



Fluid-based biomarkers to assess the effect of physical exercise in Alzheimer's disease

The ADEX trial



PhD thesis by
Camilla Steen Jensen



DANISH DEMENTIA
RESEARCH CENTRE



Rigshospitalet

Fluid-based biomarkers to assess the effect of physical exercise in Alzheimer's disease; The ADEX trial

Camilla Steen Jensen

MSc in Biology

Danish Dementia Research Centre

Rigshospitalet

Department of Clinical Medicine

Faculty of Health and Medical Science

University of Copenhagen

Principal supervisor Professor Steen Gregers Hasselbalch, MDSc

Primary supervisor Anja Hviid Simonsen, PhD

Danish Dementia Research Centre, Rigshospitalet, University of Copenhagen, Denmark

External PhD Assessor Professor Bente Finsen, MDSc, PhD

Department of Clinical Research, University of Southern Denmark

Project Supervisors

Justyna Maria Bahl, PhD. Professor Niels HH Heegaard MD, PhD. Professor Henrik Zetterberg, MD, PhD.
Professor Kaj Blennow, MD, PhD. Associated Prof. Erik Portelius, PhD. Christian Enevold, PhD

Evaluation Committee

Professor Rigmor Højland Jensen, Chair

Department of clinical Medicine

Copenhagen University, Denmark

Professor Charlotte Teunissen, PhD

Department of Clinical Chemistry - Head Neurological Laboratory and Biobank

VU University Medical Center Amsterdam, Netherlands

Professor David Brooks

Department of Clinical Medicine – Positron Emission Tomography Center

Aarhus University, Denmark

Defense July 2nd 2019, Rigshospitalet

Tryk: Red Hill

Acknowledgments

Sometimes you are lucky to meet some very special people in your life. At the Danish Dementia research center, Rigshospitalet I did just that. I owe a considerable number of people a lot of gratitude.

To the person who have as much as me invested in this Ph.D. thesis of sweat, tears, and blood, my primary supervisor Dr. Anja Hviid Simonsen. I am thankful for your guidance, I know you got my back, scientific and other wise. I fell we have developed a truly special relation during the 4 ½ years you have been my supervisor and mentor.

To my principal supervisor Prof. Steen Hasselbalch, who said 'yes' more than 'no', when I came with my crazy ideas. You took a chance and let me make the project my own and gave me a chance to work at DDRC. You gave me an insight into the exciting world of neurology and the patients we are trying to help, for that I am grateful.

A special thanks should be given to Professor Gunhild Waldemar, Chief of Danish Dementia Research Center, for the chance to work on the ADEX project and at the DDRC, and for her professional guidance.

A special thanks to Professor Bente Finsen, from university of southern Denmark, external PhD assessor on my project, for new views of the scientific questions at hand.

I wish to thank the eight memory clinics and staff from the ADEX collaboration at Roskilde, Glostrup, Slagelse, Svendborg, Aarhus, Aalborg, Copenhagen and Odense. A special thanks to our collaborators Prof. Kaj Blennow, Prof. Henrik Zetterberg, Erik Portelius, Justyna Bahl, Prof. Niles H. H. Heegaard, Christian Enevold, Pia Meinke, Prof. Ruth Frikke-Schmidt and Assoc. Prof. Volkert Siersma for collaboration and guiding on the different studies. A special thanks to the patients and their families who participated in the 'ADEX' study. Without people who are willing to donate their time and bodies in the name of science, we would be nowhere.

I am particularly grateful for my time with my colleges from the research department, also known as 'kælderen' past and present. Especially thank to partners in (PhD) crime, Dr. Anne Bie, Dr. Nina Rostgaard, Dr. Ane Nørregaard, Dr. Marie Bruun, Dr. Peter Roos, and Kathrine Bjarnø

My ever-supporting friends Dr. Rikke Darling Rasmussen, Dr. Malene Winther, Dr. Line Hjort, Dr. Camilla Holzmann Rasmussen, Cecilie Lützen Sølvtoft, and Natasja Borges Kure for life, laughter and support. To my family, my ever-patient husband, love of my life Kenneth, and my moon and stars sons Carl and Christoffer.

The studies in this thesis are supported by Ellen Mørchs fond, Savværksejer Jeppe Juhl og hustru Ovita Juhl mindelegat and Hede-Nilsen familie fond. The Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) study is supported by the Innovation Fund Denmark. The Danish Dementia Research Centre is supported by the Danish Ministry of Health, and the Danish Health Foundation. Further, studies I and II, and the 12 weeks stay abroad at Sahlgrenska Academy Gothenburg Sweden were supported by grants from the UK Dementia Research Institute at UCL, the Wellcome Trust, the Swedish Research Council, the European Research Council and the Knut and Alice Wallenberg Foundation.

Til Carl og Christoffer

List of papers

The thesis is based on the following original research papers:

Paper I

Jensen CS, Portelius E, Siersma V, Høgh P, Wermuth L, Blennow K, Zetterberg H, Waldemar G, Hasselbalch SG, Simonsen AH.

Cerebrospinal fluid amyloid beta and Tau concentration are not modulated by 16 weeks of moderate-to-high-intensity physical exercise in patients with Alzheimer's disease

Dementia and other geriatric cognitive disorders, 2016, 42,146-158.

Paper II

Jensen CS, Portelius E, Høgh P, Wermuth L, Blennow K, Zetterberg H, Hasselbalch SG, Simonsen AH.

Effect of physical exercise on markers of neuronal dysfunction in cerebrospinal fluid in patients with Alzheimer's disease.

Alzheimer's and Dementia: Translational research & clinical interventions 2017, 3, 284-290.

Paper III

Jensen CS, Bahl JM, Østergaard LB, Høgh P, Wermuth L, Heslegrave A, Zetterberg H, Heegaard NHH, Hasselbalch SG, Simonsen AH.

Exercise as a potential modulator of inflammation in patients with Alzheimer's disease measured in cerebrospinal fluid and plasma.

Experimental Gerontology 2019, 121, 91-98.

Paper IV

Jensen CS*, Simonsen AH*, Siersma V, Beyer N, Frederiksen KS, Gottrup H, Hoffman K, Høgh P, Frikke-Schmidt R, Sobol NA, Waldemar G, Wermuth L, Hasselbalch SG.

Patients with Alzheimer's Disease who carry the APOE ε4 allele benefit more from physical exercise.

Alzheimer's & Dementia: Translational Research & Clinical Interventions 2019, 5, 99-106.

*equal contribution

List of Abbreviations

AA: amino acids

A β : beta amyloid

A β 38: beta amyloid 1-38,

A β 40: beta amyloid 1-40,

A β 42: beta amyloid 1-42,

A β 42/40: beta amyloid ration between isoform 1-42/1-40,

A β long/short: ration between beta amyloid species relative expression of long isoforms (A β >17 amino acids) and short isoforms (A β <16 amino acids)

AD: Alzheimer's disease

ADL: activities of daily living

ADEX: 'Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise'

APOE: gene encoding the apolipoprotein E

APOE: the protein apolipoprotein E

APP: amyloid precursor protein

CSF: cerebrospinal fluid

ELISA: enzyme linked immunosorbent assay

EOAD: early-onset Alzheimer's disease

EOFAD: early-onset familial Alzheimer's disease

FAD: familial Alzheimer's disease

FTD: frontal temporal dementia

IFN γ : interferon gamma

IL: interleukin

PSEN1 and 2: presenilin 1 and 2

P-tau: phosphorylated-tau

ROS: reactive oxygen species

sAPP β and α : soluble amyloid precursor protein β and α

sAPP β / α : ration between soluble amyloid precursor protein β and α

SDMT: symbol digit modalities test

sTREM2: soluble triggering receptor expressed on myeloid cells 2

Tg: transgenic

TMB: tetramethyl benzidine

TNF α : tumor necrosis factor alpha

TREM2: triggering receptor expressed on myeloid cells 2

T-tau: total-tau

Content

Summary in English	3
Summary in Danish.....	5
Thesis at a glance.....	7
Introduction	9
Symptoms of Alzheimer's disease	9
Molecular pathology of Alzheimer's disease.....	9
Risk of Alzheimer's disease	11
Brain plasticity.....	14
Physical activity as a therapeutic approach	15
Interventions with physical activity and Alzheimer's disease	17
Aims and Hypotheses	23
Study I: Alzheimer's disease biomarkers – the amyloidogenic pathway.....	23
Study II: Markers of neuronal dysfunction	23
Study III: Markers of Neuroinflammation	23
Study IV: The genetic contribution – The role of <i>APOE</i>	24
Methods and Materials	25
Study population	25
Exercise regime	26
Samples	26
Statistics	26
Methods	27
Summary of results.....	34
Study cohort.....	34
Study I: Alzheimer's disease biomarkers – the amyloidogenic pathway.....	35
Study II: Markers of neuronal dysfunction	38
Study III: Markers of Neuroinflammation	40
Study IV: The genetic contribution – role of <i>APOE</i>	44
Discussion.....	48
The clinical diagnostic markers for Alzheimer's disease in CSF.....	48
Synapse stability and neurogenesis	50
Neuroinflammation	53
People who are at genetic risk of AD benefit more from exercise	55
Methodological considerations	56
Study design	56
Do biomarkers change over 16 weeks?.....	57
What have we learned and what is the next step	59

References.....63

Paper I75

Paper II91

Paper III101

Paper IV.....123

Summary in English

Worldwide prevalence of dementia is growing fast, the neurodegenerative disease Alzheimer's disease (AD) being the most common accounting for more than 50 % of dementia cases. AD is characterized by neuronal and cognitive dysfunction. The pathological hallmarks of AD include extracellular aggregation and accumulation of the protein beta amyloid ($A\beta$) into mainly senile plaques as well as the hyperphosphorylated form of the protein tau accumulating inside the neurons as neurofibrillary tangles, leading to degeneration of the neurons.

Lately, due to failure of clinical trials of disease-modifying drugs for AD treatment, researchers and physicians have turned their attention towards non-pharmacological approaches, including exercise, to slow cognitive decline. Studies of physical activity and exercise in animal models for AD have shown improved cognitive function. Furthermore, in humans higher physical activity level is associated with lower risk of developing dementia, and intervention studies report a positive effect of physical activity in patients at risk of developing AD. Although physical activity may impact AD symptomatology, the underlying biochemical changes are largely unknown

In an effort to arrest the decline in cognitive function and loss of activities of daily living, non-pharmacological approaches have been investigated. In the randomized clinical trial 'Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) study' we analyzed the effect of 16 weeks moderate-to-high intensity physical organized and monitored exercise in patients with AD on a cognitive, functional, and molecular level. The central hypothesis of the ADEX study was that patients with mild AD would benefit on cognition, physical function and executive function by an intervention of 16 weeks of moderate-to-high intensity physical exercise. We investigated the underlying molecular changes in four different studies.

In STUDY I we investigated the effect of physical activity (PA) on the markers of AD pathology. Mouse studies have shown, that beta amyloid levels are modified by PA. We analyzed beta amyloid species by ELISA and mass spectrometry, and various precursor proteins of beta amyloid, to see if PA could have a modulatory effect in patients with AD. We found no effect on beta amyloid species, long or short. Further, was there no effect on the precursor proteins for beta amyloid, indicating that PA does not modulate beta amyloid deposits and pathways in patients with AD.

In STUDY II we investigated the effect of PA on synaptic stabilization. Neuronal loss is an early event in AD and achieving a stabilizing effect on neuronal communication and structure is highly sought after. We analyzed new biomarkers related to AD. The post synaptic protein neurogranin, the axonal protein

neurofilament light chain, the microglia derived protein YKL-40 and the calcium binding protein VILIP1 were measured in CSF before and after PA. All have been associated with AD but are new markers of interest. As in STUDY I, found we no effect of PA on the selected biomarkers.

In STUDY III we investigated the effect of PA on markers of inflammation and cell stress, systemic and brain-related. Increased inflammation is well documented in AD and AD risk, and PA is a well know modulator of systemic inflammation in cognitive healthy persons. We analyzed a proinflammatory panel in CSF and in plasma, alongside sTREM2 in CSF, and 8-isoprostane in plasma and CSF. We found a stabilizing effect of PA on the pro-inflammatory cytokine IFN γ in *APOE* ϵ 4 carriers, an increase in systemic IL6 and in CSF sTREM2 levels. This indicated that PA may have a systemic modulatory effect on inflammation in patients with AD, and this effect may transfer to the CNS. Changes were however negative when correcting this exploratory study for multiple comparisons.

In STUDY IV we investigated a post hoc -hypothesis of the *APOE* genotype contribution in the effect of PA on the primary and secondary outcome measures from the ADEX study. Human and animal studies have indicated that *APOE* ϵ 4 carriers, not only are at increased risk for developing AD, but that the ϵ 4 allele may modify the effect of physical activity on AD. We found evidence suggesting that in fact *APOE* ϵ 4 carriers do benefit more from PA, than non-carriers, indicating a great therapeutic value of PA in people with genetic risk of developing AD.

Despite the many negative findings regarding AD related biomarkers and their modulation by PA, exercise may still be a valuable add-on to traditional pharmacological treatment of AD, and larger studies specifically targeting the effect of physical exercise on pathophysiological mechanisms in AD are warranted.

Summary in Danish

Forekomst af demens er hurtig voksende på verdensplan. Den neurodegenerative sygdom Alzheimers sygdom (AD) er den mest almindelige, og udgør, mere end 50% af demens tilfælde. AD er karakteriseret ved neuronalt og kognitivt tab. De patologiske kendetegn ved AD omfatter ekstracellulær aggregering og akkumulering af proteinet beta-amyloid ($A\beta$) i senile plaks, og hyperphosphorylerede form af proteinet tau, der akkumuleres i neuronerne som neurofibrillære tangles, hvilket fører til degenerering af neuronerne.

På grund af manglende succes af kliniske forsøg med sygdomsmodificerende lægemidler til AD-behandling har forskere og læger vendt deres opmærksom på ikke-farmakologiske tilgange, fx motion, for at sænke kognitiv tilbagegang. Undersøgelser af fysisk aktivitet og motion i dyremodeller for AD, har vist forbedret kognitiv funktion. Hos mennesker er højere fysisk aktivitet også forbundet med lavere risiko for at udvikle demens, og interventionsstudier rapporterer en positiv effekt af fysisk aktivitet hos patienter med risiko for at udvikle AD. Selv om fysisk aktivitet kan påvirke AD-symptomatologien, er de underliggende biokemiske årsager stort set ukendte

I et forsøg på at nedsætte hastigheden for tabet af kognitiv funktion og tabet af daglige aktiviteter, har vi her undersøgt en ikke-farmakologiske fremgangsmåder. I dette randomiserede kliniske forsøg 'Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) study', har vi analyseret effekten af 16 ugers moderat til høj intens fysisk træning i patienter med AD på kognitivt, funktionelt og molekylært niveau. Den centrale hypotese af ADEX-studiet var, at patienter med mild AD vil bedres kognitivt og øge deres fysiske funktion ved en intervention på 16 ugers moderat til høj intensitet motion. Vi undersøgte den underliggende molekulære forandring i fire forskellige studier.

I STUDIE I undersøgte vi virkningen af fysisk træning på markører for AD patologi. Tusindevis af studier har vist, at $A\beta$ niveauer kan modificeres af fysisk træning. Vi analyserede forskellige $A\beta$ varianter ved hjælp af ELISA og massespektrometri. Ydermere analyserede vi, forskellige precursor proteiner af $A\beta$ for at se, om fysisk træning kunne have en modulerende effekt hos patienter med AD. Vi fandt ingen effekt på $A\beta$ varianterne, lange eller korte. Derudover var der ingen virkning på precursor proteinerne for $A\beta$, hvilket indikerer, at fysisk træning ikke modulerer $A\beta$ aflejringer i hjerne hos patienter med AD.

I STUDIE II undersøgte vi virkningen af fysisk træning som synaptisk stabiliserende. Neuronalt tab er en tidlig begivenhed i AD og opnåelse af en stabiliserende effekt på neuron er meget eftertragtet. Vi analyserede nye biomarkører relateret til AD. Det post-synaptiske protein neurogranin, den aksonalt-relateret protein neurofilament light chain, det microglia-afledte protein YKL-40 og det calciumbindende

protein VILIP1. Alle markørerne er ny markører, og er blevet sat i forbindelse med AD. Som i STUDIE I fandt vi ingen effekt af fysisk træning på de udvalgte biomarkører.

I STUDIE III undersøgte vi effekten af fysisk træning på markører for inflammation og celle stress, systemisk og hjerne-relateret. Øget inflammation er veldokumenteret i AD udvikling og AD risiko, og fysisk træning har udviste en veldokumenteret evne til at ændre systemisk inflammation hos kognitive raske personer. Vi analyserede et pro-inflammatorisk panel i CSF og i plasma sammen med sTREM2 i CSF og 8-isoprostan i plasma og CSF. Vi fandt en stabiliserende effekt af fysisk træning på det pro-inflammatoriske cytokin IFN γ i *APOE* ϵ 4-bærere, en øgende effekt på systemisk IL6 og i CSF sTREM2-niveauer. Dette indikerede, at fysisk træning måske en systemisk ændrende effekt på inflammation hos patienter med AD, og denne effekt måske kan overføres til CNS. Studiet var som de øvrige studier eksploratorisk og ved korrektion for multiple sammenligninger var de påviste ændringer ikke længere signifikante.

I STUDIE IV undersøgte vi en post-hoc-analyse af *APOE* genets bidrag til træningseffekten på de primære og sekundære outcome punkter i ADEX-studiet. Menneske- og dyreforsøg har vist, at *APOE* ϵ 4-bærere ikke alene er i øget risiko for at udvikle AD, men at ϵ 4 allelet også er forbundet med nedsat fysisk funktion. Resultaterne kunne pege på, at *APOE* ϵ 4-bærere rent faktisk har en øget effekt af fysisk træning end ikke-bærere, hvilket indikerer en stor terapeutisk værdi af fysisk træning hos mennesker med genetisk risiko for at udvikle AD.

På trods af mange negative resultater, mener vi at motion og træning kan være en værdifuld add-on til den traditionelle farmakologisk behandling af AD, og fremtidige større studier, der er specifikt rettet mod denne problemstilling, bør afklare dette.

Thesis at a glance

Study	Question	Method	Findings	Conclusion
I Alzheimer's disease biomarkers – the amyloidogenic pathway	Does 16 weeks of physical exercise modulate the diagnostic markers of Alzheimer's disease in Cerebrospinal fluid (CSF) in patients with Alzheimer's disease?	ELISA analysis of beta amyloid species, tau and phosphorylated tau. In addition, Mass spectrometry analysis of short beta amyloid species.	We found no effect on the diagnostic markers of Alzheimer's disease beta amyloid, tau or phosphorylated tau	Physical exercise does not modulate beta amyloid, tau and phosphorylated tau in CSF in patient with Alzheimer's disease. Nor does it favor the generation of non-aggregation prone species.
I Markers of neuronal dysfunction	Does 16 weeks of physical exercise modulate possible markers of neuronal stability and dysfunction in CSF in patients with Alzheimer's disease?	ELISA analysis of the proteins neurogranin, neurofilament light chain, visinin-like protein 1, and chitinase-3-like protein 1.	We found no effect on the selected markers neurogranin, neurofilament light chain, visinin-like protein 1, and chitinase-3-like protein 1	Physical exercise does not modulate selected markers of neuronal instability.
III Markers of Neuroinflammation	Does 16 weeks of physical exercise modulate markers of inflammation in CSF and plasma in patients with Alzheimer's disease?	ELISA analysis of pro- and anti-inflammatory proteins, soluble triggering receptor expressed on myeloid cells 2, and 8-isoprostane.	Physical exercise increased Interleukin-6 in plasma and CSF sTREM2. In addition, in <i>APOE ε4</i> carriers exercise had a stabilizing effect in IFN γ . However, changes were insignificant when correcting for multiple comparisons	Indication that physical exercise might have a positive modulating effect on markers of neuroinflammation in patients with AD.
IV The genetic contribution – The role of <i>APOE</i>	Does the <i>APOE</i> genotype influence the clinical outcome of the effect of physical exercise in patients with Alzheimer's disease in relation to cognition, physical function and executive function?	Post hoc analysis of the <i>APOE ε4</i> genotype effect clinical measures from the ADEX study (cognition, physical function etc.).	We found a genotype dependent effect on walking speed and endurance, dual task, neuropsychiatric symptoms, time-up-and-go, and SDMT.	Indication that carriers of the <i>APOE ε4</i> allele benefit more from physical exercise than non-carriers of the allele, on measures of executive function, physical function, and neuropsychiatric symptoms.

Introduction

World-wide prevalence of dementia is increasing, affecting more than 80.000 people in Denmark alone[1]. Alzheimer's disease (AD) is the most common disease, and accounts for over 50% of all cases of diagnosed dementia. The American Alzheimer's Association estimates that by 2050 every 1 in 85 people will be living with the disease [2,3].

Symptoms of Alzheimer's disease

The symptoms of AD are loss of memory and other cognitive skills, as well as loss of ability to perform activities of daily living (ADL)[3]. AD is progressive, resulting in death within 4-8 years[4]. The pathological process in AD is known, however the triggering factors and the order of events in the pathological cascade are largely unknown.

Molecular pathology of Alzheimer's disease

The pathological hallmarks of AD include extracellular aggregation and accumulation of the protein beta amyloid ($A\beta$) into mainly senile plaques in the cortical grey matter, as well as the hyperphosphorylated form of the protein tau accumulating inside the neurons as neurofibrillary tangles, leading to degeneration of the neurons [5]. The degeneration starts in the hippocampus, and spreads in a predictable pattern throughout the brain as the disease progresses, see figure 1 [6].

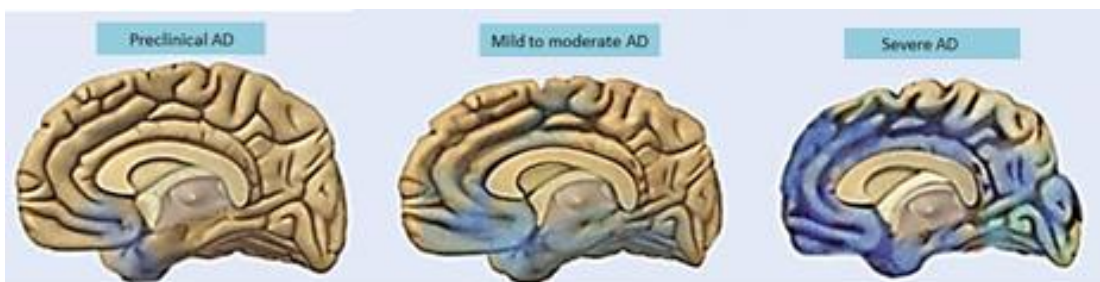


Figure 1. Alzheimer's disease spread pattern in the brain. Neurodegeneration spreads in patterns throughout the brain (blue shading) as the disease progresses from mild AD to severe AD. Picture adapted from National Institute of Ageing.

The accumulation of $A\beta$ and phosphorylation of tau, can be measured in the cerebrospinal fluid (CSF), even years before cognitive decline (see figure 2), and are used clinically as biomarkers alongside patient history and neurological examinations [7–9].

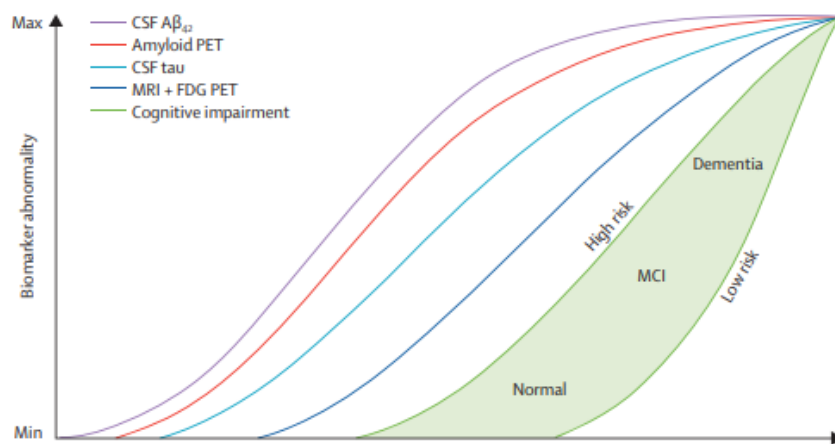


Figure 2. Model of dynamic biomarkers of the Alzheimer's disease pathological cascade. Schematic model by Jack *et al.*, 2013, that describes the diagnostic value of biomarkers even years before onset of cognitive complaint in patients with AD. A β : beta amyloid, FDG: fluorodeoxyglucose, MCI: mild cognitive impairment. Picture adapted from [9].

Amyloid beta

The major component of the amyloid plaques seen in AD is beta amyloid peptide 40-42 amino acids (aa) long referred to as A β . By proteolytic cleavage by γ -secretase, A β is derived from the protein amyloid precursor protein (APP). This is called the amyloidogenic pathway, giving rise to a variety of A β peptides ranging between 17-42aa [10–12]. APP can also undergo non-amyloidogenic processing by α -secretase, which releases 13-16 aa-long soluble A β species that do not form plaques [13], see figure 3.

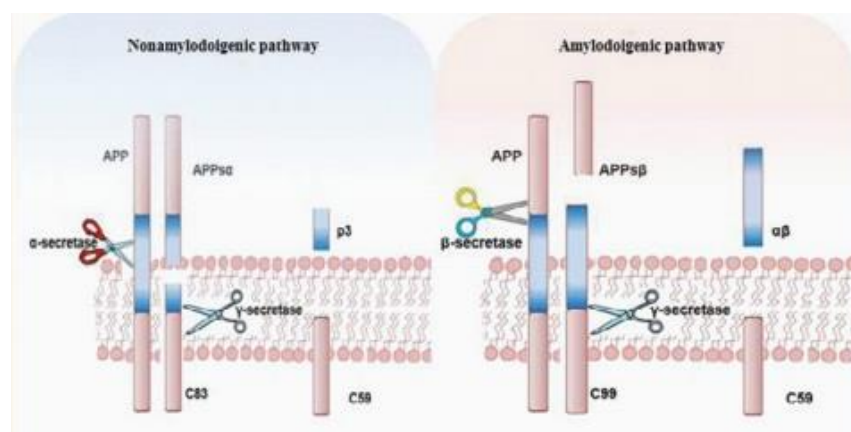


Figure 3. APP cleavage pathway. APP processing through the amyloidogenic or non-amyloidogenic pathway. Picture adapted from [12]

APP is expressed throughout the body and in the brain [14–16]. The biological function of APP has been suggested to be axonal transport, cell adhesion, cholesterol metabolism and gene transcription [17]. However, what triggers the formation of the non-soluble toxic 40-42 aa peptide form, is unknown. Point mutations and duplications of APP are causal for a subset of early onset of familial Alzheimer's disease (FAD) [11], see more under genetic risks.

Accumulation of A β aggregates in the brain can be measured as reduced levels of A β in the cerebrospinal fluid. This feature is used in the clinical diagnosis of AD, where a CSF concentration below 550 pg/mL (local cut-off, may be higher in other clinics) of A β 42 indicates AD, this however, cannot be used on its own.

Tau

The other pathological hallmark of AD is neurofibrillary tangles. A neurofibrillary tangle consists of the hyperphosphorylated protein tau (p-tau) [18]. Tau protein belongs to the microtubule-associated protein family, and functions as a structural stabilizing component of microtubules inside neurons [19]. In the diseased state, tau is hyperphosphorylated by unknown events, resulting in it losing its stabilizing ability and thereby leading to the formation of aggregates known as tangles [18]. In AD elevated CSF levels of tau and p-tau are found [20].

Elevated tau levels are not specific to AD and are seen in other neurodegenerative diseases like frontal temporal dementia (FTD) and Parkinson's disease, and in other neurological disorder like traumatic brain injury [21,22]. Like A β , tau and p-tau can be measured in CSF and are part of the clinical diagnosis of AD.

Risk of Alzheimer's disease

Alzheimer's disease is largely a sporadic disease, however, there are certain risk factors that increase the risk for AD.

Age

The prevalence of AD increases significantly with age, and incident risk rapidly accelerates with age [23,24]. The longer we live the higher likelihood we have to develop a neurodegenerative disease. The World's population is increasing in age, and more people live to be past 65 years, therefore the incidence of AD will increase too [25]. Even though AD is a disease of the older adult, AD is also seen in people younger than 65 years of age, called early-onset AD (EOAD). EOAD accounts for 1-6% of all cases of AD, however 60 % of EOAD cases have a positive family history of AD, and approximately 13% of EOAD cases have a known genetic autosomal dominant inherited type [26].

Genetic risks

Even though inherited cases of AD are rare, several genetic factors related to AD have been found. In this section, the most relevant genetic risk factors for this thesis are described briefly.

Apolipoprotein E

The gene for apolipoprotein E (*APOE*) located on chromosome 19 has three common polymorphisms named $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$. The risk of AD is associated with allele $\epsilon 4$, with the $\epsilon 4$ allele present in approximately 50% of patients who develop LOAD [27]. Having one or two copies of the $\epsilon 4$ allele increases the risk of LOAD 3- or 12-fold respectively. However, not all carriers of the $\epsilon 4$ allele develop dementia, and many carriers live well into their 90's without symptoms of the disease [28]. *APOE* encodes the protein APOE, which is a lipoprotein expressed in multiple organs, especially in the liver and brain [29]. APOE exists mainly as a component of complexes of lipoproteins in plasma and CSF. The different alleles of *APOE* encode three different polymorphic forms of the protein APOE2 (Cys-112, Cys-158), APOE3 (Cys-112, Arg-158) and APOE4 (Arg-112, Arg-158) [29]. The three different forms of the protein have been shown to have critical structural importance for the proteins, their function, and to have influence on plasma levels of APOE [30]. It has been suggested that *APOE* $\epsilon 4$ genotype can lead to AD pathogenesis by several pathways: (I) Damage of the vascular system in the brain, (II) Initiation or acceleration of A-beta accumulation, aggregation, and deposition in the brain, (III) impaired cholesterol transport, and (IV) increased neuroinflammation [31].

Triggering receptor expressed on myeloid cells 2 (TREM2)

Recent genetic studies have found rare variants of *TREM2* as a risk factor for developing AD [32,33]. The single nucleotide polymorphism (SNP) rs75932628-T, has been linked to higher levels of sTREM2 in CSF and higher risk of developing AD [34,35]. Studies in mice have shown that mutations on *TREM2* led to altered microglia function in a model for AD [36]. *TREM2* is an immune receptor, expressed on the cell surface. *TREM2* can be cleaved on the extracellular side by γ -secretase, resulting in a soluble form of the protein sTREM2. This is seen with microglial activation and stress [37]. From mouse studies, It has been found that sTREM2 acts as a microglia activator through the Akt-GSK3 β - β -cetenin pathway [38]. The elevated levels seen in AD, may be an acute survival response for the microglia. However, as AD progresses this microglia over-activation might be more harmful than useful. In addition, sTREM2 has been found to induce IL6 transcription in a mouse model [38]. A hypothesis could be that modulating the levels of sTREM2 could be an efficacious therapeutic approach to treat AD [38].

Other disease-causing mutations

APP

There have been found 32 different missense mutation in *APP*. Most of the mutations are located within the secretase cleavage sites in the transmembrane regions on exon 16 and 17. Mutations in the *APP* gene account for 10-15% of Early onset familial AD (EOFAD) [39].

Presenilin 1 og 2 (PSEN1 and 2)

Both *PSEN1* and 2, encode the homologue protein γ -secretase, that is associated with the cleavage of *APP* [39]. *PSEN1* mutations account for far most of the cases of EOFAD (18-50%), where *PSEN2* is much rarer [39,40]. Both types of mutation have been reported to increase the $A\beta_{42}$ to $A\beta_{40}$ ratio [41].

Inflammation

Inflammation is an important part of the pathophysiology of Alzheimer's disease (AD) [42,43], however, the precise pathological mechanism behind the contribution of inflammation to the pathological progression of the disease is largely unknown. Nonetheless, markers of inflammation that can be measured in CSF and even plasma have the potential to aid in understanding the development of AD [44,45].

Both pro-inflammatory cytokines (e.g. IL-6) and anti-inflammatory cytokines (e.g. IL-10, IL-18) have been found relevant in AD. It is thought that the accumulation of $A\beta$ plaque, induces prolonged stress activation of microglia that can result in the development of a harmful (inflammation-promoting) increase in cytokines, chemokines and reactive oxygen species (ROS) [46]. Previous studies have found altered cytokine profiles in patients with AD. Increased levels of brain and blood levels of IL1, have been linked to increasing $A\beta$ formation [47,48]. Furthermore, increased levels of other members of the IL1 family have been associated with increased AD risk [49]. In addition, have studies with treatments with the anti-inflammatory cytokine IL4 have shown reduced $A\beta$ accumulation in AD mouse models [50].

The pro-and anti-inflammatory cytokine IL6, has been associated with AD. Blood and CSF levels of IL6 have been found to be increased in AD compared to cognitively healthy controls, and brain tissues from AD patients shows that IL6 adhere to senile plaques [51,52].

Metabolic aspects

Retrospective population studies have identified certain lifestyle factors that increase the risk of developing AD later in life. Besides impaired metabolism homeostasis and lifestyle, low educational level [53], low physical activity throughout life [54], and smoking [55] have been connected to the risk of developing AD.

Among metabolic changes that may be implicated in AD, several studies have found an association between cognitive decline and dementia and diabetes, and even pre-diabetes[56]. Several mechanisms have been proposed to explain the association between cognition and glucose control, however there is still no consensus regarding the biological pathway [56,57]. A study indicates that an increase in glycosylated hemoglobin (HbA1c) over time, can lead to cognitive decline even in non-diabetic individuals [57,58]. A proposed link between glucose metabolism and AD, is the enzyme insulin degrading enzyme (IDE). As the name implies IDE is involved in insulin metabolism, however A β has also been found to be a substrate for degradation by IDE, thus linking AD and glucose [59].

In addition to insulin and glucose, cholesterol has been connected to AD risk, where high serum cholesterol in midlife increases AD risk later in life [60,61]. This is further underlined by observational studies finding that persons under 80 years taking statins or lipid lowering medicine, have reduced AD incidence, however this finding has been questioned, and recent randomized trials has failed to find the same connection [62,63]. The link between cholesterol and AD, has been proposed to involve the cleavage of APP. As mention earlier, APP can undergo two pathway the amyloidogenic toxic pathway, and the favorable non-amyloidogenic pathway [11]. In cultured cells, a reduction in medium cholesterol level, increases activity of α -secretase, the non-amyloidogenic pathway [64]. In contrast lowering cholesterol from cell membrane, decreases the activity of β -secretase, again favoring the non-amyloidogenic pathway [65], and in such a way links cholesterol to AD via A β . The involvement of lipids/cholesterol is further underlined by the findings that the *APOE* ϵ 4 genotype is also involved in sporadic late-onset AD [66]. It is established that lipids are implicated in AD pathogenesis 1) by regulating the trafficking and/or proteolytic activity of membrane-bound proteins involved in APP processing, and 2) by acting directly on A β and perhaps even Tau, by affecting their ability to aggregate [66]. This action could be altered by *APOE* genotype, since *APOE* is the major lipid-transporting molecule in the brain [67], and studies have shown a decrease in *APOE* levels in blood and CSF in a dose-dependent manner with the genotype E4/E4 having the lowest amount [30,68].

Brain plasticity

Certain areas of our brain shrink as we age, especially areas connected to our memory have been found to shrink 1-2% in volume per year in individuals over 50 years old [69]. These areas are further challenged in

AD, where brain atrophy is accelerated due to neurodegeneration in similar regions. The ability of the brain to generate and maintain synaptic networks throughout life is important, however plasticity is hard to define, and even harder to measure. A possible way is to measure biomarkers in CSF as pseudo-markers for synaptic stability and function, axon sprouting, and dendritic stability in the brain. New biomarkers have shown promising results, among these the protein neurogranin. Reduced levels of neurogranin have been found in AD patients' brain specimens compared to controls [70,71], and further, increased levels of neurogranin have been found in CSF from patients with AD compared to mild cognitive impaired (MCI) and controls [72]. Neurogranin is a postsynaptic protein, involved in the regulation of available calmodulin and calcium influx [73], and is thought to play a role in long term potentiation [74]. The reduced amount in brain tissue in patients with AD, and the increased concentration in CSF could be due to extracellular release as a result of AD-associated synaptic degradation. Another candidate marker, often investigated alongside neurogranin is visinin-like protein-1 (VILIP1) another calcium regulating protein, and a biomarker for neuronal cell death [75]. Other putative biomarkers suggested are the protein neurofilament light (NFL) [76] and chitinase-3-like protein 1 (YKL40) [77]. Changes in NFL are not specific to AD, however NFL has shown to be a sensitive marker of neuronal degradation [76]. Not much is, so far, known about YKL-40's involvement in AD, however a study has proposed YKL-40 as a biomarker of prognostic potential in preclinical AD [78].

Physical activity as a therapeutic approach

In this section PA is defined both as an active lifestyle (walking, leisure time activity etc.), and engaging in exercise (participating in organised exercise like playing football, handball, running etc.). In animal studies, it covers forced activity like a running wheel and subjective observed activity like engaging in its surroundings in the cage.

Lately, due to failure of clinical trials of disease-modifying drugs for AD treatment, researchers and physicians have turned their attention towards non-pharmacological approaches, including physical activity (PA), to slow cognitive decline [79–81]. Studies of PA in animal models for AD have shown improved cognitive function and reduced AD pathology [82,83]. Furthermore in humans, retrospective epidemiological studies of mid-life activity levels, have shown that higher PA level is associated with lower risk of developing dementia [54] and in a population-based longitudinal study of older adults followed over 9 years, participating in regular exercise was associated with lower odds of developing dementia [84]. In addition, intervention studies have reported a positive effect on cognition of PA in patients at risk of developing AD [85] and a beneficial effect in those already having clinical AD dementia (See table 1a).

A β and physical activity

The brain A β load seems to be directly influenced by PA [86]. Brain levels of A β measured in several studies show a significant decrease (20-66%) in AD transgenic mice subjected to PA compared to sedentary control mice[87]. Whether this reduction in A β load is due to increased degradation of A β or decreased deposition is still unknown. Studies suggest that the influence of PA on A β load is to be found in the metabolic changes of insulin and insulin degrading enzyme (IDE)[88]. IDE is found to have a proteolytic degrading effect on A β , see figure 4 [89–92], and since PA has a reducing effect on insulin levels in CSF [93], it thereby may generate more IDE available for degrading A β .

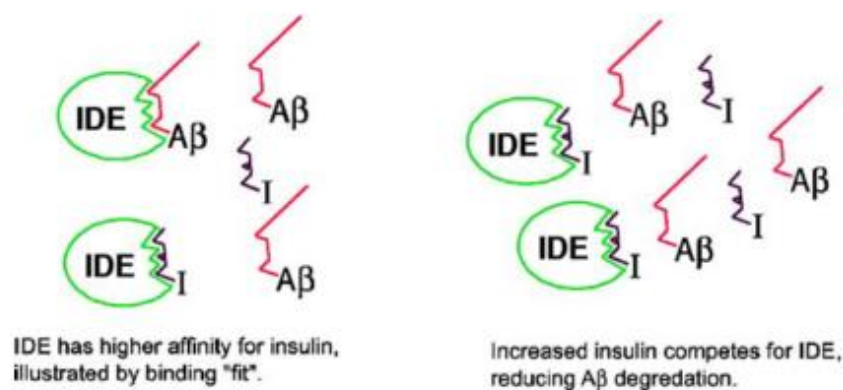


Figure 4. Insulin and A β compete for IDE activity. Adated from [88]

Modulation of metabolism and physical activity

Other metabolic changes that may be implicated in AD, can be modulated by PA [58]. It is well known that both glucose metabolism and cholesterol are modifiable by PA [94], and a general healthy life style has been found to be protective of AD [95,96], even when adjusting for lifestyle factors (education, BMI, smoking) normally connected to low PA.

One theory is that PA positively modulates the brain's glucose uptake in areas of the brain that in AD display hypometabolism [97], however the underlying pathology is unknown. Another theory concerns the findings that the cholesterol lowering effect of statins, also reduces the risk of AD, as mentioned earlier. PA has the same lowering effect on cholesterol [98], perhaps promoting the non-amyloidogenic pathway.

Neuroplasticity and physical activity

In AD, brain plasticity is markedly reduced, resulting in loss of volume in specific parts of the brain relevant for AD[99]. A study has shown that PA can increase hippocampal volume by 1-2%, reversing the age dependent loss of volume [100]. Other studies have found that greater over all fitness levels positively relate to greater white matter volume in patients with early stage AD [101].

In animal studies PA has been shown to enhance neuron-related growth factors and promote angiogenesis in the brain [102–105]. Studies in humans have also focused on promoting growth factors. Here, proteins like brain derived neurotrophic factor (BDNF), and insulin like growth factors have been found to be upregulated by PA in cognitively normal older adults [106].

Inflammation and physical activity

Systemic inflammation has been associated with accelerated cognitive decline and AD [107]. Inflammation, which can impair growth factor signaling, exacerbates the metabolic syndrome, is reduced by exercise, suggesting inflammation as a highly attractive target for prevention and arrest of AD progression [108]. In addition, PA has been shown to have a positive modulatory effect on systemic inflammation by reducing pro-inflammatory cytokines, increasing anti-inflammatory cytokines [109,110], and modulating reactive oxygen species (ROS) [111].

Interventions with physical activity and Alzheimer's disease

Physical activity as a non-pharmacological treatment has been proven beneficial to reduce the risk for many diseases including stroke, high blood pressure and mental disorders like chronic stress and depression [112]. However, compared to our understanding of PA's impact on cardiovascular health and general fitness, our knowledge of PA's impact on cognitive health is still sparse. To explore the effect of PA on cognitive function in patients with dementia and MCI, previous intervention studies have employed various strategies. In table 1a, previous studies of intervention in patients with MCI or AD have been summarized. In general, they have employed low intensity aerobic exercise (e.g. walking) with few participants and relying on self-efficacy, and self-monitoring and reporting. Also, many studies were performed in nursing-home residents with moderate to severe dementia. However, several studies have found a positive effect on cognition. In relation to biomarkers, very few intervention studies have included biomarkers in their assessment. In a systematic review, we have previously described and summarized the biochemical findings from studies of intervention with PA in MCI and AD [113]. These are summarized in table 1b. In general, the studies have been with few participants employing a low intensity PA intervention, and most significant findings were in patients with MCI.

Study	N	Population	Exercise	Duration	Controls	outcomes
Patients with MCI						
†Nascimento <i>et al.</i> , 2014 [114]	67	Community dwelling Median MoCA: MCI 20.5 cognitive normal 27	N=35 (20 MCI) Aerobic exercise (moderate-to-high intensity) 3*60 min /week	16 weeks	N=32	Improved cognition in MCI group
Suzuki <i>et al.</i> , 2013 [115]	100	Community dwelling mean MMSE: 27	N=50 Aerobic exercise 2*90 min/week	24 weeks	N=50	Increased MMSE in exercise group
Nagamatsu <i>et al.</i> , 2012 [116]	86	Community dwelling women, mean MMSE: 27	1) n=28 resistance training and 2) n=30 aerobic exercise 2*60 min/week	26 weeks	N=28 balance and tone	1 and 2: improved reaction time in spatial memory test 1: improved Stroop test, improved associative memory test. 2: improved verbal memory
†Segal <i>et al.</i> , 2012 [117]	54	No mentioning of home status Mean MMSE: aMCI not reported cognitive normal, not reported	N=26 (11 aMCI) Acute aerobic exercise (moderate-to-high intensity) 6 min	Once	N=28	Both groups improved picture recall
Varela <i>et al.</i> , 2012 [118]	48	No mentioning of home Mean MMSE: 20	1) N=17 aerobic exercise (low intensity and 2) n=16 aerobic exercise (moderate intensity) 3*30 min/week	12 weeks	N=15 recreational activity	No significant findings
†Baker <i>et al.</i> , 2010 [85]	33	No mentioning of home Mean MMSE: 28	N=23 aerobic exercise 4*45-60 min/week	24 weeks	N=10 stretching	Improved SDMT, verbal fluency, Stroop and task switching in woman, improved Trail B in both genders.
Van Uffelen <i>et al.</i> , 2008 [119]	152	Community dwelling mean MMSE: 29	1) N=71 aerobic exercise (low intensity) + dietary supplementation 2) n=78 aerobic exercise (low intensity) 2*60 min/week	52 weeks	1) N=75 with dietary supplementation 2) n=74	Improvement in attention (women), improvement in delayed recall (men)
#Lautenschlager <i>et al.</i> , 2008 [120]	170	-	N=85 stretching and strength training 3*50 min/week	24 weeks	N=85	Increase ADAS-Cog, improved in delayed recall
Scherder <i>et al.</i> , 2005 [121]	43	Nursing home MMSE range: 7-12	1)N=15 aerobic exercise (low intensity) 2) n=13 stretching training 3*30 min/week	6 weeks	N=15	Increased category naming, increase trail A and B
Patients with AD						
# Morris <i>et al.</i> , 2017 [122]	65	Community dwelling mean CDR sum of boxes 3.55	N=32 aerobic exercise 150 minutes/week distributed of 3-5 times	26 weeks	N=33	Stable effect on memory
# Holthoff <i>et al.</i> , 2015 [123]	30	Community dwelling mean MMSE: 20.6	N=15 aerobic exercise (low intensity) 3*30 min/week	12 weeks	N=15	Improvement in activities of daily living. Stable in executive function, word fluency reaction time, attention. Controls group deteriorated in all measures

Cheng <i>et al.</i> , 2014 [124]	110	Nursing home Mean MMSE: control 18.8 ± 4.1 Mahjong 19.0 ± 3.2 Tai Chi 18.7 ± 3.9	N=39 Aerobic exercise (low intensity) 3*60 min/week N=36 Cognitive activity (Mahjong) 3*60 min/week	12 weeks	N=35	Both intervention groups: Slower decline in cognitive measures
Coelho <i>et al.</i> , 2013 [125]	39	Community dwelling Mean MMSE: AD 21.0 ± 3.9 Controls 28.0 ± 2.5	N=21 acute aerobic exercise (high intensity) 17-22 min	Once	N=18 (cognitive normal)	No findings reported
Pedroso <i>et al.</i> , 2011 [126]	21	Community dwelling mean MMSE intervention: 21.1, controls: 19	N=10 aerobic exercise and dual task training 3*60 min/week	16 weeks	N=11	Improvement in balance and executive function
#Vreugdenhil <i>et al.</i> , 2012 [127]	40	Community dwelling MMSE range: 10-28	N=20 aerobic exercise (low intensity)	16 weeks	N=20	Increased MMSE, increased ADAS-Cog score
#Venturelli <i>et al.</i> , 2011 [128]	21	Nursing home, mean MMSE: 15	N=12 aerobic exercise (low intensity) 4*30 min/week	24 weeks	N=12	Control group slight decrease in MMSE
#Steinberg <i>et al.</i> , 2009 [129]	27	Community dwelling MMSE range: 15-20	N=14 aerobic exercise and strength training	12 weeks	N=13	No significant findings
†Eggermont <i>et al.</i> , 2009 [130]	97	Nursing home Mean MMSE 17.7	N=51 aerobic exercise (low intensity) 5*30 min/week	6 weeks	N=46	No significant findings
£†Akishita <i>et al.</i> , 2005 [131]	13	Nursing home women Mean MMSE: 13.9 ± 1.9	N=13 stretching and strength training 2*30 min/week	12 weeks	-	No significant findings
Van de Winckel <i>et al.</i> , 2004 [132]	25	Women Community dwelling mean MMSE 11	N=15 aerobic exercise 7*30 min/week	12 weeks	N=10	Improved MMSE and 'fluency' ADS6
Mancuso <i>et al.</i> , 2003 [133]	38	No mentioning of home status Mean MMSE: AD 17.4 ± 4.9 controls not reported	N=38 Acute resistance training (increment 10-70 % 1RM)	Once	-	No findings reported

Table 1a. Previous intervention studies of PA s in patients with MCI or AD. The cognitive findings are summarized here. For biochemical findings see table 1b. # caregiver or self-monitoring. £ supervised exercise. ¥ no description of supervision. †Biochemical markers measured (table 1b)

Study	Outcome measures	Results
Nascimento <i>et al.</i> , 2014 [114]	Cognitive markers: MoCA Biomarkers (blood): BDNF, TNF- α , IL-6	Cognitive: MCI: $\uparrow$ cognition Biomarkers: Both exercise groups: $\downarrow$ TNF- α , $\downarrow$ IL-6
Cheng <i>et al.</i> , 2014 [124]	Cognitive markers: MMSE, Call-recall, Categorical fluency, Digit span Biomarkers (blood): Cholesterol, HDL, Tri-glycerides, Glucose Genetic markers: APOE genotype	Cognitive both groups: Slower decline in cognitive measures Biomarkers: No results reported
Coelho <i>et al.</i> , 2013 [125]	Cognitive markers: - Biomarkers (blood): BDNF	Cognitive: None reported Biomarkers: Both groups $\uparrow$ BDNF
Segal <i>et al.</i> , 2012 [117]	Cognitive marker: IAPS Biomarkers (saliva): alpha-amylase	Cognitive: Both groups $\uparrow$ picture recall Biomarkers: $\uparrow$ saliva alpha-amylase
Baker <i>et al.</i> , 2010 [85]	Cognitive markers: SDMT, Verbal fluency, Trail B, Stroop, Task switching, Story recall, List-Learning Biomarkers (blood): Insulin, IGF-1, Cortisol, BDNF, Platelet factor 4, Ab ₄₂ , Cholesterol. Cardio markers: VO ₂ peak	Cognitive: ♀: $\uparrow$ executive function, $\uparrow$ performance in Stroop. Both groups: $\uparrow$ performance in trail B Biomarkers: ♀: $\uparrow$ Insulin sensitivity, $\uparrow$ Insulin, $\uparrow$ Cortisol in control group, $\uparrow$ BDNF. ♂: $\downarrow$ Cortisol in controls group, $\downarrow$ BDNF, $\uparrow$ IGF-1. Both groups: $\downarrow$ body fat, $\downarrow$ total Cholesterol $\uparrow$ VO ₂ peak
Eggermont <i>et al.</i> , 2009 [130]	Cognitive markers: MMSE, Face recognition, Picture recognition, Delayed word call, Digit span backwards, Category fluency, Letter fluency Genetic markers: APOE genotype	Cognitive: No significant results reported Biomarkers: No significant results reported
Akishita <i>et al.</i> , 2005 [131]	Cognitive markers: ADL, MMSE Biomarkers (blood): Estradiol, Testosterone, DHEA, DHEA sulfate, Sex-hormone binding globulin	Cognitive: No change in MMSE, No change in ADL Biomarkers: $\uparrow$ Testosterone, $\uparrow$ Estradiol, $\uparrow$ DHEA
Mancuso <i>et al.</i> , 2003 [133]	Cognitive markers: - Biomarkers (blood): COX, Lactate	Cognitive: Not studied Biomarkers: $\uparrow$ Lactate, $\downarrow$ Lactate recovery after exercise, $\downarrow$ COX activity

Table 1b. Results from a systematic review of interventions of exercise in patients with dementia. Adapted from [113]. ADL: Activities of Daily Living, DHEA: Dehydroepiandrosterone, AD: Alzheimer’s disease, aMCI: amnesic mild cognitive impairment, MCI: mild cognitive impairment, IGF-1: Insulin-like growth factor 1, BDNF: Brain-derived neurotrophic factor, Ab40+42: beta-amyloid isoform 40 and 42, VO₂ peak: peak oxygen uptake, HRR: Heart rate reserve, ♀: women, ♂: men, MMSE: mini-mental state examination, HDL: high-density lipoprotein. COX: Cytochrome c oxidase, MoCA: Montreal Cognitive Assessment, TNF-a: tumor necrosis factor alpha, IL-6: Interleukin-6, IPAS: International Affective Picture Set, $\uparrow$: increase, $\downarrow$ decrease, † : Diagnosed according to international guidelines, ‡ : non-diagnosed or not mentioned, $^{\epsilon}$: Supervised by caregiver, healthcare worker or not mentioned, $^{\bullet}$: supervised by trainer or physiotherapist, #: given as mean (SD).

Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) study

The projects which this thesis is part of is a multicenter, single-blinded randomized controlled trial. In an effort to arrest the decline in cognitive function and loss of activities of daily living, a non-pharmacological approach has been investigated. In the ADEX study we analyzed the effect of 16 weeks moderate-to-high intensity physical organized and monitored exercise in patients with AD on a cognitive [134], functional [135] and molecular level [136]. The main study was conducted between January 2012 to June 2014, with participation from eight memory clinics in Denmark (Aarhus, Aalborg, Odense, Svendborg, Slagelse, Glostrup, Roskilde and Copenhagen).

The ADEX study was inspired by the study by Baker *et al.*, 2010 [85] where patients with MCI were investigated. In the ADEX study, a similar design with moderate-to-high-intensity supervised exercise was applied to community-dwelling patients with mild AD. At that time, similar studies had not been performed, and as described above, evidence came largely from smaller studies in nursing-home residents or studies with unsupervised low-intensity exercise, or intensity was not monitored.

The central hypothesis of the ADEX study was that patients with mild AD would benefit on cognition, physical function and executive function by an intervention of 16 weeks of moderate-to-high intensity physical exercise. This was assessed by a comprehensive battery of tests on cognition, physical function, and neuropsychiatric symptoms. Further, all participants gave blood before and after intervention for biochemical analysis. In addition, a subgroup of participants from three out of the eight memory clinics was subjected to further brain imaging with amyloid PET scan and MRI and gave a CSF sample by lumbar puncture before and after the intervention.

The exercise intervention investigated here was an aerobic one-hour session three times a week for 16 weeks on non-consecutive days. It consisted of three times 10 minutes of moderate-to-high intensity exercise burst on either treadmills, stationary bikes or cross trainers. All sessions were supervised by 1-2 experienced physical therapist, and portable heart rate monitors were used to ensure sufficient exercise intensity. The control group received treatment as usual given by their respective clinic and carried on their normal activity levels. There was no control of the physical activity levels of patients in the control group, as monitoring of physical activity outside the exercise sessions in both groups was not possible due to lack of resources. The inclusion/exclusion criteria clearly stated however, that only patients not enrolled or participating in previous or on-going exercise regimes or structured physical exercise could be included.

Participants exercised in groups of 4-6 participants with 1-2 physical therapists as trainers and for motivation to ensure adherence and engagement in the exercise. At baseline and after 16 weeks of intervention, all participants were tested on 1) cognition and executive function, including activities of daily living by trained testers, and 2) physical function by physiotherapist. All testing was done blinded for intervention for the evaluating tester/physiotherapist.

Symbol Digit Modalities Test (SDMT) was chosen as primary outcome because it has been proven most sensitive to very early changes in executive function in AD [137] and executive function has been considered particularly sensitive for modulation by PA [138–140]. In addition, other cognitive tests, including other tests of executive function, psychiatric status and activity of daily living and quality of life were included. To assess physical function various tests to analyze walking speed, balance, and strength were applied [135].

Cognitive and physical findings in ADEX

In the ADEX study the cognitive, and physical outcomes have been reported in two previous PhD theses. The findings from the two previous theses covering the cognitive and physical findings are shortly described here.

Cognitive outcomes

Overall the cognitive findings in the ADEX study were negative. In the primary outcome SDMT, there was no significant change in score before and after intervention, however a trend for a beneficial stabilizing effect of exercise was seen [134]. Additionally, there was no change in the secondary cognitive measures (including Mini Mental State Examination and ADAS-Cog verbal memory score). However, in the secondary outcome Neuropsychiatric inventory (NPI) there was a significant change in score in favor of the exercise group. In a further analysis in a per protocol manner, where only participants who adhered to 2/3 of the exercise sessions and with a mean intensity of over 70% of maximal heart rate, a significant difference in the primary outcome SDMT in favor of the exercise group was found with a stabilizing effect, where the controls declined over the intervention period. Further, in this analysis NPI was still significantly improved in the exercise group [134].

Physical outcomes

In the intention-to-treat population, aerobic exercise improved an estimated measure of cardiorespiratory fitness (using the Astrand test) and exercise self-efficacy in patients with AD [141]. Further, in a group attending at least 2/3 of the sessions for 16 weeks, single-task physical performance and dual-task

performance was positively affected in the exercise group [141]. Also, in a subset of subjects investigated with state-of-the-art cardiorespiratory fitness ($\text{VO}_{2\text{peak}}$) measurements, changes in cardiorespiratory fitness ($\text{VO}_{2\text{peak}}$) appeared to be associated to changes in cognition and neuropsychiatric symptoms, such that increased cardiorespiratory fitness was associated with both improved performance on the SDMT, and less neuropsychiatric symptoms [142].

Aims and Hypotheses

The use of PA as a therapeutic approach for AD and other dementias is an attractive add-on to conventional medical treatment with a potential positive effect on cognition [143]. The underlying effect is however largely unknown [113], and more attention must be paid to the molecular mechanism in order to understand and optimize this type of treatment. Therefore, we sought to elucidate the molecular effects of PA in AD.

The key to this project was to find biomarkers that can be measured in CSF and/or blood, and that are thought to be affected by AD and influenced by 16 weeks of PA. Each study has its own specific share of the pathological process in AD. Figure 5 shows a graphic representation of the focus areas investigated in this PhD.

Study I: Alzheimer's disease biomarkers – the amyloidogenic pathway

This study aimed to investigate the potential of physical exercise to reduce cerebral $\text{A}\beta$. Our hypothesis was that moderate-to-high intensity PA would increase the CSF levels of $\text{sAPP}\alpha$ and less aggregation prone $\text{A}\beta$ species and reduce CSF markers of neurodegeneration (t-tau and p-tau) in patients with clinically diagnosed mild AD.

Study II: Markers of neuronal dysfunction

This study aimed to investigate the potential of physical exercise to modulate downstream molecular processes in synapses that potentially could be influenced by AD. We hypothesized that the concentrations of markers of neuronal and synaptic damage and astroglial activation would decrease in CSF in patients with AD as an effect of moderate-to-high intensity PA.

Study III: Markers of Neuroinflammation

This study aimed to investigate the potential of physical exercise to modulate inflammation markers in the brain measured in CSF. We hypothesized that PA would have an upregulating effect on anti-inflammatory

cytokines, and down-regulating effect on pro-inflammatory cytokines. Further, that PA exhibited modulating effects on markers of microglia activation and ROS, measured via sTREM2 and 8-isoprostane respectively.

Study IV: The genetic contribution – The role of *APOE*

In this post hoc analysis of the previously published ADEX results, we aimed to investigate the if the significant improvements in cognitive (SDMT) and physical outcomes were dependent on the patients' *APOE* genotype. We hypothesize that carriers of the *APOE* $\epsilon 4$ allele would benefit more from exercise, in areas of cognition and physical performance than non-carriers of the allele.

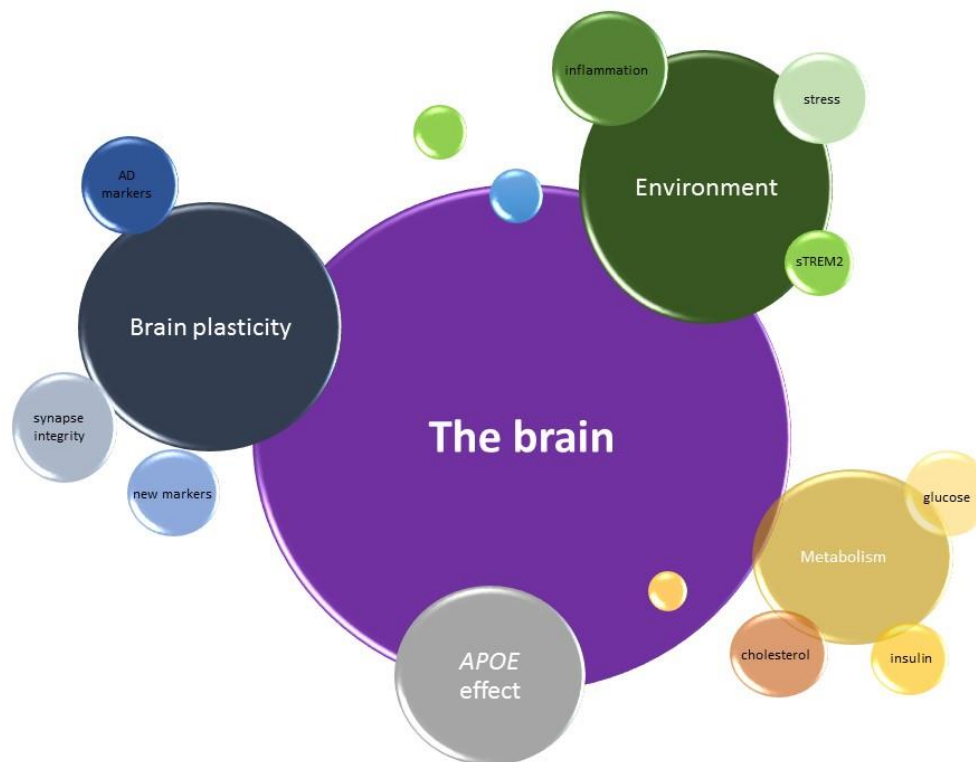


Figure 5. Project focus area. All projects derive from different pathological mechanisms seen in AD. The three main projects are indicated in blue 1) the brain plascity, in green 2) the enviroment in the brain, and lastly in grey 3) the *APOE* effect, here systemically. The last focus area, here in yellow, the metabolism has not yet resulted in published papers that are included in the thesis, however future perspectives on this focus area are touched upon in the section ‘what have we learned and what is the next step’.

Methods and Materials

Study population

The study population has been described in detail in [134]. Six-hundred and eight patients seen in eight memory clinics around Denmark, were screened for eligibility. Four-hundred and eight were rejected due to either lack of willingness to participate, they did not meet the inclusion criteria for the study (for details of inclusion and exclusion criteria and methods, see [144]), or for other reasons [134]. A total of two-hundred community-dwelling patients with clinically diagnosed mild AD according to NINCDS-ADRDA criteria [145] and a MMSE >20 were included and randomized to either a control group with treatment as usual or a 16 weeks of moderate-to-high intensity physical exercise group, figure 6 displays a schematic overview of inclusion. All participating subjects donated a blood sample before and after intervention and were tested at baseline and at 16-week follow-up with a comprehensive battery of tests of cognitive function, Activities of Daily Function, Quality of Life, physical activity and neuropsychiatric symptoms.

From the 200 participating subjects, a subgroup of 56 subjects recruited from three out of eight centers, had an additionally lumbar puncture performed to collect CSF samples before and after intervention

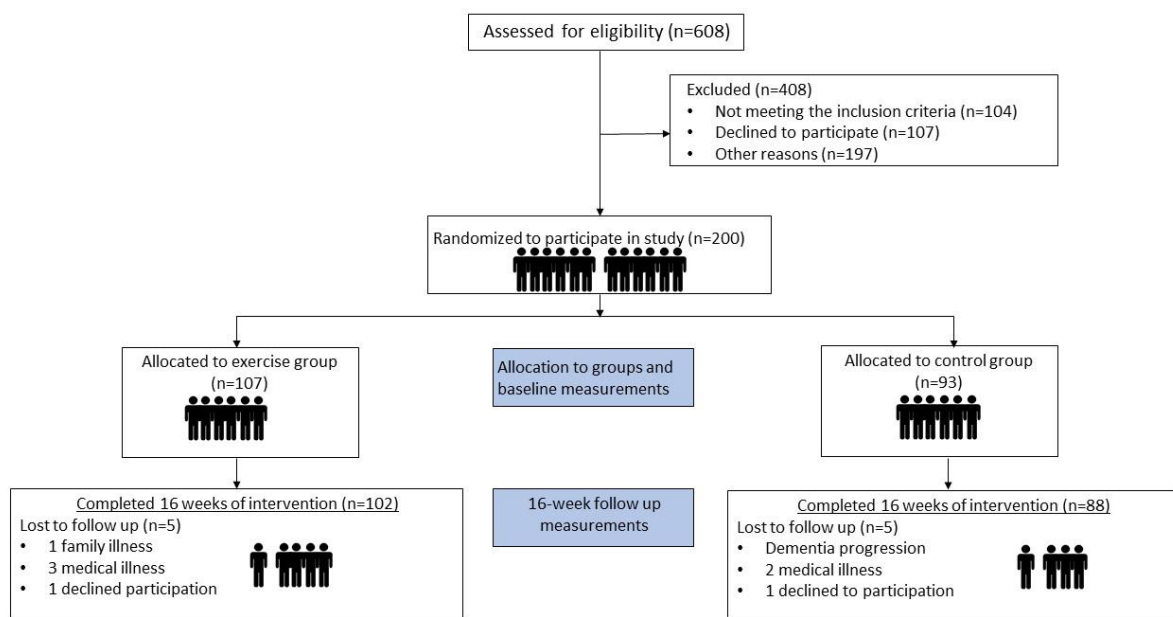


Figure 6 Enrollment flow chart in the ADEX study. A total of 608 patients were assessed for eligibility, and a total for 200 was found suitable and agreed to participate. Patients were randomized to either the controls group (n=93), or the exercise group (n=102), and baseline measures were recorded. After 16 weeks of intervention with either the exercise regime, or treatment at normal (control group), patients were tested again. A total of 102 patients completed the exercise intervention, and 88 controls participated in the follow up tests after 16 weeks.

Exercise regime

The physical exercise intervention used in this study was an aerobic exercise program consisting of three weekly sessions of one-hour group session of 2-5 participants on non-consecutive days, supervised by an experienced physiotherapist. The first four weeks of exercise consisted of an adaption period, and during the subsequent 12 weeks participants performed aerobic exercise of moderate-to-high intensity. In each session a total 3*10 minute was performed on an ergometer bicycle, cross trainer and treadmill with 2-5 min rest in between. Heart Rate (HR) was monitored during aerobic exercise, including the rest intervals. Target intensity was 70-80% of maximal HR (220 minus participants age). For participants in treatment with betablockers, the Borg scale of Perceived exertion was used to ensure intended exercise intensity. The target measurement was where the participant could not speak comfortably [146].

The participants assigned to the control group received treatment as usual. The physical activity of the control group was not monitored due to lack of funding /resources.

A subgroup of patients who attended 2/3 or more exercise sessions AND exercised with an intensity of 70% or more of maximal heart rate, was categorized as 'high exercise group'.

Samples

Blood samples were collected from all participants at baseline and 16-week follow up. Three different types of vials were collected. One vial for serum samples, two EDTA vials, one for plasma and buffycoat (white blood cells) and one for full blood. Finally, one lithium heparin for plasma samples. After collection, the blood samples were centrifuged at 2000 g at 4°C and aliquoted in to 250 µL aliquots in cryotubes, within two hours of collection, and frozen at -80°C pending analysis.

CSF was collected from a total of 56 subjects, before and after intervention. CSF was obtained by lumbar puncture between 9 a.m. and 2 p.m. and collected in polypropylene tubes. The mean delay from last days of intervention to lumbar puncture was 5.2 (3.7 SD) days for the exercise group and 6.2 (5.4 SD) days for the control group. After collection, the CSF samples were centrifuged at 2000 g at 4°C and aliquoted in to 250 µL aliquots in cryotubes, within two hours of collection, and frozen at -80°C pending analysis.

Statistics

The baseline characteristics were compared between the two groups using t-tests. The baseline and follow up concentrations of the investigated protein/peptides were analyzed using paired t-test within each group respectively, and the mean relative change from baseline in biomarker concentrations was analyzed by Student's t-test. These analyses were done both as an intention to treat (ITT) analysis where all participants

were analyzed, and as a per-protocol analysis where only the subjects that exercised with a mean intensity of > 70% of maximal heart rate (HR) and attended > 75% of the sessions (high exercise subjects), were included [134,144]. Furthermore, the *APOE* genotype contribution to the effect on the biomarkers was analyzed in non-carriers versus carriers of the AD risk relevant $\epsilon 4$ allele. The significance level was set to 0.05.

Concentration change calculations

The changes in concentration of the measured biomarkers, were presented as relative change from baseline and calculated as

$$\frac{[Follow\ up]-[Baseline]}{[Baseline]} * 100 = \text{relative change from baseline}$$

This calculation was used to take into account marginal levels at baseline (very low or very high levels). Further, it gives more emphasis on small changes in individuals with smaller baseline levels, than small changes in individuals with higher baseline levels. As a result, relative change from baseline < 0, equals lower concentration at follow up. Relative change from baseline = 0, equals no difference concentration at follow up compared to baseline. Relative change from baseline > 0, equals higher concentration at follow up.

Multiple comparison analyses

Due to the exploratory nature of the four studies included in this thesis, multiple analytes have been measured and analyzed. This increased the chance of false positive findings [147]. Although this was not addressed in the publications, in this thesis false positive findings were evaluated by applying the False Discovery Rate by Benjamini-Hochberg with a rate of 0.05.

Methods

Study I: Alzheimer's disease biomarkers – the amyloidogenic pathway

All measures were done in CSF in technical duplicates. All measurements per assay were performed in one round of experiments using one batch of reagents with double blinded samples.

A β Triplex (A β 38, A β 40 and A β 42): Concentrations of A β 38, A β 40 and A β 42 were measured using V-plex Peptide Panel 1 Kits A β 38, A β 40, A β 42 (Meso Scale Discovery system, Rockville, MD, USA) according to the manufacturer's protocol. In short, 60 μ L of CSF was diluted 2-fold in diluent provided with the kit and

calibrators and controls were prepared according to protocol. On a pre-coated 96 well MSD plates, 25 μ L of sample, calibrator or controls were added followed by addition of 25 μ L of detection antibody and incubated at room temperature for two hours. Lastly, the plates were washed in washing buffer provided with the kit and read in an MSD imager at relevant wavelength.

Total-tau: Total-tau was measured by the hTAU Ag ELISA assay (INNOTEST, Fujirebio, Japan) according to the manufacturer's protocol. In short, to an anti-human tau antibody pre-coated 96 well microtiter plate 25 μ L samples, controls and standards ranging from 50-2500 pg/mL were added followed by incubation with a biotinylated detection antibody to tau followed by peroxidase-conjugated streptavidin. The reaction was developed with tetramethyl benzidine (TMB) chromogen solution and stopped with 0.9M sulfuric acid. The plate was quantified at 450 nm in a microplate reader.

Phosphorylated tau: Tau phosphorylated at amino acid 181 (p-tau) was measured by the PHOSPHO-TAU (181p) ELISA assay (INNOTEST, Fujirebio, Japan) according to manufacturer's protocol. In short, on an anti-human p-tau antibody pre-coated 96 wells microtiter plate 75 μ L samples, controls and standards ranging from 15.6-1000 pg/mL were added followed by incubation with a biotinylated detection antibody to p-tau and addition of peroxidase-conjugated streptavidin. The reaction was developed with TMB chromogen solution, and subsequently stopped with 0.9M sulfuric acid. The plate was quantified at 450 nm in a microplate reader.

Immunoprecipitation mass spectrometry of A β : Immunoprecipitation mass spectrometry (IP-MS) of the full spectrum of A β forms present in CSF was performed as previously described [148]. In short, four μ g of the A β -specific antibodies 6E10 and 4G8 (epitope in the N-terminal region and epitope 18-22, respectively, Signet Laboratories, Inc., Dedham, MA, USA) were separately added to 25 μ L Dynabeads M-280 (Dyna). The IPs was conducted on 940 μ L CSF, with 10 μ L 2.5% Tween-20 (Bio-Rad Laboratories Inc). The beads/CSF solution (total volume 1 mL) was transferred to a KingFisher magnetic particle processor (polypropylene tubes, Thermo Scientific) for washing and elution in a 5-step procedure. The collected supernatant was dried in a vacuum centrifuge and re-dissolved in 5 μ L 0.1% formic acid in 20% acetonitrile.

The samples were analyzed by MALDI TOF/TOF operating in reflector mode. FlexAnalysis (version 3.3, Bruker Daltonics) was used for automated integration of the peaks for each spectrum. Prior to the statistical analysis, the peak areas were normalized to the sum of the integrated peaks, and the relative changes compared to baseline values were calculated. All solvents used were of HPLC quality. The A β species measured by this method: 1-14 aa, 1-15 aa, 1-16 aa, 1-17 aa, 1-19 aa, 1-20 aa, 11-40 aa, 1-28 aa, 1-

30 aa, 1-33 aa, 1-34 aa, 1-37 aa, 1-38 aa, 1-39 aa, 1-40 aa, 1-42 aa. The cut between 'long' and 'short' species, are between aa 16 and 17.

Soluble amyloid precursor protein alpha and beta: sAPP α and sAPP β were measured using Human sAPP α and Human sAPP β kits, respectively (IBL, Japan) according to the manufacturer's protocol. In short, 100 μ L of 1:40 diluted CSF, controls and standards were added to wells of anti-human sAPP α or sAPP β antibody pre-coated microtiter plates and incubated over night at 4°C. Following incubation, 100 μ L HRP-conjugated detection antibody was added to the wells. The reaction was developed with TMB chromogen solution, and subsequently stopped with 1M sulfuric acid. The plate was quantified at 450 nm in a microplate reader.

Apolipoprotein E genotyping: DNA was isolated with Promega Maxwell DNA purification kits (Promega, WI, USA), according to the manufacturer's protocol from 250 μ L of buffy coat from 6 mL EDTA vials. *ApoE* genotyping for the ϵ 2, ϵ 3, and ϵ 4 alleles was performed with a TaqMan qPCR assay as described by Koch *et al.*, 2002, and allele distribution calculated on the basis of probe fluorescence intensity by the program CFX manager (Bio-rad, CA, USA) [149]. In short, two reactions for each genotyping was set up.

1) SNP-rs429358 (334T/C). Primer set (conc. 1 μ M): 334TC-Forward: 5'-GGGCACGGCTGTCCAA-3', 334TC Reverse: 5'-ACCTCGCCGCGGTACTG-3'. And probe set (conc. 1 μ M): 334T-Probe: 5'-FAM-CGGCCGCACACGTCCTCC-TAMRA-3', 334C-Probe: 5'-JOE-CGGCCGCGCACGTCCT-TAMRA-3'. For one reaction was used: 11 μ L of TaqMan master mix (cat. 4304437, Invitrogen), 1.65 μ L of each primer, 0.55 μ L of each probe, 4.6 μ L H₂O and 2 μ L of purified genomic DNA.

2) NSP-rs7412 (472C/T). Primer set (conc. 2 μ M): 472CT-Forward: 5'-CTGCGCAAGCTGCGTAAG-3', 472CT-Forward: 5'-GCCCCGGCCTGGTACA-3'. And probe set (conc. 2 μ M): 472C-Probe: 5'-FAM-CCTGCAGAAGCGCCTGGCA-TAMRA-3', 472T-Probe: 5'-JOE-TGACCTGCAGAAGTGCCTGGCA-TAMRA-3'. For one reaction was used: 11 μ L of TaqMan master mix (cat. 4304437, Invitrogen), 3.3 μ L of each primer, 0.55 μ L of each probe, 2 μ L H₂O and 2 μ L of purified genomic DNA.

The reaction was run with the program 50 C for 2 min, 95C for 10 min and 40 *(95C for 15 sec, 60C for 60) sec with a read at appropriate wavelength after each cycle.

Study II: Markers of neuronal dysfunction

All measures were done in CSF in technical duplicates. All measurements per assay were performed in one round of experiments using one batch of reagents with double blinded samples.

Neurogranin (NG): NG concentration was measured using a previously published in-house Meso Scale Discovery assay as published in Kvarstberg *et al.*, 2011 and De Vos *et al.*, 2015 [150,151]. In short, a 96 well QUICKPLEX microtiter plates (MSD, Rockville, MD, USA) was coated with Ng7 antibody diluted in PBS over night at room temperature. Following, the plates was blocked with MSD blocking solution A (MSD), and washed in PBS. To the plates 50 ul of samples, controls and standards ranging from 31.3-4000 pg/mL was added in addition to 50 ul/well of primary antibody (ab23570, Upstate Biotechnology, NY, USA). After washing a MSD detection Antibody (Goat anti-rabbit sulfotag, MSD) was added, and the plate was lastly read with MSD Read Buffer T with surfactant in a QUICKPLEX SQ 120 reader (MSD).

Visinin-like protein 1 (VILIP1): Vilip-1 was measured using Human VILIP-1 ELISA (BioVendor GmbH, Heidelberg Germany) by following the manufactures protocol. In short, to an anti-human VILIP-1 antibody pre-coated 96 well microtiter plate 100 µL 1:3 diluted samples, controls and standards ranging from 100-5000 pg/mL were added followed by incubation with a biotinylated detection antibody to VILIP-1 followed by peroxidase-conjugated streptavidin. The reaction was developed with TMB chromogen solution and stopped with stop reagent included in the kit. The plate was quantified at 450 nm in a microplate reader.

Neurofilament light chain (NFL): NFL concentration was measured using NF-light ELISA kit (IBL international, Hamburg, Germany) by following the manufactures protocol. In short, to an anti-human NFL antibody pre-coated 96 well microtiter plate 100 µL samples, controls and standards ranging from 100-10000 pg/mL were added followed by incubation with a biotinylated detection antibody to NFL followed by peroxidase-conjugated streptavidin. The reaction was developed with TMB chromogen solution and stopped with stop reagent included in the kit. The plate was quantified at 450 nm in a microplate reader.

Chitinase 3-like 1 (YKL40): YKL40 concentration was measures by using Quantikine ELISA Human Chitinase 3-like 1 (R&D systems, MN, USA) by following the manufactures protocol. In short, to an anti-human CHI3L1 antibody pre-coated 96 well microtiter plate 100 µL 1:100 diluted samples, controls and standards ranging from 62.5-4000 pg/mL were added, followed by incubation with a horseradish peroxidase conjugated detection antibody to CHI3L1. The reaction was developed with TMB chromogen solution with hydrogen peroxide and stopped with 2N Sulfuric acid. The plate was quantified at 450 nm in a microplate reader.

Study III: Markers of Neuroinflammation

All measures were done in plasma and CSF, or CSF alone (sTREM2) in technical duplicates. All measurements per assay, except 8-isoprostane, were performed in one round of experiments using one batch of reagents with double blinded samples.

Cytokines: Concentration of selected cytokine was measured using the MSD v-plex proinflammation panel 1 human (Meso Scale Discovery, MA, USA), by following the manufacture protocol. In short, to a precoated 10-spot MSD 96 well microtiter plate 50 µL samples, controls and standards (ranging from IFN-γ: 0.37–938 pg/mL. IL-1β: 0.05–375 pg/mL. IL-2: 0.09–938 pg/mL. IL-4: 0.02–158 pg/mL. IL-6: 0.06–488 pg/mL. IL-8: 0.07–375 pg/mL. IL-10: 0.04–233 pg/mL. IL-12p70: 0.11–315 pg/mL. IL-13: 0.24–353 pg/mL. TNF-α: 0.04–248 pg/mL) were added followed by incubation with detection antibodies (Meso Scale Discovery, MA, USA), and the plate was lastly read with MSD Read Buffer T in a QUICKPLEX SQ 120 reader (MSD).

8-isoprostane: 8-isoprostane concentration was measured using 8-isoprostane ELISA kit (Cayman Chemicals, MI, USA) by following the manufacturers protocol. In short, to a precoated 96 well plates microtiter plate, 50 µL of sample, controls or standard ranging from 0.8-500 pg/mL was added, followed by incubation with tracer and antiserum solution (Cayman Chemicals, MI, USA). After, incubated with Ellemans reagent and tracer dye (Cayman Chemicals, MI, USA). The plate was quantified at 405-420 nm in a microplate reader.

sTREM2: sTREM2 concentration was measured with an in-house MSD assay. In short, a 96-well plate coated with streptavidin (Meso-Scale discovery (MSD) was incubated with biotinylated polyclonal goat anti-human TREM2 capture antibody (0.25 µg/ml R&D Systems BAF1828), and subsequently incubated with the CSF or standard solution constructed from recombinant human TREM2 protein ranging from 62.5-4000 pg/ml Sino Biological Inc. 11084-H08H). followed by addition of monoclonal mouse anti-human TREM2 detector antibody, (1µg/ml Santa Cruz Biotechnology; B-3, sc373828), and thereafter incubated with a secondary antibody (SULFO-TAG–labeled anti-mouse secondary antibody, MSD). Lastly, the plates were developed by adding MSD Read buffer and the light emission measured using the MSD SECTOR Imager 6000.

TREM2 genotype SNP rs75932628-T: TREM2 genotype for SNP rs75932628-T was analyzed using TaqMan assay (TaqMan SNP assay MTO human SMC_100657057_10, Life Technologies, CA, USA). In short, the reaction was set up with Primer and Probe provided with the kit. To each reaction 12.5 µL of TaqMan master mix (Invitrogen), 1.5 µL assay stock (primer and probes, Life Technologies, CA, USA), 3.7 µL H₂O and 7.5 purified DNA (concentration 30-100 ng). The reaction was run with the program 95C for 10 min and 40 *(95C for 15 sec., 60C for 60) sec with a read at appropriate wavelength after each cycle.

Study IV: The genetic contribution – The role of APOE

In this study a post hoc analysis was applied to the outcomes of the ADEX study previously published [134,135,141,144], where outcomes were investigated based on APOE genotype

APOE genotype – all participants: The remaining participants who did not have their genotyped assayed in study I, was genotyped, as described in study I.

Outcomes

The following tests were chosen a priori representing cognitive, neuropsychiatric and physical function on the ground that they showed significant changes following exercise in previous ADEX publications [134,135,141,144]:

Symbol Digit Modalities Test (SDMT): To assess mental speed and attention, we used SDMT, a number and symbol decoding test. Participants were asked to correctly decode several lines of symbols. Outcome measure total number of correct decodings in 120 seconds.

Neuropsychiatric inventory (NPI): To assess behavioral and psychological symptoms, as evaluated by caregivers, we applied NPI. NPI is a caregiver-rated 12-point questionnaire assessing twelve different behavioral and psychological symptoms common in AD. The caregiver rated each symptom according to frequency and severity.

Timed-Up-and-Go test (TUG): TUG test assesses basic mobility by measuring the time it takes for a person to stand up from a chair, walk 3 meters, cross a line, turn around, and walk back to the chair and sit down. Outcome measure seconds.

Timed 10-meter walk test: To assess gait speed, we applied the 10-meter walk test. Participant walk as fast as possible on a 10-meter long course. Outcome measure meter/seconds.

Timed 400-meter walk test: To assess walking endurance, we applied the 400-meter walk test. Participant were asked to walk as fast as possible 400 meters on a 20-meter long course marked with coloured cones. Outcome measure meter/seconds.

Maximal oxygen uptake (VO₂ max): As an estimate of fitness Vo₂max was calculated by the Astrand Cycle Ergometer test [152]. Calculated based on the workload and average heart rate during the last minute of a 6-minutte cycle test and corrected for age and body weight. Outcome measure ml/kg/min.

Beyond the tests described above the following secondary outcomes of the ADEX trial were not included in the analysis because they did not show significant changes with exercise in the original publications:

Mini mental state examination (MMSE) assessing global cognitive function. Stroop test assessing executive function. Alzheimer's disease assessment scale – cognitive subscale (ADAS-Cog) verbal memory test assessing memory function. Verbal fluency – letter and category task assessing lexical and categorical fluency; The physical activity scale for elderly (PASE) caregiver rated questionnaire assessing the level of physical activity; The European Quality of life-5 dimensions (EQ-5D) scale - questionnaire assessed by both caregiver and patient relating to health related quality of life; The Alzheimer's Disease Co-operative Study – activities of daily living /ADCS-ADL) scale assessing (by caregiver) competences of basic (dressing, eating, bathing) and instrumental (reading books, go shopping, cooking) activities of daily living; Hamilton Depression rating scale 17 items (HAMD-17) assessing severity of depression symptoms; Thirty-second chair stand test (STS) assessing lower-limb and endurance. Walk-talk test counting backwards while walking assessing dual-task performance; A detailed description of the tests is given in [144].

Summary of results

The results from the papers included in the thesis are summarized in this section.

Study cohort

Baseline characteristics in the control group, exercise group, and high exercise subgroup were comparable at baseline, as shown in Table 2. Body Mass Index (BMI) was slightly, but significantly higher in controls versus exercise group, and disease duration in the high exercise group was slightly shorter as compared to the control group. Table 2B displays the baseline characteristics of the subgroups with CSF samples and in these sub-groups, no differences were found between controls and exercise subjects.

Table 2	Controls	Exercise	p-value[€]	High exercise	p-value[§]
A) Whole group	(n=92)	(n=106)		(n=66)	
Age, years[¥]	71.4 (7.3)	69.9 (7.5)	0.14 [#]	70.2 (7.5)	0.31 [#]
Gender, n			0.13 [¤]		0.41 [¤]
males	57	56		34	
females	35	50		32	
Characteristics					
Disease duration, years from diagnosis[¥]	1.25 (1.0)	1.01 (1.0)	0.1 [#]	0.94 (0.9)	0.05 [#]
MMSE[¥]	24.15 (3.9)	23.8 (3.4)	0.54 [#]	24.1 (3.3)	0.96 [#]
Education, years[¥]	11.84 (2.8)	11.9 (2.7)	0.84 [#]	12.20 (2.7)	0.42 [#]
Weight, kg[¥]	71.35 (12.1)	73.0 (13.5)	0.36 [#]	72.5 (13.8)	0.18 [#]
Height, cm[¥]	172.02 (8.9)	170.2 (9.1)	0.15 [#]	170.08 (9.2)	0.18 [#]
BMI[¥]	24.08 (3.6)	25.2 (4.0)	0.04 [#]	23.03 (4.0)	0.12 [#]
APOE ε4 alleles, n			0.35 [¤]		0.22 [¤]
0	21	34		19	
1	45	45		26	
2	26	27		21	
B) CSF subgroup	(n=29)	(n=29)		(n=19)	
Age, years[¥]	68.83 (8.0)	68.83 (6.7)	0.83 [#]	68.56 (7.0)	0.91 [#]
Gender, n			0.17 [¤]		0.36 [¤]
Males	21	16		11	
Females	8	13		8	
Characteristics					
Disease duration, years from diagnosis[¥]	1.52 (1.1)	1.03 (0.9)	0.07 [#]	1.16 (1.0)	0.25 [#]
MMSE[¥]	25.07 (3.9)	25.41 (2.8)	0.70 [#]	25.05 (2.6)	0.99 [#]
Education, years[¥]	13.03 (2.9)	12.66 (2.8)	0.62 [#]	12.53 (2.5)	0.54 [#]
Weight, kg[¥]	73.28 (2.4)	73.28 (13.0)	0.51 [#]	75.37 (16.5)	0.63 [#]
Height, cm[¥]	174.76 (8.8)	172.45 (9.0)	0.33 [#]	172.74 (9.3)	0.45 [#]
BMI[¥]	23.91 (3.5)	25.39 (4.2)	0.15 [#]	25.13 (4.1)	0.28 [#]
APOE ε4 alleles, n			0.25 [¤]		0.21 [¤]
0	13	7		3	
1	10	14		9	
2	6	8		7	

Table 2 Baseline characteristics of the study cohort. A) all patients. B) CSF sub-group. [¥] Given as mean ± (Standard deviation), [#]Independent t-test, [¤] Chi-squared test, [€] Controls versus intervention, [§] Controls versus high exercise.

Study I: Alzheimer's disease biomarkers – the amyloidogenic pathway

In this study we found that A β species were not modulated by 16 weeks of physical exercise. Further, the two cleavage pathways of APP were analyzed, the amyloidogenic pathway and the non-amyloidogenic pathway, and A β sub-species. We found no result on either of the A β species. In addition, total tau concentration and p-tau concentrations were analyzed, and here we also found no effect of the intervention. Lastly, we analyzed the results on a background of *APOE* genotype, where carriers of the ϵ 4 allele were analyzed versus non-carriers and found no difference in the effect of PA on the markers investigated. The results are listed in table 3 and mean outcome in the biomarkers are shown at baseline and at follow up in figure 7.

Table 3	Baseline*			16 weeks follow-up*		Between group difference in mean change from baseline				Between group difference in mean change from baseline High exercise (n=18) versus control			
pg/mL	Control	Intervention	p-value	Control	Intervention	Intervention versus control Δ (95%CI)	p-value	High exercise versus control Δ (95%CI)	p-value	APOE ϵ 4 non-carriers High exercise control Δ (95%CI)	p-value	APOE ϵ 4 carriers High exercise control Δ (95%CI)	p-value
	(n=25)	(n=26)		(n=20)	(n=22)	(n=20, n=19)		(n=18, n=20)		(n=2, n=8)		(n=14, n=11)	
Aβ 38[#]	1735 (617)	1948 (728)	0.44	2034 (991)	1935 (628)	-68 (-271; 135)	0.50	-141 (-368; 87)	0.22	-142 (-674; 389)	0.55	-193 (484; 98)	0.18
Aβ 40[#]	3720 (1222)	4149 (1320)	0.30	4549 (1802)	4187 (1158)	-404 (-879; 71)	0.094	-489 (-102; 47)	0.072	-134 (-1513; 1245)	0.83	-703 (-1365; -41)	0.038
Aβ 42[#]	145 (69)	175 (107)	0.53	194 (80)	200 (121)	-21(-53; 11)	0.20	-29 (-63; 6)	0.10	-15 (-108; 78)	0.72	-41 (-83; 2)	0.059
Aβ 42/40	0.038 (0.012)	0.042 (0.021)	0.95	0.044 (0.013)	0.046 (0.022)	-0.0026 (-0.0062; 0.0011)	0.16	-0.0031 (-0.0070; 0.0009)	0.13	-0.0042 (-0.0143; 0.0058)	0.36	-0.0029 (-0.0080; 0.0021)	0.24
t-tau	693 (373)	722 (386)	0.69	747 (639)	868 (664)	78 (-134; 290)	0.46	113 (-136; 362)	0.36	-138 (-1131; 854)	0.76	215 (-5; 435)	0.055
p-tau	72 (30)	83 (31)	0.19	75 (32)	83 (35)	-4.8 (-12.8; 3.3)	0.24	-4.3 (-12.3; 3.6)	0.28	-10.9 (-33.1; 11.3)	0.29	-1.0 (-10.3; 8.3)	0.82
	(n=22)	(n=23)		(n=19)	(n=22)			(n=15, n=19)		(n=1, n=7)		(n=14, n=10)	
sAPP α	362 (240)	412 (198)	0.18	362 (289)	398 (151)	-34 (-142; 75)	0.53	-39 (-166; 88)	0.54	86 (-51; 223)	0.18	-93 (-260; 73)	0.26
sAPP β	859 (727)	942 (445)	0.13	742 (409)	912 (422)	15 (-198; 228)	0.89	57 (-173; 288)	0.62	179 (-939; 1298)	0.72	-32 (-268; 204)	0.78
sAPP β/α	2.14 (0.41)	2.38 (0.71)	0.17	2.29 (0.93)	2.25 (0.54)	-0.28 (-0.84; 0.28)	0.32	-0.10 (-0.69; 0.49)	0.73	-0.99 (-4.04; 2.06)	0.46	0.20 (-0.31; 0.72)	0.42
	(n=24)	(n=26)		(n=21)	(n=23)			(n=17, n=21)		(n=1, n=7)		(n=16, n=12)	
Aβ long/short[†]	6.6 (2.0)	6.6 (2.3)	0.92	8.6 (8.0)	6.8 (2.7)	-0.48 (-1.73; 0.77)	0.44	-0.38 (-1.69; 0.93)	0.56	0.61 (-5.30; 6.52)	0.81	-0.54 (-1.99; 0.91)	0.45

Table 3. Changes in CSF AD biomarkers after 16 weeks of intervention. Baseline and follow-up values of β -amyloid species, tau, phosphorylated τ -tau, and soluble APP α and β , and analysis of between group difference in mean change from baseline of β -amyloid, tau and phosphorylated τ -tau of intervention versus controls and per-protocol high-exercise versus controls. In addition, between group differences in mean change from baseline High exercise versus control depending of APOE ϵ 4 carrier status (0 alleles versus 1 or 2 alleles). Ab38: beta amyloid 1-38, Ab40: beta amyloid 1-40, Ab42: beta amyloid 1-42, Ab42/40: beta amyloid ration between isoform 1-42/1-40, T-tau: total-tau, P-tau: phosphorylated-tau, sAPP α and β : soluble amyloid precursor protein α and β , sAPP β/α : ration between soluble amyloid precursor protein β and α , Ab long/short: ration between beta amyloid species relative expression of long isoforms (A β >17 amino acids) and short isoforms (A β <16 amino acids) *mean(SD). [†] measured by MALDI-TOF/MS, # measured by MSD triplex.

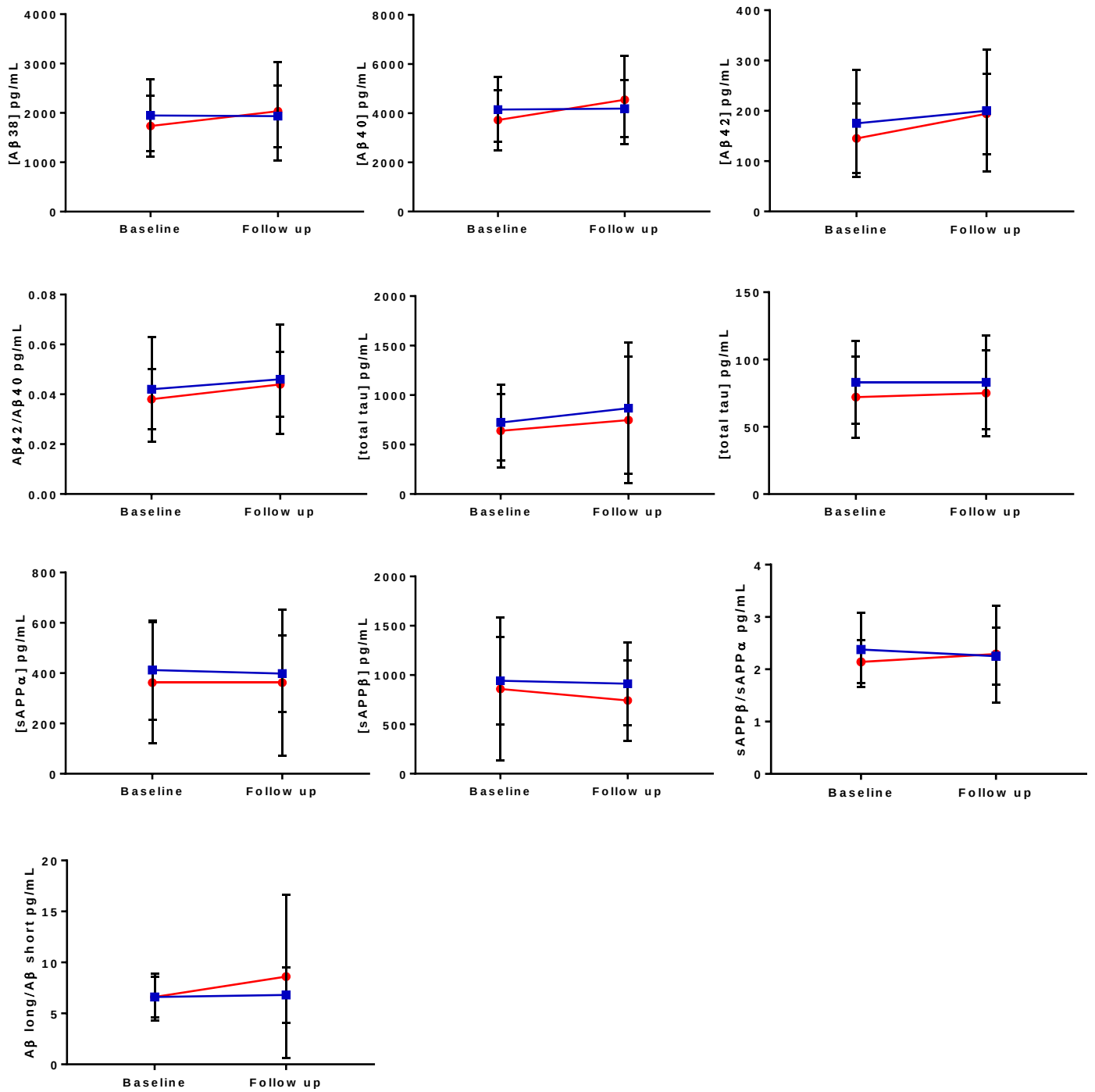


Figure 7. Exercise effect on Alzheimer's disease markers. Mean concentration of amyloid species and tau at baseline and at 16 weeks follow up. Red: control group, blue: exercise group.

Study II: Markers of neuronal dysfunction

We found no statistically significant difference in the concentration of the biomarkers (Neurofilament light, neurogranin, VILIP 1 and YKL-40) in mean change from baseline when comparing the controls and exercise group. Figure 8 displays the relative difference between the groups in percent ($((\text{follow-up} - \text{baseline}) / \text{baseline}) * 100$) in the biomarkers measured. In three out of four biomarkers analyzed, there was a trend in relative change from baseline towards an increased level in CSF after 16 weeks of intervention in the exercise group. However, the variation in the changes in both groups was large.

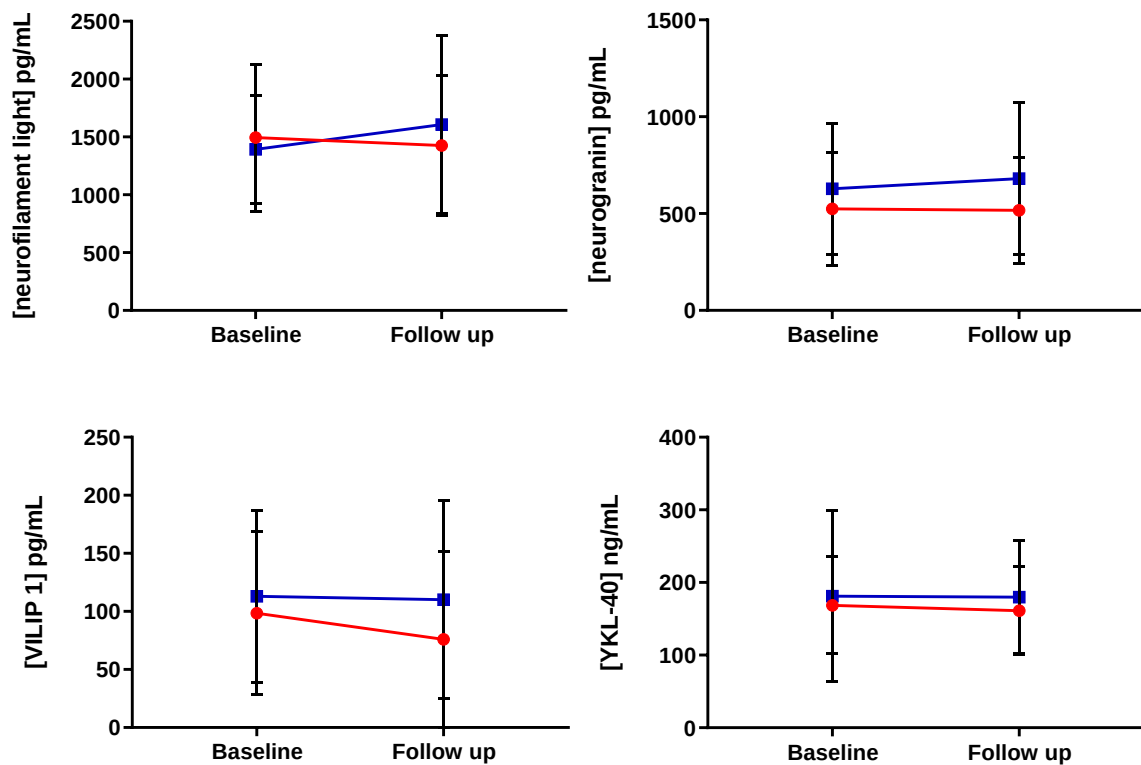


Figure 8. Exercise effect on markers of neuronal and synaptic stability. Mean concentration of neurofilament light, neurogranin, VILIP 1, and YKL-40 at baseline and at 16 weeks follow up. Red: control group, blue: exercise group

Table 4A	Baseline [‡]		16 weeks follow up [‡]		Mean relative change from baseline, ((16 week follow up – Baseline)/Baseline) * 100 ^{‡ #}				
	Controls	Exercise	Controls	Exercise	controls	Exercise	p-value	High exercise	p-value
	(n=24)	(n=25)	(n=19)	(n=21)	(n=18)	(n=21)		(n=14)	
NFL, CSF (pg/mL)	1495 (635)	1392 (467)	1427 (606)	1607 (768)	6.2 (42)	19 (71)	0.51	30 (86)	0.3
	(n=25)	(n=25)	(n=20)	(n=22)	(n=20)	(n=22)		(n=15)	
NG, CSF (pg/mL)	524 (293)	628 (339)	517 (274)	681 (383)	-1.7(14)	8.7 (29)	0.15	1.5 (15)	0.5
	(n=26)	(n=25)	(n=26)	(n=25)	(n=19)	(n=21)		(n=14)	
VILIP 1, CSF (pg/mL)	99 (70.4)	113 (74)	76 (76)	110 (85)	3.7 (59)	33 (67)	0.15	34 (62)	0.2
	(n=24)	(n=25)	(n=20)	(n=22)	(n=20)	(n=22)		(n=15)	
YKL-40, CSF (ng/mL)	168 (67)	181 (118)	161 (60)	180 (78)	-3.7 (19)	5.7 (25)	0.19	2.2 (22)	0.4

Table 4B	APOE ε4 non- carriers			APOE ε4 carriers		
	Mean relative change from baseline, ((16 week follow up – Baseline)/Baseline) * 100* #					
	Controls	Exercise	p-value	Controls	Exercise	p-value
	(n=9)	(n=5)		(n=9)	(n=16)	
NFL, CSF (pg/mL)	20 (49)	65 (142)	0.4	-7.8 (30)	4.5 (22)	0.3
	(n=10)	(n=5)		(n=10)	(n=17)	
NG, CSF (pg/mL)	-3.7 (16)	27 (54)	0.1	0.3 (11)	3.3 (15)	0.6
	(n=10)	(n=4)		(n=9)	(n=17)	
VILIP 1, CSF (pg/mL)	2.8 (60)	61 (86)	0.1	4.8 (61)	26 (63)	0.4
	(n=10)	(n=5)		(n=10)	(n=17)	
YKL-40, CSF (ng/mL)	1.2 (19)	8.7 (39)	0.6	-8.3 (19)	4.8 (22)	0.1

Table 4. Changes in CSF AD biomarkers after 16 weeks of intervention. A: Baseline and follow-up values of neurofilament light, neurogranin, visinin-like protein 1, and chitinase 3-like 1, and analysis of between group difference in mean change from baseline of intervention versus controls and per-protocol high-exercise versus controls. B: between group differences in mean change from baseline exercise versus control depending of APOE ε4 carrier status (0 alleles versus 1 or 2 alleles). NFL: neurofilament light, NG: neurogranin, VILIP1: visinin-like protein 1, and YKL40: chitinase 3-like 1 *mean (SD). # A positive value means greater positive mean change from baseline in the exercise group.

Study III: Markers of Neuroinflammation

Over all, most of the analyzed biomarkers were not affected by PA, and remained stable over the intervention period, see table 5a. However, when analyzing relative change from baseline, the IL6 plasma concentration increased significantly in the exercise group compared to the control group with a mean change from baseline at 41.03 (SD 76.7) compared to -0.97 (SD 49.4) in the control group ($p=0.049$). When analyzing the high exercise group vs controls, the same outcome was found with a mean change of 48.01 (SD 85.8) and -0.97 (SD 49.4), respectively ($p=0.047$). Similar results with borderline significance were found for sTREM2 in CSF. Here, sTREM2 mean relative change from baseline was 6.9 (SD 14.0) in the high exercise group compared to -2.4 (SD 13.8) in controls, ($p=0.05$). *TREM2* SNP assay showed no genotype that corresponded with genetically altered sTREM2 levels.

In addition, when stratifying for *APOE* genotype in carriers and non-carriers of the $\epsilon 4$ allele, we found that plasma $\text{INF}\gamma$ concentration increased more in controls who were $\epsilon 4$ carriers (114.7 [SD 188.3] compared to 7.8 (SD 42.8) in the $\epsilon 4$ -positive exercise group, $p=0.038$). The opposite was observed when analyzing sTREM2 based on *APOE* genotype. Here, there was an increase in exercising *APOE* $\epsilon 4$ carriers, 5.7 (14.4), compared to a decrease in the controls -6.4 (15.5), $p=0.037$, see table 5b.

However, when correction for multiple comparisons with a Benjamini-Hochberg adjustment with as false discovery rate of 0.05, no effect of PA was found in any of the markers in either CSF or plasma, see table 6 and 7.

Table 5A	Baseline [‡]		16 weeks follow up [‡]		Mean relative change from baseline, ((16 week follow up – Baseline)/Baseline) * 100, [‡] #				
	Controls	Exercise	Controls	Exercise	controls	Exercise	p-value	High exercise	p-value
pg/mL	(n=27)	(n=28)	(n=24)	(n=23)	(n=22)	(n=22)		(n=16)	
sTREM2, CSF	8172.51 (2656.2)	8193.32 (2992.5)	7717.16 (2488.7)	8596.5 (3267.5)	-2.4 (13.8)	5.0 (12.9)	0.075	6.9 (14.0)	0.05
	(n=27)	(n=24)	(n=27)	(n=19)	(n=26)	(n=20)		(n=14)	
IFN γ , CSF	0.56 (0.2)	0.52 (0.2)	0.55 (0.1)	0.54 (0.2)	9.0 (34.0)	1.6 (52.8)	0.57	-4.1 (51.9)	0.34
IL10, CSF	0.10 (0.0)	0.09 (0.1)	0.10 (0.1)	0.10 (0.6)	13.5 (52.4)	18.0 (67.5)	0.80	15.2 (70.8)	0.93
IL13, CSF	0.72 (0.5)	0.66 (0.3)	0.67 (0.3)	0.81 (0.5)	7.9 (68.7)	55.8 (115.4)	0.089	48.1 (107.2)	0.16
IL2, CSF	0.18 (0.2)	0.17 (0.12)	0.14 (0.1)	0.13 (0.1)	11.7 (96.1)	4.6 (69.3)	0.79	-0.1 (78.4)	0.70
IL6, CSF	1.15 (0.4)	1.09 (0.3)	1.34 (0.6)	1.08 (0.3)	21.7 (50.1)	1.9 (28.2)	0.13	3.5 (29.5)	0.22
IL8, CSF	44.75 (13.0)	42.41 (9.2)	45.05 (15.4)	40.74 (10.5)	2.7 (24.7)	-5.0 (16.6)	0.25	-2.5 (15.9)	0.48
TNF α , CSF	0.17 (0.1)	0.17 (0.1)	0.17 (0.1)	0.16 (0.1)	4.5 (31.9)	-4.7 (38.8)	0.39	-6.8 (41.5)	0.34
	(n=23)	(n=24)	(n=23)	(n=21)	(n=19)	(n=21)		(n=14)	
IFN γ , plasma	5.11 (3.4)	4.88 (2.2)	4.96 (3.2)	5.15 (2.2)	61.4 (144.6)	22.1 (63.7)	0.27	11.6 (44.2)	0.22
IL10, plasma	0.33 (0.2)	0.29 (0.2)	0.45 (0.8)	0.29 (0.2)	6.2 (58.6)	31.2 (95.6)	0.33	35.2 (109.8)	0.33
IL6, plasma	2.91 (7.4)	0.67 (0.3)	3.28 (8.6)	0.79 (0.3)	-0.97 (49.4)	41.03 (76.7)	0.049	48.0 (85.8)	0.047
IL8, plasma	4.78 (1.6)	4.76 (1.4)	5.57 (2.3)	5.40 (1.5)	27.9 (65.5)	20.7 (38.9)	0.67	25.4 (41.7)	0.90
TNF α , plasma	1.63 (0.6)	1.72 (0.4)	1.82 (0.6)	1.71 (0.4)	33.2 (83.1)	1.2 (39.6)	0.12	-3.7 (41.9)	0.14
	(n=28)	(n=27)	(n=23)	(n=22)	(n=20)	(n=22)		(n=15)	
8-isoprostane, CSF	8.7 (4.0)	9.93 (5.4)	8.97 (3.6)	16.28 (4.1)	33.2 (99.8)	80.1 (192.4)	0.33	95.4 (225.9)	0.28
	(n=87)	(n=100)	(n=80)	(n=90)	(n=75)	(n=86)		(n=55)	
8-isoprostane, plasma	70.17 (94.0)	68.36 (86.8)	70.79 (88.9)	60.25 (75.8)	213.9 (582.7)	109.9 (354.2)	0.17	98.8 (296.7)	0.18

Table 5a. *The effect of exercise on biomarker concentrations.* Baseline and follow-up values of plasma and CSF markers, and analysis of between group difference in mean change from baseline of intervention versus controls and per-protocol high-exercise versus controls. [‡] Given as mean $\pm$ (SD) # A positive value means greater positive mean change from baseline in the exercise group.

Table 5B	APOE ε4 non- carriers			APOE ε4 carriers		
Mean relative change from baseline, ((16 week follow up – Baseline)/Baseline) * 100 [‡] #						
pg/mL	Controls	Exercise	p-value	Controls	Exercise	p-value
	(n=9)	(n=5)		(n=13)	(n=17)	
sTREM2, CSF	3.5 (8.1)	2.5 (3.89)	0.81	-6.4 (15.5)	5.7 (14.4)	0.037
	(n=12)	(n=4)		(n=14)	(n=15)	
IFN γ , CSF	12.6 (38.1)	-2.4 (42.2)	0.52	5.8 (31.1)	9.4 (51.1)	0.82
IL10, CSF	25.4 (66.5)	23.6 (74.6)	0.96	3.3 (36.3)	16.5 (68.2)	0.53
IL13, CSF	29.1 (73.8)	62.3 (117)	0.31	-10.3 (61.0)	46.3 (103.4)	0.08
IL2, CSF	45.2 (129.9)	18.1 (40.4)	0.69	-17.0 (40.0)	1.0 (75.9)	0.44
IL6, CSF	39.3 (59.2)	-14.7 (26.8)	0.11	5.6 (36.3)	6.3 (27.9)	0.98
IL8, CSF	1.9 (21.8)	-11.6 (21.4)	0.30	3.4 (27.7)	-3.3 (15.5)	0.43
TNF α , CSF	16.2 (38.8)	-9.1 (33)	0.26	-5.6 (21.0)	2.9 (33.3)	0.42
	(n=10)	(n=5)		(n=9)	(n=16)	
IFN γ , plasma	13.5 (72.1)	67.9 (100.2)	0.24	114.7 (188.4)	7.8 (42.8)	0.038
IL10, plasma	-3.6 (45.5)	35.46 (68.4)	0.21	17.1 (71.7)	29.8 (104.4)	0.75
IL6, plasma	-17.15 (47.4)	1.4 (71.3)	0.56	17.0 (47.7)	53.4 (76.4)	0.21
IL8, plasma	31.2 (80.6)	3.0 (23.3)	0.46	24.2 (48)	26.2 (41.6)	0.91
TNF α , plasma	26.6 (68.3)	1.3 (46.3)	0.47	40.5 (101.1)	1.1 (39.2)	0.17
	(n=10)	(n=5)		(n=10)	(n=17)	
8-isoprostane, CSF	9.7 (94.9)	139.0 (143.8)	0.058	56.5 (101.5)	62.8 (204.9)	0.93
	(n=19)	(n=31)		(n=56)	(n=55)	
8-isoprostane, plasma	279.0 (492.1)	179.9 (479.4)	0.48	191.8 (612.9)	71.0 (255.9)	0.18

Table 5b. **The effect of exercise on biomarker concentrations stratified by APOE genotype.** Group differences in mean change from baseline, exercise versus control depending of APOE ε4 carrier status (0 alleles versus 1 or 2 alleles). [‡] Given as mean ± (SD) # A positive value means greater positive mean change from baseline in the exercise group.

Table 6 Assay	Control vs. exercise Org. p-value	B-H-adj. p-value	Sig. after adjustment	Control vs. high exercise org. p-value	B-H-adj. p-value	Sig. after adjustment
sTREM2, CSF	0.075	0.56	No	0.05	0.5	No
IFN γ , CSF	0.57	0.71	No	0.34	0.51	No
IL10, CSF	0.80	0.86	No	0.93	0.93	No
IL13, CSF	0.089	0.53	No	0.16	0.53	No
IL2, CSF	0.79	0.88	No	0.70	0.81	No
IL6, CSF	0.13	0.56	No	0.22	0.55	No
IL8, CSF	0.25	0.54	No	0.48	0.63	No
TNF α , CSF	0.39	0.53	No	0.34	0.49	No
IFN γ , plasma	0.27	0.54	No	0.22	0.51	No
IL10, plasma	0.33	0.58	No	0.33	0.55	No
IL6, plasma	0.049	0.74	No	0.047	1.41	No
IL8, plasma	0.67	0.80	No	0.90	0.93	No
TNF α , plasma	0.12	0.60	No	0.14	0.5	No
8-isoprostane, CSF	0.33	0.52	No	0.28	0.53	No
8-isoprostane, plasma	0.17	0.51	No	0.18	0.49	No

Table 6 Benjamini-Hockberg adjustment for multiple comparisons of result in study III. FDR at 0.05. Correction for finding a significant difference in the analytes due to pure chance was adjusted with Benjamini-Hockberg adjustment. None of the significant results from table 5a were significant after adjustment.

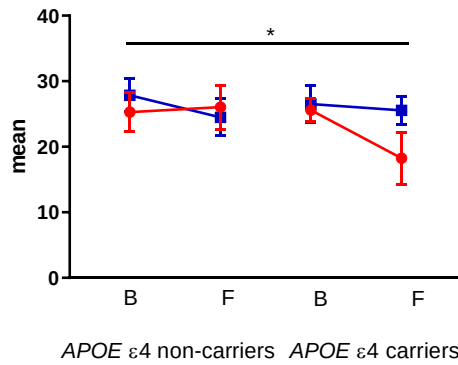
Table 7 Assay	Non-carriers Org. p-value	B-H-adj. p-value	Sig. after adjustment	Carriers org. p-value	B-H-adj. p-value	Sig. after adjustment
sTREM2, CSF	0.81	0.87	No	0.037	0.55	No
IFN γ , CSF	0.52	0.71	No	0.82	1.03	No
IL10, CSF	0.96	0.96	No	0.53	0.80	No
IL13, CSF	0.31	0.66	No	0.08	0.40	No
IL2, CSF	0.69	0.8	No	0.44	0.73	No
IL6, CSF	0.11	0.83	No	0.98	0.98	No
IL8, CSF	0.3	0.75	No	0.43	0.81	No
TNF α , CSF	0.26	0.78	No	0.42	0.90	No
IFN γ , plasma	0.24	0.90	No	0.038	0.29	No
IL10, plasma	0.21	1.05	No	0.75	1.02	No
IL6, plasma	0.56	0.70	No	0.21	0.53	No
IL8, plasma	0.46	0.86	No	0.91	1.05	No
TNF α , plasma	0.47	0.78	No	0.17	0.64	No
8-isoprostane, CSF	0.058	0.87	No	0.93	1.00	No
8-isoprostane, plasma	0.48	0.72	No	0.91	1.05	No

Table 7 Benjamini-Hockberg adjustment for multiple comparisons of result in study III, APOE stratification. FDR at 0.05. Correction for finding a significant difference in the analytes due to pure chance was adjusted with Benjamini-Hockberg adjustment. None of the significant results from table 5b were significant after adjustment.

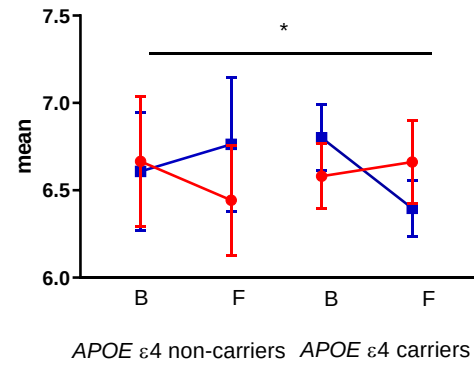
Study IV: The genetic contribution – role of *APOE*

In this post hoc analysis we found that patients who carried the *APOE* $\epsilon 4$ allele seemed to benefit more from the exercise intervention than non-carriers on a few of the outcomes (Figure 9). For the distribution of non-carriers versus carriers, see table 2a. We found that patients who were carriers of the *APOE* $\epsilon 4$ allele in the exercise group maintained their performance in the SDMT test whereas the carriers of the *APOE* $\epsilon 4$ allele in the control group deteriorated in performance, and changes with exercise differed significantly between carriers and non-carriers ($p=0.024$, Table 8). Carriers of the *APOE* $\epsilon 4$ allele who exercised experienced an improvement in neuropsychiatric symptoms compared to a worsening in the control *APOE* $\epsilon 4$ carrier group. In contrast, we could not detect an effect of the exercise intervention on neuropsychiatric symptoms in the non-carrier group. However, when comparing changes with exercise in carriers and non-carriers no significant difference was found. Further, *APOE* $\epsilon 4$ allele carriers who exercised improved their physical performance measured by walking speed compared both to non-carriers who exercised and to control *APOE* $\epsilon 4$ carriers, and changes with exercise differed significantly in one of the physical tests between carriers and non-carriers (TUG, $p=0.014$). When adjusting for multiple comparisons, exercise still showed an effect on NPI, TUG and estimated VO_{2max} in the *APOE* $\epsilon 4$ carriers, whereas differences in changes between carriers and non-carriers were no longer significant for any of the outcomes (Table 9)

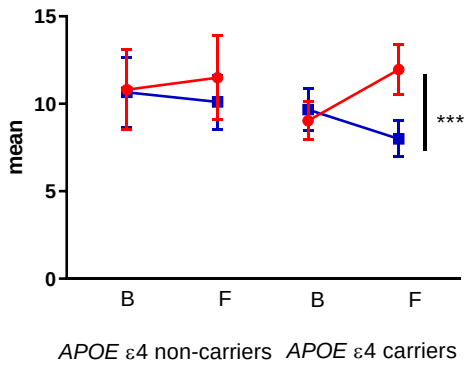
Symbol digit modalities test (# correct in 120sec)



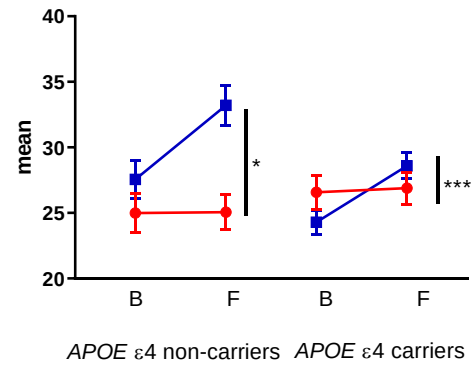
Timed up-and-go (sec)



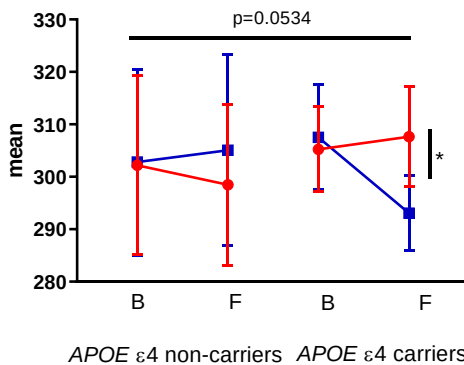
Neuropsychiatric inventory



Estimated VO2max (ml/kg/min)



400m walk (sec)



10m walk (m/s)

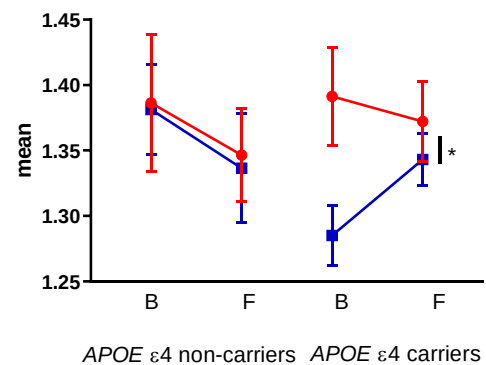


Figure 9. The effect of *APOE* ϵ 4 allele on physical exercise outcomes. Mean scores in outcome intention to treat analysis. All participants analyzed with independent t-test, respectively of *APOE* ϵ 4 carrier status and randomization group. B: baseline, F: 16 weeks follow-up, * $p < 0.05$, *** $p < 0.001$, displayed as mean score in groups with SD Red:

Table 8 <i>APOE ε4 non-carriers</i>							<i>APOE ε4 carriers</i>						
Mean (SEM)	Baseline			Follow-up			Baseline			Follow-up			p-value ³
	Controls	Exercise	P-value ¹	Controls	Exercise	p-value ²	Controls	Exercise	p-value ¹	Controls	Exercise	p-value ²	
Symbol digit modalities test, # in 90 min	25.8 (2.84)	27.8 (2.50)	0.08	26.1 (3.35)	24.5 (2.84)	0.16	25.6 (1.78)	26.5 (2.84)	0.95	18.2 (3.97)	25.6 (2.10)	0.06	0.024
Neuropsychiatric inventory, range 0-144	10.8 (2.27)	10.7 (2.02)	0.64	11.5 (2.40)	10.1 (1.54)	0.73	9.0 (1.10)	9.7 (1.19)	0.47	12 (1.45)	7.8 (1.03)	0.0002	0.16
Time up and go, sec.	6.7 (0.37)	6.6 (0.34)	0.35	6.4 (0.31)	6.8 (0.38)	0.18	6.6 (0.19)	6.8 (0.19)	0.22	6.7 (0.34)	6.4 (0.16)	0.015	0.014
Est. VO ₂ max, ml/kg/min	25 (1.51)	27.6 (1.43)	0.15	25.1 (1.31)	33.2 (1.54)	0.024	26.6 (1.28)	24.3 (0.91)	0.05	26.9 (1.22)	28.6 (0.98)	0.0002	0.88
400 m walk, sec	302.2 (17.11)	302.8 (17.72)	0.54	298.45(15.34)	305.1 (18.19)	0.54	305.2 (8.11)	307.5 (10.00)	0.69	307.6 (9.50)	293.0 (7.18)	0.042	0.07
10 m walk, m/sec	1.4 (0.05)	1.4 (0.03)	0.98	1.4 (0.04)	1.3 (0.04)	0.78	1.4 (0.04)	1.3 (0.02)	0.0061	1.4 (0.03)	1.3 (0.02)	0.027	0.13

Table 8. Effects of the intervention in APOE ε4 non-carriers and APOE ε4 carriers. ¹Baseline difference between controls versus exercise within APOE status.

Adjusted for clustering within training groups and centers. ²Follow up difference between controls versus exercise (beyond the difference present at baseline) within APOE status. Adjusted for clustering within training groups and centers. ³Effect of APOE status on the effect of exercise

Table 9	<i>Effect of exercise on APOE ε4 non-carriers</i>			<i>Effect of exercise on APOE ε4 carriers</i>			<i>The effect of APOE genotype on the effect of PA</i>		
	Org. p-value ¹	B-H-adj. p-value	Sig. after adjustment	Org. p-value ¹	B-H-adj. p-value	Sig. after adjustment	Org. p-value ²	B-H-adj. p-value	Sig. after adjustment
<i>SDMT</i>	0.16	0.24	No	0.06	0.10	No	0.024	0.07	No
<i>NPI</i>	0.73	0.80	No	0.0002	0.0024	YES	0.16	0.91	No
<i>Time up and go</i>	0.18	0.24	No	0.015	0.045	YES	0.014	0.08	No
<i>Est. VO₂ max</i>	0.024	0.06	No	0.0002	0.0012	YES	0.88	0.88	No
<i>400 m walk</i>	0.54	0.65	No	0.042	0.084	No	0.07	0.14	No
<i>10 m walk</i>	0.78	0.78	No	0.027	0.06	No	0.13	0.20	No

Table 9. Effects of the intervention in APOE ε4 non-carriers and APOE ε4 carriers. Benjamini-Hockberg adjustment for multiple comparisons of result in study III. FDR at 0.05. ¹ Follow up difference between controls versus exercise (beyond the difference present at baseline) within APOE status. Adjusted for clustering within training groups and centers. ² Effect of APOE status on the effect of exercise.

Discussion

Physical activity and exercise as a non-pharmacological treatment has proven beneficial for reducing the risk for many diseases including stroke, high blood pressure and mental disorders like chronic stress and depression [112]. However, compared to our understanding of PA's impact on cardiovascular health and general fitness, our understanding of PA's impact on cognitive health is still very much in its infancy. Very few studies have included biomarkers as an end-point in their analysis of PA's effect of dementia, which we addressed in a systematic review [113] and found a need for a molecular biomarker aspect included the analysis.

The clinical diagnostic markers for Alzheimer's disease in CSF.

In this study we analyzed whether 16 weeks of moderate-to-high intensity PA would modify the levels of A β in the brain. We found no effect in any of the relevant markers. Our findings are not in line with previous studies in animal models for AD. Both A β and p-tau have been shown to be reduced in the brain tissue as a response to moderate-intensity exercise in transgenic (Tg) mouse models [83,153]. In mouse models A β seems to disappear from the brains of the mice that are housed with access to running wheels. Figure 10 summarizes the findings in mouse models in exercise studies.

In general, Tg AD mouse models are accepted as translatable to human subjects with AD [5,154], however there have been numerous drug and intervention studies showing reduced amyloid burden in mice but not in AD patients [155]. One issue with the amyloid mouse models is that Tg mice overexpress APP [83,156,157]. The overexpression of proteins in models raises some concerns, such as that overexpression of proteins in itself could be toxic for the cells, and cell toxicity is therefore not amyloid specific. In addition, overexpression generates vast amounts of protein that could enable an unrepresentative large detectable reduction in the protein levels, due to a given intervention. Furthermore, not all the Tg mouse models develop plaques, as seen in human patients with AD [157]. Further, in many mouse models the cognitive decline precedes the formation of AD pathology in the form of A β build up [158], which is in direct contrast to humans where A β reduction in CSF can be measured decades before onset of cognitive complaint as seen in figure 2. These concerns could suggest that the difference in A β pathology between humans and mice makes direct comparison of the effect of an exercise intervention less valid and could account for the disagreement between our findings and previous animal findings on the AD biomarkers A β and tau.

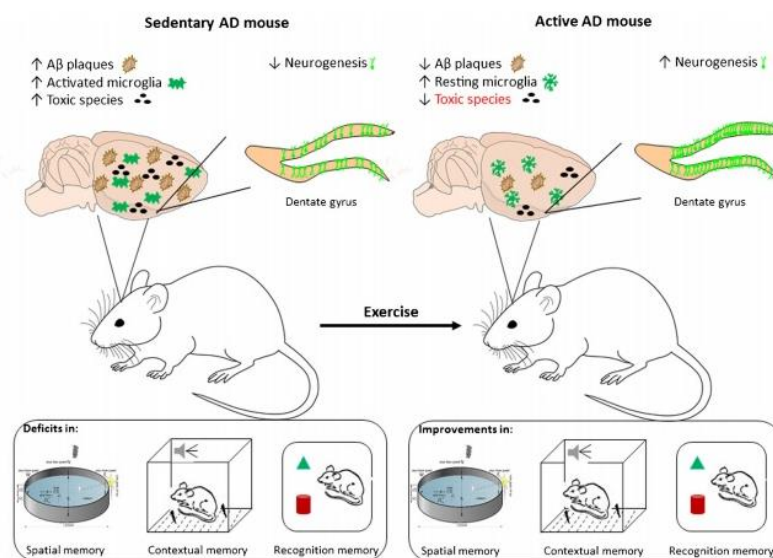


Figure 10. Mouse model findings in exercise's effect on AD pathology. In mouse models for AD a sedentary life will build up toxic species, activate microglia, and in some models, Aβ deposits. This also reflects on cognition, tested with Morris water maze, and fear response. This is however found to be positively modified with access to running wheel and living in enriched housing. Picture from [158].

Instead of assuming that a decrease in amyloid accumulation in the brain would be reflected in an increased clearance of Aβ from the brain to CSF, an alternative hypothesis could be that physical exercise may not lead to an increased clearance via the CSF, but to an increase in on-site clearance by proteases. Previous studies have found that the enzymes IDE and neprilysin may contribute to the clearance of Aβ [159,160]. A recent study found that wheel running reduced extracellular, soluble Aβ in the brains of Tg2576 mice in a dose-dependent manner through an up-regulation of Aβ clearance by activation of IDE and neprilysin [161]. Whether a similar mechanism occurs in humans remains unknown. However, mass spectrometry analysis in the present study of the relative expression of several Aβ peptides did not show any differences in the levels between controls and the intervention group. This could indicate that Aβ is not cleared on site, or that the clearance is not detectable with our methods, for instance Aβ species shorter than the Aβ1-13 aa. In this study we tried to measure IDE and NEP concentrations, and their activity in plasma and CSF, however this proved difficult. Assays were not specific or sensitive enough, and most samples fell below limit of detection. With this in mind however, measuring the effect of PA on these two proteins would be interesting, and better assays may help to resolve the involvement of IDE and NEP in the response to physical exercise in the future.

A further concern and limitation in comparing results from a PA intervention in mice and humans, is the actual PA. Unless forced PA has been employed in the mouse studies, it may be hard to monitor exactly how much each mouse is running, and studies show that mice tend to run in short bursts of high intensity many times during their active period (dark period) [162], and the duration and intensity of PA may be much higher in mice studies than in human studies. In addition, older mice run less (measured in distance) than young mice, and on top of that there is species differences, and most studies showing significant reduction in A β in mice brain have employed younger mice (one to 6 months of age), who might run for a longer time and more vigorously than the exercise undertaken by older adults humans with AD [162]. This of course could be controlled by applying forced PA e.g. motorized running wheel or swimming interventions in the animal studies. However, this seems not to be the general method chosen for analyzing impact of exercise on A β in AD mice, and forced methods may induce unwanted adverse effects, such as stress and anxiety.

In addition to the markers of specific AD pathology, we also measured the step between APP and A β formation, sAPP α and sAPP β . The hypothesis was that an intervention with exercise could shift the process from the amyloidogenic pathway, resulting in the toxic A β 42 species, to a non-amyloidogenic pathway, where soluble non-aggregable A β species are produced instead. Here we didn't find evidence that PA could cause a shift like this. We analyzed the sAPP α and β forms and the ratio between sAPP β / α . We did not find a significant difference in the sAPP β / α ratio in our study. Since the generation of the short A β peptides A β 1-15/16 is dependent on α -and β -secretase activity (non-amyloidogenic pathway) while the longer A β peptides (e.g. A β 42) are generated by combined action of β -and γ -secretase [163,164], we in addition analyzed whether the ratio between long and short A β peptides was affected by physical exercise. We found no significant difference in the mean ratios indicating that physical exercise does not seem to influence the cleavage of APP towards the non- amyloidogenic pathways. Overall no positive change was found in the A β etiology by PA.

Synapse stability and neurogenesis

As mentioned earlier, neuronal degradation and loss of synapses are normal in aging. Nonetheless, studies have shown that the hippocampus is one of the regions in the brain where neurogenesis continues throughout life [165]. However, this neurogenesis may be reduced in AD, where brain atrophy is especially severe in the hippocampus [166]. Exercise has shown to alter the progression of brain volume loss in healthy elderly subjects and in subjects with MCI or AD dementia [100,167,168], and one hypothesis could be that PA would act by stabilizing neurons and their network, measured by a decrease in release of neuronal and synaptic proteins to the CSF.

In this explorative study we investigated the effect of PA on selected biomarkers of neuronal and synaptic integrity. The biomarkers investigated in this study are all found in CSF under normal physiological conditions, and the levels found in our study corresponded to previous findings [76,77,169,170].

Increased CSF levels of the biomarkers NFL, NG, YKL-40 and VILIP-1 have been found in AD, and correlates with neurodegeneration, suggesting a potential use as surrogate markers of synaptic and neuronal loss [151,170–172]. Exercise and physical activity has been linked to cognitive preservation and structural and functional neuronal changes in humans [173]. However, the underlying neurobiology is unknown. To gain new insight into the molecular pathology in AD and possible changes with PA several pathophysiological pathways can be explored. We selected the markers in this study on the basis that they were recently investigated biomarkers of AD even though very little is known of their ability to reflect changes in the rate of neurodegeneration and if they are able to change within 16 weeks of exposure.

The main hypothesis was that PA would have a stabilizing effect on the markers, due to lesser synapse loss or even a neurogenesis effect of PA. Figure 11, is a graphical representation of the hypothesis of stimulation of neurogenesis through PA.

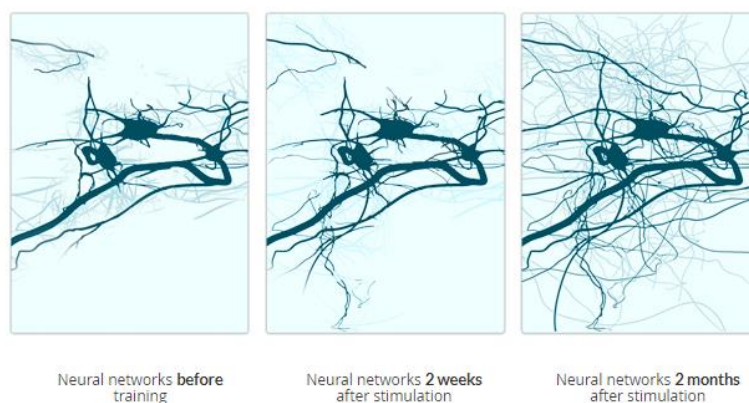


Figure 11. The hypothesis of neurogenesis in relation to exercise. We hypothesised that PA would have a stabilizing effect on synapses in patients with AD, enabling new neuronal pathways to connect. Adapted from www.cognifit.com

We found no significant effect of PA on our selected biomarkers. The most direct interpretation of these results is that they do not support any modulatory effect of PA on AD synapse integrity, as measured by selected biomarkers. However, the lack of a modulating effect of exercise on our selected biomarkers might have other explanations. Animal studies in mice suggest that environmental stimulation, including access to running wheels, improved cognition and resulted in significant higher levels of NG in brain tissue [174], however, this effect did not transfer to the present study. The synaptic protein NG is released to the CSF from the synapses [175,176]. Comparing baseline values from our study (mean concentration controls: 524 pg/ml, and exercise: 628 pg/ml) to findings from the group which assay we employed (mean concentrations in AD: 620 pg/ml) [150], shows that the concentrations found in our study are comparable with theirs. Other studies have found either considerably lower levels (mean concentration in AD: 36.6 pg/mL) of NG in CSF in patients with AD [151], or considerably higher levels (mean concentration in AD: 2381 pg/mL) of NG in CSF in patients with AD [175]. However, all have found higher levels in AD compared to cognitive healthy subjects. We would expect a lower level of NG in the CSF after an intervention with PA, if PA has a stabilizing effect on the synapses, and thereby less release of NG to the CSF by degrading neurons. As with study I, the lack of coherency with the findings in animal studies, could be due to the exercise regime employed, or that physical exercise in patients with AD dementia does not have a modulatory effect on these pathophysiologies. Finally, as in all the exploratory studies of this thesis, the question of statistical power due to low sample size must be taken into consideration.

NFL in CSF is a marker of damage to large-caliber myelinated axons, and elevated CSF levels have been associated with cognitive deterioration and structural changes in the white matter, and brain atrophy in patients with AD [76]. As a neuron cytoskeletal-function protein NFL has also been shown to be modifiable by PA in animal studies [177]. Elevated CSF NFL is not a specific AD marker, and CSF NFL levels do not correlate with A β levels [76]. Similar to NFL, CSF NG levels do not correlate with A β 42 levels in patients with MCI and AD [175,176]. We found no effect on NFL of PA. In a different study, on the same study cohort, functional MRI scans were employed to measure hippocampal and cortical thickness as a result of exercise [178]. Here no effect was found, suggesting that PA does not possess a stabilizing neuron-structural ability, although both studies suffer from low sample sizes and should be interpreted accordingly.

Just as NG, VILIP-1 has shown potential as a predictive marker for MCI progression to AD [172]. VILIP-1 is abundantly expressed in the brain, and is excessively released due to neuronal degradation [179]. Although increased CSF levels of VILIP-1 are not specific to AD, it has shown to correlate well with AD progression and pathology [172]. The microglia derived protein YKL-40's role in AD is not fully elucidated, however is thought that YKL-40 increases with neuroinflammation [169], and apart from in AD, increased levels have

been found in CSF following stroke, other neurological disorders, and in normal aging [77,169,180,181]. This indicates that YKL-40 is more a general marker of neuroinflammation/astroglial activation secondary to many different etiologies, including A β pathology [78]. The analysis of PA effect on VILIP-1 and YKL-40 was purely explorative. The biomarkers have shown potential as candidate markers for AD pathology, and no research has been published on the effect of exercise. We found no effect on the biomarkers of 16 weeks of PA. There could be several reasons for this, including lack of statistical power.

In conclusion, since synaptic integrity plays a central role in cognitive function and synaptic loss has been shown to correlate well with cognitive decline [182] biomarkers of synaptic function may be of value in monitoring neurodegeneration, not only in AD, but also in other neurodegenerative diseases [182]. However, the selected markers in this study may not be well suited to elucidate the effect of PA, or the design and/or statistical power of our study did not allow us to detect more subtle changes.

One protein that has shown great potential as neurogenesis promoting, and that can be modified by PA is Brain derived neurotrophic factor (BDNF) [183]. BDNF acts to support the survival of existing neurons, and promotes the growth and differentiation of new neurons and synapses [99,183,184]. In patients with AD significant reduced levels of BDNF in brain tissue and plasma have been found [185,186]. Both in mouse models for AD, and in humans, increased physical activity results in increased levels of BDNF and improved cognition [82,187–189]. This makes BDNF a perfect candidate for future studies. We have currently measured BDNF in CSF (data not shown), however with great difficulties due to low concentration levels, and without positive preliminary results. We are now moving on to measure BDNF in plasma. Studies in rats have shown excellent correlation between brain levels of BDNF and plasma BDNF [190] indicating that modulations in CSF BDNF levels will reflect in plasma levels where concentrations are expected to be much higher and possibly easier to analyze.

Neuroinflammation

Moving away from the AD markers and synapses, in this explorative study we investigated the influence of PA on selected biomarkers of inflammation measured in plasma and CSF, as several large population-based studies have shown that greater physical exercise levels are associated with lower levels of inflammation [191].

Inflammation is established as an important contributor to AD pathology [192], it is however, not clear if inflammation is the cause or the outcome of pathological A β plaque accumulation [193]. Nonetheless,

measuring inflammation in CSF and even plasma has the potential to aid in monitoring disease progression and help to understand the beneficial effects of treatment strategies [44,45].

Our approach resulted in mostly negative results, but we did find some moderate modulating effects of our intervention on some proteins, systemically and in CSF.

We found a mixed picture where PA increased the levels of sTREM2 in CSF and IL6 in plasma. In addition, when analyzing on the basis of *APOE* genotype, *APOE* $\epsilon 4$ carriers who exercised had stable levels of plasma IFN γ and *APOE* $\epsilon 4$ carriers in the control group increased. Further, in *APOE* $\epsilon 4$ carriers, sTREM2 increased in the exercise group, while it decreased in the control group. However, when correcting for multiple comparisons with a Benjamini-Hochberg adjustment with a false discovery rate of 0.05, no effect of PA was found in any of the markers in either CSF or plasma. This calls for studies in larger groups in order to validate these preliminary findings.

Animal studies in AD model mice, have shown increased expression of pro-inflammatory cytokines, even at young age [158]. And in addition, administration of the anti-inflammatory cytokine IL-4 reduced astrogliosis and microgliosis, amyloid plaque load, and improved memory and hippocampal neurogenesis in AD mice [194]. Very few studies have investigated the effect of PA on inflammation in AD mouse models, and the results have been mixed. Some find a modest effect on activated astrocytes and microglia activation [195,196], and another study found no effect [158].

The cytokine IL6 has commonly been perceived as a pro-inflammatory molecule, released in the acute phase of an infection. However, IL6 is increasingly being recognized to exhibit both pro-inflammatory as well as anti-inflammatory abilities. This is supported by the lack of induction of nitric oxide in response to IL6 release, as seen with cytokines IL1 and TNF α [110,197]. In relation to PA, studies have shown that exercise induces an acute phase response, similar to sepsis and trauma [110]. Especially, IL6 is produced in high amounts in response to exercise in healthy subjects, followed by a rapid decline after end of the exercise [109,110]. Additionally, IL6 is produced locally in the contracting muscle exhibiting growth factor properties [110]. These findings are in line with the present results where we also saw an increase in plasma IL6 in the exercise group compared to controls, suggesting that exercise had similar effects on IL6 in AD as in healthy subjects, however this is probably a systemic effect, rather than brain related, and as stated above needs further validation.

We found a significantly higher concentration after intervention of IFN γ among the *APOE* $\epsilon 4$ carriers in the control group. However, we are very cautious with this being a true finding due to multiple comparisons, and the limited sample size. We would expect that if IFN γ is suppressed by PA in AD we would see this

change in both *APOE* $\epsilon 4$ carriers as well as non-carriers. That said, a study in *APOE* $-/-$ mouse model has shown that these mice have an enlarged immune response, including increased $\text{IFN}\gamma$ production [198]. The closest we get to a human variant of the Human *APOE* $-/-$ mice, is the *APOE* $\epsilon 4$ carriers who have low plasma *APOE* protein levels. From our results we could postulate that PA can reverse this *APOE*/ $\text{IFN}\gamma$ negative synergy.

As described earlier, sTREM2 has been linked to AD risk. We found that exercise increased levels of sTREM2, and that non-exercise controls had a decrease in sTREM2 levels over the 16 weeks of intervention. This difference was driven by the *APOE* $\epsilon 4$ genotype, as seen in our sub-analysis. Considering the conflicting findings of the previous studies, it is hard to conclude if the increase seen in sTREM2 in the *APOE* $\epsilon 4$ exercise group, is beneficial or harmful. Nonetheless, not much is known about PA and sTREM2, and to our knowledge we are the first to investigate this.

People who are at genetic risk of AD benefit more from exercise

From population-based studies, and small explorative studies, there is an inkling that *APOE* is not only connected to the risk of AD, but that the $\epsilon 4$ allele is connected to physical function as well [199,200]. Furthermore, high fitness levels in healthy $\epsilon 4$ carriers have been associated with better cognitive performance, and preservation of hippocampal volume [120,201–203,199]. We performed a post hoc subgroup analysis study of the primary (cognitive) and secondary (neuropsychiatric symptoms and physical function) outcomes in the ADEX study. We limited our analysis to those outcomes that changed significantly with exercise and it seemed that patients who carried the *APOE* $\epsilon 4$ allele in fact benefitted more from the exercise intervention on some of these outcome measures.

In our previous investigation [22] we found that the highest effect on cognitive and physical measures were detected in the sub-group of patients who exercised at the highest intensity and who adhered most to the exercise program, described as the “high-exercise” group. In the current analysis we did not find an additional effect of high-intensity exercise after stratification by *APOE* $\epsilon 4$ carrier status. Hypothetically, this could indicate that the effect of exercise on cognitive and physical measures of carrying the *APOE* $\epsilon 4$ allele was equivalent to the effect of a higher intensity exercise regime. However, when correcting for multiple comparisons with a Benjamini-Hochberg adjustment with as false discovery rate of 0.05, only the findings in *APOE* $\epsilon 4$ carriers in the outcomes NPI, TUG, and Est. VO_2 max remained significant between controls and the exercise group. Differences in sample sized must also be taken into account, as the *APOE* $\epsilon 4$ carrier group was considerably larger and therefore had more statistical power.

The underlying mechanism for the observed *APOE*-mediated beneficial effect of exercise is unclear. First of all, it is not known whether physical exercise exerts its positive effect through disease-modifying or symptomatic mechanisms. The *APOE* genotype influences several pathways related to AD, such as vascular damage, A β accumulation, cholesterol transport, and neuroinflammation [4]. In study III we found some indications that PA had a modulating effect on inflammation, systemic and in the CNS. This could be a possible link between the *APOE* effect and exercise in this intervention study.

Methodological considerations

In this section a methodological discussion is provided covering some aspects of study design, and choice of assays used.

Study design

Limitations in study design

The ADEX study was a randomized controlled trial with 200 participants. The number of subjects was chosen based on power calculations for the primary cognitive outcome, but a larger study might have shown more robust findings. A large number of patients had to be screened, which was due to a high number of patients rejecting to participate because they had to exercise in groups located at their local hospital and not at home. For some it was a logistic problem to get to the training center (caregiver nor able to drive back and forth), for others it was a lack of willingness to exercise at all. In regard to selection bias, subjects were excluded if they had other conditions or physical problems, that prevented them from exercising, and together with willingness to exercise, this probably biased our study population towards highly motivated, reasonably fit subjects. There was a high prevalence (68%) of *APOE* $\epsilon 4$ carriers among the participating patients. Taken that all participants had been referred to a memory clinic for investigation of AD, and that *APOE* $\epsilon 4$ is a risk factor for developing AD, it was expected that the prevalence in our study would be similar to a population of AD patients in a memory clinic (approx. 50%) [204]. It is not clear why we have this high prevalence of *APOE* $\epsilon 4$ carriers in our study. To our knowledge *APOE* $\epsilon 4$ carriers are not more inclined to participate in studies, and we have no explanation for this finding.

The power calculation for the sample size for the original study was based on the primary outcome (SDMT). Due the fact that only ¼ of the participating patients donated CSF samples, all measurements in CSF were purely exploratory with small sample sizes, and when multiple comparisons with large biomarker panels were applied, the risk of both type 1 and type 2 errors must be taken into account. In study II, we tried to calculate statistical power for the biomarker analysis on the basis of change in biomarkers of 10%. This was

an unconfirmed estimate of non-validated biomarkers, and in hindsight a change of this size might have been too ambitious. In the thesis, corrections for multiple comparisons were applied, and most significant changes vanished, suggesting that the small size, especially for the CSF analyses was critical, calling for larger studies to evaluate any effects of physical exercise on AD pathophysiology.

Monitoring of physical activity:

We were not able to control the activity of the control group or in the exercise group outside the structured interventions. Both caregiver and patient were instructed to carry on as normal, but no formal control was applied due to lack of resources. As described in the method section, participants were selected based on not already being actively engaged in a structured exercise regime or having high PA levels before enrollment. One could speculate that patients in the control group would spontaneously start to exercise on their own, while subjects in the intervention groups would reduce their physical activity between training sessions. A way to monitor the general physical activity could be to have all participating subjects wear pulse trackers that could be logged. If anything, increased physical activity in the control group would have resulted in underestimation of the effect of exercise. Another issue was the impact of social contact by exercising in groups. If resources were available, we could have included some social group activity in the control group enabling us to control for the social effect of meeting peers and enrichment of social life.

Do biomarkers change over 16 weeks?

One of the big questions when analyzing the effect of an intervention on biomarkers of AD is, 'how fast does a biomarker change?'. 'Is it even possible to see any effect after 16 weeks as with this study?' From longitudinal studies of A β , tau and p-tau, A β shows a progressive decline in CSF and tau/p-tau increase, however measured over several years, see table 10, that summarizes some of the findings of longitudinal data on AD biomarkers [205]. It is unlikely that the natural progression of AD biomarkers over 16 weeks, would result in measurable difference over this time period. However, our hypothesis is that PA would generate a faster change in biomarker-kinetics. A recent, kinetic study of tau CSF turn-over [206], suggests that the turn-over rate of CSF is fast enough in order for a change in tau to be measured over weeks .

Biomarker	Annual change, normal progression pg/mL (SD)	Study
A β 42	-11.9 (28.0)	Seppälä <i>et al.</i> , 2011 [207]
	-29.48 (15.2)	Sutphen <i>et al.</i> , 2018 [205]
tau	0.55 (55.0)	Seppälä <i>et al.</i> , 2011 [207]
	-7.11 (4.2)	Sutphen <i>et al.</i> , 2018 [205]
p-tau	-2.20 (0.8)	Seppälä <i>et al.</i> , 2011 [207]
	-1.65 (0.4)	Sutphen <i>et al.</i> , 2018 [205]
VILIP-1	-6.31	Sutphen <i>et al.</i> , 2018 [205]
YKL-40	1.68 (7.1)	Sutphen <i>et al.</i> , 2018 [205]
NG	-232.43 (58.9)	Sutphen <i>et al.</i> , 2018 [205]
NFL		Sutphen <i>et al.</i> , 2018 [205]
MMSE, points	-3.00 (2.11)	Seppälä <i>et al.</i> , 2011 [207]
	-2.49 (0.3)	Sutphen <i>et al.</i> , 2018 [205]

Table 10. Annual changes in biomarkers measured in patients with AD.

In regard to the cytokines measured in study III, it is well known that cytokines have fast dynamics and can change within hours [208].

Taken together with the findings listed in table 1a and especially 1b, an intervention of 16 weeks should be sufficient to generate measurable changes in CSF biomarkers of AD (Study I) and synapse loss (Study II), if the selected intervention is powerful enough to generate such changes, and results presented in this thesis suggests it was not.

Limitation in samples and assays used

Most of the assays used in the studies have been commercially available for a long time. The assays are validated, and only assays appropriate for CSF analysis have been used. Despite that, ELISA assays are only as good as the antibodies they employ, and lower limit of detection of the assay can influence the study result immensely, since in a disease state some biomarkers may be reduced well below the limit of detection. Mass spectrometry analysis could be a way to solve this, however this is a costly and cumbersome task, unless a well-known library of peptides is used.

Pre-analytic factors

Samples used in this thesis have been collected in respect of the guidelines described in [209]. However, there were some variations in time of sampling (although all were done in the morning).

It has been reported that preanalytical factors are responsible for 40-60% of the variability in biomarkers measurements [210]. Some key issues have been identified for samples collection such as: blood contamination of the CSF samples, presence of a CSF gradient, fasting state of the patient, and laboratory procedures regarding collection and storage e.g. collection tubes, aliquot volumes, centrifugation, time delay for centrifugation, temperature at storage, numbers of freeze/thaw cycles [210]. Many of these have been addressed and standardized procedures described, and these will not be elaborated on here [209]. Not much is known about preanalytical factors influence in many of the biomarkers assayed in this thesis, however some of the established influencing factors are described below.

AD diagnostic markers ($A\beta$, tau/p-tau): most studies have found no effect of a diurnal variation on these markers however one study did find an hourly fluctuation in $A\beta$ levels. This fluctuation was partly explained due to the use of a filter in the collection procedure, where the $A\beta$ protein might have stuck to the surface of [211].

Other biomarkers: Diurnal variations have also been reported as a concern in analyzing the cytokine IL6 in plasma. In studies of non-neurogenerative related individuals have revealed daily fluctuation in plasma concentrations of IL6 [212]. In addition, the same study that analyzed the diurnal cycle influence on AD diagnostic markers, analyzed the biomarkers NG, YKL-40, and VILIP-1, which are included in Study II in the thesis, and found no effect [210].

In regards to blood samples, over all sample handling such as delayed centrifugation after collection and delays in freezing after centrifugation have been shown to markedly reduce the quality of the blood sample analyses [213].

One way to resolve the issues of preanalytical factors is to 1) unify how samples are collected, treated and stored, using standard operating procedures and 2) include a 'house-keeping analyte', a very stable biomarker that can act as a 'golden standard' for sample quality.

What have we learned and what is the next step

The overall results of the present PhD projects have been negative. However, changes were found in some of the biomarkers after PA. We found no effect on the markers for AD pathology, and no effect on synaptic markers either. Modest effects were suggested in inflammation markers, in CSF and plasma, at least indicating that PA may have an inflammation-reducing systemic effect in patients with AD, and that this

effect might be transferred to the CNS. In addition, we found some evidence that the patients with AD who are carriers of the $\epsilon 4$ allele of *APOE*, benefitted more from PA.

The reason for the negative results in our study could be because of inadequate duration of the exercise intervention or the exercise intensity/load. It could be argued that 16 weeks of PA is not a long enough intervention to permanently alter the molecular pathology in the brain. Further, any exercise-mediated effect could be so transient that the effect is not able to manifest itself permanently or that the effect wears off soon after the subjects stop exercising. We studied that effect of our intervention at the mild dementia stage, however this may be too late to expect a change in biomarkers reflecting neurodegeneration, the neurodegeneration may simply be too advanced to respond. Finally, the studies were exploratory sub studies of a larger trial, and for many of the biomarkers they were probably not powered to find significant statistical changes.

The future

Metabolism

As described in the section 'Aims' and as seen in figure 5, one focus area that is missing is the metabolic aspect of the effect of PA. We know from previous studies that PA possesses a modifying effect on metabolism. This should be investigated in our set-up, looking at plasma levels of cholesterol, insulin, and glucose. We know that elevated levels of these markers are risk factors for developing AD, and in cognitive healthy subjects, metabolism can be positively modified by PA, however whether this is the case in patients with AD is unknown and should be investigated

APOE protein

Another research question could be to investigate the protein product of *APOE* in plasma and CSF. From a large Danish cohort study, we know that APOE protein levels are *APOE* genotype dependent [30]. Figure 12 displays the relationship between APOE and protein product, and a putative effect of PA on APOE levels.

In study IV found some evidence that carriers of the $\epsilon 4$ allele benefit more from PA than non-carriers and taken together with the lower levels of APOE in $\epsilon 4$ carriers, we hypothesize that PA might upregulate the APOE levels to that of non-carriers. Therefore, we suggest measuring changes in APOE levels in plasma and in CSF, as an effect of PA in our set-up.

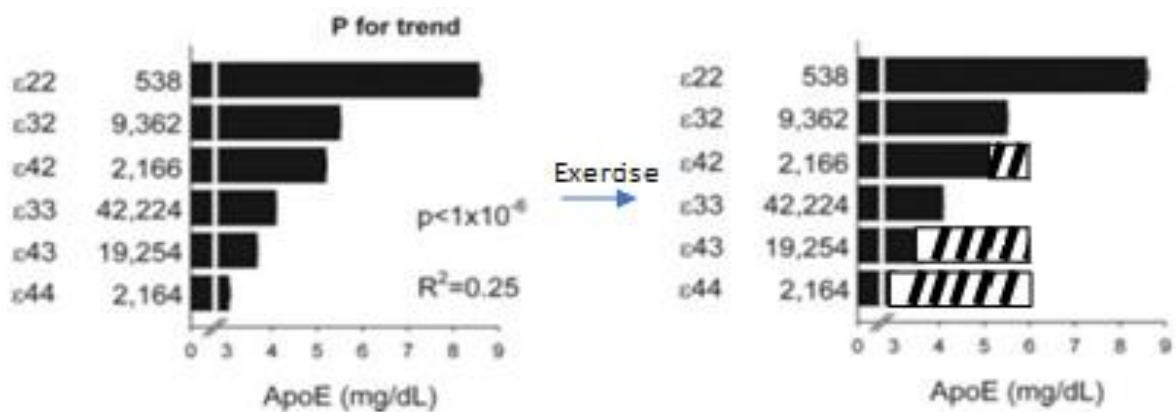


Figure 12. A putative effect pf PA on APOE levels in plasma. A hypothesis based on our finding is, that PA might upregulate the levels of APOE in plasma in ε4 carriers. Figure modified from [30].

MCI as the target

Certain biomarker profiles may be abnormal in MCI stages and may change towards normal values as the disease progresses into dementia stages [214], moreover the level of the biomarkers may vary throughout the disease.

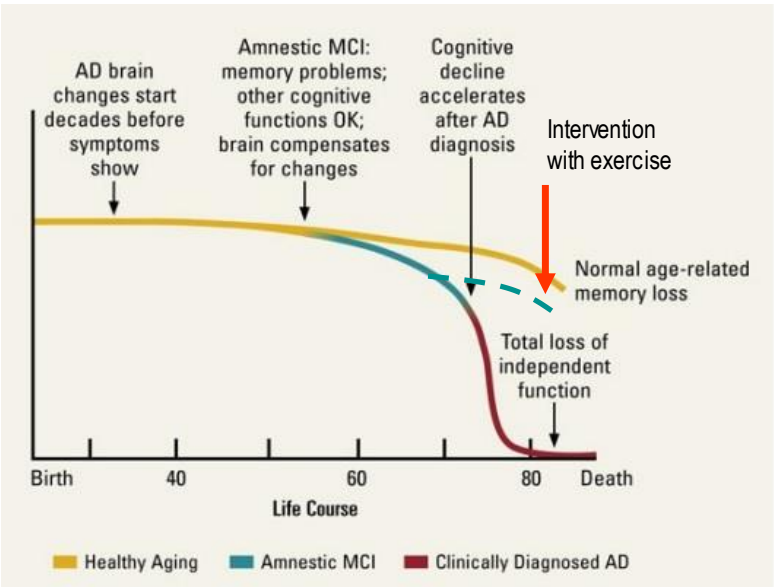


Figure 13. Hypothetical effect of an intervention at the MCI stage. Perhaps the stage we investigated were too late to see any changes. However, if an intervention was applied earlier, the decline in cognitive reserve may be easier reversed (red arrow). Modified picture from [www. NIA.org](http://www.NIA.org)

This could indicate that physical exercise intervention studies should have the MCI stage as the target, as pathophysiological events may be more amenable to change at this stage. This is further emphasized by the findings from studies of PA, where effects on cognition generally have been larger in MCI subjects than in AD subjects [215]. This could indicate that an intervention at the AD stage is too late to measure real effective results, and that the intervention should be at the MCI stage instead.

General conclusion

Despite the modest results, we still find physical activity and exercise an attractive add-on to the pharmacologic treatment for Alzheimer's disease, and our studies warrants further studies into the effect of exercise on AD symptoms and/or pathology. And even if a non-pharmacological intervention such as exercise does not alter the known pathological pathways in AD it can still be relevant for delaying disease progression by either purely symptomatic effects or by affecting some of the downstream biological processes. If exercise interventions could delay progression of AD and nursing home placement this would be of benefit for patients and caregivers but also for society as a whole.

References

- [1] Www.alzheimer.dk. Alzheimer foreningen 2014.
- [2] Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi H. Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement* 2007;3:186–9. doi:10.1016.
- [3] Holtzman DM, Morris JC, Goate AM. Alzheimer's disease: the challenge of the second century. *Sci Transl Med* 2011;6.
- [4] Mölsä P, Marttila R, Rinne U. Long-term survival and prediction of mortality in Alzheimer's disease and multi-infarct dementia. *Acta Neurol Scand* 1995;91:159–64.
- [5] Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. *Lancet* 2006;368:387–403. doi:10.1016/S0140-6736(06)69113-7.
- [6] Jellinger KA, Bancher C. Neuropathology of Alzheimer's disease: a critical update. *J Neural Transm Suppl* 1998;54:77–95.
- [7] Dowling NM, Johnson SC, Gleason CE, Jagust WJ. The mediational effects of FDG hypometabolism on the association between cerebrospinal fluid biomarkers and neurocognitive function. *Neuroimage* 2015;105:357–68. doi:10.1016/j.neuroimage.2014.10.050.
- [8] Wallin ÅK, Blennow K, Andreasen N, Minthon L. CSF Biomarkers for Alzheimer's Disease: Levels of β -Amyloid, Tau, Phosphorylated Tau Relate to Clinical Symptoms and Survival. *Dement Geriatr Cogn Disord* 2006;21:131–8. doi:10.1159/000090631.
- [9] Ord C, Jack R, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol* 2013;12.
- [10] Portelius E, Price E, Brinkmalm G, Stiteler M, Olsson M, Persson R, et al. A novel pathway for amyloid precursor protein processing. *Neurobiol Aging* 2011;32:1090–8. doi:10.1016/j.neurobiolaging.2009.06.002.
- [11] Zheng H, Koo EH. The amyloid precursor protein: beyond amyloid. *Mol Neurodegener* 2006;1:5. doi:10.1186/1750-1326-1-5.
- [12] Dislich B, Lichtenthaler SF. The membrane-bound aspartyl protease BACE1: molecular and functional properties in Alzheimer's disease and beyond. *Front Physiol* 2012;3. doi:10.3389/fphys.2012.00008.
- [13] Portelius E, Mattsson N, Andreasson U, Blennow K, Zetterberg H. Novel $\alpha\beta$ isoforms in Alzheimer's disease - their role in diagnosis and treatment. *Curr Pharm Des* 2011;17:2594–602.
- [14] Selkoe DJ. The cell biology of β -amyloid precursor protein and presenilin in Alzheimer's disease. *Trends Cell Biol* 1998;8:447–53.
- [15] Selkoe DJ, Berman Podlisny M, Joachim CL, Vickers EA, Lee G, Fritz LC, et al. β -Amyloid precursor protein of Alzheimer disease occurs as 110- to 135-kilodalton membrane-associated proteins in neural and nonneural tissues (cDNA transfection/senile plaques/amyloidosis). *Proc Natl Acad Sci USA* 1988;85:7341–5.
- [16] Tanaka S, Shiojiri S, Takahashi Y, Kitaguchi N, Ito H, Kameyama M, et al. Tissue-specific expression of three types of β -protein precursor mRNA: Enhancement of protease inhibitor-harboring types in Alzheimer's disease brain. *Biochem Biophys Res Commun* 1989;165:1406–14. doi:10.1016/0006-291X(89)92760-5.
- [17] Turner PR, O'Connor K, Tate WP, Abraham WC. Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. *Prog Neurobiol* 2003;70:1–32. doi:10.1016/S0301-0082(03)00089-3.
- [18] Ping Chong F, Yen Ng K, Yian Koh R, Moi Chye S. Tau Proteins and Tauopathies in Alzheimer's Disease. *Cell Mol Neurobiol* 2018;38:965–80. doi:10.1007/s10571-017-0574-1.
- [19] Duan AR, Jonasson EM, Alberico EO, Li C, Scripture JP, Miller RA, et al. Interactions between Tau and Different Conformations of Tubulin: Implications for Tau Function and Mechanism. *J Mol Biol* 2017;429:1424–38. doi:10.1016/j.jmb.2017.03.018.
- [20] Andreasen N, Sjögren M, Blennow K. CSF markers for Alzheimer's disease: total tau, phospho-tau and A β 42. *World J Biol Psychiatry* 2003;4:147–55.

- [21] Gao Y-L, Wang N, Sun F-R, Cao X-P, Zhang W, Yu J-T. Tau in neurodegenerative disease. *Ann Transl Med* 2018;6. doi:10.21037/atm.2018.04.23.
- [22] Zanier ER, Bertani I, Sammal E, Pischiutta F, Chiaravalloti MA, Vegliante G, et al. Induction of a transmissible tau pathology by traumatic brain injury. *Brain* 2018;141:2685–99. doi:10.1093/brain/awy225.
- [23] Kukull WA, Higdon R, Bowen JD, McCormick WC, Teri L, Schellenberg GD, et al. Dementia and Alzheimer Disease Incidence. *Arch Neurol* 2002;59:1737. doi:10.1001/archneur.59.11.1737.
- [24] Rocca WA, Hofman A, Brayne C, Breteler MMB, Clarke M, Copeland JRM, et al. Frequency and distribution of Alzheimer's disease in Europe: A collaborative study of 1980-1990 prevalence findings. *Ann Neurol* 1991;30:381–90. doi:10.1002/ana.410300310.
- [25] He W, Goodkind D, Kowal P. *An Aging World*: 2015. n.d.
- [26] Campion D, Dumanchin C, Hannequin D, Dubois B, Belliard S, Puel M, et al. Early-Onset Autosomal Dominant Alzheimer Disease: Prevalence, Genetic Heterogeneity, and Mutation Spectrum. vol. 65. 1999.
- [27] Ward A, Crean S, Mercaldi CJ, Collins JM, Boyd D, Cook MN, et al. Systematic Review Prevalence of Apolipoprotein E4 Genotype and Homozygotes (APOE e4/4) among Patients Diagnosed with Alzheimer's Disease: A Systematic Review and Meta-Analysis. *Neuroepidemiology* 2012;38:1–17. doi:10.1159/000334607.
- [28] Verghese PB, Castellano JM, Holtzman DM. Apolipoprotein E in Alzheimer's disease and other neurological disorders. *Lancet Neurol* 2011;10:241–52. doi:10.1016/S1474-4422(10)70325-2.
- [29] Mahley RW, Rall SC. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet* 2000;1:507–37. doi:10.1146/annurev.genom.1.1.507.
- [30] Rasmussen KL, Tybjaerg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. Plasma levels of apolipoprotein E and risk of dementia in the general population. *Ann Neurol* 2015;77:301–11. doi:10.1002/ana.24326.
- [31] Liu C-C, Liu C-C, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol* 2013;9:106–18. doi:10.1038/nrneurol.2012.263.
- [32] Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, et al. TREM2 Variants in Alzheimer's Disease. *N Engl J Med* 2013;368:117–44. doi:10.1056/NEJMoa1211851.
- [33] Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson P V., Snaedal J, et al. Variant of TREM2 Associated with the Risk of Alzheimer's Disease. *N Engl J Med* 2013;368:107–16. doi:10.1056/NEJMoa1211103.
- [34] Ruiz A, Dols-Icardo O, Bullido MJ, Pastor P, Rodríguez-Rodríguez E, López De Munain A, et al. Assessing the role of the TREM2 p.R47H variant as a risk factor for Alzheimer's disease and frontotemporal dementia on behalf of The dementia genetic Spanish consortium (DEGESCO). *Neurobiol Aging* 2014;35:444.e1-444.e4. doi:10.1016/j.neurobiolaging.2013.08.011.
- [35] Piccio L, Deming Y, Del-Águila JL, Ghezzi L, Holtzman DM, Fagan AM, et al. Cerebrospinal fluid soluble TREM2 is higher in Alzheimer disease and associated with mutation status. *Acta Neuropathol* 2016;131:925–33. doi:10.1007/s00401-016-1533-5.
- [36] Rivest S. TREM2 enables amyloid β clearance by microglia. *Cell Res* 2015;25:535–6. doi:10.1038/cr.2015.37.
- [37] Wunderlich P, Glebov K, Kemmerling N, Tien NT, Neumann H, Walter J. Sequential proteolytic processing of the triggering receptor expressed on myeloid cells-2 (TREM2) protein by ectodomain shedding and γ -secretase-dependent intramembranous cleavage. *J Biol Chem* 2013;288:33027–36. doi:10.1074/jbc.M113.517540.
- [38] Zhong L, Chen X-F, Wang T, Wang ZZ, Liao C, Wang ZZ, et al. Soluble TREM2 induces inflammatory responses and enhances microglial survival. *J Exp Med* 2017;214:597–607. doi:10.1084/jem.20160844.
- [39] Bekris LM, Yu C-E, Bird TD, Tsuang DW. Genetics of Alzheimer Disease. *J Geriatr Psychiatry Neurol* 2010;23:213–27. doi:10.1177/0891988710383571.
- [40] Theuns J, Del-Favero J, Dermaut B, van Duijn CM, Backhovens H, Van den Broeck M V, et al. Genetic variability in the regulatory region of presenilin 1 associated with risk for Alzheimer's disease and variable expression. *Hum Mol Genet* 2000;9:325–31.

- [41] Citron M, Westaway D, Xia W, Carlson G, Diehl T, Levesque G, et al. Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. *Nat Med* 1997;3:67–72.
- [42] Cameron B, Landreth GE. Inflammation, microglia, and Alzheimer's disease. *Neurobiol Dis* 2010;37:503–9. doi:10.1016/j.nbd.2009.10.006.
- [43] Calsolaro V, Edison P. Neuroinflammation in Alzheimer's disease: Current evidence and future directions. *Alzheimer's Dement* 2016;12:719–32. doi:10.1016/j.jalz.2016.02.010.
- [44] Galasko D, Montine TJ. Biomarkers of oxidative damage and inflammation in Alzheimer's disease. *Biomark Med* 2010;4:27–36. doi:10.2217/BMM.09.89.
- [45] Thambisetty M, Lovestone S. Blood-based biomarkers of Alzheimers disease: Challenging but feasible. *Biomark Med* 2010;4:65–79. doi:10.2217/bmm.09.84.
- [46] Tozzi A, Hannan AJ, Skaper SD, Facci L, Zusso M, Giusti P. An Inflammation-Centric View of Neurological Disease: Beyond the Neuron 2018;12. doi:10.3389/fncel.2018.00072.
- [47] Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, et al. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc Natl Acad Sci U S A* 1989;86:7611–5.
- [48] Licastro F, Pedrini S, Caputo L, Annoni G, Davis LiJ, Ferri C, et al. Increased plasma levels of interleukin-1, interleukin-6 and a-1-antichymotrypsin in patients with Alzheimer's disease: peripheral inflammation or signals from the brain? *J Neuroimmunol* 2000;103:97–102.
- [49] Su F, Bai F, Zhang Z. Inflammatory Cytokines and Alzheimer's Disease: A Review from the Perspective of Genetic Polymorphisms. *Neurosci Bull* 2016;32:469–80. doi:10.1007/s12264-016-0055-4.
- [50] Kawahara K, Suenobu M, Yoshida A, Koga K, Hyodo A, Ohtsuka H, et al. Intracerebral microinjection of interleukin-4/interleukin-13 reduces β -amyloid accumulation in the ipsilateral side and improves cognitive deficits in young amyloid precursor protein 23 mice. *Neuroscience* 2012;207:243–60. doi:10.1016/j.neuroscience.2012.01.049.
- [51] Hüll M, Fiebich BL, Lieb K, Strauss S, Berger M, Volk B, et al. Interleukin-6-associated inflammatory processes in Alzheimer's disease: New therapeutic options. *Neurobiol Aging* 1996;17:795–800. doi:10.1016/0197-4580(96)00107-8.
- [52] Wennström M, Hall S, Nägga K, Londos E, Minthon L, Hansson O. Cerebrospinal fluid levels of IL-6 are decreased and correlate with cognitive status in DLB patients. *Alzheimers Res Ther* 2015;7:63. doi:10.1186/s13195-015-0145-y.
- [53] Stern Y, Gurland B, Tatemichi TK, Tang MX, Wilder D, Mayeux R. Influence of Education and Occupation on the Incidence of Alzheimer's Disease. *JAMA J Am Med Assoc* 1994;271:1004. doi:10.1001/jama.1994.03510370056032.
- [54] Rovio S, Kåreholt I, Helkala E-L, Viitanen M, Winblad B, Tuomilehto J, et al. Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. *Lancet Neurol* 2005;4:705–11. doi:10.1016/S1474-4422(05)70198-8.
- [55] Ott A, Slioter A, Hofman A, van Harskamp F, Witteman J, Van Broeckhoven C, et al. Smoking and risk of dementia and Alzheimer's disease in a population-based cohort study: the Rotterdam Study. *Lancet* 1998;351:1840–3. doi:10.1016/S0140-6736(97)07541-7.
- [56] Yaffe K, Blackwell T, Whitmer R, Krueger K, Barrett-Connor E. Glycosylated hemoglobin level and development of mild cognitive impairment or dementia in older woman. *J Nutr Heal Aging* 2006;10:293–5.
- [57] Ravona-Springer R, Lou X, Schmeidler J, Wysocki M, Lesser G, Rapp M, et al. Diabetes is associated with increased rate of cognitive decline in questionably demented elderly. *Dement Geriatr Cogn Disord* 2010;29:68–74.
- [58] Mavros Y, Kay S, Anderberg K, Banker M, Wang Y, Zhao R, et al. Changes in Insulin Resistance and HbA 1c Are Related to Exercise-Mediated. *Cardiovasc Metab Risk* 2013;36:2372–9. doi:10.2337/dc12-2196.

- [59] Bennett RG, Duckworth WC, Hamel FG. Degradation of Amylin by Insulin-degrading Enzyme* 2000. doi:10.1074/jbc.M006170200.
- [60] Notkola IL, Sulkava R, Pekkanen J, Erkinjuntti T, Ehnholm C, Kivinen P, et al. Serum total cholesterol, apolipoprotein E epsilon 4 allele, and Alzheimer's disease. *Neuroepidemiology* 1998;17:14–20. doi:10.1159/000026149.
- [61] Kivipelto M, Ngandu T, Fratiglioni L, Viitanen M, Kåreholt I, Winblad B, et al. Obesity and Vascular Risk Factors at Midlife and the Risk of Dementia and Alzheimer Disease. *Arch Neurol* 2005;62:1556–60. doi:10.1001/archneur.62.10.1556.
- [62] Jick H, Zornberg G, Jick S, Seshadri S, Drachman D. Statins and the risk of dementia. *Lancet* 2000;356:1627–31. doi:10.1016/S0140-6736(00)03155-X.
- [63] McGuinness B, Passmore P. Can Statins Prevent or Help Treat Alzheimer's Disease? *J Alzheimer's Dis* 2010;20:925–33. doi:10.3233/JAD-2010-091570.
- [64] Kojro E, Gimpl G, Lammich S, Marz W, Fahrenholz F. Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the γ -secretase ADAM 10. *Proc Natl Acad Sci* 2001;98:5815–20. doi:10.1073/pnas.081612998.
- [65] Sjögren M, Blennow K. The link between cholesterol and Alzheimer's disease The link between cholesterol and Alzheimer's disease. *World J Biol Psychiatry* 2005;6:85–97. doi:10.1080/15622970510029795.
- [66] Di Paolo G, Kim T-W. Linking lipids to Alzheimer's disease: cholesterol and beyond. *Nat Rev Neurosci* 2011;12:284–96. doi:10.1038/nrn3012.
- [67] Liao F, Yoon H, Kim J. Apolipoprotein E metabolism and functions in brain and its role in Alzheimer's disease. *Curr Opin Lipidol* 2017;28:60–7. doi:10.1097/MOL.0000000000000383.
- [68] Cruchaga C, Kauwe JSK, Nowotny P, Bales K, Pickering EH, Mayo K, et al. Cerebrospinal fluid APOE levels: an endophenotype for genetic studies for Alzheimer's disease. *Hum Mol Genet* 2012;21:4558–71. doi:10.1093/hmg/dd296.
- [69] Raz N, Rodrigues K, Head D, Kennedy K, Acker J. Differential aging of the medial temporal lobe. *Neurology* 2004;62:433–8.
- [70] Davidsson P, Blennow K. Neurochemical dissection of synaptic pathology in Alzheimer's disease. *Int Psychogeriatrics* 1998;10:11–23.
- [71] Reddy PH, Mani G, Park BS, Jacques J, Murdoch G, Whetsell W, et al. Differential loss of synaptic proteins in Alzheimer's disease: implications for synaptic dysfunction. *J Alzheimers Dis* 2005;7:103-17-80.
- [72] Thorsell A, Bjerke M, Gobom J, Brunhage E, Vanmechelen E, Andreasen N, et al. Neurogranin in cerebrospinal fluid as a marker of synaptic degeneration in Alzheimer's disease. *Brain Res* 2010;1362:13–22. doi:10.1016/j.brainres.2010.09.073.
- [73] Cohen RW, Margulies JE, Coulter PM, Watson JB. Functional consequences of expression of the neuron-specific, protein kinase C substrate RC3 (neurogranin) in *Xenopus* oocytes. vol. 627. 1993.
- [74] Hayashi Y. Long-term potentiation: two pathways meet at neurogranin. *EMBO J* 2009;28:2859–60. doi:10.1038/emboj.2009.273.
- [75] Groblewska M, Muszynski P, Wojtulewska-Supron A, Kulczynska-Przybik A, Morczko B. The Role of Visinin-Like Protein-1 in the pathophysiology of Alzheimer's Disease. *J Alzheimer's Dis* 2015;47:17–32.
- [76] Zetterberg H, Skillbäck T, Mattsson N, Trojanowski JQ, Portelius E, Shaw LM, et al. Association of Cerebrospinal Fluid Neurofilament Light concentration with Alzheimer Disease Progression. *JAMA Neurol* 2016;73:60–7. doi:10.1001/jamaneurol.2015.3037.
- [77] Kester MI, Teunissen CE, Sutphen C, Herries EM, Ladenson JH, Xiong C, et al. Cerebrospinal fluid VILIP-1 and YKL-40, candidate biomarkers to diagnose, predict and monitor Alzheimer's disease in a memory clinic cohort 2011. doi:10.1186/s13195-015-0142-1.
- [78] Craig-Schapiro R, Perrin RJ, Roe CM, Xiong C, Carter D, Cairns NJ, et al. YKL-40: A Novel Prognostic Fluid Biomarker for Preclinical Alzheimer's Disease. *Biol Psychiatry* 2010;68:903–12.

doi:10.1016/j.biopsycho.2010.08.025.

- [79] Hogervorst E, Riedel W, Jeukendrup A, Jolles J. Cognitive performance after strenuous physical exercise. *Percept Mot Ski* 1996;83:479–88.
- [80] Kramer AFA, Erickson KI, Colcombe SJS. Exercise, cognition, and the aging brain. *J Appl Physiol* 2006;101:1237–42. doi:10.1152/jappphysiol.00500.2006.
- [81] Hosseini N, Alaei H, Reisi P, Radahmadi M. The effect of treadmill running on passive avoidance learning in animal model of Alzheimer's disease. *Int J Prev Med* 2013;4:187–92.
- [82] Um H, Kang E, Leem Y, Cho I, Yang C, Chae K, et al. Exercise training acts as a therapeutic strategy for reduction of the pathogenic phenotypes for Alzheimer's disease in an NSE / APPsw-transgenic model. *Int J Mol Med* 2008;4:529–39. doi:10.3892/ijmm.
- [83] Adlard PA, Perreau VM, Pop V, Cotman CW. Voluntary Exercise Decreases Amyloid Load in a Transgenic Model of Alzheimer's Disease. *J Neurosci* 2005;25:4217–21. doi:10.1523/JNEUROSCI.0496-05.2005.
- [84] Zhou Z, Fu J, Hong JA, Wang P. Association between exercise and the risk of dementia: results from a nationwide longitudinal study in China. *BMJ Open* 2017;7. doi:10.1136/bmjopen-2017-017497.
- [85] Baker LD, Frank LL, Foster-Schubert K, Green PS, Wilkinson CW, McTiernan A, et al. Effects of aerobic exercise on mild cognitive impairment: a controlled trial. *JAMA Neurol* 2010;67:71–9. doi:10.1001/archneurol.2009.307.
- [86] Baker LD, Bayer-Carter JL, Skinner J, Montine TJ, Cholerton BA, Callaghan M, et al. High-intensity physical activity modulates diet effects on cerebrospinal amyloid- β levels in normal aging and mild cognitive impairment. *J Alzheimers Dis* 2012;28:137–46.
- [87] Maesako M, Uemura K, Iwata A, Kubota M, Watanabe K, Uemura M, et al. Continuation of exercise is necessary to inhibit high fat diet-induced β -amyloid deposition and memory deficit in amyloid precursor protein transgenic mice. *PLoS One* 2013;8:e72796.
- [88] Farris W, Mansourian S, Chang Y, Lindsley L, Eckman E, Frosch M, et al. Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. *PNAS* 2003;100:4162–7.
- [89] Qiu WQ, Walsh DM, Ye Z, Vekrellis K, Zhang J, Podlisny MB, et al. Insulin-degrading enzyme regulates extracellular levels of amyloid β -protein by degradation. *J Biol Chem* 1998;273:32730–8. doi:10.1074/jbc.273.49.32730.
- [90] Kurochkin I, Goto S. Alzheimer's beta-amyloid peptide specifically interacts with and is degraded by insulin degrading enzyme. *FEBS Lett* 1994;345:33–7.
- [91] Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, Frosch MP, et al. Insulin-degrading enzyme regulates the levels of insulin, amyloid-protein, and the-amyloid precursor protein intracellular domain in vivo. vol. 100. 2003.
- [92] Qiao Qiu W, Folstein MF, Friedman DR. Insulin, insulin-degrading enzyme and amyloid-peptide in Alzheimer's disease: review and hypothesis. *Neurobiol Aging* 2006;27:190–8. doi:10.1016/j.neurobiolaging.2005.01.004.
- [93] Schön M, Kovaničová Z, Košťutká Z, Nemec M, Tomková M, Jacková L, et al. Effects of running on adiponectin, insulin and cytokines in cerebrospinal fluid in healthy young individuals. *Sci Rep* 2019;9:1959. doi:10.1038/s41598-018-38201-2.
- [94] Warburton DER, Nicol CW, Bredin SSD. Health benefits of physical activity: the evidence Review. *CMAJ* 2006;174:801. doi:10.1503/cmaj.051351.
- [95] Friedland RP, Fritsch T, Smyth KA, Koss E, Lerner AJ, Chen CH, et al. Patients with Alzheimer's disease have reduced activities in midlife compared with healthy control-group members. *Proc Natl Acad Sci USA* 2001;98:3440–5.
- [96] Lindsay J, Laurin D, Verreault R, Hébert R, Helliwell B, Hill GB, et al. Risk factors for Alzheimer's disease: a prospective analysis from the Canadian Study of Health and Aging. *Am J Epidemiol* 2002;156:445–53.
- [97] Dougherty RJ, Schultz SA, Kirby TK, Boots EA, Oh JM, Edwards D, et al. Moderate Physical Activity is Associated

with Cerebral Glucose Metabolism in Adults at Risk for Alzheimer's Disease HHS Public Access. *J Alzheimers Dis* 2017;58:1089–97. doi:10.3233/JAD-161067.

- [98] Haskell WL. The influence of exercise on the concentrations of triglyceride and cholesterol in human plasma. *Exerc Sport Sci Rev* 1984;12:205–44.
- [99] Wenk G. neuropathologic changes in Alzheimer's disease. *J Clin Psychiatry* 2003;64:7–10.
- [100] Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, et al. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci U S A* 2011;108:3017–22. doi:10.1073/pnas.1015950108.
- [101] Burns JM, Cronk BB, Anderson HS, Donnelly JE, Thomas GP, Harsha A, et al. Cardiorespiratory Fitness and Brain Atrophy in Early Alzheimer's Disease. *Neurology* 2008;71:210–6. doi:10.1212/01.wnl.0000317094.86209.cb.
- [102] Carro E, Trejo JL, Busiguina S, Torres-Aleman I. Circulating Insulin-Like Growth Factor I Mediates the Protective Effects of Physical Exercise against Brain Insults of Different Etiology and Anatomy. *J Neuroscience* 2001;21:5678–84.
- [103] Black JE, Isaacs KR, Anderson BJ, Alcantara AA, Greenough WT. Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats (paramedian lobule/neural plasticity/exercise). *Proc Natl Acad Sci USA* 1990;87:5568–72.
- [104] Isaacs KR, Anderson J, Alcantara AA, Black JE, Greenough T. Exercise and the Brain: Angiogenesis in the Adult Rat Cerebellum After Vigorous Physical Activity and Motor Skill Learning. *J Cereb Blood Flow Metab* 1992;12:110–9.
- [105] Van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc Natl Acad Sci USA* 1999;96:13427–31. doi:10.1073/PNAS.96.23.13427.
- [106] Voss MW, Erickson KI, Prakash RS, Chaddock L, Kim JS, Alves H, et al. Neurobiological markers of exercise-related brain plasticity in older adults. *Brain Behav Immun* 2013;28:90–9. doi:10.1016/J.BBI.2012.10.021.
- [107] Yaffe K, Kanaya A, Lindquist K, Simonsick EM, Harris T, Shorr RI, et al. The metabolis syndrom, inflammation, and risk of cognitive decline. *JAMA* 2004;292:2237–42.
- [108] Cotman CW, Berchtold NC, Christie L-A. Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci* 2007;30:464–72. doi:10.1016/j.tins.2007.06.011.
- [109] Petersen AMW, Pedersen BK. The anti-inflammatory effect of exercise. *J Appl Physiol* 2005;98:1154–62.
- [110] Pedersen BKL. Exercise and cytokines. *Immunol Cell Biol* 2000;78.
- [111] Radak Z, Suzuki K, Higuchi M, Balogh L, Boldogh I, Koltai E. Physical exercise, reactive oxygen species and neuroprotection. *Free Radic Biol Med* 2016;98:187–96. doi:10.1016/J.FREERADBIOMED.2016.01.024.
- [112] Lee C Do, Folsom AR, Blair SN. Physical Activity and Stroke Risk A meta-Analysis. *Stroke* 2003;34:2475–82.
- [113] Jensen CS, Hasselbalch SG, Waldemar G, Simonsen AH. Biochemical Markers of Physical Exercise on Mild Cognitive Impairment and Dementia: Systematic Review and Perspectives. *Front Neurol* 2015;6. doi:10.3389/fneur.2015.00187.
- [114] Nascimento CMC, Pereira JR, de Andrade LP, Garuffi M, Talib LL, Forlenza OV, et al. Physical exercise in MCI elderly promotes reduction of pro-inflammatory cytokines and improvements on cognition and BDNF peripheral levels. *Curr Alzheimer Res* 2014;11:799–805.
- [115] Suzuki T, Shimada H, Makizako H, Doi T, Yoshida D, Ito K, et al. A Randomized Controlled Trial of Multicomponent Exercise in Older Adults with Mild Cognitive Impairment. *PLoS One* 2013;8:e61483. doi:10.1371/journal.pone.0061483.
- [116] Nagamatsu LS, Handy TC, Hsu CL, Voss M, Liu-Ambrose T. Resistance training promotes cognitive and functional brain plasticity in seniors with probable mild cognitive impairment: A 6-month randomized controlled trial. *Arch Intern Med* 2013;172:666–8. doi:10.1001/archinternmed.2012.379.Resistance.
- [117] Segal SK, Cotman CW, Cahill LF. Exercise-induced noradrenergic activation enhances memory consolidation in both normal aging and patients with amnesic mild cognitive impairment. *J Alzheimers Dis* 2012;32:1011–8.

doi:10.3233/JAD-2012-121078.

- [118] Varela S, Ayán C, Cancela JM, Martín V. Effects of two different intensities of aerobic exercise on elderly people with mild cognitive impairment: a randomized pilot study. *Clin Rehabil* 2012;26:442–50. doi:10.1177/0269215511425835.
- [119] van Uffelen JGZ, Chinapaw MJM, van Mechelen W, Hopman-Rock M. Walking or vitamin B for cognition in older adults with mild cognitive impairment? A randomised controlled trial. *Br J Sports Med* 2008;42:344–51. doi:10.1136/bjsm.2007.044735.
- [120] Lautenschlager NT, Cox KL, Flicker L, Foster JK, van Bockxmeer FM, Xiao J, et al. Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. *JAMA* 2008;300:1027–37. doi:10.1001/jama.300.9.1027.
- [121] Scherder EJA, Van Paasschen J, Deijen J-B, Van Der Knokke S, Orlebeke JFK, Burgers I, et al. Physical activity and executive functions in the elderly with mild cognitive impairment. *Aging Ment Health* 2005;9:272–80. doi:10.1080/13607860500089930.
- [122] Morris JK, Vidoni ED, Johnson DK, Van Sciver A, Mahnken JD, Honea RA, et al. Aerobic exercise for Alzheimer's disease: A randomized controlled pilot trial. *PLoS One* 2017;12. doi:10.1371/journal.pone.0170547.
- [123] Holthoff VA, Marschner K, Scharf M, Steding J, Meyer S, Koch R, et al. Effects of Physical Activity Training in Patients with Alzheimer's Dementia: Results of a pilot RTC study. *PLoS One* 2015;10. doi:10.1371/journal.pone.0121478.
- [124] Cheng S-T, Chow PK, Song Y-Q, Yu ECS, Chan ACM, Lee TMC, et al. Mental and physical activities delay cognitive decline in older persons with dementia. *Am J Geriatr Psychiatry* 2014;22:63–74. doi:10.1016/j.jagp.2013.01.060.
- [125] Coelho FGDM, Gobbi S, Andreatto CAA, Corazza DI, Pedroso RV, Santos-Galduróz RF. Physical exercise modulates peripheral levels of brain-derived neurotrophic factor (BDNF): A systematic review of experimental studies in the elderly. *Arch Gerontol Geriatr* 2013;56:10–5.
- [126] Pedroso RV, Via Gomes De Melo Coelho F, Ferreira Santos-Galduróz Z R, Luiz J, Costa R, Gobbi S, et al. Balance, executive functions and falls in elderly with Alzheimer's disease (AD): A longitudinal study 2011. doi:10.1016/j.archger.2011.05.029.
- [127] Vreugdenhil A, Cannell J, Davies A, Razay G. A community-based exercise programme to improve functional ability in people with Alzheimer's disease: a randomized controlled trial. *Scand J Caring Sci* 2012;26:12–9. doi:10.1111/j.1471-6712.2011.00895.x.
- [128] Venturelli M, Scarsini R, Schena F. Six-month walking program changes cognitive and ADL performance in patients with Alzheimer. *Am J Alzheimers Dis Other Dement* 2011;26:381–8. doi:10.1177/1533317511418956.
- [129] Steinberg M, Leoutsakos J-MS, Podewils LJ, Lyketsos CG. Evaluation of a home-based exercise program in the treatment of Alzheimer's disease: The Maximizing Independence in Dementia (MIND) study. *Int J Geriatr Psychiatry* 2009;24:680–5. doi:10.1002/gps.2175.
- [130] Eggermont LHP, Swaab DF, Hol EM, Scherder EJ a. Walking the line: a randomised trial on the effects of a short term walking programme on cognition in dementia. *J Neurol Neurosurg Psychiatry* 2009;80:802–4. doi:10.1136/jnnp.2008.158444.
- [131] Akishita M, Yamada S, Nishiya H, Sonohara K, Nakai R, Toba K. Effects of physical exercise on plasma concentrations of sex hormones in elderly women with dementia. *J Am Geriatr Soc* 2005;53:1076–7. doi:10.1111/j.1532-5415.2005.53338_4.x.
- [132] Van de Winckel A, Feys H, De Weerd W, Dom R. Cognitive and behavioural effects of music-based exercises in patients with dementia. *Clin Rehabil* 2004;18:253–60. doi:10.1191/0269215504cr750oa.
- [133] Mancuso M, Filosto M, Bosetti F, Ceravolo R, Rocchi A, Tognoni G, et al. Decreased platelet cytochrome c oxidase activity is accompanied by increased blood lactate concentration during exercise in patients with Alzheimer disease. *Exp Neurol* 2003;182:421–6.
- [134] Hoffmann K, Sobol NA, Frederiksen KS, Beyer N, Vogel A, Vestergaard K, et al. Moderate-to-high Intensity Physical Exercise in Patients with Alzheimer's Disease: A Randomised Controlled Trial. *J Alzheimer's Dis* 2015.

- [135] Sobol NA, Hoffmann K, Vogel A, Lolk A, Gottrup H, Høgh P, et al. Associations between physical function, dual-task performance and cognition in patients with mild Alzheimer's disease. *Aging Ment Health* 2015;1–8. doi:10.1080/13607863.2015.1063108.
- [136] Steen Jensen C, Portelius E, Siersma V, Høgh P, Wermuth L, Blennow K, et al. Cerebrospinal Fluid Amyloid Beta and Tau Concentrations Are Not Modulated by 16 Weeks of Moderate- to High-Intensity Physical Exercise in Patients with Alzheimer Disease. *Dement Geriatr Cogn Disord* 2016;42:146–58. doi:10.1159/000449408.
- [137] Bennett DA, Wilson RS, Schneider JA, Evans DA, Beckett LA, Aggarwal NT, et al. Natrual histiry og mild cognitive impairment in older persons. *Neurology* 2002;56:1133–42. doi:10.1212/wnl.59.2.198.
- [138] Netz Y, Wu M-J, Becker BJ, Tenenbaum G. Physical Activity and Psychological Well-Being in Advanced Age: A Meta-Analysis of Intervention Studies. *Psychol Aging* 2005;20:272–84. doi:10.1037/0882-7974.20.2.272.
- [139] Wilbur J, Marquez DX, Fogg LX, Wilson RS, Staffileno BA, Hoyem RL, et al. The relationship between physical activity and cognition in older Latinos. *Journals Gerontol Ser B Psychol Sci Soc Sci* 2012;67:525–34. doi:10.1093/geronb/gbr137.
- [140] Sink KM, Espeland MA, Castro CM, Church T, Cohen R, Dodson JA, et al. Effect of a 24-month physical activity intervention compared to health education on cognitive outcomes in sedentary older adults: the LIFE Randomized Trial HHS Public Access. *JAMA* 2015;314:781–90. doi:10.1001/jama.2015.9617.
- [141] Sobol NA, Hoffmann K, Frederiksen KS, Vogel A, Vestergaard K, Brændgaard H, et al. Effect of aerobic exercise on physical performance in patients with Alzheimer's disease. *Alzheimer's Dement* 2016;12:1207–15. doi:10.1016/j.jalz.2016.05.004.
- [142] Sobol NA, Dall CH, Høgh P, Hoffmann K, Steen Frederiksen K, Vogel A, et al. Change in Fitness and the Relation to Change in Cognition and Neuropsychiatric Symptoms After Aerobic Exercise in Patients with Mild Alzheimer's Disease. *J Alzheimer's Dis* 2018;65:137–45. doi:10.3233/JAD-180253.
- [143] Groot C, Hooghiemstra AM, Raijmakers PGHM, van Berckel BNM, Scheltens P, Scherder EJA, et al. The effect of physical activity on cognitive function in patients with dementia: A meta-analysis of randomized control trials. *Ageing Res Rev* 2016;25:13–23. doi:10.1016/j.arr.2015.11.005.
- [144] Hoffmann K, Frederiksen KS, Sobol NA, Beyer N, Vogel A, Simonsen AH, et al. Preserving cognition, quality of life, physical health and functional ability in Alzheimer's disease: the effect of physical exercise (ADEX trial): rationale and design. *Neuroepidemiology* 2013;41:198–207.
- [145] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. 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;34:939–44.
- [146] Borg G. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med* 1970;2:92–8.
- [147] Glickman ME, Rao SR, Schultz MR. False discovery rate control is a recommended alternative to Bonferroni-type adjustments in health studies- ClinicalKey. *J Clin Epidemiol* 2014;67:850–7.
- [148] Portelius E, Tran AJ, Andreasson U, Persson R, Brinkmalm G, Zetterberg H, et al. Characterization of amyloid beta peptides in cerebrospinal fluid by an automated immunoprecipitation procedure followed by mass spectrometry. *J Proteome Res* 2007;6:4433–9. doi:10.1021/pr0703627.
- [149] Koch W, Ehrenhaft A, Griesser K, Pfeufer A, Müller J, Schömig A, et al. TaqMan systems for genotyping of disease-related polymorphisms present in the gene encoding apolipoprotein E. *Clin Chem Lab Med* 2002;40:1123–31. doi:10.1515/CCLM.2002.197.
- [150] Kvartsberg H, Portelius E, Andreasson U, Brinkmalm G, Hellwig K, Lelental N, et al. Characterization of the postsynaptic protein neurogranin in paired cerebrospinal fluid and plasma samples from Alzheimer's disease patients and healthy controls. *Alzheimers Res Ther* 2011;7. doi:10.1186/s13195-015-0124-3.
- [151] De Vos A, Jacobs D, Struyfs H, Fransen E, Andersson K, Portelius E, et al. C-terminal neurogranin is increased in cerebrospinal fluid but unchanged in plasma in Alzheimer's disease. *Alzheimer's Dement* 2015;11:1461–9. doi:10.1016/j.jalz.2015.05.012.
- [152] Astrand PO, Ryhming I. A nomogram for calculation of aerobic capacity (Physical Fitness) from pulse rate during sunmaximal worh. *J Appl Physiol* 1954;7:218–21.

- [153] Leem Y-H, Lim H-J, Shim S-B, Cho J-Y, Kim B-S, Han P-L. Repression of tau hyperphosphorylation by chronic endurance exercise in aged transgenic mouse model of tauopathies. *J Neurosci Res* 2009;87:2561–70. doi:10.1002/jnr.22075.
- [154] Zahs KR, Ashe KH. “Too much good news” - are Alzheimer mouse models trying to tell us how to prevent, not cure, Alzheimer’s disease? *Trends Neurosci* 2010;33:381–9. doi:10.1016/j.tins.2010.05.004.
- [155] Castello MA, Jeppson JD, Soriano S. Moving beyond anti-amyloid therapy for the prevention and treatment of Alzheimer’s disease. *BMC Neurol* 2014;14:169. doi:10.1186/s12883-014-0169-0.
- [156] Elder GA, Gama Sosa MA, De Gasperi R. Transgenic mouse models of Alzheimer’s disease. *Mt Sinai J Med* 2010;77:69–81. doi:10.1002/msj.20159.
- [157] Hwang DY, Cho JS, Lee SH, Chae KR, Lim HJ, Min SH, et al. Aberrant expressions of pathogenic phenotype in Alzheimer’s diseased transgenic mice carrying NSE-controlled APPsw. *Exp Neurol* 2004;186:20–32. doi:10.1016/j.expneurol.2003.09.021.
- [158] Ryan SM, Kelly ÁM. Exercise as a pro-cognitive, pro-neurogenic and anti-inflammatory intervention in transgenic mouse models of Alzheimer’s disease. *Ageing Res Rev* 2016;27:77–92. doi:10.1016/j.arr.2016.03.007.
- [159] Qiu W, Walsh D, Ye Z, Vekrellis K, Zhang J, Podlisny M, et al. Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J Biol Chem* 1998;273:32730–8.
- [160] Kanemitsu H, Tomiyama T, Mori H. Human neprilysin is capable of degrading amyloid beta peptide not only in the monomeric form but also the pathological oligomeric form. *Neurosci Lett* 2003;350:113–6. doi:10.1016/S0304-3940(03)00898-X.
- [161] Moore KM, Girens RE, Larson SK, Jones MR, Restivo JL, Holtzman DM, et al. A spectrum of exercise training reduces soluble A β in a dose-dependent manner in a mouse model of Alzheimer’s disease. *Neurobiol Dis* 2015;85:218–24. doi:10.1016/j.nbd.2015.11.004.
- [162] Goh J, Ladiges W. Voluntary Wheel Running in Mice. *Curr Protoc Mouse Biol* 2015;5:283–90. doi:10.1002/9780470942390.mo140295.
- [163] Esch FS, Keim PS, Beattie EC, Blacher RW, Culwell AR, Oltersdorf T, et al. Cleavage of amyloid β peptide during constitutive processing of its precursor. *Science* (80-) 1990;248:1122–4.
- [164] Zhang X, Song W. The role of APP and BACE1 trafficking in APP processing and amyloid- β generation. *Alzheimers Res Ther* 2013;5:46. doi:10.1186/alzrt211.
- [165] Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, et al. Dynamics of Hippocampal Neurogenesis in Adult Humans. *Cell* 2013;153:1219–27. doi:10.1016/j.cell.2013.05.002.
- [166] Halliday G. Pathology and hippocampal atrophy in Alzheimer’s disease. *Lancet Neurol* 2017;16:862–4.
- [167] Ten Brinke LF, Bolandzadeh N, Nagamatsu LS, Liang Hsu C, Davis JC, Miran-Khan K, et al. Aerobic Exercise Increases Hippocampal Volume in Older Women with Probable Mild Cognitive Impairment: A 6-Month Randomized Controlled Trial n.d. doi:10.1136/bjsports-2013-093184.
- [168] Morris JK, Vidoni ED, Johnson DK, Van Sciver A, Mahnken JD, Honea RA, et al. Aerobic exercise for Alzheimer’s disease: A randomized controlled pilot trial. *PLoS One* 2017;12. doi:10.1371/journal.pone.0170547.
- [169] Bonneh-Barkay D, Wang G, Starkey A, Hamilton RL, Wiley CA. In vivo CHI3L1 (YKL-40) expression in astrocytes in acute and chronic neurological diseases. *J Neuroinflammation* 2010;7:34. doi:10.1186/1742-2094-7-34.
- [170] Janelidze S, Hertze J, Zetterberg H, Landqvist Waldö M, Santillo A, Blennow K, et al. Cerebrospinal fluid neurogranin and YKL-40 as biomarkers of Alzheimer’s disease. *Ann Clin Transl Neurol* 2016;3:12–20. doi:10.1002/acn3.266.
- [171] Wildsmith KR, Schauer SP, Smith AM, Arnott D, Zhu Y, Haznedar J, et al. Identification of longitudinally dynamic biomarkers in Alzheimer’s disease cerebrospinal fluid by targeted proteomics. *Mol Neurodegener* 2014;9:22. doi:10.1186/1750-1326-9-22.
- [172] Tarawneh R, Lee J-M, Ladenson JH, Morris JC, Holtzman DM. CSF VILIP-1 predicts rates of cognitive decline in early Alzheimer disease. *Neurology* 2012;78:709–19. doi:10.1212/WNL.0b013e318248e568.

- [173] Erickson KI, Weinstein AM, Lopez OL. Physical Activity, Brain Plasticity, and Alzheimer's Disease. *Arch Med Res* 2012;43:615–21. doi:10.1016/j.arcmed.2012.09.008.
- [174] Huang FL, Huang K-P, Wu J, Boucheron C. Environmental Enrichment Enhances Neurogranin Expression and Hippocampal Learning and Memory But Fails to Rescue the Impairments of Neurogranin Null Mutant Mice. *J Neurosci* 2006;26:6230–7. doi:10.1523/JNEUROSCI.1182-06.2006.
- [175] Kester MI, Teunissen CE, Crimmins DL, Herries EM, Ladenson JH, Scheltens P, et al. Neurogranin as a Cerebrospinal Fluid Biomarker for Synaptic Loss in Symptomatic Alzheimer Disease. *JAMA Neurol* 2015;72:1275–80. doi:10.1001/jamaneurol.2015.1867.
- [176] Portelius E, Zetterberg H, Skillbäck T, Törnqvist U, Andreasson U, Trojanowski JQ, et al. Cerebrospinal fluid neurogranin: relation to cognition and neurodegeneration in Alzheimer's disease. *Brain* 2015;138:3373–85. doi:10.1093/brain/awv267.
- [177] Ding Q, Vaynman S, Souda P, Whitelegge JP, Gomez-Pinilla F. Exercise affects energy metabolism and neural plasticity-related proteins in the hippocampus as revealed by proteomic analysis. *Eur J Neurosci* 2006;24:1265–76. doi:10.1111/j.1460-9568.2006.05026.x.
- [178] Steen Frederiksen K, Thode Larsen C, Gregers Hasselbalch S, Nymark Christensen A, Høgh P, Wermuth L, et al. A 16-Week Aerobic Exercise Intervention Does Not Affect Hippocampal Volume and Cortical Thickness in Mild to Moderate Alzheimer's Disease. *Front Aging Neurosci* 2018;Sep 25. doi:10.3389/fnagi.2018.00293.
- [179] Lee J-M, Blennow K, Andreasen N, Laterza O, Modur V, Olander J, et al. The brain injury biomarker VLP-1 is increased in the cerebrospinal fluid of Alzheimer disease patients. *Clin Chem* 2008;54:1617–23. doi:10.1373/clinchem.2008.104497.
- [180] Hjalmarsson, Bjerke M, Andersson B, Blennow K, Zetterberg H, Aberg D, et al. Neuronal and Glia-Related Biomarkers in Cerebrospinal Fluid of Patients with Acute Ischemic Stroke. *J Cent Nerv Syst Dis* 2014;51. doi:10.4137/JCNSD.S13821.
- [181] Sutphen CL, Jasielec MS, Shah AR, Macy EM, Xiong C, Vlassenko AG, et al. Longitudinal Cerebrospinal Fluid Biomarker Changes in Preclinical Alzheimer Disease During Middle Age. *JAMA Neurol* 2015;72:1029–42. doi:10.1001/jamaneurol.2015.1285.
- [182] Scheff SW, Price DA, Schmitt FA, DeKosky ST, Mufson EJ. Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. *Neurology* 2007;68:1501–8. doi:10.1212/01.wnl.0000260698.46517.8f.
- [183] Huang E, Reichardt L. Neurotrophins roles in neurodegenerative in diseases. *Annu Rev Neurosci* 2009;677–736. doi:10.1146/annurev.neuro.24.1.677.Neurotrophins.
- [184] Ravona-Springer R, Moshier E, Schmeidler J, Godbold J, Akrivos J, Rapp M, et al. Changes in glycemic control are associated with chnages in cognition in non-diabetic alderly. *J Alzheimers Dis* 2012;30:299–309. doi:10.3233/JAD-2012-120106.Changes.
- [185] Coelho FGDM, Vital TM, Stein AM, Arantes FJ, Rueda AV, Camarini R, et al. Acute aerobic exercise increases brain-derived neurotrophic factor levels in elderly with Alzheimer's disease. *J Alzheimer's Dis* 2014;39:401–8. doi:10.3233/JAD-131073.
- [186] Erickson KI, Miller DLD, Roecklein KA. the aging hippocampus:Interactions between exercise, depression, and BDNF. *Neuroscientist* 2012;18:82–97. doi:10.1177/1073858410397054.
- [187] Nascimento C, Pereira J, Pires de Andrade L, Garuffi M, Ayan C, Kerr D, et al. physical exercise improves peripheral BDNF levels and cognitive fenctions in Elderly mild cognitive impairment individuals with different BDNF Val166Met genotypes. *J Alzheimers Dis* 20154;43:81–91.
- [188] Berchtold NC, Castello N, Cotman CW. Exercise and time-dependent benefits to learning and memory. *Neuroscience* 2010;167:588–97.
- [189] Zoladz J, Pilc A, Majerczak J, Grandys M, Zapart-Bukowska J, Duda K. ENdurance training encreases plamsa brain.derived neurotrophic factor concentration in young healthy men. *J Physiol Pharmacol* 2008;59:119–32.
- [190] Klein AB, Williamson R, Santini MA, Clemmensen C, Ettrup A, Rios M, et al. Blood BDNF concentrations reflect brain-tissue BDNF levels across species. *Int J Neuropsychopharmacol* 2011;14:347–53. doi:10.1017/S1461145710000738.

- [191] Beavers KM, Brinkley TE, Nicklas BJ. Effect of exercise training on chronic inflammation. *Clin Chim Acta* 2010;411:785–93. doi:10.1016/j.cca.2010.02.069.
- [192] Ransohoff RM. How neuroinflammation contributes to neurodegeneration. *Science* (80-) 2016;353:777–83. doi:10.1126/science.aag3194.
- [193] Bagyinszky E, Giau V Van, Shim K, Suk K, Soo S, An A, et al. Role of inflammatory molecules in the Alzheimer's disease progression and diagnosis ☆ 2017. doi:10.1016/j.jns.2017.03.031.
- [194] Kiyota T, Okuyama S, Swan RJ, Jacobsen MT, Gendelman HE, Ikezu T. CNS expression of anti-inflammatory cytokine interleukin-4 attenuates Alzheimer's disease-like pathogenesis in APP+PS1 bigenic mice. *FASEB J* 2010;24:3093–102. doi:10.1096/fj.10-155317.
- [195] Tapia-Rojas C, Aranguiz F, Varela-Nallar L, Inestrosa NC. Voluntary Running Attenuates Memory Loss, Decreases Neuropathological Changes and Induces Neurogenesis in a Mouse Model of Alzheimer's Disease. *Brain Pathol* 2016;26:62–74. doi:10.1111/bpa.12255.
- [196] Ke H-C, Huang H-J, Liang K-C, Hsieh-Li HM. Selective improvement of cognitive function in adult and aged APP/PS1 transgenic mice by continuous non-shock treadmill exercise. *Brain Res* 2011;1403:1–11. doi:10.1016/j.brainres.2011.05.056.
- [197] Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6 2011. doi:10.1016/j.bbamcr.2011.01.034.
- [198] Getz GS, Reardon CA. Apoprotein E as a lipid transport and signaling protein in the blood, liver, and artery wall. *J Lipid Res* 2009;50 Suppl:S156–61. doi:10.1194/jlr.R800058-JLR200.
- [199] Doi T, Shimada H, Makizako H, Tsutsumimoto K, Uemura K, Suzuki T. Apolipoprotein E genotype and physical function among older people with mild cognitive impairment. *Geriatr Gerontol Int* 2015;15:422–7. doi:10.1111/ggi.12291.
- [200] Luck T, Riedel-Heller SG, Lupp M, Wiese B, Köhler M, Jessen F, et al. Apolipoprotein E epsilon 4 genotype and a physically active lifestyle in late life: analysis of gene-environment interaction for the risk of dementia and Alzheimer's disease dementia. *Psychol Med* 2014;44:1319–29. doi:10.1017/S0033291713001918.
- [201] Schuit AJ, Feskens EJ, Launer LJ, Kromhout D. Physical activity and cognitive decline, the role of the apolipoprotein e4 allele. *Med Sci Sports Exerc* 2001;33:772–7.
- [202] Smith JC, Nielson KA, Woodard JL, Seidenberg M, Rao SM. Physical activity and brain function in older adults at increased risk for Alzheimer's disease. *Brain Sci* 2013;3:54–83. doi:10.3390/brainsci3010054.
- [203] Deeny SP, Poeppel D, Zimmerman JB, Roth SM, Brandauer J, Witkowski S, et al. Exercise, APOE, and working memory: MEG and behavioral evidence for benefit of exercise in epsilon4 carriers. *Biol Psychol* 2008;78:179–87. doi:10.1016/j.biopsycho.2008.02.007.
- [204] Ward A, Crean S, Mercaldi CJ, Collins JM, Boyd D, Cook MN, et al. Prevalence of Apolipoprotein E4 Genotype and Homozygotes (APOE e4/4) among Patients Diagnosed with Alzheimer's Disease: A Systematic Review and Meta-Analysis. *Neuroepidemiology* 2012;38:1–17. doi:10.1159/000334607.
- [205] Sutphen CL, McCue L, Herries EM, Xiong C, Ladenson JH, Holtzman DM, et al. Longitudinal decreases in multiple cerebrospinal fluid biomarkers of neuronal injury in symptomatic late onset Alzheimer's disease n.d. doi:10.1016/j.jalz.2018.01.012.
- [206] Sato C, Barthé Lemy NR, Mawuenyega KG, Miller TM, Karch CM, Bateman RJ. Tau Kinetics in Neurons and the Human Central Nervous System. *Neuron* 2018;97:1284–98. doi:10.1016/j.neuron.2018.02.015.
- [207] Seppälä TT, Koivisto AM, Hartikainen P, Helisalmi S, Soininen H, Herukka S-K. Longitudinal Changes of CSF Biomarkers in Alzheimer's Disease. *J Alzheimer's Dis* 2011;25:583–94. doi:10.3233/JAD-2011-101911.
- [208] Togo F, Natelson BH, Adler GK, Ottenweller JE, Goldenberg DL, Struzik ZR, et al. Plasma Cytokine Fluctuations over Time in Healthy Controls and Patients with Fibromyalgia. *Exp Biol Med* 2009;234:232–40. doi:10.3181/0808-RM-254.
- [209] del Campo M, Mollenhauer B, Bertolotto A, Engelborghs S, Hampel H, Simonsen AH, et al. Recommendations to standardize preanalytical confounding factors in Alzheimer's and Parkinson's disease cerebrospinal fluid

biomarkers: an update. *Biomark Med* 2012;6:419–30. doi:10.2217/bmm.12.46.

- [210] Cicognola C, Chiasserini D, Eusebi P, Andreasson U, Vanderstichele H, Zetterberg H, et al. No diurnal variation of classical and candidate biomarkers of Alzheimer's disease in CSF. *Mol Neurodegener* 2016;11:65. doi:10.1186/s13024-016-0130-3.
- [211] Slats D, Claassen JAHR, Spies PE, Borm G, Besse KTC, Van Aalst W, et al. Hourly variability of cerebrospinal fluid biomarkers in Alzheimer's disease subjects and healthy older volunteers. *NBA* 2012;33:831.e1-831.e9. doi:10.1016/j.neurobiolaging.2011.07.008.
- [212] A. D-R, P. A-G, J.C. K. Inflammatory systemic biomarkers in setting acute coronary syndromes - Effects of the diurnal variation. *Curr Drug Targets* 2009;10:1001–8.
- [213] Ju Kang H, Young Jeon S, Park J-S, Young Yun J, Na Kil H, Kyung Hong W, et al. Identification of Clinical Biomarkers for Pre-Analytical Quality Control of Blood Samples n.d. doi:10.1089/bio.2012.0051.
- [214] Duits FH, Brinkmalm G, Teunissen CE, Brinkmalm A, Scheltens P, Van Der Flier WM, et al. Synaptic proteins in CSF as potential novel biomarkers for prognosis in prodromal Alzheimer's disease. *Alzheimer's Res Ther* 2018;10:1–9. doi:10.1186/s13195-017-0335-x.
- [215] Öhman H, Savikko N, Strandberg TE, Pitkälä KH. Effect of physical exercise on cognitive performance in older adults with mild cognitive impairment or dementia: a systematic review. *Dement Geriatr Cogn Disord* 2014;38:347–65. doi:10.1159/000365388.

Paper I

Original Research Article

Cerebrospinal Fluid Amyloid Beta and Tau Concentrations Are Not Modulated by 16 Weeks of Moderate- to High-Intensity Physical Exercise in Patients with Alzheimer Disease

Camilla Steen Jensen^a Erik Portelius^b Volkert Siersma^c
Peter Høgh^d Lene Wermuth^e Kaj Blennow^b Henrik Zetterberg^{b,f}
Gunhild Waldemar^a Steen Gregers Hasselbalch^a Anja Hviid Simonsen^a

^aDanish Dementia Research Centre, Department of Neurology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; ^bDepartment of Psychiatry and Neurochemistry, Sahlgrenska University Hospital, Gothenburg University, Mölndal, Sweden; ^cResearch Unit for General Practice and Section of General Practice, Department of Public Health, University of Copenhagen, Copenhagen; ^dRegional Dementia Research Centre, Department of Neurology, Zealand University Hospital and Copenhagen University Hospital, Roskilde, and ^eDementia Clinic, Department of Neurology, Odense University Hospital, Odense, Denmark; ^fDepartment of Molecular Neuroscience, UCL Institute of Neurology, London, UK

Key Words

Dementia · Alzheimer disease · Amyloid beta · Amyloid · Physical activity

Abstract

Background: Physical exercise may have some effect on cognition in patients with Alzheimer disease (AD). However, the underlying biochemical effects are unclear. Animal studies have shown that amyloid beta (A β), one of the pathological hallmarks of AD, can be altered with high levels of physical activity. **Aim:** The objective of this study was to elucidate the effect of 16 weeks of moderate- to high-intensity physical exercise on the biomarkers of AD, with special emphasis on the amyloidogenic pathway. **Methods:** From a total of 53 patients with AD participating in the Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) study we analyzed cerebrospinal fluid samples for A β species, total tau (t-tau), phosphorylated tau (p-tau) and soluble amyloid precursor protein (sAPP) species. We also assessed the patients for apolipoprotein E ϵ 4 (ApoE ϵ 4) genotype. **Results:** We found no effect of 16 weeks of physical exercise on the selected biomarkers, and no effect of ApoE ϵ 4 genotype. **Conclusion:** Our findings suggest that the possible effect of physical exercise on cognition in patients with AD is not due to modulation of A β , t-tau, p-tau and sAPP species.

© 2016 S. Karger AG, Basel

Camilla Steen Jensen
Danish Dementia Research Centre, Department of Neurology
Rigshospitalet, University of Copenhagen, Blegdamsvej 9
DK-2100 Copenhagen (Denmark)
E-Mail Camilla.steen.jensen@regionh.dk

Introduction

Dementia prevalence is increasing, affecting more than 45 million people worldwide, where Alzheimer disease (AD) accounts for approximately 60% of all cases of diagnosed dementia [1]. AD is characterized by progressive cognitive deterioration, and symptoms include loss of memory and other cognitive skills, as well as loss of the ability to perform activities of daily living [2]. Pathological hallmarks of AD include extracellular aggregation and accumulation of amyloid beta (A β) peptides into mainly senile plaques in the cortical grey matter, as well as the hyperphosphorylated form of the protein tau accumulating inside the neurons as neurofibrillary tangles, signifying subsequent neurodegeneration [3]. Both deposits spread throughout the brain as the disease progresses [4]. Abnormal biomarkers can be detected before the emergence of cognitive decline and suggest a causal sequence of events [5, 6].

The effect of pharmacological treatments has so far been limited to symptomatic benefits, with no new drugs approved in the last decade [7, 8]. Several potentially disease-modifying compounds targeting A β are currently in the pipeline, some of which are in phase III clinical trials. However, acknowledging the continuing lack of effective treatment, there is also a crucial need to investigate non-pharmacological treatment opportunities.

In animal studies, physical activity seems to improve cognitive performance [9] and even reduce the deposition of A β in the brain [10–12], and previous human longitudinal cohort studies have established a connection between physical exercise and reduced risk of AD [13, 14]. Increased physical activity could therefore be a non-pharmacological approach to reduce A β in the human brain.

The 42 amino acid (aa)-long aggregation-prone A β peptide (A β 42) is derived from amyloid precursor protein (APP), which is a type I transmembrane protein expressed in the cell membrane of neurons and several other cell types, by proteolytic processing involving β - and γ -secretase. This processing pathway is sometimes referred to as ‘the amyloidogenic pathway’, which may be regarded a misnomer since it also gives rise to a variety of A β peptides ranging between 17 and 40 aa [15], along with β -cleaved soluble APP (sAPP β), all of which are secreted from the cell. APP can also undergo non-amyloidogenic processing by α -secretase, which results in the release of sAPP α , and by β - and α -secretase acting on the same APP molecule, which results in the release of 13–16 aa-long soluble A β species that do not form plaques [16]. The stoichiometry of sAPP α and sAPP β has not been established; however, it is speculated that they are generated in a 1:1 manner [17]. Physical exercise may exert its effect by shifting the proteolytic pathway towards the non-amyloidogenic pathway. This can be expressed as the ratio between sAPP β and sAPP α (sAPP β / α) and the ratio between the relative expression of the long A β species generated by the amyloidogenic pathway (17–42 aa) in relation to the short A β species generated by the non-amyloidogenic pathway (13–16 aa). Furthermore, the ratio between cerebrospinal fluid (CSF) A β 42 and A β 40 (A β 42/40) can be regarded as a marker of the total amyloid load in the brain reflected by amyloid positron emission tomography (PET) [18–20].

Possession of one or more apolipoprotein E ϵ 4 (ApoE ϵ 4) alleles is a known risk factor for developing AD. However, very little is known about the relationship between ApoE genotypes and the effect of physical exercise on cognition and physical function. Previous studies have indicated that individuals with one or two ApoE ϵ 4 alleles have a higher beneficial effect of physical exercise than ApoE ϵ 4 non-carriers [21]. Therefore, we included ApoE genotype in our study to elucidate the specific genotype contribution to the effect of physical exercise on A β species, total tau (t-tau) and phosphorylated tau (p-tau).

In a multicentre randomized controlled trial, the Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer’s Disease: The Effect of Physical Exercise (ADEX) study, we have recently established that physical exercise can decrease the progression

of cognitive dysfunction and neuropsychiatric symptoms in patients with mild AD [22]. However, the underlying molecular mechanisms are not known [23]. A potential mechanism for the effect of physical exercise in AD could be a reduction in A β plaque formation by modulating the APP processing by α -, β - and γ -secretases. In this study, we investigated the potential of physical exercise to reduce cerebral A β load in persons with AD. Our hypothesis was that moderate- to high-intensity physical exercise would increase the CSF levels of sAPP α and less aggregation-prone A β species and reduce CSF markers of neurodegeneration (t-tau and p-tau) in patients with clinically diagnosed mild AD.

Materials and Methods

Participating Subjects

Two-hundred community-dwelling patients with clinically diagnosed mild AD according to NINCDS-ADRDA criteria [24] and a MMSE >19 were included and randomized to either a control group with treatment as usual or a group with 16 weeks of three weekly sessions of 1 h duration of moderate- to high-intensity physical exercise. All subjects donated a blood sample before and after the intervention, and were tested at baseline and at the 16-week follow-up with a comprehensive battery of tests of cognitive function, activities of daily function, quality of life, physical activity and neuropsychiatric symptoms. For details of inclusion and exclusion criteria and methods, see Hoffmann et al. [25]. From the 200 participating subjects, a subgroup of 56 subjects, recruited from three out of eight centres, had a lumbar puncture performed to collect CSF samples before and after the intervention. For this sub-study, only subjects with biomarker-positive (CSF A β 42 <550 pg/ml) AD were included.

Intervention

The physical exercise intervention used in this study was an aerobic exercise program consisting of three weekly sessions of 1 h duration in groups of 2–5 participants, supervised by an experienced physiotherapist. The first 4 weeks of exercise consisted of an adaption period, and during the subsequent 12 weeks, participants performed aerobic exercise of moderate to high intensity (in each session a total 3 $\times$ 10 min on an ergometer bicycle, cross trainer and treadmill with 2–5 min rest in between). Heart rate (HR) was monitored during aerobic exercise, including the rest intervals. Target intensity was 70–80% of maximal HR (220 minus age).

The participants assigned to the control group received treatment as usual. The physical activity of the control group was not monitored.

Statistics

The baseline characteristics age, MMSE, weight, height and BMI, and the outcome measures at baseline were compared between the two groups using t tests. The difference in change in outcome from baseline to the 16-week follow-up between the groups was analyzed in a linear mixed model with a patient random effect to account for repeated measurement on the same individual. These analyses were done both as intention-to-treat analysis where all participants were analyzed, and as a per-protocol analysis where only the subjects that exercised with a mean intensity of >70% of maximal HR and attended >80% of the sessions (hereafter called high exercise subjects) were included. The significance level was set to 0.05.

Outcomes

The primary outcome measures were group differences in changes from baseline in the CSF concentration of A β , t-tau, p-tau, and the two proteolytic isoforms of the A β precursor protein APP α and β .

Sample Material

CSF was collected from a total of 56 subjects, before and after the intervention. CSF was obtained by lumbar puncture between 9 a.m. and 2 p.m. and collected in polypropylene tubes. The mean delay from last day of intervention to lumbar puncture was 5.2 (3.7 SD) days for the exercise group and 6.2 (5.4 SD) days for the control group. After collection, the CSF samples were centrifuged at 2,000 *g* at 4°C and aliquoted into 250- μ l aliquots in cryotubes, within 2 h of collection, and frozen at –80°C pending analysis as recommended [26]. Furthermore, ApoE genotype for polymorphism 334 and 472 was included in the analysis according to ApoE ϵ 4 allele possession, by dividing the subjects into ApoE ϵ 4 non-carriers (0 ϵ 4 alleles) or ApoE ϵ 4 carriers (1 or 2 alleles).

Aβ Triplex

CSF concentrations of Aβ38, Aβ40, and Aβ42 were measured using V-plex Peptide Panel 1 Kits Aβ38, Aβ40, and Aβ42 (Meso Scale Discovery system, Rockville, Md., USA) according to the manufacturer's protocol. In brief, 60 µl of CSF was diluted 2-fold in diluent provided with the kit. Calibrators and controls were prepared according to protocol. On a capture-antibody pre-coated 96-well MSD plate, 25 µl of sample, calibrator or controls were added followed by addition of 25 µl of detection antibody and incubation at room temperature for 2 h. Finally, the plate was washed in washing buffer provided with the kit and read in a MSD imager at appropriate wavelength.

Total Tau

CSF t-tau was measured by the hTAU Ag ELISA assay (INNOTEST, Fujirebio, Japan) according to the manufacturer's protocol. On an anti-human tau antibody pre-coated 96-well microtiter plate, 25-µl samples, controls and standards ranging from 50 to 2,500 pg/ml were added followed by incubation with a biotinylated detection antibody to tau and addition of peroxidase-conjugated streptavidin. The reaction was developed with tetramethyl benzidine (TMB) chromogen solution and subsequently stopped with 0.9 M sulfuric acid and quantified at 450 nm in a microplate reader.

Phosphorylated Tau

CSF tau phosphorylated at amino acid 181 (p-tau) was measured by the PHOSPHO-TAU (181p) ELISA assay (INNOTEST) according to the manufacturer's protocol. On an anti-human p-tau antibody pre-coated 96-well microtiter plate, 75-µl samples, controls and standards ranging from 15.6 to 1,000 pg/ml were added followed by incubation with a biotinylated detection antibody to p-tau and addition of peroxidase-conjugated streptavidin. The reaction was developed with TMB chromogen solution and subsequently stopped with 0.9 M sulfuric acid and quantified at 450 nm in a microplate reader.

Immunoprecipitation Mass Spectrometry of Aβ

Immunoprecipitation mass spectrometry of the full spectrum of Aβ forms present in CSF was performed as previously described [27]. Briefly, 4 µg of the Aβ-specific antibodies 6E10 and 4G8 (epitope in the N-terminal region and epitope 18–22, respectively; Signet Laboratories, Inc., Dedham, Mass., USA) were separately added to 25 µl Dynabeads M-280 (Dyna). The immunoprecipitation was conducted on 940 µl CSF, to which 10 µl 2.5% Tween-20 (Bio-Rad Laboratories Inc.) had been added. The beads/CSF solution (total volume 1 ml) was transferred to a KingFisher magnetic particle processor (polypropylene tubes, Thermo Scientific) for washing and elution in a 5-step procedure. The collected supernatant was dried in a vacuum centrifuge and re-dissolved in 5 µl 0.1% formic acid in 20% acetonitrile.

The samples were analyzed by MALDI TOF/TOF operating in reflector mode. FlexAnalysis (version 3.3, Bruker Daltonics) was used for automated integration of the peaks for each spectrum. Prior to the statistical analysis, the peak areas were normalized to the sum of the integrated peaks, and the relative changes compared to baseline values were calculated. All solvents used were of HPLC quality. Relative expressions of the ratio between long Aβ species (e.g., 17–42 aa) and short Aβ species (e.g., 13–16 aa) were analyzed as a surrogate marker for the ratio between aggregation-prone species produced by the amyloidogenic pathway and the non-aggregation-prone species produced by the non-amyloidogenic pathway.

Soluble Amyloid Precursor Protein α and β

sAPPα and sAPPβ were measured using Human sAPPα and Human sAPPβ kits, respectively (IBL, Japan) according to the manufacturer's protocol. In brief, 100 µl of 1:40 diluted CSF, controls and standards (ranging from 0.78 to 50 ng/ml) were added to wells of anti-human sAPPα or sAPPβ antibody pre-coated microtiter plates and incubated overnight at 4°C. Following incubation, 100 µl HRP-conjugated detection antibody was added to the wells. The reaction was developed with TMB chromogen solution and subsequently stopped with 1 M sulfuric acid and quantified at 450 nm in a microplate reader.

Apolipoprotein E Genotyping

DNA was isolated with Promega Maxwell DNA purification kits (Promega, Wis., USA), according to the manufacturer's protocol from 250 µl of buffy coat from 6 ml EDTA vials. *ApoE* genotyping for the ε2, ε3, and ε4 alleles was performed with a TaqMan qPCR assay as described by Koch et al. [28].

Table 1. Subjects' baseline characteristics

	Controls (n = 27)	Intervention (n = 26)	p value ^a	High exercise (n = 18)	p value ^b
Age, years	69.2 ± 3.9	68.1 ± 6.8	0.59 ^c	68.7 ± 7.6	0.84 ^c
Gender			0.06 ^d		0.18 ^d
Male	21 (60)	14 (40)		10 (32)	
Female	6 (33.3)	12 (66.6)		8 (57)	
Disease duration, years	1.5 ± 1.0	1.1 ± 1.0	0.13 ^c	1.2 ± 1.0	0.28 ^c
MMSE	25.1 ± 3.9	25.5 ± 2.3	0.62 ^c	25.0 ± 2.6	0.94 ^c
Characteristics					
Weight, kg	72.7 ± 13.7	76.2 ± 16.1	0.40 ^c	75.6 ± 17.0	0.54 ^c
Height, cm	175.7 ± 8.3	173.4 ± 9.0	0.33 ^c	173.3 ± 8.6	0.37 ^c
BMI	23.5 ± 3.7	25.3 ± 4.4	0.11 ^c	25.0 ± 4.2	0.20 ^c
ApoE ε4			0.22 ^d		0.23 ^d
0 alleles	11 (20.8)	5 (9.4)		2 (3.8)	
1 allele	9 (17.0)	13 (24.5)		9 (17.0)	
2 alleles	7 (13.2)	8 (15.1)		7 (13.2)	

Data given as mean ± standard deviation or n (%). ^a Controls versus intervention; ^b controls versus high exercise; ^c independent t test; ^d χ^2 test.

Results

Baseline Characteristics

From the 200 randomized subjects included in the intervention study, CSF samples were obtained from 56 subjects in addition to blood samples from all participants. Of the 56 subjects with a CSF sample available, 53 met the criteria of CSF A β 42 levels below 550 pg/ml. For the three patients with levels >550 pg/ml, an amyloid PET scan was performed. In all three cases, the amyloid PET scan was negative, and the patients were excluded. There was no significant difference in baseline characteristics between the intervention group and control group in any of the parameters (table 1). Of the 53 subjects, 18 were female (34%), the mean age was 68.7 years, and the mean MMSE score was 25.3. In the intervention group, 18 subjects (69.2%) fulfilled the criteria for high exercise subjects (see Materials and Methods). There was no significant difference at baseline between the control and the high exercise group.

Baseline CSF Concentrations of AD Biomarkers

The concentrations of A β 38/40/42/, t-tau, p-tau, sAPP α , and sAPP β are shown in tables 2 and 3. There was no statistical significant difference in baseline mean concentrations (pg/ml) of these markers when comparing controls and intervention groups. Furthermore, we did not find any difference in baseline ratios (A β 42/40 or sAPP β / α) when comparing controls and intervention group, as seen in tables 2 and 3. Analysis of the relative expression of a variety of A β species (long/short ratio analyzed by immunoprecipitation mass spectrometry) revealed no statistical significant difference at baseline (see table 4).

Effect of Intervention: Intention-to-Treat Analysis

Mean between-group differences in outcomes after 16 weeks of intervention are shown in tables 2–4. There was no significant difference in changes from baseline to follow-up between the control and intervention groups in any of the outcome measures. Figure 1 displays the between-group difference (follow-up minus baseline) in outcome measure.

Table 2. Changes in CSF AD biomarkers after 16 weeks of intervention

	Baseline		16-week follow-up		Between-group difference in mean change from baseline		Between-group difference in mean change from baseline, high exercise (n=18) versus control		ApoE ε4 carriers, high exercise (n = 14), control (n = 11)	
	control (n = 25)	intervention (n = 26)	control (n = 20)	intervention (n = 22)	intervention versus control (n = 18)	high exercise (n = 20)	high exercise (n = 2)	high exercise (n = 2)	high exercise (n = 2)	high exercise (n = 2)
	mean ± SD ^a	mean ± SD ^a	mean ± SD ^a	mean ± SD ^a	Δ (95% CI)	Δ (95% CI)	p value	Δ (95% CI)	p value	p value
Aβ38	1,735 ± 617	1,948 ± 728	2,034 ± 991	1,935 ± 628	-68 (-271; 135)	-141 (-368; 87)	0.50	-142 (-674; 389)	0.55	0.18
Aβ40	3,720 ± 1,222	4,149 ± 1,320	4,549 ± 1,802	4,187 ± 1,158	-404 (-879; 71)	-489 (-1,024; 47)	0.094	-134 (-1,513; 1,245)	0.83	0.038
Aβ42	145 ± 69	175 ± 107	194 ± 80	200 ± 121	-21 (-53; 11)	-29 (-63; 6)	0.20	-15 (-108; 78)	0.72	0.059
Aβ42/40	0.038 ± 0.012	0.042 ± 0.021	0.044 ± 0.013	0.046 ± 0.022	-0.0026 (-0.0062; 0.0011)	-0.0031 (-0.0070; 0.0009)	0.16	-0.0042 (-0.0143; 0.0058)	0.36	0.24
t-tau	693 ± 573	722 ± 586	747 ± 639	868 ± 664	78 (-134; 290)	113 (-136; 362)	0.46	138 (-1,131; 954)	0.76	0.055
p-tau	72 ± 30	83 ± 31	75 ± 32	83 ± 35	-48 (-128; 33)	-43 (-123; 36)	0.24	-109 (-331; 113)	0.29	0.82

Baseline and follow-up values of β-amyloid, tau and p-tau and analysis of between-group difference in mean change from baseline of β-amyloid, tau and p-tau of intervention versus controls and per-protocol high exercise versus controls. In addition, between-group differences in mean change from baseline high exercise versus control depending on ApoE ε4 carrier status (0 alleles vs. 1 or 2 alleles). Aβ38 = beta-amyloid 1–38; Aβ40 = beta-amyloid 1–40; Aβ42 = beta-amyloid 1–42; Aβ42/40 = beta-amyloid ratio between isoforms 1–42 and 1–40. ^aGiven as mean pg/ml.

Table 3. Changes in CSF AD biomarkers after 16 weeks of intervention

	Baseline		16-week follow-up		Between-group difference in mean change from baseline		Between-group difference in mean change (n = 15) versus control		Between-group difference in mean change from baseline, high exercise (n = 10)	
	control (n = 22)		intervention (n = 22)		intervention versus control		high exercise (n = 15) versus control (n = 19)		ApoE ε4 carriers; high exercise (n = 14), control (n = 10)	
	mean ± SD ^a	p value	mean ± SD ^a	p value	Δ (95%CI)	p value	Δ (95%CI)	p value	Δ (95%CI)	p value
sAPP _α	362 ± 240	0.18	398 ± 151	0.53	-34 (-142; 75)	0.54	-39 (-166; 88)	0.18	-93 (-260; 73)	0.26
sAPP _β	859 ± 227	0.13	912 ± 409	0.89	15 (-198; 228)	0.62	57 (-173; 288)	0.72	-32 (-268; 204)	0.78
sAPP _β /α	2.14 ± 0.41	0.17	2.29 ± 0.54	0.32	-0.28 (-0.84; 0.28)	0.73	-0.10 (-0.69; 0.49)	0.46	0.20 (-0.31; 0.72)	0.42

Baseline and follow-up values of sAPP_α, sAPP_β and sAPP_β/α ratio and analysis of between-group difference in mean change from baseline of sAPP_α, sAPP_β and sAPP_β/α ratio of intervention versus controls and per-protocol high exercise versus controls. In addition, between-group differences in mean change from baseline high exercise versus control depending on ApoE ε4 carrier status (0 alleles vs. 1 or 2 alleles). ^aGiven as mean pg/ml.

Table 4. Ratio between relative expressions of long versus short forms of Aβ

	Baseline		16-week follow-up		Between-group difference in mean change from baseline		Between-group difference in mean change (n = 17) versus control		Between-group difference in mean change from baseline, high exercise (n = 12)	
	control (n = 24)		intervention (n = 23)		intervention versus control		high exercise (n = 17) versus control (n = 21)		ApoE ε4 carriers; high exercise (n = 16), control (n = 12)	
	mean ± SD	p value	mean ± SD	p value	Δ (95%CI)	p value	Δ (95%CI)	p value	Δ (95%CI)	p value
Aβ long/short ^a	6.6 ± 2.0	0.92	6.8 ± 2.7	0.44	-0.48 (-1.73; 0.77)	0.56	-0.38 (-1.69; 0.93)	0.81	-0.54 (-1.99; 0.91)	0.45

Baseline and follow-up values of Aβ long/short and analysis of between-group difference in mean change from baseline of Aβ long/short of intervention versus controls and per-protocol high exercise versus controls. In addition, between-group differences in mean change from baseline high exercise versus control depending on ApoE ε4 carrier status (0 alleles vs. 1 or 2 alleles). ^aRelative expression of long isoforms of Aβ >17 amino acids, short isoforms of Aβ <16 amino acids.

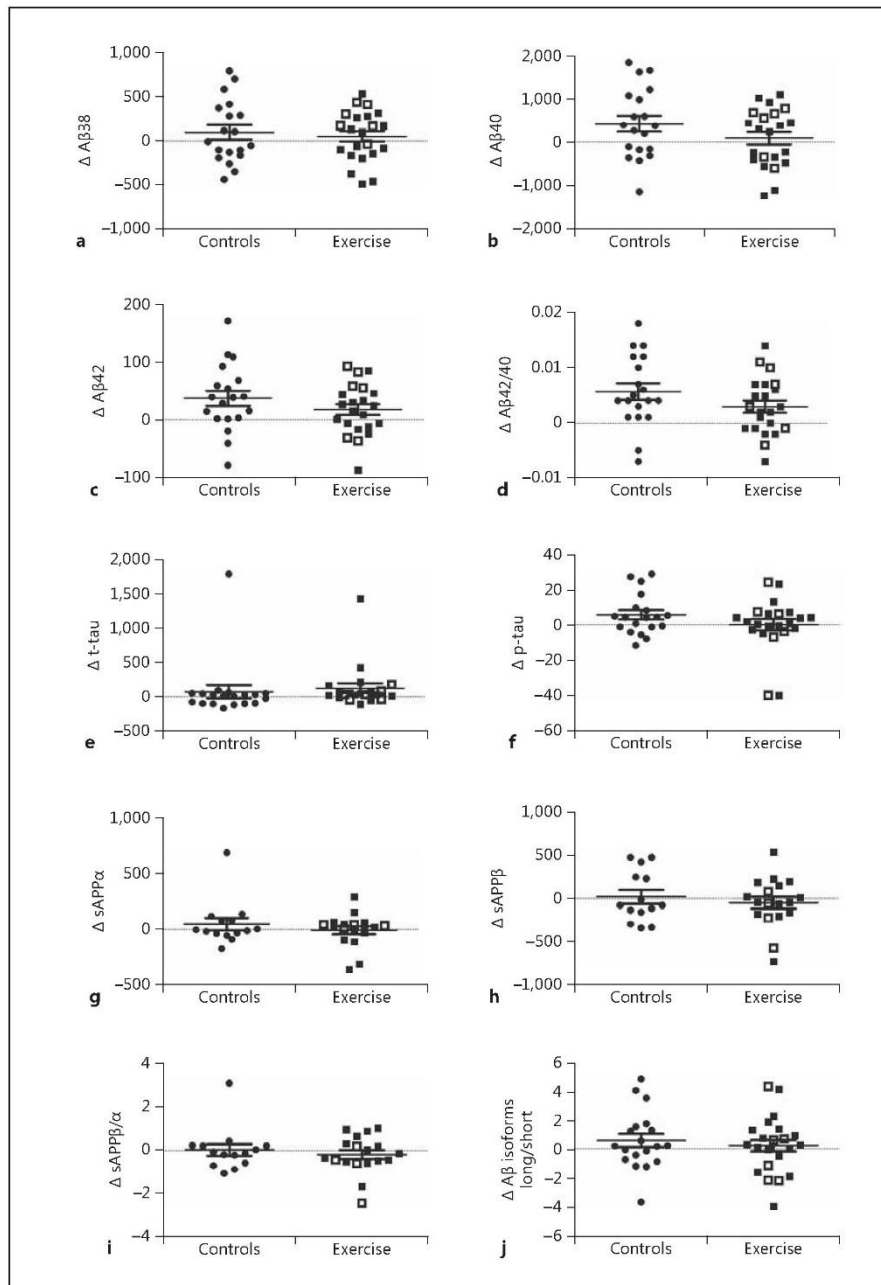
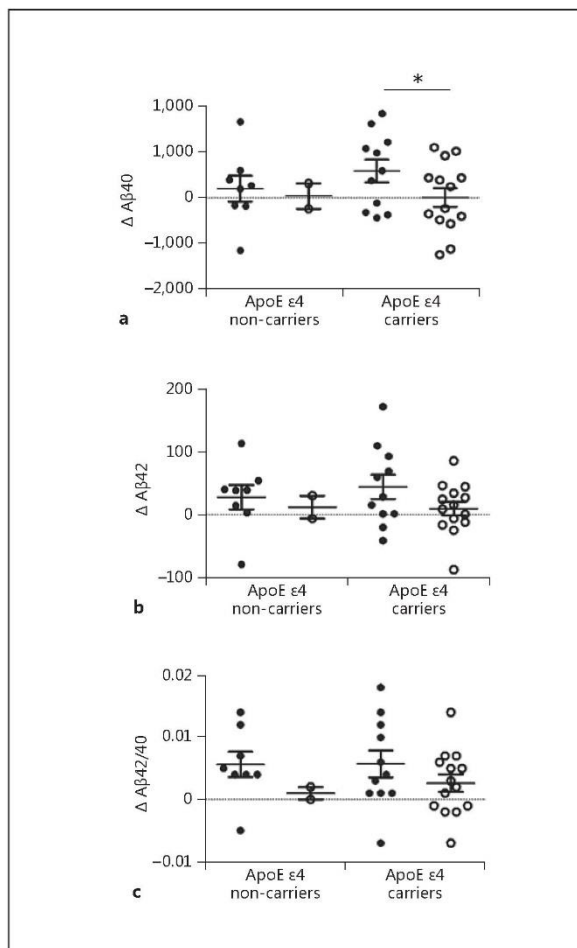


Fig. 1. Scatterplot of changes from baseline to the 16-week follow-up in outcome measure. **a** β -Amyloid 1–38. **b** β -Amyloid 1–40. **c** β -Amyloid 1–42. **d** β -Amyloid 42/40 ratio. **e** Total tau. **f** Phosphorylated tau. **g** sAPP α . **h** sAPP β . **i** sAPP β/α . **j** β -Amyloid long/short isoforms. Controls: closed circles, exercise group: closed squares. The open squares in the exercise group belong to the low exercise group (HR <70% of maximal HR).

Fig. 2. Scatterplot of changes from baseline to the 16-week follow-up according to ApoE ϵ 4 alleles in controls and high exercise groups. **a** β -Amyloid 1–40. Significant higher mean change from baseline in A β 40 levels in CSF in the control ApoE ϵ 4 carriers compared to high exercise ApoE ϵ 4 carriers (* $p = 0.038$, independent t test). **b** β -Amyloid 1–42. **c** β -Amyloid 42–40 ratio. Controls: closed circles, high exercise: open circles.



Effect of Intervention: Per-Protocol Analysis

Dividing the intervention group into low and high exercise subjects did not alter the results. At follow-up, the high exercise subjects ($n = 18$) did not differ from the control group in any of the outcome measures (tables 2–4).

Further, analyzing the outcome measures according to ApoE genotype (ApoE ϵ 4 non-carriers versus carriers [1 or 2 alleles]) revealed that only A β 40 was significantly altered in response to physical exercise in the ApoE ϵ 4 carriers controls versus high exercise (see figure 2). The high exercise group had a mean of 4,165.7 pg/ml (SD 1,421.9) at baseline and 4,020.8 pg/ml (SD 1,247.0) at 16 weeks, while the control group increased from a mean of 3,720.2 pg/ml (SD 1,221.5) at baseline to a mean of 4,549.3 pg/ml (SD 1,801.9) at 16 weeks.

Discussion

The current study investigated the effects of physical exercise on A β peptides, cleaved APP subtypes, and the neuronal injury markers t-tau and p-tau. Our study is the first to investigate the effect of physical exercise on biomarkers of AD in humans in a controlled setting of 16 weeks of moderate- to high-intensity exercise. We found no significant effect of physical exercise on any of the most frequently applied CSF AD biomarkers, either in an intention-to-treat analysis of all subjects, or in a per-protocol analysis, where only subjects that adhered to the exercise program were included.

A limitation of the current study is the sample size. Only 53 subjects were analyzed for levels of biomarkers in CSF. This low sample size may not have generated enough statistical power to calculate a significant difference in mean levels. Another issue is the duration of the exercise intervention. In a recent review, multiple exercise interventions in dementia or mild cognitive impairment (MCI) patients were reviewed [23]. The intervention ranged from a single burst of exercise to an exercise period duration of 26 weeks, with various, mostly non-significant effects on biomarkers. In our study the intervention was 16 weeks, which might not be long enough to see a significant alteration in A β and tau. Furthermore, there was a small time lag from the last exercise session to the date of lumbar puncture of 5.2 days (SD 3.7) and 6.2 days (SD 5.4) for the exercise group and control groups, respectively. If the effect of physical exercise on AD pathology is transient or only present when the individuals exercise regularly, the effect may have been attenuated at the time of sampling.

One of the strengths of our study was the good adherence to the study in general (96%) and a good per-protocol adherence (69%) to the targeted exercise intensity of over 70% of maximal HR. All exercise was supervised by professional and experienced trainers, and the levels of activity were additionally monitored by pulse watches.

When the ApoE ϵ 4 genotype status was included in the analysis, the mean change from baseline in CSF A β 40 concentration was significantly lower in the high exercise ApoE ϵ 4 carrier group compared to ApoE ϵ 4 carrier controls. ApoE status has been associated with higher amyloid deposition in the brain and is a known risk factor for developing AD [29]. Previous studies of the effect of exercise according to ApoE genotype have indicated that cognitively healthy ApoE ϵ 4 carriers (with low expression of the APOE protein) have greater benefit of a physically active lifestyle [29] and a protective effect on hippocampal atrophy [30]. However, a recent study in ApoE knockout mice suggested that exercise could not reverse age-related neurovascular dysfunction in contrast to mice with normal levels of APOE expression [31]. Our results indicated that high exercise ApoE ϵ 4 carriers had no changes in levels of A β 40 in CSF after 16 weeks of exercise, compared to control ApoE ϵ 4 carriers. This indicates that in ApoE ϵ 4-positive subjects, compliant exercise may protect against a generic increase in A β 40. Although this single significant finding should be treated with caution, it contradicts the notion that moderate- to high-intensity physical exercise increases the amyloid excretion to the CSF in ApoE carriers. If physical exercise is more effective in reducing brain amyloid burden in healthy aged ApoE ϵ 4 carriers, our study suggests that other mechanisms should account for any clinical effects of exercise in AD.

Our findings are in disagreement with previous studies in animal models for AD. Both A β and p-tau have been shown to be reduced in the brain tissue as a response to moderate-intensity exercise in transgenic (Tg) mice models for A β and tau, respectively [10, 32]. Tg AD mouse models have in general acceptable translatability to AD in humans [3, 33]; however, there have been numerous drugs and intervention studies showing reduced amyloid burden in mice but not in AD patients. The amyloid mouse models that were used in the studies of Um et al. [12] and Adlard et al. [10] are Tg overexpressing APP [34, 35]. The overexpression of proteins in models raises some concerns regarding protein integrity, such that overex-

pression of proteins in itself could be toxic for the cells, and cell toxicity therefore is not protein specific. In addition, overexpression generates vast amounts of protein that could enable an unrepresentative large detectable reduction in protein levels in intervention studies. Furthermore, the Tg NSE/APPsw mouse model used by Um et al. [12] does not develop plaques, as seen in human patients with AD [36]. The abovementioned concern suggests that the different pathology of A β in the mouse makes direct comparison of the effect of an exercise intervention between humans and animal models less valid and could account for the disagreement between our findings and previous animal findings.

In the present study, we assumed that decreased amyloid accumulation in the brain would be reflected in an increased clearance of A β from the brain to CSF. However, physical exercise may not lead to an increased clearance via the CSF, but to an increase in on-site clearance by proteases. Previous studies have found that the enzymes insulin-degrading enzyme and neprilysin may contribute to the clearance of A β [37, 38]. A recent study found that exercise training reduced extracellular, soluble A β in the brains of Tg2576 mice in a dose-dependent manner through an upregulation of A β clearance caused by an induction of insulin-degrading enzyme and neprilysin [39]. Whether a similar mechanism occurs in humans remains to be investigated. However, mass spectrometry analysis of the relative expression of several A β peptides did not show any differences in the levels between controls and the intervention group. This could indicate that A β is not cleared on site, or that the clearance is not detectable with our methods (e.g., A β species shorter than the A β 1–13).

Lately, trials with antibody treatment and immunizations against A β have tried to prevent plaque formation, although without overwhelmingly positive results [40]. A target in preventing A β plaque formation is the prevention of production of A β peptides that are prone to aggregate. We analyzed whether physical exercise had the potential to shift APP processing towards the non-amyloidogenic pathway by analyzing the sAPP α and β forms and the ratio between sAPP β and α . We did not find a significant difference in the sAPP β / α ratio in our study. Since the generation of the short A β peptides A β 1–15/16 are dependent on α - and β -secretase activity (non-amyloidogenic pathway), while the longer A β peptides (e.g., A β 42) are generated by combined action of β - and γ -secretase [41, 42], we also analyzed whether the ratio between long and short A β peptides was affected by physical exercise. We found no significant difference in the mean ratios indicating that physical exercise does not seem to influence the cleavage of APP towards the non-amyloidogenic pathways. Further, there was no relative increase in C-terminally truncated A β peptides, e.g., A β 1–37 and A β 1–38, that are thought to be markers of beneficial γ -secretase modulation.

The subjects in this study and in previous studies had already reached the dementia stage (see Jensen et al. [23] for review). An intervention to reduce amyloid plaque formation might be more effective several years before the fulfillment of criteria for dementia, since A β plaque formation precedes neurodegeneration and cognitive dysfunction. In a study by Baker et al. [43], there was a trend indicating that physical exercise could alter the levels of A β in plasma in MCI subjects, though this finding was not significant. However, Nascimento et al. [44] found that physical exercise had a positive effect on markers of inflammation and on cognition in patients with MCI. One could speculate that the beneficial effect of physical exercise on cognition is related to alterations in the inflammation profile or pathophysiological pathway in very early AD.

In conclusion, the molecular mechanisms behind the clinical effects of a 16-week exercise intervention in patients with mild AD could not be explained by modulation of A β or tau levels in the CSF. More studies investigating other pathological pathways, including neuroinflammation, synaptic plasticity dysfunction, and oxidative stress, will hopefully establish whether physical exercise has a direct disease-modifying effect in AD.

Acknowledgements

We thank Ulrika Törnqvist, Åsa Källen, and Annie Westerlund from the Department of Psychiatry and Neurochemistry, Sahlgrenska University Hospital, Gothenburg University, and Kathrine Bjarnø and Andrea Albrechtsen from the Danish Dementia Research Centre, Department of Neurology, Rigshospitalet University of Copenhagen for technical support. The study is supported by The Swedish Research Council, the European Research Council, the Knut and Alice Wallenberg Foundation, the Torsten Söderberg Foundation, and Swedish State Support for Clinical Research. The Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) study is supported by the Innovation Fund Denmark (J No. 10-092814). The Danish Dementia Research Centre is supported by grants from the Danish Ministry of Health (J No. 2007-12143-112, project 59506/J No. 0901110, project 34501) and the Danish Health Foundation (J No. 2007B004).

Disclosure Statement

The authors declare no competing financial interests.

References

- 1 Prince M, Wilmo A, Guerchet M, Ali G, Wu Y: Alzheimer's Disease International: World Alzheimer Report 2015. <https://www.alz.co.uk/research/WorldAlzheimerReport2015.pdf> (accessed November 23, 2015).
- 2 Holtzman DM, Morris JC, Goate AM: Alzheimer's disease: the challenge of the second century. *Sci Transl Med* 2011;3:77sr1.
- 3 Blennow K, de Leon MJ, Zetterberg H: Alzheimer's disease. *Lancet* 2006;368:387–403.
- 4 Jellinger KA, Bancher C: Neuropathology of Alzheimer's disease: a critical update. *J Neural Transm Suppl* 1998;54:77–95.
- 5 Dowling NM, Johnson SC, Gleason CE, Jagust WJ: The mediational effects of FDG hypometabolism on the association between cerebrospinal fluid biomarkers and neurocognitive function. *Neuroimage* 2015;105:357–368.
- 6 Wallin ÅK, Blennow K, Andreasen N, Minthon L: CSF biomarkers for Alzheimer's disease: levels of β -amyloid, tau, phosphorylated tau relate to clinical symptoms and survival. *Dement Geriatr Cogn Disord* 2006;21:131–138.
- 7 Castellani RJ, Perry G: Pathogenesis and disease-modifying therapy in Alzheimer's disease: the flat line of progress. *Arch Med Res* 2012;43:694–698.
- 8 van de Glind EMM, van Enst WA, van Munster BC, Olde Rikkert MGM, Scheltens P, Scholten RJPM, et al: Pharmacological treatment of dementia: a scoping review of systematic reviews. *Dement Geriatr Cogn Disord* 2013;36:211–228.
- 9 Nichol KE, Parachikova AI, Cotman CW: Three weeks of running wheel exposure improves cognitive performance in the aged Tg2576 mouse. *Behav Brain Res* 2007;184:124–132.
- 10 Adlard PA, Perreau VM, Pop V, Cotman CW: Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. *J Neurosci* 2005;25:4217–4221.
- 11 Souza LC, Filho CB, Goes ATR, Fabbro L Del, de Gomes MG, Savegnago L, et al: Neuroprotective effect of physical exercise in a mouse model of Alzheimer's disease induced by β -amyloid_{1–40} peptide. *Neurotox Res* 2013;24:148–163.
- 12 Um H, Kang E, Leem Y, Cho I, Yang C, Chae K, et al: Exercise training acts as a therapeutic strategy for reduction of the pathogenic phenotypes for Alzheimer's disease in an NSE/APPsw-transgenic model. *Int J Mol Med* 2008;4:529–539.
- 13 Kulmala J, Solomon A, Käreholt I, Ngandu T, Rantanen T, Laatikainen T, et al: Association between mid- to late life physical fitness and dementia: evidence from the CAIDE study. *J Intern Med* 2014;276:296–307.
- 14 Sofi F, Valecchi D, Bacci D, Abbate R, Gensini GF, Casini A, et al: Physical activity and risk of cognitive decline: a meta-analysis of prospective studies. *J Intern Med* 2011;269:107–117.
- 15 Portelius E, Price E, Brinkmalm G, Stiteler M, Olsson M, Persson R, et al: A novel pathway for amyloid precursor protein processing. *Neurobiol Aging* 2011;32:1090–1098.
- 16 Portelius E, Mattsson N, Andreasson U, Blennow K, Zetterberg H: Novel $\alpha\beta$ isoforms in Alzheimer's disease – their role in diagnosis and treatment. *Curr Pharm Des* 2011;17:2594–2602.
- 17 Checler F: Processing of the beta-amyloid precursor protein and its regulation in Alzheimer's disease. *J Neurochem* 1995;65:1431–1444.
- 18 Spies PE, Slats D, Sjögren M, Kremer BPH, Verhey FRJ, Rikkert MGM, et al: The cerebrospinal fluid amyloid beta 42/40 ratio in the differentiation of Alzheimer's disease from non-Alzheimer's dementia. *Curr Alzheimer Res* 2010;7:470–476.

- 19 Janelidze S, Zetterberg H, Mattsson N, Palmqvist S, Vanderstichele H, Lindberg O, et al: CSF A β 42/A β 40 and A β 42/A β 38 ratios: better diagnostic markers of Alzheimer disease. *Ann Clin Transl Neurol* 2016;3:154–165.
- 20 Koivunen J, Pirttilä T, Kemppainen N, Aalto S, Herukka S-K, Jauhianen AM, et al: PET amyloid ligand [11C]PIB uptake and cerebrospinal fluid beta-amyloid in mild cognitive impairment. *Dement Geriatr Cogn Disord* 2008;26:378–383.
- 21 Smith JC, Nielson KA, Woodard JL, Seidenberg M, Rao SM: Physical activity and brain function in older adults at increased risk for Alzheimer's disease. *Brain Sci* 2013;3:54–83.
- 22 Hoffmann K, Sobol NA, Frederiksen KS, Beyer N, Vogel A, Vestergaard K, et al: Moderate-to-high intensity physical exercise in patients with Alzheimer's disease: a randomised controlled trial. *J Alzheimers Dis* 2015;50:443–453.
- 23 Jensen CS, Hasselbalch SG, Waldemar G, Simonsen AH: Biochemical markers of physical exercise on mild cognitive impairment and dementia: systematic review and perspectives. *Front Neurol* 2015;6:187.
- 24 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM: 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;34:939–944.
- 25 Hoffmann K, Frederiksen KS, Sobol NA, Beyer N, Vogel A, Simonsen AH, et al: Preserving cognition, quality of life, physical health and functional ability in Alzheimer's disease: the effect of physical exercise (ADEX trial): rationale and design. *Neuroepidemiology* 2013;41:198–207.
- 26 del Campo M, Mollenhauer B, Bertolotto A, Engelborghs S, Hampel H, Simonsen AH, et al: Recommendations to standardize preanalytical confounding factors in Alzheimer's and Parkinson's disease cerebrospinal fluid biomarkers: an update. *Biomark Med* 2012;6:419–430.
- 27 Portelius E, Tran AJ, Andreasson U, Persson R, Brinkmalm G, Zetterberg H, et al: Characterization of amyloid beta peptides in cerebrospinal fluid by an automated immunoprecipitation procedure followed by mass spectrometry. *J Proteome Res* 2007;6:4433–4439.
- 28 Koch W, Ehrenhaft A, Griesser K, Pfeufer A, Müller J, Schömig A, et al: TaqMan systems for genotyping of disease-related polymorphisms present in the gene encoding apolipoprotein E. *Clin Chem Lab Med* 2002;40:1123–1131.
- 29 Head D, Bugg JM, Goate AM, Fagan AM, Mintun M, Benzinger T, et al: Exercise engagement as a moderator of the effects of APOE genotype on amyloid deposition. *Arch Neurol* 2012;69:636–643.
- 30 Smith JC, Nielson KA, Woodard JL, Seidenberg M, Durgerian S, Hazlett KE, et al: Physical activity reduces hippocampal atrophy in elders at genetic risk for Alzheimer's disease. *Front Aging Neurosci* 2014;6:61.
- 31 Soto I, Graham LC, Richter HJ, Simeone SN, Radell JE, Grabowska W, et al: APOE stabilization by exercise prevents aging neurovascular dysfunction and complement induction. *PLoS Biol* 2015;13:e1002279.
- 32 Leem Y-H, Lim H-J, Shim S-B, Cho J-Y, Kim B-S, Han P-L: Repression of tau hyperphosphorylation by chronic endurance exercise in aged transgenic mouse model of tauopathies. *J Neurosci Res* 2009;87:2561–2570.
- 33 Zahs KR, Ashe KH: 'Too much good news' – are Alzheimer mouse models trying to tell us how to prevent, not cure, Alzheimer's disease? *Trends Neurosci* 2010;33:381–389.
- 34 Philpott O, Lord A, Gumucio A, O'Callaghan P, Lannfelt L, Nilsson LG: Animal models of amyloid-beta-related pathologies in Alzheimer's disease. *FEBS J* 2010;277:1389–1409.
- 35 Elder GA, Gama Sosa MA, De Gasperi R: Transgenic mouse models of Alzheimer's disease. *Mt Sinai J Med* 2010;77:69–81.
- 36 Hwang DY, Cho JS, Lee SH, Chae KR, Lim HJ, Min SH, et al: Aberrant expressions of pathogenic phenotype in Alzheimer's diseased transgenic mice carrying NSE-controlled APPsw. *Exp Neurol* 2004;186:20–32.
- 37 Qiu W, Walsh D, Ye Z, Vekrellis K, Zhang J, Podlisny M, et al: Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J Biol Chem* 1998;273:32730–32738.
- 38 Kanemitsu H, Tomiyama T, Mori H: Human neprilysin is capable of degrading amyloid beta peptide not only in the monomeric form but also the pathological oligomeric form. *Neurosci Lett* 2003;350:113–116.
- 39 Moore KM, Girens RE, Larson SK, Jones MR, Restivo JL, Holtzman DM, et al: A spectrum of exercise training reduces soluble A β in a dose-dependent manner in a mouse model of Alzheimer's disease. *Neurobiol Dis* 2015;85:218–224.
- 40 Castello MA, Jeppson JD, Soriano S: Moving beyond anti-amyloid therapy for the prevention and treatment of Alzheimer's disease. *BMC Neurol* 2014;14:169.
- 41 Esch FS, Keim PS, Beattie EC, Blacher RW, Culwell AR, Oltersdorf T, et al: Cleavage of amyloid β peptide during constitutive processing of its precursor. *Science* 1990;248:1122–1124.
- 42 Zhang X, Song W: The role of APP and BACE1 trafficking in APP processing and amyloid- β generation. *Alzheimers Res Ther* 2013;5:46.
- 43 Baker LD, Frank LL, Foster-Schubert K, Green PS, Wilkinson CW, McTiernan A, et al: Effects of aerobic exercise on mild cognitive impairment: a controlled trial. *Arch Neurol* 2010;67:71–79.
- 44 Nascimento CMC, Pereira JR, de Andrade LP, Garuffi M, Talib LL, Forlenza OV, et al: Physical exercise in MCI elderly promotes reduction of pro-inflammatory cytokines and improvements on cognition and BDNF peripheral levels. *Curr Alzheimer Res* 2014;11:799–805.

Paper II

Featured Article

Effect of physical exercise on markers of neuronal dysfunction in cerebrospinal fluid in patients with Alzheimer's disease

Camilla Steen Jensen^{a,*}, Erik Portelius^{b,c}, Peter Høgh^d, Lene Wermuth^e, Kaj Blennow^{b,c}, Henrik Zetterberg^{b,c,f}, Steen Gregers Hasselbalch^a, Anja Hviid Simonsen^a

^aDanish Dementia Research Centre, Department of Neurology, Rigshospitalet University of Copenhagen, Copenhagen, Denmark

^bDepartment of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

^cClinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

^dRegional Dementia Research Centre, Department of Neurology, Zealand University Hospital and Copenhagen University Hospital, Roskilde, Denmark

^eDementia Clinic, Department of Neurology, Odense University Hospital, Odense, Denmark

^fDepartment of Molecular Neuroscience, UCL Institute of Neurology, London, UK

Abstract

Introduction: Physical exercise has gained increasing focus as a potential mean to maintain cognitive function in patients with Alzheimer's disease (AD). Alongside the markers of specific AD pathology (amyloid β and tau), other pathologies such as neuronal damage and synaptic loss have been proposed as markers of the disease. Here, we study the effect of physical exercise on biomarkers of neuronal and synaptic integrity.

Methods: Cerebrospinal fluid (CSF) from 51 AD subjects who participated in the randomized controlled trial Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) was analyzed for the concentration of neurofilament light (NFL), neurogranin (Ng), visinin-like protein-1 (VILIP-1), and chitinase-3-like protein 1 (YKL-40). Participants were subjected to either 16 weeks of moderate- to high-intensity exercise ($n = 25$) or treatment as usual (control group, $n = 26$), and CSF was collected before and after intervention.

Results: No significant differences in CSF concentrations of VILIP-1, YKL-40, NFL, and Ng were observed when comparing mean change from baseline between the exercise and control groups. Similarly, when classifying subjects based on their exercise levels, no significant changes were observed for the biomarkers in the control group compared with the high-exercise group (attending 80% of the exercise sessions with an intensity of 70% of maximum heart rate or above).

Discussion: These results are not supportive of a modulatory effect of physical exercise on the selected biomarkers of neuronal and synaptic integrity in patients with AD.

© 2017 The Authors. Published by Elsevier Inc. on behalf of the Alzheimer's Association. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Keywords:

Alzheimer's disease; Physical exercise; Biomarkers; Synapses; Neurons; Stability

1. Introduction

The molecular pathological mechanisms behind Alzheimer's disease (AD) have been studied for decades. Although the exact mechanisms are still not quite clear, accumulation of amyloid β 42 (A β 42) is an early pathological

hallmark that interacts with and/or promotes neurodegeneration without being directly linked to clinical symptoms [1]. One key feature of neurodegeneration in AD is neuronal and synaptic loss that better reflects AD progression and cognitive decline [1–3]. Studies in transgenic animals suggest effects of physical exercise on neurogenesis, cognition, and amyloid deposition [4]. Further, epidemiological studies suggest that higher physical activity may reduce the risk of dementia in late life [5], and some intervention

*Corresponding author. Tel.: 0045-35455573; Fax: 0045-35452446.

E-mail address: camilla.steen.jensen@regionh.dk

<http://dx.doi.org/10.1016/j.trci.2017.03.007>

2352-8737/© 2017 The Authors. Published by Elsevier Inc. on behalf of the Alzheimer's Association. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

studies in elderly suggest that participation in physical activity and cognitive training may reduce future cognitive decline [6]. However, these studies have investigated possible effects on risk of dementia and cognitive decline in general and have not used any biomarkers to diagnose AD or specifically study effects on AD pathophysiology. Furthermore, in an effort to arrest the decline in cognitive function (CF) and loss of activities of daily living, nonpharmacological approaches have been investigated. In the randomized clinical trial “Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer’s Disease: The Effect of Physical Exercise (ADEX) study,” we analyzed the effect of 16 weeks moderate- to high-intensity physical exercise (EXE) in patients with AD on a cognitive [7], functional [8], and molecular level [9]. Despite some effect on clinical symptoms such as processing speed and neuropsychiatric symptoms [7], we found no effect of EXE on levels of A β , tau, and phosphorylated tau (p-tau) in cerebrospinal fluid (CSF) [9]. Therefore, we sought to investigate downstream molecular processes that potentially could be influenced by EXE. In this study, we investigated the effect of EXE on molecular markers of AD neurodegeneration in CSF from patients with AD participating in the ADEX study. This is not a study on the potential diagnostic value of these markers as in cross-sectional study, however an explorative study on modulating effects of selected markers on exercise. Neurofilament light (NFL) is a marker of damage to large-caliber myelinated axons, and elevated CSF levels have been associated with cognitive deterioration and structural changes in the white matter and brain atrophy in patients with AD [10]. Elevated CSF NFL is not a specific AD marker, and CSF NFL levels do not correlate with A β 42 levels, indicating that the changes in NFL are not driven by A β 42 pathology [10]. Similar to NFL, CSF neurogranin (Ng) levels do not correlate with A β 42 levels in patients with mild cognitive impairment (MCI) and AD [11,12]. The synaptic protein Ng is released to the CSF from the synapses, which is an early event in the pathogenesis of AD. This also makes Ng a candidate marker for early AD diagnosis. Alongside this, Ng possesses the ability to differentiate patients with MCI who progress to AD (elevated Ng levels) and patients with MCI who remain stable (low levels of Ng) [11,12]. Just as Ng, the biomarker visinin-like protein-1 (VILIP-1) has shown potential as a marker of MCI progression to AD [3]. VILIP-1 is abundantly expressed in the brain and is excessively released because of neuronal degradation [13]. Although increased CSF levels of VILIP-1 are not specific to AD, they have shown to correlate well with AD progression and pathology [3]. The microglia derived protein YKL-40’s role in AD is not fully elucidated. It is speculated that YKL-40 increases with neuroinflammation [14]. However, apart from AD, increased levels have been found in CSF after stroke, other neurological disorders, and normal aging [14–17]. This indicates that YKL-40 is more

a general marker of neuroinflammation/astroglial activation secondary to many different etiologies, including A β pathology [18].

We hypothesized that the concentrations of markers of neuronal and synaptic damage and astroglial activation would decrease in CSF in patients with AD as an effect of moderate- to high-intensity EXE, because of stabilizing effects of EXE on neurons, synapses, and astrocytes, resulting in less neurodegeneration and inflammation and thereby lead to less release of the selected biomarkers in CSF.

2. Methods

2.1. Study population

Two-hundred community-dwelling patients with clinically diagnosed mild AD according to National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria [19] and a Mini-Mental State Examination >19 were included and randomized to either a control group with treatment as usual or a 16-week 60-minute three times per week moderate- to high-intensity aerobic physical exercise (treadmill, stationary bike, and cross-trainer) group, in groups of four to six subjects per group with an educated trainer. For detailed description of the intervention used and study participant enrollment, see Hoffmann et al. [7, 20]. All subjects donated a blood sample before and after intervention and were tested at baseline and at 16 week follow-up with a comprehensive battery of tests of CF, activities of daily function, quality of life, physical activity, and neuropsychiatric symptoms. For details of inclusion and exclusion criteria, methods for inclusion, and cognitive and physical tests, see Steen Jensen et al. [9]. A subgroup of 56 subjects recruited from three of eight centers had a lumbar puncture performed to collect CSF samples before and after intervention. Samples were centrifuged on acquisition at 2000 g and stored in 250 μ L aliquots at -80°C until use. The baseline characteristics of the study population can be seen in Table 1.

2.2. Samples

CSF samples from 51 patients of the 56 possible CSF samples, subjected to 16 weeks of moderate- to high-intensity EXE (exercise group) or treatment as usual (control group), were analyzed for the concentration of Ng (UniProt #: Q92686), VILIP-1 (UniProt #: P62760), NFL (UniProt #: P07196), and YKL-40 (UniProt #: P36222), at baseline and 16 weeks follow-up.

2.3. Assays

CSF Ng concentration was measured using a previously published in-house Meso Scale Discovery assay as published in Kvastberg et al. [21] and De Vos et al. [22]. Commercially available kits were used for measuring the

Table 1
Baseline characteristics of the study cohort

	Controls (n = 26)	Intervention (n = 25)	P-value*	High exercise (n = 16)	P-value [†]
Age, y [‡]	68.9 (8.05)	68.2 (6.94)	.211 [§]	68.3 (7.71)	.60 [§]
Gender, n (%)			.15		.13
Male	19 (73)	13 (52)		8 (50)	
Female	7 (27)	12 (48)		8 (50)	
Characteristics					
Disease duration, y [‡]	1.7 (1.09)	1.1 (0.97)	.11 [§]	1.3 (1.00)	.23 [§]
MMSE [‡]	25.4 (3.66)	25.4 (2.96)	.29 [§]	24.9 (2.80)	.16 [§]
Education level, y [‡]	13.0 (2.96)	13.4 (2.81)	.622 [§]	13.4 (2.73)	.665 [§]
BMI	23.3 (3.56)	25.3 (4.42)	.24 [§]	25.1 (4.37)	.21 [§]
Medication					
Alzheimer's medication users	23	25	.08	16	.216
Antidepressive users	6	5	.789	3	.945
Antipsychotic users	1	0	.322	0	.612
Baseline outcome measure [‡]					
NFL (pg/mL)	1494.9 (634.85)	1392.1 (467.19)	.42 [§]	1487.3 (469.89)	.45 [§]
Ng (pg/mL)	524.4 (292.66)	628.2 (338.68)	.42 [§]	660.3 (371.27)	.25 [§]
YKL-40 (ng/mL)	168.4 (66.7)	181.0 (11.8)	.28 [§]	187.7 (17.2)	.16 [§]
VILIP-1 (pg/mL)	98.5 (70.3)	113.1 (74.1)	.95 [§]	111.1 (73.9)	.83 [§]

Abbreviations: BMI, body mass index; MMSE, Mini-Mental State Examination; NFL, neurofilament light; Ng, neurogranin; YKL-40, chitinase-3-like protein 1; VILIP-1, visinin-like protein-1.

*Controls versus intervention.

[†]Controls versus high exercise.

[‡]Given as mean $\pm$ standard deviation.

[§]Independent *t*-test.

^{||}Chi-squared test.

concentrations of VILIP-1 (Human VILIP-1 ELISA; BioVendor GmbH, Heidelberg, Germany), NFL (NF-light ELISA; IBL international, Hamburg, Germany), and YKL-40 (Quantikine ELISA Human Chitinase-3-like 1; R&D systems, MN, USA) by following the manufactures enclosed procedure, in technical duplicates. All measurements per assay were performed in one round of experiments using one batch of reagents for each biomarker by board-certified laboratory technicians who were blinded to clinical data.

2.4. Statistics

The baseline characteristics were compared between the two groups using *t*-tests. The baseline versus follow-up concentrations were analyzed using paired *t*-test within each group, respectively, and the mean change from baseline in CSF biomarker concentrations was analyzed by Student *t*-test. These analyses were done both as an intention-to-treat analysis where all participants were analyzed and as a per-protocol analysis where only the subjects that exercised with a mean intensity of >70% of maximal heart rate (HR) and attended > 80% of the sessions (high-exercise subjects) were included. The significance level was set to .05. No post hoc statistics were used.

3. Results

There were no significant differences in baseline characteristics between control and intervention groups (both all and high-exercise subjects) and in concentrations of

VILIP-1, NFL, Ng, and YKL-40 in CSF between the intervention group and control group (Table 1). Furthermore, there was no significant difference at baseline between control and the high-exercise group with regard to the concentrations of VILIP-1, NFL, Ng, and YKL-40, as shown in Table 1. There was no statistical significant difference in the concentration of the biomarkers in CSF between baseline and 16 week follow-up in the two groups, respectively, see Fig. 1, or in means change from baseline comparing the two groups. Fig. 2 displays the relative difference between the groups in percent ([follow-up minus baseline]/baseline) $\times$ 100 in the biomarkers measured. In three out of four biomarkers analyzed, there was a trend in relative change from baseline toward an increased level in CSF after 16 weeks of intervention in the exercise group. However, the variation in the changes in both groups was large. From the controls, group coefficient of variation (CV) was calculated for each assay, to check assay stability. The assay CV ranged from 3% to >30% (NFL: 17.9, Ng: 8.6, YKL-40: 2.9, and VILIP-1: 32.3).

4. Discussion

In this study, we investigated the effect of an intervention of 16 weeks of moderate- to high-intensity EXE in patients with AD on selected biomarkers of neuronal and synaptic integrity and neuroinflammation. The biomarkers investigated in this study are all found in CSF under normal physiological conditions, and the levels found correspond well with findings in previous studies [10,14,16,23].

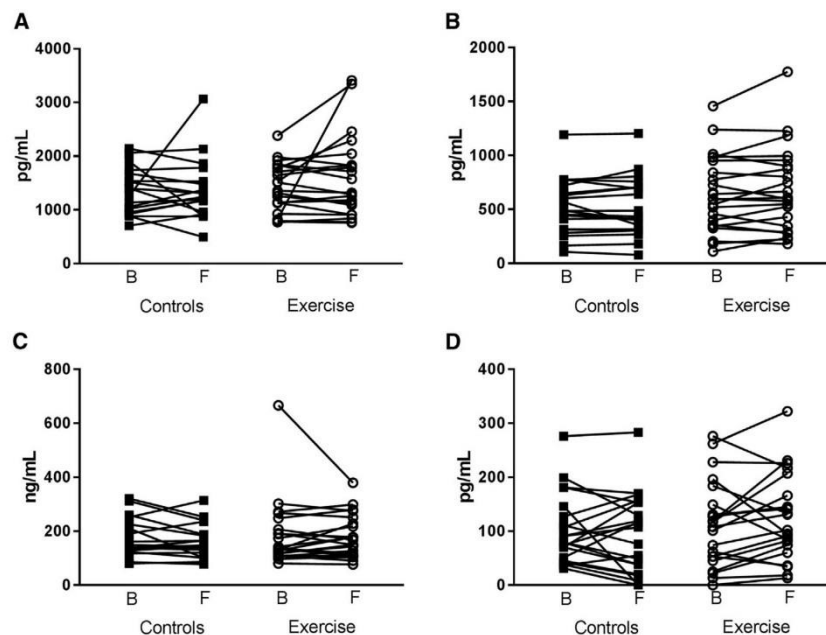


Fig. 1. Spaghetti plots of concentration of biomarkers in cerebrospinal fluid at baseline and at 16 weeks follow-up in outcome measures. (A) Neurofilament light (NFL), (B) neurogranin (Ng), (C) chitinase-3-like protein 1 (YKL-40), and (D) visinin-like protein-1 (VILIP-1). B: Baseline and F: 16-week follow-up.

Increased CSF levels of the biomarkers NFL, Ng, YKL-40, and VILIP-1 have been found in AD and correlate with neurodegeneration, suggesting a potential use as a surrogate marker of synaptic and neuronal loss [3,22–24]. Exercise and physical activity have been linked to cognitive preservation and structural and functional neuronal changes in humans [25]. However, the underlying neurobiology is unknown. To gain new insight into the molecular pathology in AD and possible changes with physical exercise, several pathophysiological pathways can be explored. The markers selected in this study are recently investigated biomarkers of AD, and very little is known of their ability to reflect changes in the rate of neurodegeneration.

We hypothesized that EXE would have a stabilizing effect on the neurons and a dampening effect of astroglial activation resulting in less release to the CSF of the biomarkers investigated. However, we found no significant effect of EXE on our selected biomarkers. In a previous study, we also did not find effect on A β species and tau/p-tau [9]. These markers, however, may provide novel insight into AD pathology and its progression [16,18,26]. The biomarkers reflecting specific AD pathology in CSF (A β and tau/p-tau) change over the disease course in a pattern that does not reflect neurodegeneration in a simple way making them less suitable for longitudinal markers of disease progression [27,28]. Because synaptic integrity plays a central role in CF and synaptic loss has been shown to correlate well with cognitive decline [29], biomarkers of

synaptic function may be of value in monitoring neurodegeneration, not only in AD but also in other neurodegenerative diseases [29].

As mentioned earlier, the studied biomarkers could be target biomarkers for measuring neurodegeneration in patients with AD, including effects of drug candidates and interventions on AD pathophysiology. However, no changes were found in biomarker levels in our 16-week aerobic exercise intervention. The most straightforward interpretation of these results is that they do not support any modulatory effect of physical exercise on AD pathophysiology, as measured by selected biomarkers of neuronal and synaptic integrity. The lack of a modulating effect of exercise on our selected biomarkers might have other explanations. Animal studies in mice suggest that environmental stimulation, including access to running wheels, improved cognition and resulted in significant higher levels of brain Ng [30]. This discrepancy may be because of full-length Ng is measured in the brain, whereas in the CSF, there are several endogenous cleavage products of Ng measured [21]. Also YKL-40 has previously been studied in relation to exercise. In a human study, the postexercise serum concentration of YKL-40 was found to be upregulated both in RNA expression and in circulating protein concentration [31]. However, in that setting, the elevated YKL-40 levels may have been because of muscle contraction. One animal study found that synapse-plasticity proteins, including NFL, were upregulated in exercising rats [32], and previous studies have shown that exercise promotes neurogenesis in the adult

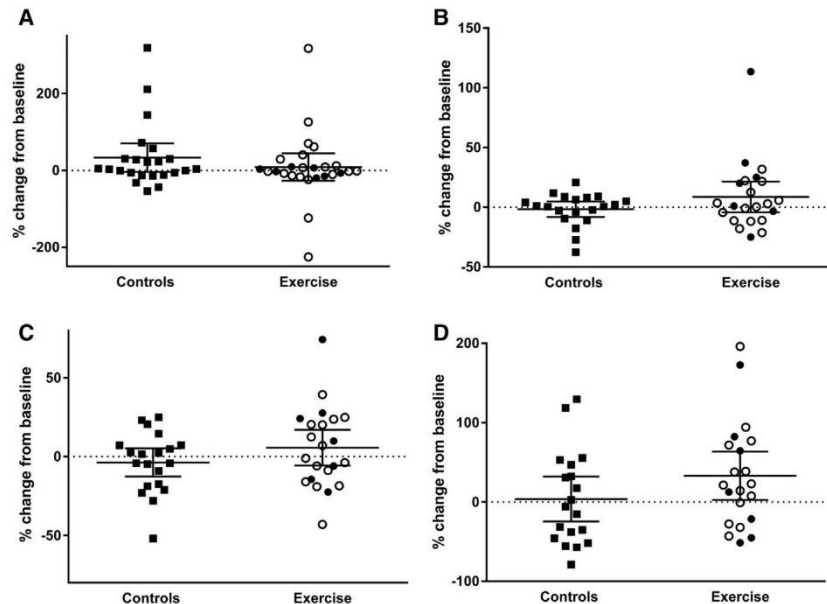


Fig. 2. Scatterplot of relative changes from baseline to 16 weeks follow-up in outcome measure in percent. (A) Neurofilament (NFL), (B) neurogranin (Ng), (C) chitinase-3-like protein 1, and (D) visinin-like protein-1 (VILIP-1). Closed squares: controls, open circles: exercise group, and closed circles: high exercise. Displayed as mean with 95% confidential interval.

mouse brain [33,34]. Taken together these animal studies suggest that exercise has a beneficial effect on synaptic and neuronal integrity thereby promoting brain plasticity. The reason for the negative results in our study could be because of insufficient duration of the exercise. It could be argued that 16 weeks of EXE is not a long enough intervention to permanently alter the synaptic function in the brain. Furthermore, any exercise mediated effect on the synaptic level in AD could be so transient that the effect is not able to manifest itself permanently or that the effect wears off as soon as the subjects stop exercising. In addition, information on changes of these biomarkers with interventions or longitudinal changes is scarce. At the stage of the disease in which the subjects included in this study were studied, we do not know if it is reasonable to expect changes in biomarkers reflecting neurodegeneration. The neurodegeneration may be too advanced for a signal to be picked up, if only subpopulations of neurons respond to EXE.

A further limitation of our study is the small number of subjects. We had CSF available from 51 patients, of which 26 were randomized to the control group. Of the 25 exercise participants, 16 met the criteria “high-exercise subjects.” Restricting the analysis to high-exercise subjects compared with controls, did, however, not change the overall negative findings. However, a power calculation (80% power) showed that we had sufficient subjects in each group for detecting a 10% change in CSF concentration, based on the previous studies, for Ng (eight subjects needed), YKL-40 (four subjects needed), and NFL (nine subjects needed). Only our

VILIP-1 measurement is underpowered because 37 subjects were needed to display a 10% changes in CSF concentration. From Fig. 1 and the interassay CV, we can conclude that the assays are fairly stable, and results are reproducible.

The use of EXE as a therapeutic approach for AD and other dementias is an attractive add-on to conventional medical treatment, and there seems to be a positive effect on cognition [35]. The underlying effect is, however, largely unknown [36], and more attention must be paid to the molecular mechanism to understand and optimize this type of treatment. Therefore, more studies investigating the effect of type and length of physical exercise are needed to elucidate the molecular effect of EXE in AD.

Acknowledgments

We thank Ulrika Törnqvist and Åsa Källen from the Department of Psychiatry and Neurochemistry, Sahlgrenska University Hospital, Gothenburg University, and Kathrine Bjarnø from the Danish Dementia Research Centre, Department of Neurology, Rigshospitalet University of Copenhagen for technical support. The study is supported by The Swedish Research Council, the European Research Council, the Knut and Alice Wallenberg Foundation, the Torsten Söderberg Foundation, and Swedish State Support for Clinical Research. The Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) study is supported by the Innovation Fund Denmark (J No.

10-092814). The Danish Dementia Research Centre is supported by grants from the Danish Ministry of Health (J No. 2007-12143-112, project 59506/J No. 0901110, project 34501) and the Danish Health Foundation (J No. 2007B004).

RESEARCH IN CONTEXT

1. Systematic review: Based on our previous finding in other areas of the effects of physical exercise biomarkers, we know that there is a beneficial effect of physical exercise on memory, neuropsychiatric symptoms, and physical function. However, we have not found the underlying molecular mechanism.
2. Interpretation: We interpret our results as a clue to finding the molecular mechanism, in understanding effect of exercise in patients with Alzheimer's disease (AD). Although our results are not a positive find, we now know more exercise as a stabilizing agent on synapse stability and integrity.
3. Future directions: The overall conclusion is that exercise does have a beneficial effect on the selected biomarkers; however, we recommend further studies in other areas affected by AD that could be implicated in the effect of physical exercise.

References

- [1] Ossenkoppele R, van der Flier WM, Verfaillie SC, Vrenken H, Versteeg A, van Schijndel RA, et al. Long-term effects of amyloid, hypometabolism, and atrophy on neuropsychological functions. *Neurology* 2014;82:1768–75.
- [2] West M, Coleman P, Flood D, Troncoso J. Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet* 1994;344:769–72.
- [3] Tarawneh R, Lee JM, Ladenson JH, Morris JC, Holtzman DM. CSF VILIP-1 predicts rates of cognitive decline in early Alzheimer disease. *Neurology* 2012;78:709–19.
- [4] Ryan SM, Kelly AM. Exercise as a pro-cognitive, pro-neurogenic and anti-inflammatory intervention in transgenic mouse models of Alzheimer's disease. *Ageing Res Rev* 2016;27:77–92.
- [5] Rovio S, Käreholt I, Helkala EL, Viitanen M, Winblad B, Tuomilehto J, et al. Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. *Lancet Neurol* 2005;4:705–11.
- [6] Ngandu T, Lehtisalo J, Solomon A, Levälahti E, Ahtiluoto S, Antikainen R, et al. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. *Lancet* 2015;385:2255–63.
- [7] Hoffmann K, Sobol NA, Frederiksen KS, Beyer N, Vogel A, Vestergaard K, et al. Moderate-to-High Intensity Physical Exercise in Patients with Alzheimer's Disease: A Randomised Controlled Trial. *J Alzheimers Dis* 2016;50:443–53.
- [8] Sobol NA, Hoffmann K, Vogel A, Lolk A, Gottrup H, Høgh P, et al. Associations between physical function, dual-task performance and cognition in patients with mild Alzheimer's disease. *Ageing Ment Health* 2016;20:1139–46.
- [9] Steen Jensen C, Portelius E, Siersma V, Høgh P, Wermuth L, Blennow K, et al. Cerebrospinal Fluid Amyloid Beta and Tau Concentrations Are Not Modulated by 16 Weeks of Moderate- to High-Intensity Physical Exercise in Patients with Alzheimer Disease. *Dement Geriatr Cogn Disord* 2016;42:146–58.
- [10] Zetterberg H, Skillbäck T, Mattsson N, Trojanowski JQ, Portelius E, Shaw LM, et al. Association of Cerebrospinal Fluid Neurofilament Light concentration with Alzheimer Disease Progression. *JAMA Neurol* 2016;73:60–7.
- [11] Kester MI, Teunissen CE, Crimmins DL, Herries EM, Ladenson JH, Scheltens P, et al. Neurogranin as a Cerebrospinal Fluid Biomarker for Synaptic Loss in Symptomatic Alzheimer Disease. *JAMA Neurol* 2015;72:1275–80.
- [12] Portelius E, Zetterberg H, Skillbäck T, Törnqvist U, Andreasson U, Trojanowski JQ, et al. Cerebrospinal fluid neurogranin: relation to cognition and neurodegeneration in Alzheimer's disease. *Brain* 2015;138:3373–85.
- [13] Lee JM, Blennow K, Andreasen N, Laterza O, Modur V, Olander J, et al. The brain injury biomarker VLP-1 is increased in the cerebrospinal fluid of Alzheimer disease patients. *Clin Chem* 2008;54:1617–23.
- [14] Bonneh-Barkay D, Wang G, Starkey A, Hamilton RL, Wiley CA. In vivo CHIL1 (YKL-40) expression in astrocytes in acute and chronic neurological diseases. *J Neuroinflammation* 2010;7:34.
- [15] Hjalmarsson C, Bjerke M, Andersson B, Blennow K, Zetterberg H, Aberg D, et al. Neuronal and Glia-Related Biomarkers in Cerebrospinal Fluid of Patients with Acute Ischemic Stroke. *J Cent Nerv Syst Dis* 2014;6:51–8.
- [16] Kester MI, Teunissen CE, Sutphen C, Herries EM, Ladenson JH, Xiong C, et al. Cerebrospinal fluid VILIP-1 and YKL-40, candidate biomarkers to diagnose, predict and monitor Alzheimer's disease in a memory clinic cohort. *Alzheimers Res Ther* 2015;7:59.
- [17] Sutphen CL, Jasielec MS, Shah AR, Macy EM, Xiong C, Vlassenko AG, et al. Longitudinal Cerebrospinal Fluid Biomarker Changes in Preclinical Alzheimer Disease During Middle Age. *JAMA Neurol* 2015;72:1029–42.
- [18] Craig-Schapiro R, Perrin RJ, Roe CM, Xiong C, Carter D, Cairns NJ, et al. YKL-40: A Novel Prognostic Fluid Biomarker for Preclinical Alzheimer's Disease. *Biol Psychiatry* 2010;68:903–12.
- [19] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. 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;34:939–44.
- [20] Hoffmann K, Frederiksen KS, Sobol NA, Beyer N, Vogel A, Simonsen AH, et al. Preserving cognition, quality of life, physical health and functional ability in Alzheimer's disease: the effect of physical exercise (ADEX trial): rationale and design. *Neuroepidemiology* 2013;41:198–207.
- [21] Kvartsberg H, Portelius E, Andreasson U, Brinkmalm G, Hellwig K, Lelental N, et al. Characterization of the postsynaptic protein neurogranin in paired cerebrospinal fluid and plasma samples from Alzheimer's disease patients and healthy controls. *Alzheimers Res Ther* 2015;7:40.
- [22] De Vos A, Jacobs D, Struyfs H, Franssen E, Andersson K, Portelius E, et al. C-terminal neurogranin is increased in cerebrospinal fluid but unchanged in plasma in Alzheimer's disease. *Alzheimers Dement* 2015;11:1461–9.
- [23] Janelidze S, Hertze J, Zetterberg H, Landqvist Waldö M, Santillo A, Blennow K, et al. Cerebrospinal fluid neurogranin and YKL-40 as biomarkers of Alzheimer's disease. *Ann Clin Transl Neurol* 2016;3:12–20.
- [24] Wildsmith KR, Schauer SP, Smith AM, Arnott D, Zhu Y, Haznedar J, et al. Identification of longitudinally dynamic biomarkers in

- Alzheimer's disease cerebrospinal fluid by targeted proteomics. *Mol Neurodegener* 2014;9:22.
- [25] Erickson KI, Weinstein AM, Lopez OL. Physical Activity, Brain Plasticity, and Alzheimer's Disease. *Arch Med Res* 2012;43:615–21.
- [26] Mattsson N, Rütschi U, Pijnenburg YA, Blankenstein MA, Podust VN, Li S, et al. Novel cerebrospinal fluid biomarkers of axonal degeneration in frontotemporal dementia. *Mol Med Rep* 2008;1:667–71.
- [27] Zhou B, Teramukai S, Yoshimura K, Fukushima M. Validity of cerebrospinal fluid biomarkers as endpoints in early-phase clinical trials for Alzheimer's disease. *J Alzheimers Dis* 2009;18:89–102.
- [28] Daniela B, Dirk LK, Scheltens P, Visser PJ. Alzheimer's Disease Neuroimaging Initiative. Temporal evolution of biomarkers and cognitive markers in the asymptomatic, MCI, and dementia stage of Alzheimer's disease. *Alzheimers Dement* 2015;11:511–22.
- [29] Scheff SW, Price DA, Schmitt FA, DeKosky ST, Mufson EJ. Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. *Neurology* 2007;68:1501–8.
- [30] Huang FL, Huang KP, Wu J, Boucheron C. Environmental Enrichment Enhances Neurogranin Expression and Hippocampal Learning and Memory But Fails to Rescue the Impairments of Neurogranin Null Mutant Mice. *J Neurosci* 2006;26:6230–7.
- [31] Görgens SW, Jensen J, Holven KB, Tennagels N, Eckardt K, Eckel J. The novel myokine YKL-40 is increased by exercise and inhibits TNFalpha-induced inflammation and insulin resistance in human skeletal muscle cells. n.d.
- [32] Ding Q, Vaynman S, Souda P, Whitelegge JP, Gomez-Pinilla F. Exercise affects energy metabolism and neural plasticity-related proteins in the hippocampus as revealed by proteomic analysis. *Eur J Neurosci* 2006;24:1265–76.
- [33] van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc Natl Acad Sci U S A* 1999;96:13427–31.
- [34] van Praag H, Shubert T, Zhao C, Gage FH. Exercise Enhances Learning and Hippocampal Neurogenesis in Aged Mice. *J Neurosci* 2005;25:8680–5.
- [35] Groot C, Hooghiemstra AM, Raijmakers PG, van Berckel BN, Scheltens P, Scherder EJ, et al. The effect of physical activity on cognitive function in patients with dementia: a meta-analysis of randomized control trials. *Ageing Res Rev* 2016;25:13–23.
- [36] Jensen CS, Hasselbalch SG, Waldemar G, Simonsen AH. Biochemical Markers of Physical Exercise on Mild Cognitive Impairment and Dementia: Systematic Review and Perspectives. *Front Neurol* 2015;6:187.

Paper III

Exercise as a potential modulator of inflammation in patients with Alzheimer's disease measured in cerebrospinal fluid and plasma

Experimental Gerontology. (In press)

Camilla Steen Jensen^{1,2}, Justyna Maria Bahl³, Lærke Borg Østergaard¹, Peter Høgh^{2,4}, Lene Wermuth⁵, Amanda Heslegrave^{6,7}, Henrik Zetterberg^{6,7,8,9}, Niels H. H. Heegaard^{10,11,†}, Steen Gregers Hasselbalch^{1,2}, Anja Hviid Simonsen¹

1 Danish Dementia Research Centre, Department of Neurology, Rigshospitalet University of Copenhagen, DK-2100 Copenhagen Denmark.

2 Department of clinical medicine, University of Copenhagen

3 Department of bioanalysis, Lundbeck, 2500 Valby, Denmark.

4 Regional Dementia Research Centre, Department of Neurology, Zealand University Hospital, DK-4000 Roskilde Denmark,

5 Dementia Clinic, Department of Neurology, Odense University Hospital DK-5000 Odense Denmark,

6 Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, WC1N London UK.

7 UK Dementia Research Institute at UCL, London WC1E 6BT, UK

8 Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, S-431 80 Mölndal Sweden,

9 Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, S-431 80 Mölndal, Sweden.

10 Department of Clinical Biochemistry, Immunology and Genetics, Statens Serum Institut, Copenhagen, Denmark.

11 Department of Clinical Biochemistry, University of Southern Denmark, Odense, Denmark

† Deceased

Corresponding author: Camilla Steen Jensen, Danish Dementia Research Centre, Department of Neurology, Rigshospitalet University of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

Email: Camilla.steen.jensen@regionh.dk. Tele: +45 35457662.

Email to all authors: Justyna Maria Bahl: Jumb@lundbeck.com; Lærke Borg Østergaard:

Lærke.borg.ostergaard@regionh.dk; Peter Høgh: pjh@regionsjaelland.dk; Lene Wermuth:

Lene.Wermuth@rsyd.dk; Amanda Heslegrave: a.heslegrave@ucl.ac.uk; Henrik Zetterberg:

henrik.zetterberg@clinchem.gu.se; Steen Gregers Hasselbalch: Steen.gregers.hasselbalch@regionh.dk;

Anja Hviid Simonsen: anja.hviid.simonsen@regionh.dk

Acknowledgements

We thank Karina Liebmann Madsen and Kristin Skogstrand from the section of Immunoassay, Department of Diagnostics and Infection controls at Statens Serum Institut, Denmark for technical support. Further, we would like to thank Dr. Anne Bie, Danish Dementia Research Center, Copenhagen University Hospital Rigshospitalet, Denmark for technical and academic supervision for the qPCR assay.

The study is supported by Ellen Mørchs fond. The Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) study is supported by the Innovation Fund Denmark (J No. 10-092814). The Danish Dementia Research Centre is supported by grants from the Danish Ministry of Health (J No. 2007-12143-112, project 59506/J No. 0901110, project 34501) and the Danish Health Foundation (J No. 2007B004). HZ is supported by grants from the UK Dementia Research Institute at UCL, the Wellcome Trust, the Swedish Research Council, the European Research Council and the Knut and Alice Wallenberg Foundation.

The authors have nothing to declare

Abstract

Background: Neuroinflammation is recognized as part of the pathological progression of Alzheimer's disease (AD), but the molecular mechanisms are still not entirely clear. Systemically, physical exercise has shown to have a positive modulating effect on markers of inflammation. It is not known if this general effect also takes place in the central nervous system in AD. The aim of this study was to investigate the effect of 16 weeks of moderate to high-intensity physical exercise on selected biomarkers of inflammation both systemically and in the CNS, in patients with AD.

Methods: Plasma and cerebrospinal fluid (CSF) from 198 patients with Alzheimer's disease participating in the Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) study were analyzed for concentrations of 8-isoprostane, soluble trigger receptor expressed on myeloid cells 2 (sTREM2), and the MSD v-plex proinflammation panel 1 human containing interferon gamma (IFN γ), Interleukin-10 (IL10), IL12p70, IL13, IL1 β , IL2, IL4, IL6, IL8, and tumor necrosis factor alpha (TNF α), before and after a 16-week intervention with physical exercise, and we studied whether changes were modulated by the patients' *APOE* genotype

Results: Most inflammatory markers remained unchanged after exercise. We found an increasing effect of 16 weeks of physical exercise on sTREM2 measured in CSF. Further, IL6 in plasma increased in the exercise group after physical exercise (mean relative change 41.03, SD 76.7), compared to controls (-0.97, SD 49.4). In a sub-analysis according to *APOE* genotype, we found that in ϵ 4 carriers, exercise had a stabilizing effect on IFN γ concentration with a mean relative change of 7.84 (SD 42.6), as compared to controls (114.7 (SD 188.3), $p=0.038$).

Conclusion: Our findings indicate an effect of physical exercise on markers of neuroinflammation in CSF measured by an increase in sTREM2 in patients with AD. Further, there may be a small inflammatory systemic effect related to physical exercise in patients with AD.

Keyword:

Alzheimer's disease, physical activity, exercise, biomarkers, inflammation, CSF, plasma,

Background

Physical activity (PA) has been shown to have a positive modulatory effect on systemic inflammation by reducing pro-inflammatory cytokines, increasing anti-inflammatory cytokines [1,2], and modulating reactive oxygen species (ROS) both in young and older adults [3–6]. [7]. Inflammation is an important part of the pathophysiology of Alzheimer's disease (AD) [8,9], however, the precise pathological mechanism behind the contribution of inflammation to the pathological progression of the disease is largely unknown. It is thought that prolonged stress activation of microglia due to amyloid β ($A\beta$) plaque formation, can result in the development of a harmful (inflammation-promoting) increase in cytokines, chemokines and ROS [10]. Whereas longitudinal cohort studies show an association between higher levels of PA and a reduced risk of cognitive decline and dementia [11], there is limited evidence for an effect of PA on AD biomarkers [12,13]. However, PA has been shown to slow down hippocampal atrophy in healthy elderly [14], change specific AD biomarkers in a favorable way in healthy subjects at risk of AD [15], and improve cognition in older adults with mild cognitive impairment [16]. It is therefore likely that PA may slow down the clinical progression of AD by reducing harmful inflammation, promoting beneficial myokines and reducing systemic metabolic risk factors of AD, e.g., insulin resistance and hypercholesterolemia [17]

In this study, we wished to analyze the effect of 4 months of moderate to high-intensity physical exercise in patients with AD, on markers of different inflammatory processes in cerebrospinal fluid (CSF) and plasma. We employed a general pro-inflammatory cytokine panel, which also includes anti-inflammatory cytokines and growth factors. Furthermore, we chose a marker of microglia activation due to cellular stress, which has been linked to AD risk, soluble trigger receptor expressed on myeloid cells 2 (sTREM2) [18–20] and 8-isoprostane, a marker of oxidative stress and lipid peroxidation [21,22]. In addition, we determined the single nucleotide polymorphism (SNP) rs75932628-T, which has been linked to genetic higher levels of sTREM2 in CSF and higher risk of developing AD [23,24].

We hypothesized that PA would have an upregulating effect on anti-inflammatory cytokines, and down-regulating effect on pro-inflammatory cytokines. Further, we analyzed whether PA exhibited modulating effects on markers of microglia activation and ROS, measured via sTREM2 and 8-isoprostane respectively. Finally, we analyzed whether the apolipoprotein E (*APOE*) genotype influenced the impact of PA on inflammation.

Material and methods

Study population

One hundred and eighty eight home-dwelling patients with clinically diagnosed mild AD according to NINCDS-ADRDA criteria [25] and an MMSE >19 were enrolled in the multicenter 'Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise' or in short, the ADEX study. For a detailed description of the study, please see [26]. In short, patients with mild to moderate AD were randomized to either a control group with treatment as usual or a 16-week 60 min three times per week moderate-to-high intensity aerobic physical exercise (treadmill, stationary bike, cross trainer) group. Subjects were tested at baseline and at 16-week follow-up with a comprehensive battery of tests of cognitive function, Activities of Daily Function, Quality of Life, physical activity and neuropsychiatric symptoms.

Primary and secondary clinical outcomes have previously been published showing an effect of physical exercise on neuropsychiatric symptoms as well as on cognitive and physical function [27,28]. Before and after the intervention, blood samples were collected from all subjects, and CSF was collected from a subgroup of patients. The baseline characteristics of the whole study population and the CSF subgroup are shown in table 1.

Samples

A total of 198 patients participated in the study, all gave blood samples, and 58 patients donated CSF samples. Due to technical reasons, not all analytes were measured in all the patients. CSF sTREM2 was measured in 55 samples (27 controls and 28 exercise). Paired plasma and CSF cytokine profile was measured in 51 samples (27 controls and 24 exercise) with the MSD v-plex proinflammation panel 1 human. CSF 8-isoprostane was measured in 55 samples (28 controls and 27 exercise) and in 187 plasma samples (87 controls and 100 exercise). All samples were collected and processed according to international guidelines [29].

Assays

Commercially available kits were used for measuring the concentrations of plasma and CSF cytokines containing interferon gamma (IFN γ), Interleukin-10 (IL10), IL12p70, IL13, IL1 β , IL-2, IL4, IL6, IL8, tumor necrosis factor alpha (TNF α) (MSD v-plex proinflammation panel 1 human, Meso Scale Discovery, MA, USA), and plasma and CSF 8-isoprostane (8-isoprostane ELISA kit, Cayman Chemicals, MI, USA) by following the manufacturers' enclosed procedure, in technical duplicates.

CSF sTREM2 was measured with an in-house MSD assay. In short, to streptavidin-coated 96-well plates (Meso-Scale discovery (MSD) a biotinylated polyclonal goat anti-human TREM2 capture antibody (0.25

µg/ml R&D Systems BAF1828) was bound, and subsequently incubated with the CSF or a standard protein solution constructed from recombinant human TREM2 protein (4000 -62.5pg/ml Sino Biological Inc. 11084-H08H). Further, a detector antibody, monoclonal mouse anti-human TREM2 antibody (1µg/ml Santa Cruz Biotechnology; B-3, sc373828), was added, and thereafter incubated with a secondary antibody (SULFO-TAG-labeled anti-mouse secondary antibody, MSD). Lastly, the plates were developed by adding MSD Read buffer and the light emission measured using the MSD SECTOR Imager 6000. The concentration of sTREM2 was calculated using a five-parameter logistic curve fitting method with the MSD Workbench software package. Intra-assay coefficients of variation (CVs) were < 10%, and all samples were measured on the same day using the same reagents. *TREM2* genotype for SNP rs75932628-T was analyzed using TaqMan assay (TaqMan SNP assay MTO human SMC_100657057_10, Life Technologies, CA, USA).

Concentration change calculations

The changes in concentration of the biomarkers measures, were presented as relative change from baseline and calculated as

$$\frac{[Follow\ up]-[Baseline]}{[Baseline]} * 100 = relative\ change\ from\ baseline$$

This calculation was used to take into account marginal levels at baseline (very low or very high levels). Further, it gives more emphasis on small changes in individuals with smaller baseline levels, than small changes in individuals with higher baseline levels. As a result, relative change from baseline < 0, equals lower concentration at follow up. Relative change from baseline = 0, equals no difference concentration at follow up compared to baseline. Relative change from baseline > 0, equals higher concentration at follow up.

Statistics

The baseline characteristics were compared between the two groups using t-tests. The baseline and follow up concentrations were analyzed using paired t-test within each group respectively, and the mean relative change from baseline in biomarker concentrations was analyzed by Student's t-test. These analyses were done both as an intention to treat (ITT) analysis where all participants were analyzed, and as a per-protocol analysis where only the subjects that exercised with a mean intensity of > 70% of maximal heart rate (HR) and attended > 80% of the sessions, were included, this sub-group is called high exercise group, and consists of 66 subjects from the exercise group [26,27]. Additionally, linear regression between BMI at follow up and change from baseline was performed for the cytokine panel. Furthermore, the *APOE*

genotype contribution to the effect on the biomarkers was analyzed in non-carriers versus carriers of the AD risk relevant $\epsilon 4$ allele. The significance level was set to 0.05 and outlier analysis was performed.

Results

Baseline characteristics in the control group, exercise group, and high exercise sub-group were comparable, as shown in Table 1. Body Mass Index (BMI) was slightly, but significantly higher in controls versus exercise group, and disease duration in the high exercise sub-group was slightly shorter as compared to the control group. Table 1B displays the baseline characteristics of the groups and high exercise sub-group with CSF samples and in these sub-groups, no differences were found between controls and exercise subjects. Further, there was no difference in baseline levels of any of the analytes when comparing controls to exercise, and controls to the high exercise group (data not shown).

Over all, most of the analyzed biomarkers were not affected by PA, and remained stable over the intervention period, see table 2. However, when analyzing relative change from baseline, the IL6 plasma concentration increased significantly in the exercise group compared to the control group with a mean change from baseline at 41.03 (SD 76.7) compared to -0.97 (SD 49.4) in the control group ($p = 0.049$). When analyzing the high exercise group vs controls, the same outcome was found with a mean change of 48.01 (SD 85.8) and -0.97 (SD 49.4), respectively ($p = 0.047$). Similar results with borderline significance were found for sTREM2 in CSF. Here, sTREM2 mean relative change from baseline was 6.9 (SD 14.0) in the high exercise group compared to -2.4 (SD 13.8) in controls, ($p = 0.05$). *TREM2* SNP assay showed no genotype that corresponds with genetically altered sTREM2 levels.

Furthermore, analysis whether BMI at follow up and relative change from baseline were correlated showed significant correlations between BMI and relative change in IFN γ in CSF and in TNF α in plasma. Further analysis with exercise as a groups variable showed that in CSF IFN γ the correlation was due to BMI and independent of randomization group ($p = 0.017$). The same result was in plasma TNF α ($p = 0.03$). In plasma IL6 there was not an overall correlation between BMI and relative change in IL6 however, analysis with groups variable showed a significant effect of exercise and not BMI ($p = 0.04$) on the relative change from baseline, see figure 1.

In addition, when stratifying for *APOE* genotype in carriers and non-carriers of the $\epsilon 4$ allele, we found that plasma IFN γ concentration increased more in controls who were $\epsilon 4$ carriers (114.7 [SD 188.3] compared to 7.8 (SD 42.8) in the $\epsilon 4$ -positive exercise group, $p = 0.038$). The opposite was observed when analyzing sTREM2 based on *APOE* genotype. Here, there was an increase in exercising *APOE* $\epsilon 4$ carriers, 5.7 (14.4), compared to a decrease in the controls -6.4 (15.5), $p = 0.037$, see table 3.

Difference in patient numbers in the reported results, and in follow up versus mean relative change from baseline, is due to samples falling below limit of detection in either baseline or follow up measurements, or due to technical reasons e.g. lysis of the sample.

Discussion

In this explorative sub-study, we investigated the effect of an intervention of 16 weeks of moderate-to-high intensity exercise in patients with AD on selected biomarkers of inflammation measured in plasma and CSF, to attempt to elucidate the biochemical effect of PA, as several large population-based studies have shown that greater physical exercise is associated with lower levels of inflammation [30]. We have previously published the effect on cognition [27] and physical measures [28] Here, we have measured a variety of known biomarkers of inflammation to investigate molecular underpinnings of the previously reported beneficial effect on cognition and physical parameters in this population. The approach resulted in many negative results, but we did find some moderate modulating effects of our intervention on a number of inflammatory proteins. Besides this study we have published our results on cognition, neuropsychiatric [27], physical parameters [28,31], and other relevant biomarkers [32,33]

We found PA to increase the levels of sTREM2 in CSF and IL6 in plasma. In a subgroup analysis of *APOE* ϵ 4 carriers, plasma IFN γ increased in the control group whereas it remained unchanged in the exercise group. Additionally, in *APOE* ϵ 4 carriers, sTREM2 increased in the exercise group, while decreased in the control group. Furthermore, analysis of BMI correlated with relative change from baseline with randomization groups as a covariate showed, that some of the relative change from baseline detected was due to increased BMI. Both negatively and positively. However only a very small percentage (ranging from 13-18%) of the relative change from baseline in the biomarkers could be explained by BMI.

Inflammation has been established as an important contributor to AD pathology [34]. It is however not clear if inflammation is the cause or the outcome of pathological A β plaque accumulation, in other words, whether A β plaques cause inflammation or whether neuroinflammation increases A β buildup [35].

Nonetheless, markers of inflammation that can be measured in CSF and even plasma have the potential to aid in diagnosis, monitor disease progression and help to understand the beneficial effects of treatment strategies [36,37].

Many previous studies have found altered cytokine levels in patients with AD. IL1 has been investigated in brains from AD patients and in blood, where Increased levels in brain and blood have been linked to A β formation [38,39]. Furthermore, increased levels of other members of the IL1 family, IL18, IL33, and IL12 have been linked to increased AD risk [40]. In addition to findings of increased pro-inflammatory cytokines,

treatments with the anti-inflammatory cytokine IL4 have shown reduced A β accumulation in AD mice models [41].

The cytokine IL6 has historically been viewed as a pro-inflammatory molecule, released in the acute phase of an infection. However, IL6 is increasingly being recognized to exhibit both pro-inflammatory as well as anti-inflammatory abilities, supported by the lack of induction of among others nitric oxide with IL6 release, as seen with the release of the pro-inflammatory cytokines IL1 and TNF α [2,42]. AD research has focused on IL6 both systemically and in the CNS. Blood levels of IL6 have been found to be increased in AD as compared to cognitively healthy controls, and brain tissues from AD patients stained for IL6 showed increased levels of IL6 adhesive to senile plaque inclusion [39,43,44]. In relation to exercise and physical activity, studies have shown that exercise induces an acute phase response, similar to sepsis and trauma [2]. Especially, IL6 is produced in high amounts in response to exercise in healthy subjects, with a rapid decline after end of the exercise [1,2]. In addition, IL6 is also produced locally in the muscles exhibiting growth factor properties [2]. These findings are in line with the present results where we also see an increase in plasma IL6 in the exercise group, compared to controls, suggesting that exercise have similar effects on IL6 in AD as in healthy subjects.

IFN γ is a key player in the adaptive immune system, in promoting T1-helper cells and clear infections [45]. IFN γ has been shown to increase A β and tau pathology in a mouse model for AD [46], but on the other hand to improve cognition in AD patients [47]. We found a significantly higher concentration at follow up in IFN γ among the *APOE* ϵ 4 carriers in the control group. However, this result should be interpreted with great caution, due to the very small sample number, as it could be a false positive result. We would assume that if IFN γ is suppressed by PA in AD, we would see a change in both *APOE* ϵ 4 carriers as well as non-carriers. However, studies in apoE -/- mice models, have shown that these mice have an increased immune response including increased IFN γ production [48]. Human *APOE* ϵ 4 carriers have low plasma apoE protein levels [49], similar to the apoE -/- mice, and studies have shown apoE to be tightly related to the immune system response [50], suggesting that apoE protein helps suppress the activity of natural killer t-cells (NKT) cells, and the IFN γ release [50]. In other words, lower levels of apoE protein could result in elevated levels of IFN γ . This, it seems, is inhibited by exercise, displaying an anti-inflammatory effect of PA in *APOE* ϵ 4 carriers, but not in non-carriers.

In the gene encoding TREM2, SNPs have been linked to AD risk, and studies have found conflicting results in the analysis of sTREM2 in CSF, some with higher amount of sTREM2 in CSF in patients with AD, and some with lower levels, compared to healthy controls [18,51–53]. We found that exercise increased levels of

sTREM2, and that non-exercise controls had a decrease in sTREM2 levels over the 16 weeks of intervention. This difference was driven by the *APOE* ϵ 4 genotype, as seen in our sub-analysis according to *APOE* genotype. As with the results found with IFN γ , the stratification in *APOE* genotype generates very small sample sizes, and the results should be interpreted with caution. Considering the conflicting findings of the previous studies, it is hard to conclude if the increase seen in sTREM2 in the *APOE* ϵ 4 exercise group, is beneficial or harmful.

TREM2 expressed on the cell surface acts as an immune receptor, a soluble form of the protein sTREM2 can be cleaved from the cell surface under conditions of microglial activation and stress [54]. It has been found in mice studies that sTREM2 acts as a microglia activator through the Akt-GSK3 β - β -cetenin pathway [55]. The elevated levels seen in AD, may be due to the need for an acute survival response for the microglia. However, later in the process of AD sTREM2 might be more harmful than useful. In addition to its proinflammatory function, sTREM2 has been shown to induce IL6 transcription in a mouse model [55]. One study even suggests that modulating sTREM2 levels could be an efficacious therapeutic approach to treat AD [55]. This is further underlined with the finding that loss of function mutation in the gene for TREM2, leads to the early onset dementia disease Nasu-Hakola disease [24]. In that case we could postulate that exercise may in fact be such a therapeutic approach, since the exercise group displays increased sTREM2 in CSF after 16 weeks of physical exercise.

A general limitation of this study is the size of the CSF group, and subgrouping these patients according to *APOE* ϵ 4 genotype generated even smaller groups. The very small group sizes increase chances of a statistical type II error [56]. This is only solved by having big sample sizes or very small variations in data [57]. However, variations in biomarkers were high, due to biological or analytical variations. In addition, the disease stage may have impacted our results. Certain biomarker profiles may be abnormal in MCI stages and may change towards normal values as the disease progress into dementia stages [58], more over the level of inflammation may vary throughout the disease. This could indicate that physical exercise intervention studies should have the MCI stage as the target, as pathophysiological events may be more amenable to change at this stage. This is further emphasized by the findings from studies of PA, where effects on cognition generally have been larger in MCI subjects than in AD subjects [59].

Conclusion

In conclusion, IL6 and sTREM2 were modulated in patients with AD by 16 weeks of PA. In addition, IFN γ was increased in *APOE* ϵ 4 carriers who exercised. However, most other inflammatory markers in plasma and CSF remained unchanged following PA. The use of PA as a therapeutic approach for AD and other

dementias is an attractive add-on to conventional medical treatment [60], but the underlying biochemical effects remain largely unknown [12]. Our study addresses part of this and reports promising pilot data on a number of inflammatory/microglia-related biomarkers from a hypothesis-generating explorative analysis, not corrected for multiple comparisons. More studies, preferably also examining effects of type and length of the physical exercise, are needed to replicate these findings and elucidate more of the molecular effects of PA in AD. In larger patient cohorts, it would be relevant to stratify according to disease severity and analyze the impact of PA on inflammation in subgroups with subjective cognitive impairment MCI, mild, moderate and severe AD.

List of abbreviations

AD: Alzheimer's disease. ADEX: Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's disease: The Effect of Physical Exercise. APOE: apolipoprotein E. CNS: central nervous system. CSF: cerebrospinal fluid. TREM2: trigger receptor expressed on myeloid cells 2. sTREM2: soluble trigger receptor expressed on myeloid cells 2. IFN γ : interferon gamma, IL: interleukin. TNF α : tumor necrosis factor alpha. APOE: apolipoprotein E. PA: physical activity. ROS: reactive oxygen species. A β : amyloid beta. SNP: single nucleotide polymorphism. NINCDS-ADRDA: National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association. MMSE: mini mental status examination. MSD: Meso Scale Discovery. ITT: intention to treat. HR: heart rate. BMI: body mass index. SD: standard deviation. NKT: natural killer t cells. MCI: mild cognitive impaired. CV: coefficient of variation.

References

- [1] Petersen AMW, Pedersen BK. The anti-inflammatory effect of exercise. *J Appl Physiol* 2005;98:1154–62.
- [2] Pedersen BK. Exercise and cytokines. *Immunol Cell Biol* 2000;78.
- [3] Kohut ML, McCann DA, Russell DW, Konopka DN, Cunnick JE, Franke WD, et al. Aerobic exercise, but not flexibility/resistance exercise, reduces serum IL-18, CRP, and IL-6 independent of β -blockers, BMI, and psychosocial factors in older adults. *Brain Behav Immun* 2006;20:201–9. doi:10.1016/J.BBI.2005.12.002.
- [4] Woods JA, Wilund KR, Martin SA, Kistler BM. Exercise, Inflammation and Aging. *Aging Dis* 2012;3:130–40.
- [5] Pedersen BK, Bruunsgaard H. Possible beneficial role of exercise in modulating low-grade inflammation in the elderly. *Scand J Med Sci Sport* 2003;13:56–62. doi:10.1034/j.1600-0838.2003.20218.x.
- [6] Wärnberg J, Cunningham K, Romeo J, Marcos A. Role of physical activity on immune function Physical activity, exercise and low-grade systemic inflammation. *Nutr Soc* 2010;69:400–6. doi:10.1017/S0029665110001928.
- [7] Radak Z, Suzuki K, Higuchi M, Balogh L, Boldogh I, Koltai E. Physical exercise, reactive oxygen species and neuroprotection. *Free Radic Biol Med* 2016;98:187–96. doi:10.1016/J.FREERADBIOMED.2016.01.024.
- [8] Cameron B, Landreth GE. Inflammation, microglia, and Alzheimer’s disease. *Neurobiol Dis* 2010;37:503–9. doi:10.1016/j.nbd.2009.10.006.
- [9] Calsolaro V, Edison P. Neuroinflammation in Alzheimer’s disease: Current evidence and future directions. *Alzheimer’s Dement* 2016;12:719–32. doi:10.1016/j.jalz.2016.02.010.
- [10] Tozzi A, Hannan AJ, Skaper SD, Facci L, Zusso M, Giusti P. An Inflammation-Centric View of Neurological Disease: Beyond the Neuron 2018;12. doi:10.3389/fncel.2018.00072.
- [11] Blondell SJ, Hammersley-Mather R, Veerman JL. Does physical activity prevent cognitive decline and dementia?: A systematic review and meta-analysis of longitudinal studies. *BMC Public Health* 2014;14. doi:10.1186/1471-2458-14-510.
- [12] Jensen CS, Hasselbalch SG, Waldemar G, Simonsen AH. Biochemical Markers of Physical Exercise on Mild Cognitive Impairment and Dementia: Systematic Review and Perspectives. *Front Neurol* 2015;6. doi:10.3389/fneur.2015.00187.
- [13] Steen Frederiksen K, Gjerum L, Waldemar G, Gregers Hasselbalch S. Effects of Physical Exercise on Alzheimer’s Disease Biomarkers: A Systematic Review of Intervention Studies. *J Alzheimer’s Dis* 2018;61:359–72. doi:10.3233/JAD-170567.
- [14] Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, et al. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci U S A* 2011;108:3017–22. doi:10.1073/pnas.1015950108.
- [15] Law LL, Rol RN, Schultz SA, Dougherty RJ, Edwards DF, Kosciak RL, et al. Moderate intensity physical

- activity associates with CSF biomarkers in a cohort at risk for Alzheimer's disease. *Alzheimer's Dement (Amsterdam, Netherlands)* 2018;10:188–95. doi:10.1016/j.dadm.2018.01.001.
- [16] Baker LD, Frank LL, Foster-Schubert K, Green PS, Wilkinson CW, McTiernan A, et al. Effects of aerobic exercise on mild cognitive impairment: a controlled trial. *JAMA Neurol* 2010;67:71–9. doi:10.1001/archneurol.2009.307.
 - [17] Chakrabarti S, Khemka VK, Banerjee A, Chatterjee G, Ganguly A, Biswas A. Metabolic Risk Factors of Sporadic Alzheimer's Disease: Implications in the Pathology, Pathogenesis and Treatment. *Aging Dis* 2015;6:282–99. doi:10.14336/AD.2014.002.
 - [18] Suárez-Calvet M, Kleinberger G, Ángel M, Caballero A, Brendel M, Rominger A, et al. sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. *EMBO Mol Med* 2016;8:466–76. doi:10.15252/emmm.
 - [19] Colonna M, Wang Y. TREM2 variants: new keys to decipher Alzheimer disease pathogenesis. *Nat Rev Neurosci* 2016;17:201–7. doi:10.1038/nrn.2016.7.
 - [20] Cantoni C, Bollman B, Licastro D, Xie M, Mikesell R, Schmidt R, et al. TREM2 regulates microglial cell activation in response to demyelination in vivo. *Acta Neuropathol* 2015;129:429–47. doi:10.1007/s00401-015-1388-1.
 - [21] Praticò D. The neurobiology of isoprostanes and Alzheimer's disease. *Biochim Biophys Acta - Mol Cell Biol Lipids* 2010;1801:930–3. doi:10.1016/j.bbalip.2010.01.009.
 - [22] Praticò D, Clark C, Lee V, Trojanowski J, Rokach J, FitzGerald G. Increased 8,12-iso-iPF₂α-VI in Alzheimer's disease: correlation of a noninvasive index of lipid peroxidation with disease severity. *Ann Neurol* 2000;48:809–12.
 - [23] Ruiz A, Dols-Icardo O, Bullido MJ, Pastor P, Rodríguez-Rodríguez E, López De Munain A, et al. Assessing the role of the TREM2 p.R47H variant as a risk factor for Alzheimer's disease and frontotemporal dementia on behalf of The dementia genetic Spanish consortium (DEGESCO). *Neurobiol Aging* 2014;35:444.e1–444.e4. doi:10.1016/j.neurobiolaging.2013.08.011.
 - [24] Piccio L, Deming Y, Del-Águila JL, Ghezzi L, Holtzman DM, Fagan AM, et al. Cerebrospinal fluid soluble TREM2 is higher in Alzheimer disease and associated with mutation status. *Acta Neuropathol* 2016;131:925–33. doi:10.1007/s00401-016-1533-5.
 - [25] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. 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;34:939–44.
 - [26] Hoffmann K, Frederiksen KS, Sobol NA, Beyer N, Vogel A, Simonsen AH, et al. Preserving cognition, quality of life, physical health and functional ability in Alzheimer's disease: the effect of physical exercise (ADEX trial): rationale and design. *Neuroepidemiology* 2013;41:198–207.
 - [27] Hoffmann K, Sobol NA, Frederiksen KS, Beyer N, Vogel A, Vestergaard K, et al. Moderate-to-high Intensity Physical Exercise in Patients with Alzheimer's Disease: A Randomised Controlled Trial. *J Alzheimer's Dis* 2015.
 - [28] Sobol NA, Hoffmann K, Frederiksen KS, Vogel A, Vestergaard K, Brændgaard H, et al. Effect of aerobic exercise on physical performance in patients with Alzheimer's disease. *Alzheimer's Dement*

- 2016;12:1207–15. doi:10.1016/j.jalz.2016.05.004.
- [29] Teunissen CE, Tumani H, Engelborghs S, Mollenhauer B. Biobanking of CSF: International standardization to optimize biomarker development. *Clin Biochem* 2014;47:288–92. doi:10.1016/J.CLINBIOCHEM.2013.12.024.
 - [30] Beavers KM, Brinkley TE, Nicklas BJ. Effect of exercise training on chronic inflammation. *Clin Chim Acta* 2010;411:785–93. doi:10.1016/j.cca.2010.02.069.
 - [31] Sobol NA, Hoffmann K, Vogel A, Lolk A, Gottrup H, Høgh P, et al. Associations between physical function, dual-task performance and cognition in patients with mild Alzheimer’s disease. *Aging Ment Health* 2015:1–8. doi:10.1080/13607863.2015.1063108.
 - [32] Jensen CS, Portelius E, Høgh P, Wermuth L, Blennow K, Zetterberg H, et al. Effect of physical exercise on markers of neuronal dysfunction in cerebrospinal fluid in patients with Alzheimer’s disease. *Alzheimer’s Dement Transl Res Clin Interv* 2017;3:284–90.
 - [33] Steen Jensen C, Portelius E, Siersma V, Høgh P, Wermuth L, Blennow K, et al. Cerebrospinal Fluid Amyloid Beta and Tau Concentrations Are Not Modulated by 16 Weeks of Moderate- to High-Intensity Physical Exercise in Patients with Alzheimer Disease. *Dement Geriatr Cogn Disord* 2016;42:146–58. doi:10.1159/000449408.
 - [34] Ransohoff RM. How neuroinflammation contributes to neurodegeneration. *Science* (80-) 2016;353:777–83. doi:10.1126/science.aag3194.
 - [35] Bagyinszky E, Giau V Van, Shim K, Suk K, Soo S, An A, et al. Role of inflammatory molecules in the Alzheimer’s disease progression and diagnosis ☆ 2017. doi:10.1016/j.jns.2017.03.031.
 - [36] Galasko D, Montine TJ. Biomarkers of oxidative damage and inflammation in Alzheimer’s disease. *Biomark Med* 2010;4:27–36. doi:10.2217/BMM.09.89.
 - [37] Thambisetty M, Lovestone S. Blood-based biomarkers of Alzheimers disease: Challenging but feasible. *Biomark Med* 2010;4:65–79. doi:10.2217/bmm.09.84.
 - [38] Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, et al. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc Natl Acad Sci U S A* 1989;86:7611–5.
 - [39] Licastro F, Pedrini S, Caputo L, Annoni G, Davis LJ, Ferri C, et al. Increased plasma levels of interleukin-1, interleukin-6 and a-1-antichymotrypsin in patients with Alzheimer’s disease: peripheral inflammation or signals from the brain? *J Neuroimmunol* 2000;103:97–102.
 - [40] Su F, Bai F, Zhang Z. Inflammatory Cytokines and Alzheimer’s Disease: A Review from the Perspective of Genetic Polymorphisms. *Neurosci Bull* 2016;32:469–80. doi:10.1007/s12264-016-0055-4.
 - [41] Kawahara K, Suenobu M, Yoshida A, Koga K, Hyodo A, Ohtsuka H, et al. Intracerebral microinjection of interleukin-4/interleukin-13 reduces β -amyloid accumulation in the ipsilateral side and improves cognitive deficits in young amyloid precursor protein 23 mice. *Neuroscience* 2012;207:243–60. doi:10.1016/j.neuroscience.2012.01.049.
 - [42] Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6 2011. doi:10.1016/j.bbamcr.2011.01.034.

- [43] Eriksson UK, Pedersen NL, Reynolds CA, Hong M-G, Prince JA, Gatz M, et al. Associations of Gene Sequence Variation and Serum Levels of C-Reactive Protein and Interleukin-6 with Alzheimer's Disease and Dementia. *J Alzheimer's Dis* 2011;23:361–9. doi:10.3233/JAD-2010-101671.
- [44] Strauss S, Bauer J, Ganter U, Jonas U, Berger M, Volk B. Detection of interleukin-6 and alpha 2-macroglobulin immunoreactivity in cortex and hippocampus of Alzheimer's disease patients. *Lab Invest* 1992;66:223–30.
- [45] Zhu J, Paul WE. Peripheral CD4 T cell differentiation regulated by networks of cytokines and transcription factors. *Immunol Rev* 2010;238:247–62. doi:10.1111/j.1600-065X.2010.00951.x.
- [46] Janelins MC, Mastrangelo MA, Park KM, Sudol KL, Narrow WC, Oddo S, et al. Chronic Neuron-Specific Tumor Necrosis Factor-Alpha Expression Enhances the Local Inflammatory Environment Ultimately Leading to Neuronal Death in 3xTg-AD Mice. *Am J Pathol* 2008;173:1768–82. doi:10.2353/ajpath.2008.080528.
- [47] Tobinick E, Gross H, Weinberger A, Cohen H. TNF-alpha modulation for treatment of Alzheimer's disease: a 6-month pilot study. *MedGenMed* 2006;8:25.
- [48] Getz GS, Reardon CA. Apoprotein E as a lipid transport and signaling protein in the blood, liver, and artery wall. *J Lipid Res* 2009;50 Suppl:S156–61. doi:10.1194/jlr.R800058-JLR200.
- [49] Rasmussen KL, Tybjaerg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. Plasma levels of apolipoprotein E and risk of dementia in the general population. *Ann Neurol* 2015;77:301–11. doi:10.1002/ana.24326.
- [50] Zhang H-L, Wu J, Zhu J. The immune-modulatory role of apolipoprotein E with emphasis on multiple sclerosis and experimental autoimmune encephalomyelitis. *Clin Dev Immunol* 2010;2010:186813. doi:10.1155/2010/186813.
- [51] Heslegrave A, Heywood W, Paterson R, Magdalinou N, Svensson J, Johansson P, et al. Increased cerebrospinal fluid soluble TREM2 concentration in Alzheimer's disease. *Mol Neurodegener* 2016;11:3. doi:10.1186/s13024-016-0071-x.
- [52] Kleinberger G, Yamanishi Y, Suárez-Calvet M, Czirr E, Lohmann E, Cuyvers E, et al. TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. *Sci Transl Med* 2014;6:243ra86. doi:10.1126/scitranslmed.3009093.
- [53] Finelli D, Rollinson S, Harris J, Jones M, Richardson A, Gerhard AI, et al. TREM2 analysis and increased risk of Alzheimer's disease. *Neurobiol Aging* 2015;36:546.e9–546.e13.
- [54] Wunderlich P, Glebov K, Kemmerling N, Tien NT, Neumann H, Walter J. Sequential proteolytic processing of the triggering receptor expressed on myeloid cells-2 (TREM2) protein by ectodomain shedding and γ -secretase-dependent intramembranous cleavage. *J Biol Chem* 2013;288:33027–36. doi:10.1074/jbc.M113.517540.
- [55] Zhong L, Chen X-F, Wang T, Wang Z, Liao C, Wang Z, et al. Soluble TREM2 induces inflammatory responses and enhances microglial survival. *J Exp Med* 2017;214:597–607. doi:10.1084/jem.20160844.
- [56] Banerjee A, Chitnis UB, Jadhav SL, Bhawalkar JS, Chaudhury S. Hypothesis testing, type I and type II errors. *Ind Psychiatry J* 2009;18:127–31. doi:10.4103/0972-6748.62274.

- [57] Biau DJ, Kernéis S, Porcher R. The Importance of Sample Size in the Planning and Interpretation of Medical Research. *CLin Orthop Relat Res* 2008;466:2282–8. doi:10.1007/s11999-008-0346-9.
- [58] Duits FH, Brinkmalm G, Teunissen CE, Brinkmalm A, Scheltens P, Van Der Flier WM, et al. Synaptic proteins in CSF as potential novel biomarkers for prognosis in prodromal Alzheimer's disease. *Alzheimer's Res Ther* 2018;10:1–9. doi:10.1186/s13195-017-0335-x.
- [59] Öhman H, Savikko N, Strandberg TE, Pitkälä KH. Effect of physical exercise on cognitive performance in older adults with mild cognitive impairment or dementia: a systematic review. *Dement Geriatr Cogn Disord* 2014;38:347–65. doi:10.1159/000365388.
- [60] Groot C, Hooghiemstra AM, Raijmakers PGHM, van Berckel BNM, Scheltens P, Scherder EJA, et al. The effect of physical activity on cognitive function in patients with dementia: A meta-analysis of randomized control trials. *Ageing Res Rev* 2016;25:13–23. doi:10.1016/j.arr.2015.11.005.

Table 1	Controls (n=92)	Exercise (n=106)	p-value [€]	High exercise sub-group (n=66)	p-value [§]
A) Whole group					
Age, years [¥]	71.4 (7.3)	69.9 (7.5)	0.14 [#]	70.2 (7.5)	0.31 [#]
Gender, n			0.13 [¤]		0.41 [¤]
males	57	56		34	
females	35	50		32	
Characteristics					
Disease duration, years from diagnosis [¥]	1.3 (1.0)	1.0 (1.0)	0.1 [#]	0.94 (0.9)	0.05 [#]
MMSE [¥]	24.2 (3.9)	23.8 (3.4)	0.54 [#]	24.1 (3.3)	0.96 [#]
Education, years [¥]	11.8 (2.8)	11.9 (2.7)	0.84 [#]	12.2 (2.7)	0.42 [#]
Weight, kg [¥]	71.4 (12.1)	73.0 (13.5)	0.36 [#]	72.5 (13.8)	0.18 [#]
Height, cm [¥]	172.0 (8.9)	170.2 (9.1)	0.15 [#]	170.1 (9.2)	0.18 [#]
BMI [¥]	24.1 (3.6)	25.2 (4.0)	0.04 [#]	23.0 (4.0)	0.12 [#]
APOE ε4 alleles, n			0.35 [¤]		0.22 [¤]
0	21	34		19	
1	45	45		26	
2	26	27		21	
B) CSF subgroup					
	(n=29)	(n=29)		(n=19)	
Age, years [¥]	68.8 (8.0)	68.83 (6.7)	0.83 [#]	68.6 (7.0)	0.91 [#]
Gender, n			0.17 [¤]		0.36 [¤]
Males	21	16		11	
Females	8	13		8	
Characteristics					
Disease duration, years from diagnosis [¥]	1.5 (1.1)	1.0 (0.9)	0.07 [#]	1.2 (1.0)	0.25 [#]
MMSE [¥]	25.1 (3.9)	25.4 (2.8)	0.70 [#]	25.1 (2.6)	0.99 [#]
Education, years [¥]	13.0 (2.9)	12.7 (2.8)	0.62 [#]	12.5 (2.5)	0.54 [#]
Weight, kg [¥]	73.3 (2.4)	73.3 (13.0)	0.51 [#]	75.4 (16.5)	0.63 [#]
Height, cm [¥]	174.8 (8.8)	172.5 (9.0)	0.33 [#]	172.8 (9.3)	0.45 [#]
BMI [¥]	23.9 (3.5)	25.4 (4.2)	0.15 [#]	25.1 (4.1)	0.28 [#]
APOE ε4 alleles, n			0.25 [¤]		0.21 [¤]
0	13	7		3	
1	10	14		9	
2	6	8		7	

Table 1 Baseline characteristics of the study cohort. A) all patients. B) CSF sub-group. [¥] Given as mean ± (Standard deviation), [#]Independent t-test, [¤] Chi-squared test, [€] Controls versus exercise, [§] Controls versus high exercise. From the exercise group, participants who participated more than 80% of the session and had a mean intensity of 70% or high, was further analyzed as a 'high exercise group'.

Table 2	Baseline ^x		16 week follow up ^y		Mean relative change from baseline, ((16 week follow up – Baseline)/Baseline)*100, ^z #				
	Controls	Exercise	Controls	Exercise	controls	Exercise	p-value	High exercise	p-value
pg/mL	(n=27)	(n=28)	(n=24)	(n=23)	(n=22)	(n=22)		(n=16)	
sTREM2, CSF	8172.51 (2656.2)	8193.32 (2992.5)	7717.16 (2488.7)	8596.5 (3267.5)	-2.4 (13.8)	5.0 (12.9)	0.075	6.9 (14.0)	0.05
	(n=27)	(n=24)	(n=27)	(n=19)	(n=26)	(n=19)		(n=14)	
IFN γ , CSF	0.6 (0.2)	0.5 (0.2)	0.6 (0.1)	0.5 (0.2)	9.0 (34.0)	1.6 (52.8)	0.57	-4.1 (51.9)	0.34
IL10, CSF	0.1 (0.0)	0.1 (0.1)	0.1 (0.1)	0.1 (0.6)	13.5 (52.4)	18.0 (67.5)	0.80	15.2 (70.8)	0.93
IL13, CSF	0.7 (0.5)	0.7 (0.3)	0.7 (0.3)	0.8 (0.5)	7.9 (68.7)	55.8 (115.4)	0.09	48.1 (107.2)	0.16
IL2, CSF	0.2 (0.2)	0.2 (0.12)	0.1 (0.1)	0.1 (0.1)	11.7 (96.1)	4.6 (69.3)	0.79	-0.1 (78.4)	0.70
IL6, CSF	1.2 (0.4)	1.1 (0.3)	1.3 (0.6)	1.1 (0.3)	21.7 (50.1)	1.9 (28.2)	0.13	3.5 (29.5)	0.22
IL8, CSF	44.8 (13.0)	42.4 (9.2)	45.1 (15.4)	40.7 (10.5)	2.7 (24.7)	-5.0 (16.6)	0.25	-2.5 (15.9)	0.48
TNF α , CSF	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	4.5 (31.9)	-4.7 (38.8)	0.39	-6.8 (41.5)	0.34
	(n=23)	(n=24)	(n=23)	(n=21)	(n=19)	(n=21)		(n=14)	
IFN γ , plasma	5.1 (3.4)	4.9 (2.2)	5.0 (3.2)	5.2 (2.2)	61.4 (144.6)	22.1 (63.7)	0.27	11.6 (44.2)	0.22
IL10, plasma	0.3 (0.2)	0.3 (0.2)	0.5 (0.8)	0.3 (0.2)	6.2 (58.6)	31.2 (95.6)	0.33	35.2 (109.8)	0.33
IL6, plasma	2.9 (7.4)	0.7 (0.3)	3.3 (8.6)	0.8 (0.3)	-1.0 (49.4)	41.0 (76.7)	0.049	48.0 (85.8)	0.047
IL8, plasma	4.8 (1.6)	4.8 (1.4)	5.6 (2.3)	5.4 (1.5)	27.9 (65.5)	20.7 (38.9)	0.67	25.4 (41.7)	0.90
TNF α , plasma	1.6 (0.6)	1.7 (0.4)	1.8 (0.6)	1.7 (0.4)	33.2 (83.1)	1.2 (39.6)	0.12	-3.7 (41.9)	0.14
	(n=28)	(n=27)	(n=23)	(n=22)	(n=20)	(n=22)		(n=15)	
8-isoprostane, CSF	8.7 (4.0)	9.93 (5.4)	8.97 (3.6)	16.28 (4.1)	33.2 (99.8)	80.1 (192.4)	0.33	95.4 (225.9)	0.28
	(n=87)	(n=100)	(n=80)	(n=90)	(n=75)	(n=86)		(n=55)	
8-isoprostane, plasma	70.2 (94.0)	68.4 (86.8)	70.8 (88.9)	60.3 (75.8)	213.9 (582.7)	109.9 (354.2)	0.17	98.8 (296.7)	0.18

Table 2. Changes in plasma and CSF biomarkers after 16 weeks of intervention. Baseline and follow-up values of sTREM2, IFN γ , IL10, IL6, IL8, IL13, IL2, TNF α , and 8-isoprostane. In addition, analysis of between group difference in mean change from baseline exercise versus controls and per-protocol high-exercise versus controls.

^x Given as mean $\pm$ (Standard deviation). # A positive value means greater positive mean change from baseline in the exercise group.

Table 3	APOE e4 non- carriers			APOE e4 carriers		
Mean relative change from baseline, ((16 week follow up – Baseline)/Baseline) * 100 [‡] #						
pg/mL	Controls	Exercise	p-value	Controls	Exercise	p-value
	(n=9)	(n=5)		(n=13)	(n=17)	
sTREM2, CSF	3.5 (8.1)	2.5 (3.89)	0.81	-6.4 (15.5)	5.7 (14.4)	0.037
	(n=12)	(n=4)		(n=14)	(n=15)	
IFN γ , CSF	12.6 (38.1)	-2.4 (42.2)	0.52	5.8 (31.1)	9.4 (51.1)	0.82
IL10, CSF	25.4 (66.5)	23.6 (74.6)	0.96	3.3 (36.3)	16.5 (68.2)	0.53
IL13, CSF	29.1 (73.8)	62.3 (117)	0.31	-10.3 (61.0)	46.3 (103.4)	0.08
IL2, CSF	45.2 (129.9)	18.1 (40.4)	0.69	-17.0 (40.0)	1.0 (75.9)	0.44
IL6, CSF	39.3 (59.2)	-14.7 (26.8)	0.11	5.6 (36.3)	6.3 (27.9)	0.98
IL8, CSF	1.9 (21.8)	-11.6 (21.4)	0.30	3.4 (27.7)	-3.3 (15.5)	0.43
TNF α , CSF	16.2 (38.8)	-9.1 (33)	0.26	-5.6 (21.0)	2.9 (33.3)	0.42
	(n=10)	(n=5)		(n=9)	(n=16)	
IFN γ , plasma	13.5 (72.1)	67.9 (100.2)	0.24	114.7 (188.4)	7.8 (42.8)	0.038
IL10, plasma	-3.6 (45.5)	35.46 (68.4)	0.21	17.1 (71.7)	29.8 (104.4)	0.75
IL6, plasma	-17.15 (47.4)	1.4 (71.3)	0.56	17.0 (47.7)	53.4 (76.4)	0.21
IL8, plasma	31.2 (80.6)	3.0 (23.3)	0.46	24.2 (48)	26.2 (41.6)	0.91
TNF α , plasma	26.6 (68.3)	1.3 (46.3)	0.47	40.5 (101.1)	1.1 (39.2)	0.17
	(n=10)	(n=5)		(n=10)	(n=17)	
8-isoprostane, CSF	9.7 (94.9)	139.0 (143.8)	0.058	56.5 (101.5)	62.8 (204.9)	0.93
	(n=19)	(n=31)		(n=56)	(n=55)	
8-isoprostane, plasma	279.0 (492.1)	179.9 (479.4)	0.48	191.8 (612.9)	71.0 (255.9)	0.18

Table 3, *The effect of exercise on biomarker concentrations, stratified according to ApoE genotype*. Group differences in mean change from baseline High exercise versus control depending of ApoE ϵ 4 carrier status (0 alleles versus 1 or 2 alleles). [‡] Given as mean $\pm$ (Standard deviation). # A positive value means greater positive mean change from baseline in the exercise group.

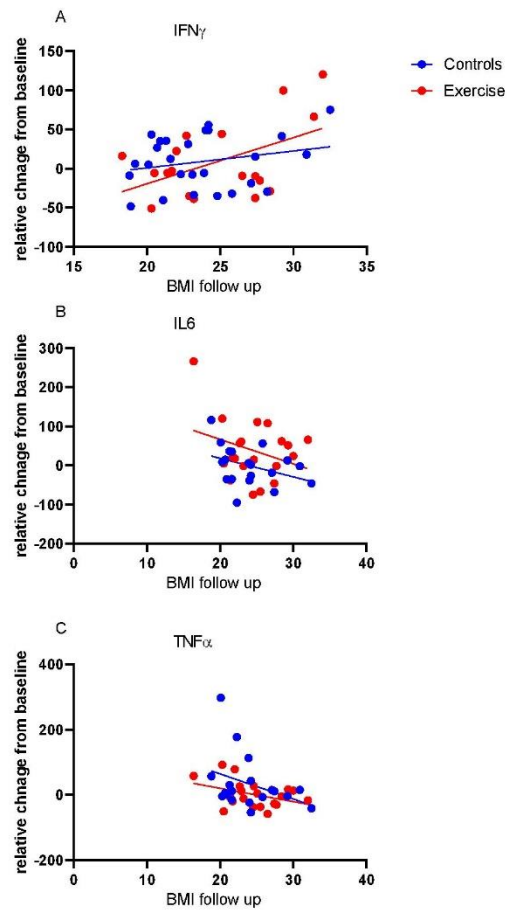


Figure 1. correlation between BMI at follow up and relative change in biomarkers, with randomization groups as a covariate A) relative change in CSF IFN γ , B) relative change in plasma IL6, and C) relative change in plasma TNF α .

Paper IV



Featured Article

Patients with Alzheimer's disease who carry the *APOE* ϵ 4 allele benefit more from physical exercise

Camilla Steen Jensen^{a,1}, Anja Hviid Simonsen^{a,*}, Volkert Siersma^b, Nina Beyer^c,
Kristian Steen Frederiksen^a, Hanne Gottrup^d, Kristine Hoffman^a, Peter Høgh^{e,f},
Ruth Frikke-Schmidt^{f,g}, Nanna Aue Sobol^c, Gunhild Waldemar, Professor^{a,f}, Lene Wermuth^h,
Steen Gregers Hasselbalch, Professor^{a,f}

^aDepartment of Neurology, Danish Dementia Research Centre, Rigshospitalet Copenhagen University Hospital, Denmark

^bDepartment of Public Health, Research Unit for General Practice and Section of General Practice, University of Copenhagen, Denmark

^cDepartment of Physical and Occupational Therapy, Institute of Sports Medicine, Bispebjerg and Frederiksberg Hospital, University of Copenhagen, Denmark

^dDementia Clinic, Department of Neurology, Aarhus University Hospital, Aarhus, Denmark

^eRegional Dementia Research Centre, Department of Neurology, Zealand University Hospital, Roskilde, Denmark

^fDepartment of Clinical Medicine, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark

^gDepartment of Clinical Biochemistry, Rigshospitalet Copenhagen University Hospital, Denmark

^hDementia Clinic, Department of Neurology, Odense University Hospital, Denmark

Abstract

Introduction: Our group has completed an exercise study of 200 patients with mild Alzheimer's disease. We found improvements in cognitive, neuropsychiatric, and physical measures in the participants who adhered to the protocol. Epidemiological studies in healthy elderly suggest that exercise preserves cognitive and physical abilities to a higher extent in *APOE* ϵ 4 carriers.

Methods: In this post hoc subgroup analysis study, we investigated whether the beneficial effect of an exercise intervention in patients with mild AD was dependent on the patients' *APOE* genotype.

Results: We found that patients who were *APOE* ϵ 4 carriers benefitted more from the exercise intervention by preservation of cognitive performance and improvement in physical measures.

Discussion: This exploratory study establishes a possible connection between the beneficial effects of exercise in AD and the patients' *APOE* genotype. These findings, if validated, could greatly impact the clinical management of patients with AD and those at risk for developing AD.

© 2019 The Author. Published by Elsevier Inc. on behalf of the Alzheimer's Association. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Keywords: Alzheimer's disease; Exercise; *APOE*; Genetic; Intervention

1. Introduction

Cognitive decline in late life and dementia are associated with decreases in activities of daily living, quality of life, and increased mortality. There are an estimated 46.8 million people living with dementia worldwide [1], and with an aging population, dementia and Alzheimer's disease (AD)

constitute a growing health and economic burden [1,2]. Several genetic, environmental, and clinical risk factors for developing AD have been identified. The presence of the apolipoprotein-E (*APOE*) ϵ 4 allele is the most important genetic risk factor, whereas physical inactivity is a major lifestyle risk factor for the development of AD [3].

The ϵ 4 allele of the *APOE* gene located on chromosome 19 is associated with higher risk of developing AD and at an earlier age, whereas the ϵ 2 allele has a protective effect against AD, and the ϵ 3 allele is considered neutral [4]. The neuropathological hallmarks of AD are intracellular neurofibrillary tangles and extracellular plaques composed of tau and

The authors declare no conflicts of interest.

¹These authors contributed equally to the manuscript.

*Corresponding author. Tel.: +45 3545 6911; Fax: +45 3545 2446.

E-mail address: anja.hviid.simonsen@regionh.dk

<https://doi.org/10.1016/j.trci.2019.02.007>

2352-8737/ © 2019 The Author. Published by Elsevier Inc. on behalf of the Alzheimer's Association. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

amyloid beta₄₂ (A-beta), respectively [5]. Further neuropathological findings include increased inflammation and oxidative stress [6,7]. It has been suggested that *APOE* ε4 genotype can lead to AD pathogenesis by several pathways: (1) damage of the vascular system in the brain; (2) initiation or acceleration of A-beta accumulation, aggregation, and deposition in the brain; (3) impaired cholesterol transport; and (4) increased neuroinflammation [4].

In animal and human studies, exercise has shown a neuroprotective effect, notably in the hippocampus and the dentate gyrus, both areas showing pathological changes in AD [8–11]. Observational studies in healthy elderly persons show that higher levels of physical activity are associated with lower risk of cognitive decline, better cognitive performance, and preservation of hippocampal volume and that the effect was stronger in *APOE* ε4 carriers when compared to noncarriers [12–14]. Furthermore, higher levels of physical activity have been associated with faster reaction time in *APOE* ε4 carriers compared to noncarriers [15]. In subjects with MCI, 1 study investigated the effect of *APOE* genotype on physical function and found that walking speed was reduced in the *APOE* ε4 carriers, compared to noncarriers [16]. Despite evidence from observational studies in healthy elderly and MCI subjects of associations between physical inactivity, cognitive decline, and *APOE* ε4 carrier status, there is a paucity of knowledge of these associations in subjects with AD dementia. Similarly, in exercise intervention studies, positive effect of *APOE* ε4 carrier status on cognition has been found in healthy elderly subjects [17,18], but to the best of our knowledge, this effect has not been evaluated in patients with AD.

Because the search for pharmacological treatments to slow down or reverse AD has not proven successful so far [19], researchers have turned their attention toward nonpharmacological approaches, including exercise, to try to slow the cognitive decline [20,21]. Our group has previously investigated the effect of exercise in patients with mild AD in the “Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer’s disease: The Effect of Physical Exercise (ADEX) study” [22]. In that study, for subjects who adhered to the protocol, we found a significant improvement in cognitive performance measured by the Symbol Digit Modalities Test (SDMT), neuropsychiatric symptoms [22], as well as in physical function [23].

Results of all cognitive tests (SDMT, ADAS-Cog, Stroop Color and Word test, verbal fluency, and MMSE) have been published [22]; we found a significant effect of exercise on our primary outcome measure, SDMT, but no effect of the exercise intervention was detected on the other cognitive tests. SDMT was chosen as primary outcome because this test is sensitive to very early changes in aspects of executive function (mental speed and attention) in AD [24]. Executive function is considered to be particularly sensitive to the effect of physical exercise [25,26], which recently was

corroborated in two trials of moderate- to high-intensity exercise in sedentary older adults [27] and patients with MCI [28] where executive function and performance on SDMT was improved by exercise.

In this post hoc subgroup analysis of patients with mild AD from the ADEX study, we aimed to investigate if the significant improvements in cognitive and physical outcomes were dependent on the patients’ *APOE* genotype.

2. Materials and methods

2.1. Patient cohort and baseline characteristics

A total of 200 patients from eight memory clinics in Denmark were randomized to either a control group (n = 93) or an intervention group (n = 107), and 190 patients completed follow-up [29]. The inclusion/exclusion criteria have been described previously in the study by Hoffmann et al. [30]. Briefly, the patients were diagnosed with AD according to the NINCDS-ADRDA criteria [29] before enrollment in the trial, were between 50 and 90 years of age, and had a MMSE score ≥20.

Patient baseline characteristics have been summarized in Table 1. All patients gave blood samples before and after the intervention. The patients’ *APOE* genotype was not known at the time of the exercise intervention because *APOE* genotyping was not part of the routine diagnostic procedure and was only analyzed after the end of the trial.

The study was approved by the ethical committee of the Capital Region of Denmark (H-3-2011-128) and the Danish Data protection agency (Capital Region of Denmark, 30-0718). All patients gave informed consent before enrollment (ClinicalTrials.gov registration number: NCT01681602).

2.2. Exercise intervention

The physical exercise intervention was supervised aerobic exercise at moderate-to-high intensity (70–80% of maximal heart rate [HR] measured by pulse watches) 1 hour, three times weekly for 16 weeks in groups of two to five participants. The exercise protocol has been described in detail in the study by Sobol et al. [31] and consisted of a short warm-up session followed by aerobic exercise on treadmill, stationary bike, and cross-trainer.

2.3. *APOE* genotype

DNA was isolated with Promega Maxwell DNA purification kits (Promega, Madison, WI), according to the manufacturer’s protocol from 250 μL of buffy coat from 6 mL EDTA vials. *APOE* genotyping for the ε2, ε3, and ε4 alleles was performed with a TaqMan qPCR assay as described by Koch et al. [32].

Table 1
Baseline characteristics of the study cohort

	Controls			Exercise		
	<i>APOE</i> ε4 noncarriers	<i>APOE</i> ε4 carriers	<i>P</i> value	<i>APOE</i> ε4 noncarriers	<i>APOE</i> ε4 carriers	<i>P</i> value
n	21	72 (27 homozygotes)		34	72 (27 homozygotes)	
Gender			0.45*			0.23*
Males, n (%)	11 (52.4)	41 (56.9)		17 (50)	43 (59.7)	
Females, n (%)	10 (47.6)	31 (43.1)		17 (50)	29 (40.3)	
Age, years [†]	68.0 (8.8)	72.5 (6.5)	0.01 [‡]	67.9 (9.1)	70.8 (6.4)	0.06 [‡]
MMSE [†]	25.0 (3.6)	23.9 (3.9)	0.27 [‡]	24.4 (3.5)	23.6 (3.3)	0.26 [‡]
Education, years [‡]	11.5 (2.9)	11.9 (2.7)	0.55 [‡]	12.0 (2.9)	11.9 (2.6)	0.83 [‡]

P values are *APOE* ε4 carriers versus *APOE* ε4 noncarriers. Bold value denotes statistical significance *P* < 0.05.

*Chi-squared test.

[†]Given as mean ± (standard deviation).

[‡]Independent t-test.

2.4. Clinical outcomes

We have previously published the results of our exercise intervention on cognitive [22] and physical [23] outcomes. We found a significant effect of exercise on neuropsychiatric symptoms measured by the Neuropsychiatric Inventory (NPI) in the intention-to-treat group. Patients who reached the target intervention intensity of 70% of maximum HR and participated in at least two-thirds of the exercise sessions (per-protocol group, termed “high exercise group,” *n* = 66 of 107 patients) improved significantly on NPI as well as on a cognitive test of attention and mental speed (SDMT). We therefore chose these two outcomes for analysis in the present study to investigate whether the effect was different in *APOE* ε4 carriers compared to noncarriers.

2.4.1. Cognitive outcome

SDMT assesses mental speed and attention. Using the number and symbol key at the top of the test page, participants were asked to correctly decode several lines of symbols [33]. The total number of correct decodings in 120 s was used as outcome.

2.4.2. Neuropsychiatric outcome

NPI is a caregiver-rated 12-point questionnaire assessing 12 different behavioral and psychological symptoms common in AD. The primary caregiver rates each symptom according to frequency and severity [34].

2.4.3. Physical outcomes

Timed-Up-and-Go test assesses basic mobility by measuring the time it takes for a person to stand up from a chair, walk 3 meters, cross a line, turn around, and walk back to the chair and sit down [35].

Timed 10-meter walk test assesses gait speed on a 10-meter long course [36].

Timed 400-meter walk test assesses walking endurance measured on a 20-meter long course marked with colored cones [37].

Dual task performance is measured with the timed 10-meter walk test combined with counting backward from 50 [36].

Maximal oxygen uptake (VO₂max): the submaximal test for estimating maximal oxygen uptake is considered to be valid for assessing changes in cardiovascular fitness (aerobic fitness) and is estimated based on the workload and average HR during the last minute of a 6-minute cycle test, corrected for age and body weight [38].

2.5. Statistical analyses

Baseline characteristics were compared between randomization groups and between *APOE* genotypes with chi-square tests (categorical characteristics) and t-tests (continuous characteristics). The effect of the exercise intervention was assessed separately for *APOE* ε4 carriers and noncarriers in a linear regression model for each outcome as intention-to-treat analyses including all patients were performed and per-protocol analyses where only patients who reached the target intervention intensity of 70% of maximum HR and participated in at least two-thirds of the exercise sessions were included. Possible differential dropout between the randomization groups was adjusted for by weighting the available observations by the inverse of the probability of being in the data, estimated in logistic regression models including baseline characteristics of the patients. The analysis was performed with generalized estimating equations to account for the weighting and for repeated measurements. Analyses were done with SAS v9.4. A significance level of 5% was used.

3. Results

3.1. Patient cohort

Baseline characteristics of the patient cohort are summarized in Table 1. There was an even distribution of *APOE* ε4 carriers versus noncarriers in the control and intervention groups, with approximately 70% being carriers of the *APOE* ε4 allele. We did not find significant differences in baseline characteristics between the randomization groups. When analyzed by the patients' *APOE* genotype, the *APOE* ε4 noncarriers were significantly younger than

Table 2
Effects of the intervention in *APOE* ε4 noncarriers and *APOE* ε4 carriers

Mean (SEM)	<i>APOE</i> ε4 noncarriers						<i>APOE</i> ε4 carriers					
	Baseline			Follow-up			Baseline			Follow-up		
	Controls	Exercise	<i>P</i> value [‡]	Controls	Exercise	<i>P</i> value [‡]	Controls	Exercise	<i>P</i> value [‡]	Controls	Exercise	<i>P</i> value [‡]
Symbol Digit Modalities Test, # in 90 min	25.78 (2.84)	27.84 (2.50)	0.0770	26.05 (3.35)	24.49 (2.84)	0.1637	25.58 (1.78)	26.51 (2.84)	0.9446	18.24 (3.97)	25.55 (2.10)	0.0236
Neuropsychiatric Inventory, range 0–144	10.81 (2.27)	10.66 (2.02)	0.6413	11.49 (2.40)	10.10 (1.54)	0.7331	9.03 (1.10)	9.67 (1.19)	0.4722	11.96 (1.45)	7.80 (1.03)	0.1633
Time-Up-and-Go, sec.	6.67 (0.37)	6.61 (0.34)	0.3491	6.44 (0.31)	6.76 (0.38)	0.1794	6.58 (0.19)	6.80 (0.19)	0.2247	6.66 (0.34)	6.40 (0.16)	0.0143
Estimated VO ₂ max, ml/kg/min	24.99 (1.51)	27.55 (1.43)	0.1502	25.06 (1.31)	33.21 (1.54)	0.0240	26.57 (1.28)	24.30 (0.91)	0.0541	26.89 (1.22)	28.60 (0.98)	0.0002
400 m walk, sec	302.19 (17.11)	302.80 (17.72)	0.5428	298.48 (15.34)	305.06 (18.19)	0.5381	305.23 (8.11)	307.53 (10.00)	0.6896	307.63 (9.50)	293.04 (7.18)	0.0425
10 m walk, m/sec	1.39 (0.05)	1.38 (0.03)	0.9814	1.35 (0.04)	1.34 (0.04)	0.7779	1.39 (0.04)	1.29 (0.02)	0.0061	1.37 (0.03)	1.34 (0.02)	0.0269

Bold values denote statistical significance $P < 0.05$.

[‡]Baseline difference between controls versus exercise within *APOE* status. Adjusted for clustering within training groups and centers.

[†]Follow-up difference between controls versus exercise (beyond the difference present at the baseline) within *APOE* status. Adjusted for clustering within training groups and centers.

[‡]Effect of *APOE* status on the effect of exercise.

the *APOE* ε4 carriers in the control group. There was no significant difference in gender distribution, MMSE, or years of education between the *APOE* ε4 carriers and the *APOE* ε4 noncarriers.

3.2. Clinical outcomes

Carriers of the *APOE* ε4 allele who participated in the exercise arm maintained their performance on SDMT while *APOE* ε4 carriers who did not exercise had a decline in performance (Table 2). Exercise did not influence SDMT scores in the *APOE* ε4 noncarrier patients. There was a stabilizing effect of exercise on SDMT in *APOE* ε4 carriers compared to noncarriers, $P = .0236$ (Table 2, Fig. 1).

Similarly, *APOE* ε4 carriers who exercised had a significant and marked improvement in neuropsychiatric symptoms as evaluated by NPI compared to the control *APOE* ε4 carriers ($P = .0002$), although the effect of exercise on neuropsychiatric symptoms was absent in the *APOE* ε4 noncarriers (Table 2, Fig. 1).

3.3. Physical outcomes

We found that exercise improved Timed-Up-and-Go test in the *APOE* ε4 carriers versus noncarriers, $P = .0143$. Furthermore, we found significant improvement only in *APOE* ε4 carriers for timed 10-meter walk test ($P = .0269$), and timed 400-meter walk test ($P = .0425$), and these outcomes did not change significantly in noncarriers. Estimated VO₂max improved significantly after exercise in both carriers and noncarriers (see Table 2 and Fig. 1).

4. Discussion

In this post hoc subgroup analysis study, we investigated whether the beneficial effect of an exercise intervention in patients with mild AD was driven by the patients' *APOE* genotype.

We found that patients who carried the *APOE* ε4 allele benefitted more from the exercise intervention than noncarriers on cognitive, neuropsychiatric, and physical measures. These findings are in line with the results from epidemiological studies showing that exercise improved cognitive and physical performance especially in carriers of the *APOE* ε4 allele [13,16].

We found that patients who were carriers of the *APOE* ε4 allele in the exercise group maintained their performance in the SDMT test, whereas the carriers of the *APOE* ε4 allele in the control group deteriorated in performance. The effect of exercise on SDMT seen in this study is in line with the studies in patients with MCI [28] and in older adults [27] where executive function and performance on SDMT was improved by exercise. Furthermore, carriers of the *APOE* ε4 allele who exercise experienced an improvement in neuropsychiatric symptoms compared to a worsening in the control *APOE* ε4 carrier group. By contrast, we could

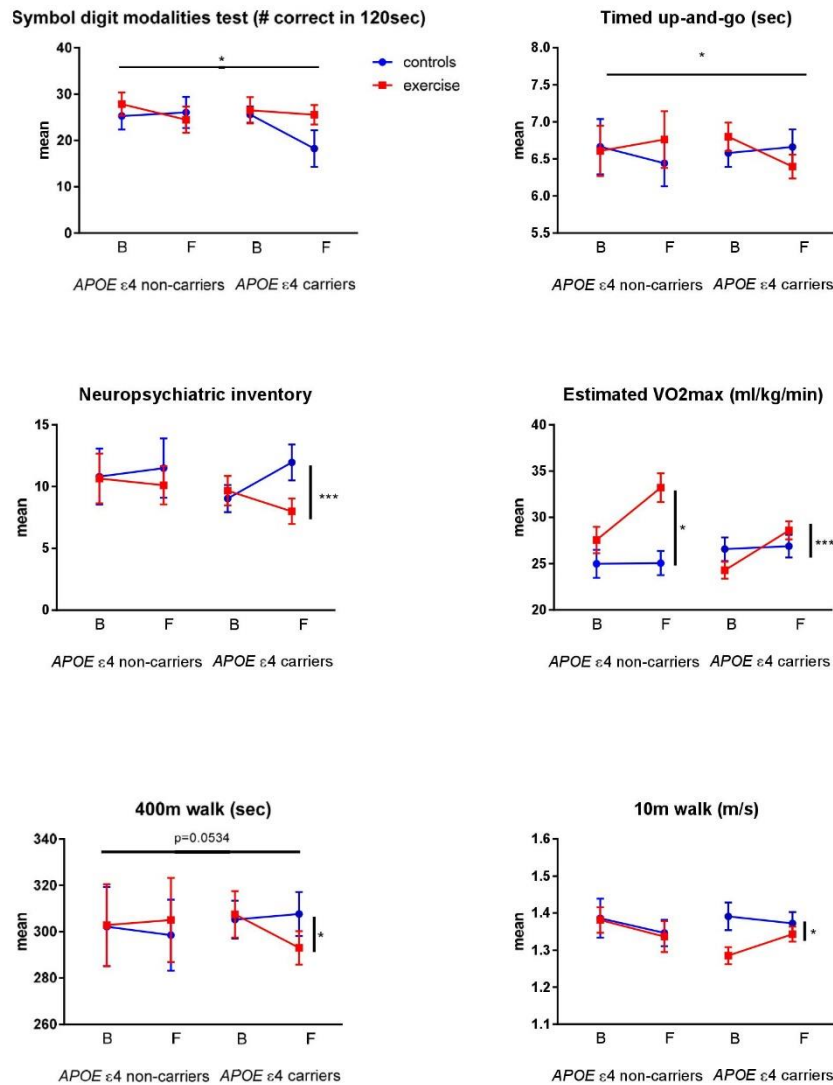


Fig. 1. Mean scores in outcome intention-to-treat analysis. All participants analyzed with independent t-test, respectively of ApoE ε4 carrier status and randomization group. B: baseline, F: 16-week follow-up. * $P < .05$, *** $P < .001$, displayed as mean score in groups with SD.

not detect an effect of the exercise intervention on neuropsychiatric symptoms in the noncarrier group.

Carriers of the *APOE* ε4 allele who exercised improved their physical performance measured by walking speed compared both to noncarriers who exercised and to carriers in the control group. By contrast, we found that the noncarriers improved their cardiovascular fitness measured by estimated VO_{2max} but this improvement was not reflected on their performance on the walking tests. All the participants in the intervention group had exercised on a stationary bike and most of them preferred this way of training

endurance. Some participants did not perform treadmill walking exercises because they felt unsafe on the treadmill, and other than the treadmill-workout walking exercises were not part of the exercise intervention. Sitting on a test bike, where the movement is very simple and controlled, and where the assessor ensures that the cadence is kept, is probably less demanding than walking. Walking requires among other trunk stability, balance, and coordination. This will have an impact on the variation of spatiotemporal gait parameters, which has been shown to be greater in people with cognitive impairment [39].

In our previous work [22], we discovered that the highest effect on cognitive and physical end points was present in the subgroup of patients who exercised at the highest intensity and with most adherence to the exercise program, termed the “high-exercise” group. In the current analysis, we did not find an additional effect of high exercise after segregating by *APOE* ϵ 4 carrier status (data not shown). This could indicate that the effect exercise on cognitive and physical measures of carrying the *APOE* ϵ 4 allele is equivalent to the effect of a higher intensity exercise regime.

There are several theories regarding why the *APOE* ϵ 4 allele evolved in humans, and why this allele has been preserved during evolution up to modern times. Some have suggested that the *APOE* ϵ 4 allele evolved in response to shifts in diet, response to a highly infectious environment, and need of increased absorption and reuptake of vitamin D [40,41], cases where the *APOE* ϵ 4 allele could be beneficial. The detrimental effects of the *APOE* ϵ 4 allele, namely increased risk of atherosclerosis, stroke, and AD, were counteracted by the higher physical activity of the hunter-gatherers [42]. In modern societies, where high levels of physical activity are no longer required, this balance is skewed toward the adverse effects of the *APOE* ϵ 4 allele. This highly speculative hypothesis would fit with the observation in the present study that physical exercise counteracts AD-related symptoms to a higher extent in *APOE* ϵ 4 carriers than in noncarriers.

The mechanism for our observed *APOE*-mediated beneficial effect of exercise is unclear. It is not known whether physical exercise exerts its positive effect through disease-modifying or symptomatic mechanisms. The *APOE* genotype influences several pathways related to AD, such as vascular damage, A-beta accumulation, cholesterol transport, and neuroinflammation [4]. In a small subsample of the ADEX patients, we could not detect an effect of exercise on CSF levels of biomarkers of AD pathology [43] or synaptic function [44], regardless of the patients' *APOE* genotype, and although this could be due to the small sample size, effects on other downstream pathways could be involved, such as positive effects on cerebral vasculature, improved cholesterol transport, or decreased neuroinflammation.

A limitation of this study is its exploratory nature because the ADEX study was not designed to elucidate the effects of the *APOE* genotype on an exercise intervention. However, this study is a large study of 200 patients from a genetically homogeneous population. All exercise sessions were supervised by qualified physical therapists; the exercise intensity was high as was the adherence to the exercise program. In our sample, 70% of the patients were carriers of the *APOE* ϵ 4 allele; this is slightly higher than the background population of patients with AD with approximately 60% being carriers of one or two alleles of the *APOE* ϵ 4 allele in northern Europe [45]. We have no simple explanation for this difference other than a possible random selection bias. This imbalance could influence our results because of the smaller

number of subjects not carrying the *APOE* ϵ 4 allele giving rise to a statistical type I error thus potentially not detecting significant changes that might happen in the noncarrier group.

Even if a nonpharmacological intervention such as exercise does not alter the known pathological pathways in AD, it can still be relevant for delaying disease progression by either purely symptomatic effects or by affecting some of the downstream biological processes. If exercise interventions could delay progression of AD and nursing home placement, this would be of benefit for patients and caregivers but also for society.

In conclusion, this exploratory study establishes a possible connection between the beneficial effects of exercise in AD and the patients' *APOE* genotype. These findings need to be replicated in larger studies specifically designed to investigate a potential causality. If replicated, these findings will contribute to a targeted and more personalized treatment approach in AD and give new hope for approximately 14% of the population with an increased genetic risk for AD.

Acknowledgments

The Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) study is supported by the Innovation Fund Denmark (J No. 10-092814). The Danish Dementia Research Centre is supported by grants from the Danish Ministry of Health (J No. 2007-12143-112, project 59506/J No. 0901110, project 34501) and the Danish Health Foundation (J No. 2007B004). The authors thank Kathrine Bjarnoe for technical support.

RESEARCH IN CONTEXT

1. Systematic review: Review of the literature shows self-reported physical activity is associated with better cognitive and physical performance in healthy elderly who carry the *APOE* ϵ 4 allele.
2. Interpretation: In a post hoc analysis of a large multicenter study, we find that patients with Alzheimer's disease who carry the *APOE* ϵ 4 allele benefit more from an exercise intervention on cognitive, neuropsychiatric, and physical measures compared to patients with Alzheimer's disease who do not carry the *APOE* ϵ 4 allele.
3. Future directions: These results need to be validated in larger studies. If positive, this knowledge could greatly impact the clinical management of Alzheimer's disease.

References

- [1] Prince M, Wimo A, Guerchet M, Ali G-C, Wu Y-T, Prina M, et al. International, World Alzheimer Report 2015. London: The global impact of dementia; 2015. p. 1–87.
- [2] Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement* 2007;3:186–91.
- [3] Livingston G, Sommerlad A, Orgeta V, Costafreda SG, Huntley J, Ames D, et al. Dementia prevention, intervention, and care. *Lancet* 2017;390:2673–734.
- [4] Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol* 2013; 9:106–18.
- [5] Jellinger KA, Bancher C. Neuropathology of Alzheimer's disease: a critical update. *J Neural Transm Suppl* 1998;54:77–95.
- [6] Luque-Contreras D, Carvajal K, Toral-Rios D, Franco-Bocanegra D, Campos-Pena V. Oxidative stress and metabolic syndrome: cause or consequence of Alzheimer's disease? *Oxid Med Cell Longev* 2014; 2014:497802.
- [7] Heneka MT, Carson MJ, El KJ, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol* 2015;14:388–405.
- [8] Pereira AC, Huddleston DE, Brickman AM, Sosunov AA, Hen R, McKhann GM, et al. An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc Natl Acad Sci U S A* 2007; 104:5638–43.
- [9] Erickson KI, Miller DL, Roecklein KA. The aging hippocampus: interactions between exercise, depression, and BDNF. *Neuroscientist* 2012;18:82–97.
- [10] West MJ, Coleman PD, Flood DG, Troncoso JC. Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet* 1994;344:769–72.
- [11] Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, et al. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci U S A* 2011;108:3017–22.
- [12] Schuit AJ, Feskens EJ, Launer LJ, Kromhout D. Physical activity and cognitive decline, the role of the apolipoprotein e4 allele. *Med Sci Sports Exerc* 2001;33:772–7.
- [13] Lautenschlager NT, Cox KL, Flicker L, Foster JK, van Bockxmeer FM, Xiao J, et al. Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. *JAMA* 2008;300:1027–37.
- [14] Smith JC, Nielson KA, Woodard JL, Seidenberg M, Durgerian S, Hazlett KE, et al. Physical activity reduces hippocampal atrophy in elders at genetic risk for Alzheimer's disease. *Front Aging Neurosci* 2014;6:61.
- [15] Deeny SP, Poeppel D, Zimmerman JB, Roth SM, Brandauer J, Witkowski S, et al. Exercise, APOE, and working memory: MEG and behavioral evidence for benefit of exercise in epsilon4 carriers. *Biol Psychol* 2008;78:179–87.
- [16] Doi T, Shimada H, Makizako H, Tsutsumimoto K, Uemura K, Suzuki T. Apolipoprotein E genotype and physical function among older people with mild cognitive impairment. *Geriatr Gerontol Int* 2015;15:422–7.
- [17] Etner JL, Caselli RJ, Reiman EM, Alexander GE, Sibley BA, Tessier D, et al. Cognitive performance in older women relative to ApoE-epsilon4 genotype and aerobic fitness. *Med Sci Sports Exerc* 2007;39:199–207.
- [18] Smith JC, Nielson KA, Woodard JL, Seidenberg M, Rao SM. Physical activity and brain function in older adults at increased risk for Alzheimer's disease. *Brain Sci* 2013;3:54–83.
- [19] Khoury R, Patel K, Gold J, Hinds S, Grossberg GT. Recent Progress in the Pharmacotherapy of Alzheimer's Disease. *Drugs & Aging* 2017; 34:811–20.
- [20] Herholz SC, Herholz RS, Herholz K. Non-pharmacological interventions and neuroplasticity in early stage Alzheimer's disease. *Expert Rev Neurother* 2013;13:1235–45.
- [21] Horr T, Messinger-Rapport B, Pillai JA. Systematic review of strengths and limitations of randomized controlled trials for non-pharmacological interventions in mild cognitive impairment: focus on Alzheimer's disease. *J Nutr Health Aging* 2015;19:141–53.
- [22] Hoffmann K, Sobol NA, Frederiksen KS, Beyer N, Vogel A, Vestergaard K, et al. Moderate-to-High Intensity Physical Exercise in Patients with Alzheimer's Disease: A Randomized Controlled Trial. *J Alzheimers Dis* 2016;50:443–53.
- [23] Sobol NA, Hoffmann K, Frederiksen KS, Vogel A, Vestergaard K, Braendgaard H, et al. Effect of aerobic exercise on physical performance in patients with Alzheimer's disease. *Alzheimers Dement* 2016;12:1207–15.
- [24] Bennett DA, Wilson RS, Schneider JA, Evans DA, Beckett LA, Aggarwal NT, et al. Natural history of mild cognitive impairment in older persons. *Neurology* 2002;59:198–205.
- [25] Wilbur J, Marquez DX, Fogg L, Wilson RS, Staffileno BA, Hoyem RL, et al. The relationship between physical activity and cognition in older Latinos. *J Gerontol B Psychol Sci Soc Sci* 2012;67:525–34.
- [26] Netz Y, Wu MJ, Becker BJ, Tenenbaum G. Physical activity and psychological well-being in advanced age: a meta-analysis of intervention studies. *Psychol Aging* 2005;20:272–84.
- [27] Sink KM, Espeland MA, Castro CM, Church T, Cohen R, Dodson JA, et al. Effect of a 24-Month Physical Activity Intervention vs Health Education on Cognitive Outcomes in Sedentary Older Adults: The LIFE Randomized Trial. *JAMA* 2015;314:781–90.
- [28] Baker LD, Frank LL, Foster-Schubert K, Green PS, Wilkinson CW, McTiernan A, et al. Effects of aerobic exercise on mild cognitive impairment: a controlled trial. *Arch Neurol* 2010;67:71–9.
- [29] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. 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;34:939–44.
- [30] Hoffmann K, Frederiksen KS, Sobol NA, Beyer N, Vogel A, Simonsen AH, et al. Preserving cognition, quality of life, physical health and functional ability in Alzheimer's disease: the effect of physical exercise (ADEX trial): rationale and design. *Neuroepidemiology* 2013;41:198–207.
- [31] Sobol NA, Hoffmann K, Vogel A, Lolk A, Gottrup H, Hogh P, et al. Associations between physical function, dual-task performance and cognition in patients with mild Alzheimer's disease. *Aging Ment Health* 2016;20:1139–46.
- [32] Koch W, Ehrenhaft A, Griesser K, Pfeufer A, Muller J, Schomig A, et al. TaqMan systems for genotyping of disease-related polymorphisms present in the gene encoding apolipoprotein E. *Clin Chem Lab Med* 2002;40:1123–31.
- [33] Smith A. Symbol Digit Modalities Test (SDMT). California, USA: Western Psychological Services; 1973.
- [34] Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. *Neurology* 1994;44:2308–14.
- [35] McGough EL, Kelly VE, Logsdon RG, McCurry SM, Cochrane BB, Engel JM, et al. Associations between physical performance and executive function in older adults with mild cognitive impairment: gait speed and the timed "up & go" test. *Phys Ther* 2011;91:1198–207.
- [36] Lamoth CJ, van Deudekom FJ, van Campen JP, Appels BA, de Vries OJ, Pijnappels M. Gait stability and variability measures show effects of impaired cognition and dual tasking in frail people. *J Neuroeng Rehabil* 2011;8:2.
- [37] Simonsick EM, Fan E, Fleg JL. Estimating cardiorespiratory fitness in well-functioning older adults: Treadmill validation of the long distance corridor walk. *J Am Geriatr Soc* 2006;54:127–32.

- [38] Cink RE, Thomas TR. Validity of the Astrand-Ryhming nomogram for predicting maximal oxygen intake. *Br J Sports Med* 1981;15:182–5.
- [39] Allali G, Annweiler C, Blumen HM, Callisaya ML, De Cock AM, Kressig RW, et al. Gait phenotype from mild cognitive impairment to moderate dementia: Results from the GOOD initiative. *Eur J Neurol* 2016;23:527–41.
- [40] Finch CE. Evolution of the human lifespan, past, present, and future: phases in the evolution of human life expectancy in relation to the inflammatory load. *Proc Am Philos Soc* 2012;156:9–44.
- [41] Eisenberg DT, Kuzawa CW, Hayes MG. Worldwide allele frequencies of the human apolipoprotein E gene: Climate, local adaptations, and evolutionary history. *Am J Phys Anthropol* 2010;143:100–11.
- [42] Raichlen DA, Alexander GE. Exercise, APOE genotype, and the evolution of the human lifespan. *Trends Neurosci* 2014;37:247–55.
- [43] Steen Jensen C, Portelius E, Siersma V, Hogh P, Wermuth L, Blennow K, et al. Cerebrospinal Fluid Amyloid Beta and Tau Concentrations Are Not Modulated by 16 Weeks of Moderate- to High-Intensity Physical Exercise in Patients with Alzheimer Disease. *Dement Geriatr Cogn Disord* 2016;42:146–58.
- [44] Jensen CS, Portelius E, Hogh P, Wermuth L, Blennow K, Zetterberg H, et al. Effect of physical exercise on markers of neuronal dysfunction in cerebrospinal fluid in patients with Alzheimer's disease. *Alzheimers Dement (N Y)* 2017;3:284–90.
- [45] Ward A, Crean S, Mercaldi CJ, Collins JM, Boyd D, Cook MN, et al. Prevalence of apolipoprotein E4 genotype and homozygotes (APOE e4/e4) among patients diagnosed with Alzheimer's disease: a systematic review and meta-analysis. *Neuroepidemiology* 2012; 38:1–17.



DECLARATION OF CO-AUTHORSHIP

Information on PhD student:	
Name of PhD student	Camilla Steen Jensen
E-mail	camillajensen@comkeen.dk
Date of birth	28-08-1985
Work place	Danish Dementia Research centre, Department of Neurology, Rigshospitalet
Principal supervisor	Steen G Hasselbalch

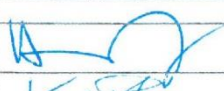
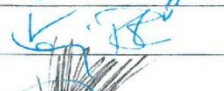
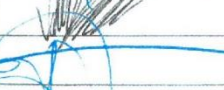
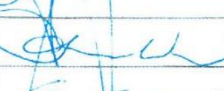
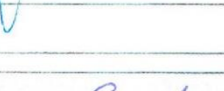
Title of PhD thesis:
Fluid-based biomarkers ^{for AD} on the effect of physical exercise in Alzheimer's disease; the ADEX trial

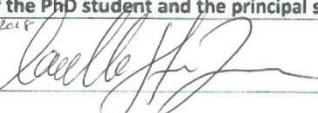
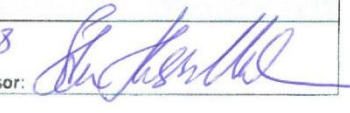
This declaration concerns the following article:
Cerebrospinal fluid amyloid beta and Tau concentration are not modulated by 16 weeks of moderate-to-high-intensity physical exercise in patients with Alzheimer's disease, 2016, dementia and other geriatric cognitive disorders.

The PhD student's contribution to the article: (please use the scale (A,B,C) below as benchmark*)	(A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable by experiments	C
2. Planning of the experiments and methodology design, including selection of methods and method development	C
3. Involvement in the experimental work	C
4. Presentation, interpretation and discussion in a journal article format of obtained data	C

*Benchmark scale of the PhD student's contribution to the article		
A. refers to:	Has contributed to the co-operation	0-33 %
B. refers to:	Has contributed considerably to the co-operation	34-66 %
C. refers to:	Has predominantly executed the work independently	67-100 %

Signature of the co-authors:			
Date:	Name:	Title:	Signature:
7/9-2018	Steen Gregers Hasselbalch	Professor, overlæge	
1/9-18	Erik Portelius	ph.d.	
7/sep-2018	Anja Hviid Simonsen	ph.d.	

1 st Sep 18	Henrik Zetterberg	professor, overlæge	
1 st Sep 18	Kaj Blenow	professor, overlæge	
5/11/18	Volkert Siersma	lektor, ph.d.	
23/10/18	Peter Høgh	overlæge	
5/10/18	Lene Wermuth	overlæge	
	Gunhild Waldemar	Professor, overlæge	

Signature of the PhD student and the principal supervisor:	
Date: 28-11-2018	Date: 29/11-18
PhD student: 	Principal supervisor: 



DECLARATION OF CO-AUTHORSHIP

Information on PhD student:	
Name of PhD student	Camilla Steen Jensen
E-mail	camillajensen@comkeen.dk
Date of birth	28-08-1985
Work place	Danish Dementia Research centre, Department of Neurology, Rigshospitalet
Principal supervisor	Steen G Hasselbalch

Title of PhD thesis:
Fluid-based biomarkers ^{for ADSS} on the effect of physical exercise in Alzheimer's disease; the ADEX trial

This declaration concerns the following article:
Effect of physical exercise on markers of neuronal dysfunction in cerebrospinal fluid in patients with Alzheimer's disease, 201, Alzheimer's and Dementia: Translational research & clinical interventions.

The PhD student's contribution to the article: (please use the scale (A,B,C) below as benchmark*)	(A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable by experiments	C
2. Planning of the experiments and methodology design, including selection of methods and method development	C
3. Involvement in the experimental work	C
4. Presentation, interpretation and discussion in a journal article format of obtained data	C

*Benchmark scale of the PhD student's contribution to the article		
A. refers to:	Has contributed to the co-operation	0-33 %
B. refers to:	Has contributed considerably to the co-operation	34-66 %
C. refers to:	Has predominantly executed the work independently	67-100 %

Signature of the co-authors:			
Date:	Name:	Title:	Signature:
7/6-2018	Steen Gregers Hasselbalch	Professor, overlæge	
1/9-18	Erik Portelius	ph.d.	
7/sep-2018	Anja Hviid Simonsen	ph.d.	

1st 8/9/18	Henrik Zetterberg	professor, overlæge	
1st 8/9/18	Kaj Blenow	professor, overlæge	
9/10/18	Lene Wermuth	overlæge	
23/10/18	Peter Høgh	overlæge	

Signature of the PhD student and the principal supervisor:	
Date: 29-11-2018	Date: 29/11-18
PhD student: 	Principal supervisor: 



DECLARATION OF CO-AUTHORSHIP

Information on PhD student:	
Name of PhD student	Camilla Steen Jensen
E-mail	camillajensen@comkeen.dk
Date of birth	28-08-1985
Work place	Danish Dementia Research centre, Department of Neurology, Rigshospitalet
Principal supervisor	Steen G Hasselbalch

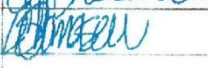
Title of PhD thesis:
Fluid-based biomarkers ^{for AD} the effect of physical exercise in Alzheimer's disease; the ADEX trial

This declaration concerns the following article:
Exercise as a potential modulator of inflammation in patients with Alzheimer's Disease measured in cerebrospinal fluid and plasma

The PhD student's contribution to the article: (please use the scale (A,B,C) below as benchmark*)	(A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable by experiments	C
2. Planning of the experiments and methodology design, including selection of methods and method development	C
3. Involvement in the experimental work	C
4. Presentation, interpretation and discussion in a journal article format of obtained data	C

*Benchmark scale of the PhD student's contribution to the article		
A. refers to:	Has contributed to the co-operation	0-33 %
B. refers to:	Has contributed considerably to the co-operation	34-66 %
C. refers to:	Has predominantly executed the work independently	67-100 %

Signature of the co-authors:			
Date:	Name:	Title:	Signature:
23/11-18	Justyna Maria Bahl	PhD	
10/11-18	Lærke Borg Østergaard	Bioanalytiker	
23/10/18	Peter Høgh	Overlæge	
3/10/18	Lene Wermuth	Overlæge	

2/11/18	Amanda Heslegrave	PhD	
1st Sep 18	Henrik Zetterberg	Professor, Overlæge	
-	Niels H.H. Heegaard	Professor (deceased) Atket	
7/11 2018	Steen Gregers Hasselbalch	Professor, overlæge	
7/sep-2018	Anja Hviid Simonsen	PhD	

Signature of the PhD student and the principal supervisor:	
Date: 29/11-2018	Date: 29/11-18
PhD student: 	Principal supervisor: 



DECLARATION OF CO-AUTHORSHIP

Information on PhD student:	
Name of PhD student	Camilla Steen Jensen
E-mail	camillajensen@comeen.dk
Date of birth	28-08-1985
Work place	Danish Dementia Research Centre, Department of Neurology, Rigshospitalet
Principal supervisor	Steen G Hasselbalch

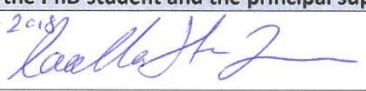
Title of PhD thesis:
Fluid-based biomarkers ^{for AD} in the effect of physical exercise in Alzheimer's disease; the DAEX trial

This declaration concerns the following article:
Patients with Alzheimer's Disease who carry the APOE ε4 allele benefit more from physical exercise.

The PhD student's contribution to the article: (please use the scale (A,B,C) below as benchmark*)	(A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable by experiments	C
2. Planning of the experiments and methodology design, including selection of methods and method development	C
3. Involvement in the experimental work	B
4. Presentation, interpretation and discussion in a journal article format of obtained data	B

*Benchmark scale of the PhD student's contribution to the article		
A. refers to:	Has contributed to the co-operation	0-33 %
B. refers to:	Has contributed considerably to the co-operation	34-66 %
C. refers to:	Has predominantly executed the work independently	67-100 %

Signature of the co-authors:			
Date:	Name:	Title:	Signature:
21/11-2018	Camilla Steen Jensen	MSc	
21/11-2018	Anja Hviid Simonsen	PhD	
21/11-2018	Steen Gregers Hasselbalch	Professor (principal Supervisor)	

Signature of the PhD student and the principal supervisor:	
Date: 21/11-2018 PhD student: 	Date: 21/NOV/2018 Principal supervisor: 



Do. Or do not. There is no try.

Yoda – the Empire strikes back