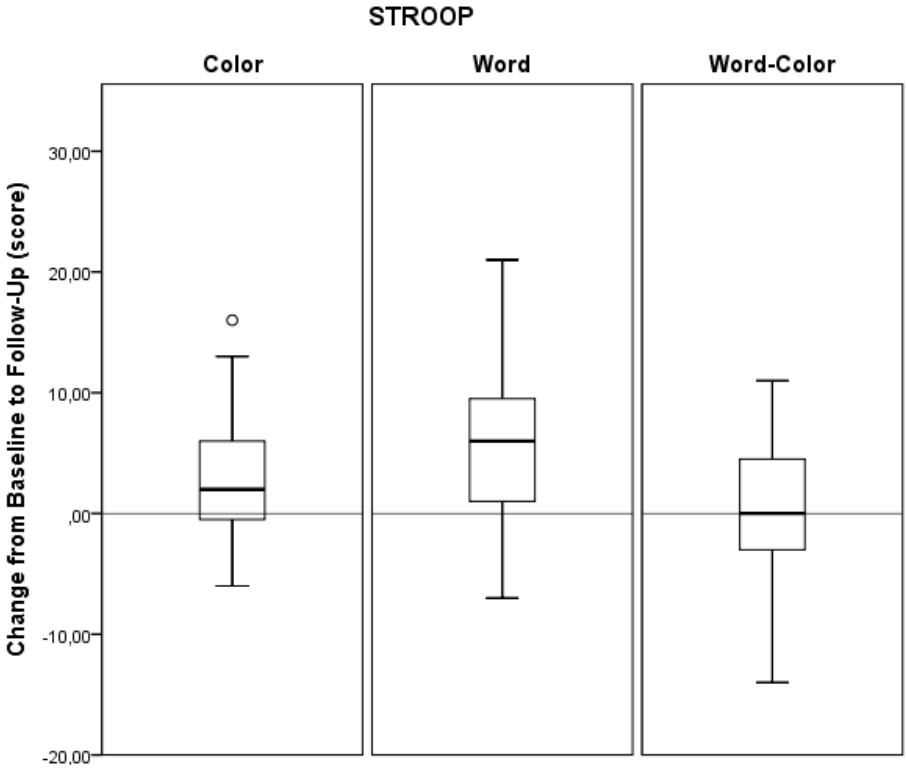

STROOP
Color
Word
Word-Color
Change from Baseline to Follow-Up (score)
30,00
20,00
10,00
,00
-10,00
-20,00

Table S1 Changes from baseline to 16 weeks follow-up in serum variables, for participants with supplementation (active) and for control participants.

	Active (n = 27) $\overline{x}$ (SD, range)	P^*	**Control (n = 20)** $\overline{x}$ (SD, range)	P^*	Active vs Control $p^\$$
Cholesterol			**Cholesterol**		
Pre	4.3 (1.4, 1.3 to 6.7)		5.0 (0.8, 3.4 to 6.5)		
Post	4.7 (1.4, 2.6 to 7.3)		5.2 (0.8, 3.7 to 6.6)		
Diff	0.4 (0.8, -0.7 to 3.0)	0.01	0.1 (0.5, -0.8 to 1.1)	0.25	0.14$^\#$
HDL			**HDL**		
Pre	1.2 (0.3, 0.8 to 1.9)		1.5 (0.5, 1 to 2.8)		
Post	1.2 (0.3, 0.8 to 1.8)		1.5 (0.5, 0.9 to 2.8)		
Diff	0.0 (0.1, -0.1 to 0.2)	0.81	0.1 (0.2, -0.3 to 0.4)	0.20	0.27$^\#$
LDL			**LDL$^{n=19}$**		
Pre	2.8 (1.3, 1.2 to 5.1)		3.3 (0.9, 1.3 to 4.7)		
Post	3.1 (1.4, 0.9 to 5.4)		3.2 (0.8, 1.6 to 4.6)		
Diff	0.2 (0.8, -1.1 to 3)	0.16	0.1 (0.4, -0.6 to 1)	0.54	0.37$^\#$
Triglycerides			**Triglycerides**		
Pre	1.1 (0.5, 0.6 to 2.4)		0.8 (0.4, 0.4 to 2)		
Post	1.4 (0.5, 0.5 to 5.3)		1.2 (0.7, 0.4 to 3.2)		
Diff	0.2 (0.6, -3.5 to 2.9)	0.05	0.2 (0.4, -0.4 to 1.2)	0.067	0.74
Fasting glucose			**Fasting glucose**		
Pre	5.6 (0.8, 4.5 to 7.3)		5.3 (0.5, 4.6 to 6.6)		
Post	5.7 (0.7, 4.6 to 7.2)		5.2 (0.5, 4.3 to 6.3)		
Diff	0.1 (0.4, -0.6 to 0.8)	0.07	-0.2 (0.2, -0.5 to 0.3)	0.005	0.002$^\#$
HbA1c			**HbA1c**		
Pre	5.8 (0.5, 4.9 to 7.9)		5.5 (0.3, 4.8 to 6.2)		
Post	5.8 (0.5, 5.3 to 7.3)		5.4 (0.4, 4.6 to 6.2)		
Diff	-0.0 (0.2, -0.6 to 0.5)	0.88	-0.1 (0.1, -0.2 to 0.3)	0.076	0.33
IL-8			**IL-8**		
Pre	9.0 (2.1, 5.2 to 13.3)		7.9 (1.9, 5.54 to 13.86)		

Post	8.9 (2.5, 3.8 to 13.6)		8.1 (2.0, 4.7 to 12.9)		
Diff	-0.1 (1.7, -3.5 to 2.9)	0.75	0.1 (1.7, -2.5 to 3.6)	0.72	0.63
MCP-1			**MCP-1**		
Pre	42.7 (31.4, 5.8 to 152.5)		56.4 (24.4, 19.5 to 119)		
Post	42.1 (29.6, 6.0 to 133.1)		65.2 (31.2, 20.6 to 139)		
Diff	-0.7 (8.3, -19.4 to 24.6)	0.69	8.8 (16.8, -20 to 51.6)	0.030	0.028[#]
RANTES			**RANTES**		
Pre	9045 (1324, 5483 to 11846)		8971 (1098, 6855 to 11410)		
Post	9012 (1238, 6327 to 12559)		9159 (1297, 5527 to 11643)		
Diff	-33 (1118, -2705 to 3469)	0.88	189 (994, -1428 to 1867)	0.41	0.49
TNFa			**TNFa$^{n=19}$**		
Pre	11.1 (4.4, 3.3 to 24.1)		8.5 (3.6, 3.7 to 16.1)		
Post	11.4 (8.0, 4.2 to 46.1)		9.8 (4.7, 0.98 to 23)		
Diff	0.3 (6.5, -12.7 to 22)	0.85	0.7 (4.9, -8.54 to 12.7)	0.56	0.82

Table S2 Changes from baseline to 16 weeks follow-up in cognitive variables, for participants with supplementation (active).

	Active (n = 27)	
	$\overline{x}$ (SD, range)	P^*
CERAD Learning		
Pre	19.4 (4.6, 8 to 27)	
Post	20.8 (5.4, 8 to 29)	
Diff.	1.4 (3.0, -6 to 9)	0.021
CERAD Recall		
Pre	5.8 (2.2, 1 to 9)	
Post	6.9 (2.6, 1 to 10)	
Diff.	1.1 (1.8, -1 to 4)	< 0.001
CERAD Recognition		
Pre	18.7 (1.7, 14 to 20)	
Post	19.2 (1.6, 13 to 20)	
Diff.	0.5 (1.4, -4 to 4)	0.091
TMT A		
Pre	35.5 (12.5, 15 to 66)	
Post	32.3 (12.2, 13 to 64)	
Diff.	-3.2 (8.4, -26 to 10)	0.059
TMT B		
Pre	96.9 (47.2, 48 to 226)	
Post	91.0 (63.7, 47 to 321)	
Diff.	-5.9 (31.3, -70 to 98)	0.35
STROOP Word		
Pre	83.4 (17.16, 48 to 115)	
Post	88.8 (17.9, 49 to 119)	
Diff.	5.4 (6.1, -7 to 21)	< 0.001
STROOP Color		
Pre	58.9 (12.9, 26 to 83)	
Post	61.4 (12.9, 38 to 88)	
Diff.	2.5 (5.5, -6 to 16)	0.025
STROOP Word-Color		
Pre	34.0 (11, 11 to 56)	
Post	34.2 (12, 14 to 60)	
Diff.	0.2 (5.8, -14 to 11)	0.84

10. Appendices

10.1 Ethical approval

10.2 Consent form for Anthocyanin group

10.3 Consent form for normal controls

10.1 Ethical approval for Anthocyanin Study

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Gjøril Bergva	22845529	17.12.2014	2014/1966 REK sør-øst D
			Deres dato:	Deres referanse:
			28.10.2014	

Vår referanse må oppgis ved alle henvendelser

Dag Årsland
Stavanger universitetssjukehus
Pb 8100 Forus
4068 Stavanger

2014/1966 Effekten av Medox på blodlipider, inflammasjon og oksidativt stress

Forskningsansvarlig: Helse Stavanger HF
Prosjektleder: Dag Årsland

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst) i møtet 26.11.2014. Vurderingen er gjort med hjemmel i helseforskningsloven (hfl.) § 10, jf. forskningsetikkloven § 4.

Prosjektomtale
Teste hypotesen; kardiovaskulære fysiologiske mekanismer, og markører for inflammasjon og oksidasjon i blod, endrer seg i gunstig retning etter behandling med antocyaner (kosttilskudd) hos personer med økt risiko for demens, dvs med koronar hjertesykdom og/eller mild kognitiv svikt. Forskningen; i 2 faser: Fase 1 (Åpen pilotstudie) Inkluderer 30 pasienter som blir gitt Antocyaner i 16 uker. Blodprøve + kognitiv test + fysiologiske tester(Kondisjonstest, Flow-mediert endotelfunksjon, koronar gjennomstrømningsreserve og kontinuerlig rytmeovervåkning av hjertet i 24 timer) v/ baseline og etter 16 uker Fase 2: Randomisert placebo-kontrollert paralellgruppestudie. 150 pasienter-som ovenfor. Endepunkt; blodprøver som har vist vist effekt av antocyaner i fase 1. Blodprøver + kognitive tester tas v/baseline og etter 6 mnd. Subgruppe-Kondisjonstet, Undersøke blodåre på underarmen, gjort hjertefunksjonsvurdering via ultralyd av hjertet og kontinuerlig rytmeovervåkning av hjertet i 24 timer.

Vurdering
I denne studien skal 30 personer med økt risiko for demens inkluderes i en åpen pilotstudie der de får antocyaner i 16 uker og skal evalueres for gunstig effekt på inflammasjon og oksidasjon ved hjelp av en rekke tester. I fase 2 skal 150 pasienter randomiseres til placebo eller antocyan. Endepunkt er blodprøver som har vist effekt i fase 1.

Komiteen er ikke innstilt på å godkjenne fase 2 av studien før fase 1 er gjennomført. Når fase 1 er gjennomført ber komiteen om en redegjørelse for resultatene, før komiteen vurderer fase 2.

Komiteen har ingen innvendinger mot at fase 1 studien gjennomføres som beskrevet i søknad og protokoll. Kosttilskuddet er allerede godkjent og anbefalt brukt hos pasientgruppen. Det er ingen kjente bivirkninger. Deltagelse innebærer en rekke tester, men ingen er forbundet med risiko.

Det opplyses om at blodprøver skal lagres i en generell biobank for forskning om aldersrelaterte hjernesykdommer (SESAM-biobanken), REK-referanse 2014/328. Blodprøvene skal sendes til Karolinsk

Institutt, Sverige, for analyse. Komiteen ber om at deltagerne får informasjonsskriv og samtykkeerklæring for avgivelse av materiale til den generelle forskningsbiobanken, i tillegg til informasjonsskriv og samtykkeerklæring for denne konkrete studien. Komiteen mener at deltagerne bør gis mulighet til å reservere seg mot at materialet inngår i en generell forskningsbiobank, men at de likevel kan delta i studien.

Komiteen setter følgende vilkår for godkjenning av fase 1:
-Deltagerne skal få informasjonsskriv/samtykkeerklæring for avgivelse av materiale til den generelle forskningsbiobanken, i tillegg til informasjonsskriv og samtykkeerklæring for denne konkrete studien.
- Deltagerne skal gis mulighet til å reservere seg mot at materialet inngår i en generell forskningsbiobank, men at de likevel kan delta i studien.

Vedtak
Med hjemmel i helseforskningsloven § 9 jf. 33 godkjenner komiteen at fase 1 av studien gjennomføres under forutsetning av at ovennevnte vilkår oppfylles.

I tillegg til vilkår som fremgår av dette vedtaket, er godkjenningen gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknad og protokoll, og de bestemmelser som følger av helseforskningsloven med forskrifter.

Med hjemmel i helseforskningsloven § 29 tillater komiteen at humant biologisk materiale utføres til utlandet.

Tillatelsen gjelder til 20.11.2016. Av dokumentasjonshensyn skal opplysningene likevel bevares inntil 20.11.2021. Forskningsfilen skal oppbevares avidentifisert, dvs. atskilt i en nøkkel- og en opplysningsfil. Opplysningene skal deretter slettes eller anonymiseres, senest innen et halvt år fra denne dato.

Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder for «Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse og omsorgssektoren».

Dersom det skal gjøres vesentlige endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK.

Prosjektet skal sende sluttmelding på eget skjema, senest et halvt år etter prosjektslutt.

Klageadgang
REKs vedtak kan påklages, jf. forvaltningslovens § 28 flg. Klagen sendes til REK sør-øst D. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK sør-øst D, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Vi ber om at alle henvendelser sendes inn på korrekt skjema via vår saksportal: http://helseforskning.etikkom.no. Dersom det ikke finnes passende skjema kan henvendelsen rettes på e-post til: post@helseforskning.etikkom.no.

Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen

Finn Wisløff
Professor em. dr. med.
Leder

Gjøril Bergva
Rådgiver

Kopi til:stein.tore.nilsen@sus.no; Stavanger universitetssjukehus ved øverste administrative ledelse: post@helse-stavanger.no

10.2 Consent form for Anthocyanin group

Forespørsel om deltakelse i forskningsprosjekt

"ANTOCYAN-STUDIEN"

Bakgrunn og hensikt

Forskere tilknyttet SESAM (Regionalt kompetansesenter for eldremedisin og samhandling) og kardiologisk avdeling ved Stavanger universitetssjukehus ønsker å finne ut om antocyaner, gitt i form av Medox® kapsler.har positive helseeffekter.

Mer presist ønsker vi å undersøke om antocyaner kan påvirke mekanismer som vi tror bidrar til utviklingen av både hjerte-/kar-sykdom og hukommelsesproblemer.

Virkestoffet i Medox kapsler er antocyaner, som er stoffer som finnes i mørke bær (som blåbær og solbær), frukt og flere andre plantevekster. De utgjør plantenes «immunforsvar». Disse stoffene har vist seg å ha antioksidative egenskaper, d.v.s. at de kan beskytte mot skadelige kjedereaksjoner i kroppens celler som potensielt ville kunne bidra til utvikling av karsykdom og hukommelsesproblemer.

Resultatene fra ulike tidligere studier tyder også på at antocyaner reduserer kronisk inflammasjon, altså en indre betennelsestilstand i kroppen, som er knyttet til de aller fleste kroniske sykdommer. Betennelsesnivået øker med alderen og bidrar til at risikoen for å få flere sykdommer øker etter hvert som man blir eldre. Andre resultater tyder på at antocyaner kan bidra til å heve det «gode» kolesterolet på bekostning av det «dårlige». Påvirkning av disse disse faktorene i gunstig retning kan tenkes å redusere faren for å utvikle hukommelsesproblemer.

Hva innebærer studien?

Aktuelle studiedeltakere er personer over 50 år som har stabil koronar hjertesykdom. I tillegg vil vi inkludere personer som har begynnende hukommelsesproblemer.

Praktisk gjennomføring
Studiedeltakerne vil bli gitt Medox kapsler i 16 uker.

De som skal delta i studien må si seg villig til å bli tatt blodprøver av, bli intervjuet og få utført generell medisinsk undersøkelse, , samt å gjennomgå enkle hukommelsestester.

Undersøkelsene vil bli gjort ved studiestart, så etter 16 uker. Ved begge tilfellene må deltakerne møte to ganger. Først hos lege så hos sykepleier.Man får utlevert Medox kapsler som man skal ta daglig, 2 om morgenen og 2 om kvelden i 16 uker. Etter 8 uker vil deltakerne bli kontaktet av studieleder. Etter endt studie skal deltakerne levere inn de tomme Medox-eskene.
Forskerne vil deretter undersøke om det er forskjeller i målingene og testene mellom starttidspunkt og etter 16 uker med inntak av Medox.

Medox er et norsk produkt, utviklet og produsert i Norge. Produsenten, BioLink, vil være involvert i prosjektet med å levere Medox kapsler, men prosjektet er planlagt og vil gjennomføres av forskere ved SUS uten påvirkning fra BioLink.

Mulige fordeler og ulemper

Det vil ikke være økonomiske kostnader forbundet med deltakelse i studien. Eventuelle utgifter vil bli refundert.
Medox er et naturprodukt og har ingen kjente potensielt farlige bivirkninger. Utover ubehag i forbindelse med blodprøver og undersøkelser er det derfor ingen risiko ved å delta.

Hva skjer med prøvene og informasjonen om deg? Personvern

Prøvene tatt av deg og informasjonen som registreres om deg (relevante helseopplysninger, prøvesvar, og resultat på undersøkelsene samt alder og kjønn) skal kun brukes som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer og andre opplysninger som kan brukes for å identifisere deg. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Det betyr at all informasjon om deg er avidentifisert.

Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg.
Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Frivillig deltakelse
Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien. Dette vil ikke få konsekvenser for din videre behandling. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke uten at det påvirker din øvrige behandling. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte;

Overlege/forsker Anne Katrine Bergland anhe@sus.no tlf 97430694
Prosjektleder Dag Årsland dag.arsland@sus.no tlf 51516052

Biobank, økonomi og forsikring

Biobank
Blodprøvene som blir tatt vil bli lagret i en forskningsbiobank ved SUS (SESAM-biobanken). Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Dag Årsland er ansvarshavende for forskningsbiobanken. Det biologiske materialet kan bare brukes etter godkjenning fra Regional komité for medisinsk og helsefaglig forskningsetikk (REK).

Utlevering av materiale og opplysninger til andre
Hvis du sier ja til å delta i studien, gir du også ditt samtykke til at prøver og avidentifiserte opplysninger utleveres til våre samarbeidspartnere ved Karolinska Institutet i Stockholm.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver
Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Økonomi og BioSynth Biolink? rolle
Studien og biobanken er finansiert gjennom forskningsmidler fra Sandnes Sparebank. I tillegg bidrar produsenten av Medox ved å produsere Medox og placebo gratis til prosjektet. Utover dette har sponsor ingen innvirkning på planlegging og gjennomføring av studien

Forsikring
Deltakerne er forsikret gjnnom Pasientforsikringsloven

Informasjon om utfallet av studien
Deltakere har rett til å få informasjon om utfallet/resultatet av studien.

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

_____
(Signert av prosjektdeltaker, dato)

Jeg ønsker å reservere meg mot at mine blodprøver lagres i en generell forskningsbiobank

_____
(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

_____

(Signert, rolle i studien, dato)

10.3 Consent form for normal controls

Forespørsel om deltakelse i forskningsprosjekt

Bakgrunn og hensikt

Forskere tilknyttet SESAM (Regionalt kompetansesenter for eldremedisin og samhandling), og Kardiologisk avdeling ved Stavanger universitessykehus, ønsker nå å finne ut om Medox® kapsler har positive effekter på hjernen. I den forbindelse er flere pasienter inkludert i en studie hvor dette undersøkes, bl.a gjennom blodprøve analyser før og etter Medox-bruk.

Forskningsgruppen ønsker nå å rekruttere personer til å delta i en kontrollgruppe.

Hva innebærer studien?

Aktuelle studiedeltakere er personer over 50 år som er villige til å avgi fastende blodprøver to ganger, med fire måneders mellomrom.

Mulige fordeler og ulemper

Det vil ikke være økonomiske kostnader forbundet med deltakelse i studien. Eventuelle utgifter vil bli refundert. Blodprøvetakingen vil kunne være forbundet med noe ubehag. Dog ikke utover det man forventer ved standard blodprøvetaking.

Hva skjer med prøvene og informasjonen om deg? Personvern

Prøvene tatt av deg og informasjonen som registreres om deg (relevante helseopplysninger, prøvesvar, samt alder og kjønn) skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer og andre opplysninger som kan brukes for å identifisere deg. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Det betyr at all informasjon om deg er avidentifisert.

Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg.
Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Frivillig deltakelse
Det er helt frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien. Dette vil ikke få konsekvenser for deg. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke når som helst. Dersom du senere ønsker å trekke deg eller har spørsmål om studien, kan du kontakte

Overlege/forsker Anne Katrine Bergland anhe@sus.no tlf 97430694
Prosjektleder professor og forskningsleder Dag Årsland dag.aarsland@sus.no tlf 51516052

Biobank, økonomi og forsikring

Biobank
Blodprøvene som blir tatt vil bli lagret i en såkalt forskningsbiobank ved SUS (SESAM-biobanken). Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Professor Dag Årsland er ansvarshavende for forskningsbiobanken. Det biologiske materialet kan bare brukes etter godkjenning fra Regional komité for medisinsk og helsefaglig forskningsetikk (REK).

Utlevering av materiale og opplysninger til andre
Hvis du sier ja til å delta i studien, gir du også ditt samtykke til at prøver og avidentifiserte opplysninger utleveres til våre samarbeidspartnere ved Karolinska Institutet i Stockholm.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver
Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Forsikring
Deltakerne er forsikret gjennom Pasientforsikringsloven

Informasjon om utfallet av studien
Deltakere har rett til å få informasjon om utfallet/resultatet av studien.

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

_____
(Signert av prosjektdeltaker, dato)

Jeg ønsker å reservere meg mot at mine blodprøver lagres i en generell forskningsbiobank

_____
(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

_____
(Signert, rolle i studien, dato)

11. Errata

Paper II, Page 1121; Corrected “PLS-DA” to “Partial least squares discriminant analysis (PLS-DA)”.

Paper II, Page 1122, Table 1; Incorrect number for VOSP T-score Mean (SD) for Controls. Corrected to **52.9.**

Page 7 Incorrect words: “was found for the cytokine CCL-5/“regulated on activation, normal T-cell expressed and secreted” (RANTES). – corrected to “was found for monocyte chemoattractant protein (MCP-1) and fasting glucose.”

Page 10 Missing word: “Vascular risk and dementia progression” – corrected to “Vascular risk factors and dementia progression”

Page 34 Missing word: “Vascular risk and dementia progression” – corrected to “Vascular risk factors and dementia progression”

Page 35 Missing word: “progression due AD” -corrected to “progression due to AD”

Page 43 Incorrect numbers: Table 2 “CAD 8” – corrected to “CAD: 19”

Page 43 Incorrect numbers: Table 2 “MCI: 19” – corrected to “MCI: 8”

Page 60 Misspelling: “finding” – corrected to “findings“

Page 63 Incorrect words: “difference was for ΔRANTES (difference from baseline to study end) which decreased in the supplementation group and increased in the NC group (p=0.003).” – corrected to “difference were for ΔMCP-1 (difference from baseline to study end) (p=0.011) and Δfasting glucose (p=0.003).”

Page 80 Missing word: “analysis showed significant differences.” – corrected to “analysis showed no significant differences.”

Page 85 Incorrect words: “was with respect to the within-group difference of RANTES (difference from baseline to study end), which decreased non-significantly in the anthocyanin group and increased non-significantly in the normal control group.”– corrected to “was with respect to the within-group difference of MCP-1 and fasting glucose (difference from baseline to study end).”

Page 100 Incorrect reference: “Evans, R.M., et al., Serum cholesterol, APOE genotype, and the risk of Alzheimer's disease: a population-based study of African Americans. Neurology, 2000. 54(1): p. 240-2.” - corrected to “Evans, R.M., et al., Cholesterol and APOE genotype interact to influence Alzheimer disease progression. Neurology, 2004. 62(10): p. 1869-71.”

Paper III, Page 5 Incorrect number: Table 2 Fasting glucose Active vs. control $p^{\#}$ = 0.71 Should read $p^{\#}$ = 0.003

Paper III, Page 5 Incorrect number: Table 2 HbA1c Active vs. control $p^{\#}$ = 0.11 Should read $p^{\#}$ = 0.26

Paper III, Page 5 Incorrect number: Table 2 IL-8 Active vs. control $p^{\#}$ = 0.41 Should read $p^{\#}$ = 0.71

Paper III, Page 5 Incorrect number: Table 2 MCP-1 Active vs. control $p^{\#}$ = 0.0.95 Should read $p^{\#}$ = 0.011

Paper III, Page 5 Incorrect number: Table 2 RANTES Active vs. control $p^{\#}$ = 0.0.003 Should read $p^{\#}$ = 0.41

Paper III, Page 5 Incorrect number: Table 2 TNFa Active vs. control $p^{\#}$ = 0.26 Should read $p^{\#}$ = 0.95

Grafisk design: Kommunikasjonsavdelingen, UiB / Trykk: Skipnes Kommunikasjon AS

uib.no

9788230859254 (print)
9788230866948 (PDF)