

Intervening in Brain Aging

Evidence from Neuroimaging,
Vascular Health, and Lifestyle Factors



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Mark R. van Loenen

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Intervening in Brain Aging

Evidence from Neuroimaging, Vascular Health, and Lifestyle Factors

Mark Ruben van Loenen

Intervening in Brain Aging: Evidence from Neuroimaging, Vascular Health, and Lifestyle Factors

Mark van Loenen

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Intervening in Brain Aging

Evidence from Neuroimaging, Vascular Health, and Lifestyle Factors

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*“Je moet een hindernis pas nemen
als je er voor staat”*
mijn opa, P.G. Speijdel

Voor mijn ouders,
mijn gezin,
mijn familie

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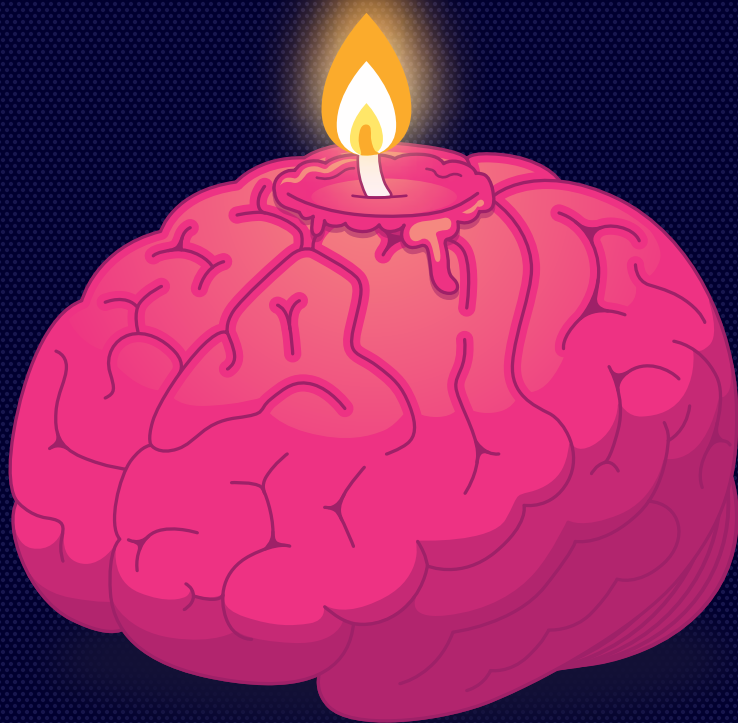
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PART I

Introduction and Thesis Outline

Chapter 1

General Introduction

For many, cognitive decline and dementia are among the most frightening prospects of older age. As we move through life, we come to expect that our bodies will eventually wear out in certain ways. We accept that our joints may stiffen, our muscles may weaken, and that we might one day need a cane or walker to support us over longer distances. We can even take comfort in the fact that, thanks to modern day medicine, a worn-out hip or knee can often be replaced. These age-related challenges are changes we can see, measure, and respond to. Yet the aging brain presents an entirely different challenge. Perhaps this is because we so closely associate the brain with our very sense of self. It is the seat of our consciousness, our personal identity, and the countless memories and experiences that shape how we perceive and understand the world around us. Through the brain we learn, laugh, and love. It allows us to recognize familiar faces, engage in conversations, and find our way in the world. Where a prosthetic joint replacement or a mobility aid can support or restore independence in movement, there exists no replacement for the intricate and highly complex neural networks of the brain. Cognitive decline threatens not only our independence in daily life but also our sense of cognitive autonomy and individuality, which are the qualities that make us who we are.

While cognitive decline and dementia are concerning at the individual level, its consequences become even more urgent when considered in the broader context of a rapidly aging population. With increasing life expectancy and demographic shifts in age-groups resulting in a growing proportion of adults aged 65 and older, the total number of people living with aging-related cognitive decline and dementia is expected to rise significantly. If trends continue, current estimates project more than 150 million cases of dementia worldwide by the year 2050 [1, 2]. This projected increase will translate to heavy societal and economic consequences, as it will place emotional and practical burdens on patients and caregivers and will demand resources to facilitate healthcare systems and long-term care [3]. In order to effectively prevent or delay aging-related cognitive decline, it is essential to develop interventions that preserve cognitive functioning. Achieving this requires a mechanistic understanding of the processes underlying aging-related cognitive decline and insight into how these processes may be slowed, prevented, or even reversed.

Structural and functional brain aging

Aging is a longitudinal process which affects various structural and functional properties of the brain across the lifespan. Structurally, it is most predominantly reflected in the gradual deterioration of gray and white matter, as well as in the vasculature that supports these tissues [4].

The gray matter is primarily comprised of neuronal cell bodies and forms the outer layer of the brain (i.e., the cortex) as well as several deep-brain subcortical nucleic structures (e.g., basal ganglia, thalamus, hippocampus). Longitudinal and cross-sectional studies following the trajectory of global gray matter volume change from adulthood into older age consistently report an approximate annual volume decrease of 0.2% from early adulthood until 70 years of age [5, 6], rising to around 0.5% per year thereafter [5-7]. This volume loss is only partly explained by neuronal cell death, but instead, is primarily driven by cortical thinning: a process reflecting neuronal body shrinkage, reductions in synaptic spine count, and decreased synaptic connectivity [4]. Similar patterns of structural deterioration, affecting the interconnectivity between neurons rather than causing complete cell death, are also observed in the white matter. The white matter consists of myelinated fiber connections called axons, linking neurons within the gray matter and establishing connectivity between different brain regions [8]. Myelin membranes serve as an insulating layer around axons, improving impulse transmission between neurons and thereby supporting brain connectivity and function. Aging-related white matter volume loss is primarily attributed to degradation of myelin integrity, resulting in reduced axonal tail lengths and functional connectivity [4]. Interestingly, while gray matter decreases gradually and consistently across the lifespan, white matter shows an inverted-U trajectory: volumes increase up until approximately 50 years of age and incrementally decline thereafter [9-11].

Multiple longitudinal studies have demonstrated change-change associations between measures of structural integrity and cognitive functioning [12-14], hinting at a direct link between gray and white matter deterioration and cognitive decline in older adults. Additional evidence shows that aging-related structural changes are often region-specific, affecting brain regions that support cognitive domains such as executive functioning, working memory, episodic memory, and processing speed [15-19]. These domains are fundamental to higher-order cognitive functions, including planning, decision-making, and critical thinking, which are essential for maintaining independent functioning in daily life [20].

The aging-related structural and functional brain changes summarized above reflect the typical course of normal aging in healthy older adults. A critical next step is to distinguish these normative changes from those observed in pathological aging conditions, such as mild cognitive impairment (MCI) and Alzheimer's disease (AD). MCI is a transitional clinical phase between normal aging and dementia, characterized by cognitive decline that exceeds what is expected for a person's age, but without significant enough disruption of instrumental activities of daily living (IADL) to meet the diagnostic criteria of AD or other forms of dementia such as

vascular, frontotemporal, or Lewy Body dementia [21, 22]. Interestingly, when considering transitional phases of neurodegenerative conditions and the shared biological characteristics of gray and white matter atrophy, functional connectivity, and cognitive decline, normal and pathological aging could be viewed as existing on a continuous scale [23]. On this scale, the changes in brain structure and cognitive functioning seen in normal aging are qualitatively similar to those in pathological aging but differ in rate of progression, magnitude, and regional specificity [24]. Pathological aging, however, also involves distinct additional factors such as protein aggregation (e.g., amyloid- β and tau in AD [25]) or pronounced progression of cerebrovascular small vessel disease hallmarks (e.g., cerebral microbleeds, lacunar infarctions, white matter hyperintensities [26]), which push these changes beyond what is considered within the normal range of brain aging [24]. To effectively distinguish normal from pathological aging, it is essential to visualize current brain health markers *in vivo*, particularly in at-risk cohorts which may show early signs of brain integrity changes. This approach may enable the assessment of brain deterioration along this proposed continuous brain aging scale.

Advancements in neuroimaging for aging and dementia

Although the concept of aging-related cognitive decline dates back to Greek and Roman times, significant efforts in identifying pathological markers of dementia only intensified around the 19th century [27]. Clinical neuroscientists and pathologists primarily relied on post-mortem neuroanatomy and histology studies of deceased dementia patients to uncover the possible biological markers underlying this condition. This research led to the discovery and description of senile plaques and fat deposits within the brain vasculature, highlighting the potential vascular contributions to aging-related brain pathology as early as the late 19th century. While these studies, leading to Alois Alzheimer's first clinical definition of AD in 1906 [28], were fundamental in elucidating dementia pathology, they were limited to patients in advanced stages of the disease, when brain damage and cognitive symptoms were already extensive and irreversible.

In the decades following Alois Alzheimer's discovery of AD, clinical imaging technology began to emerge. In 1946, nuclear magnetic resonance (NMR) was discovered and described by Bloch and Purcell [29], and by 1980, the principles of NMR were applied to develop the first magnetic resonance imaging (MRI) scanners for clinical use [30]. In short, MRI uses a strong, external magnetic field to align water molecules in the body. Pulsed radio frequency (RF) waves are applied to temporarily

knock these water protons out of their original aligned position. As the unaligned protons realign with the external magnetic field (relaxation), energy is released in the form of RF waves, which are detected by the MRI scanner with receiver coils and subsequently converted into interpretable images [29]. Building on this principle, MRI has since been adapted and applied to assess both structural and functional measures of the brain [31]. These technological advances now allow the acquisition of intricate, high-resolution in-vivo images of the brain, which enable us to more accurately follow brain aging trajectories, even during or before prodromal stages of disease.

Within the context of aging-related cognitive decline and dementia research, multiple different MR imaging modalities are used to assess the structural and functional brain changes associated with aging as discussed earlier. High-contrast structural MRI is commonly used to accurately quantify brain region volumes [32, 33]. Due to region- or tissue-specific variations in water content and NMR relaxation, distinct signal intensities are formed, producing MR-images with clear contrast and allowing for volumetric quantification. These imaging techniques provide valuable insight into region-specific gray and white matter atrophy associated with aging and dementia, including the frontal and medial-temporal lobes [34-36]. Functional MRI (fMRI) is used to assess regional neural activity in the brain, either at rest (resting-state fMRI; rs-fMRI) or during performance of a specific neuropsychological task (task-based fMRI) [37]. In fMRI, neural activation is indirectly measured through changes in blood oxygenation, known as the blood-oxygen-level dependent (BOLD) signal. Increased neural activity locally elevates cerebral blood flow (CBF) to meet increased metabolic demands, leading to a higher ratio of oxygenated to deoxygenated hemoglobin. This local shift alters NMR relaxation and thereby produces a signal contrast that can be mapped to specific brain regions [37]. Task-based fMRI is extensively utilized to assess normal and pathological aging-related neural activity decline or compensatory mechanisms, especially in cognitive domains predominantly affected by aging such as executive functioning, working memory, episodic memory, and processing speed [38]. However, while BOLD fMRI provides a proxy of neural activity through hemodynamic shifts, it does not provide a direct numerical measure of CBF. In contrast, perfusion imaging techniques such as arterial spin labelling (ASL) allow for the absolute quantification of CBF [39-41]. With ASL, water protons in oxygen-rich arterial blood are unaligned to the external magnetic field as they travel to the brain. Due to brain region-specific differences in arterial transit time, caused by the vascular structures of the brain, and the subsequent variation in NMR relaxation, contrasting RF signal intensities are generated. These contrasts are then compared to a control image, wherein all water protons are aligned

to the external magnetic field, to create perfusion maps and calculate absolute quantified CBF [41, 42]. ASL provides crucial insights into whole-brain and region-specific CBF, which show distinct patterns of hypo- and hyperperfusion in normal aging [43] and in MCI [44] and AD [45]. These aging-related perfusion effects closely reflect the many established cardiovascular risk factors associated with aging-related cognitive decline and dementia [2].

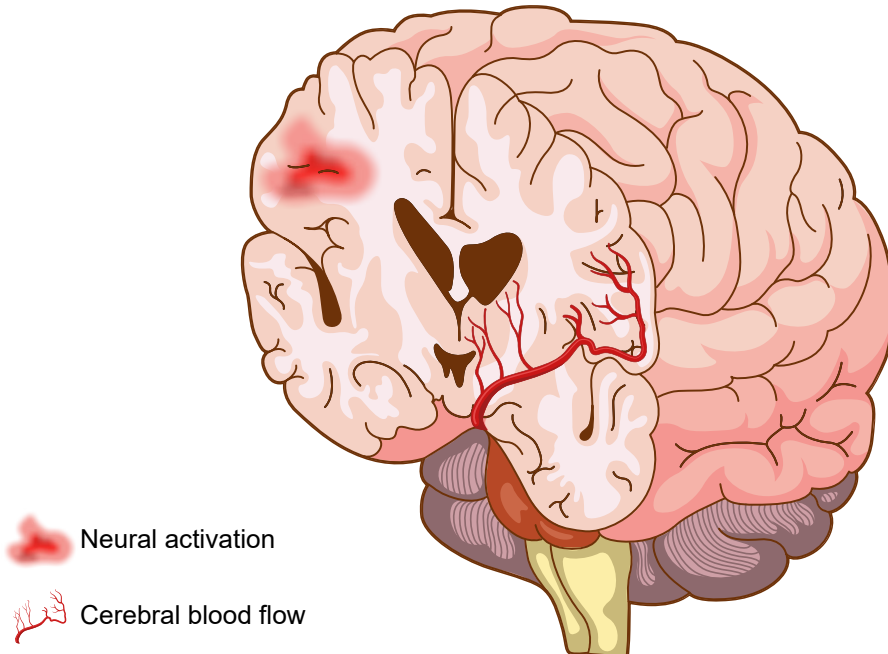


Figure 1. Functional brain imaging measures sensitive to ageing-related decline. Local patterns of hyper- and hypoactivation of neurons during rest or performance of a task can be measured using BOLD fMRI. Cerebral blood flow can be visualized and quantified using ASL perfusion imaging.

Structural markers of aging-related cognitive decline and dementia are broadly used to diagnose, follow, and predict disease progression [46, 47]. However, changes in functional markers, such as BOLD fMRI, can be detected in earlier, preclinical phases of disease [48, 49]. This suggests that functional markers of brain deterioration are more sensitive to the process of aging [50], and may serve as promising targets for early intervention strategies aimed at preventing or slowing cognitive decline before irreversible damage has occurred.

Prevention of aging-related cognitive decline

The urgency for early intervention strategies, before irreversible damage to brain structure and function occurs, is underlined by the current lack of curative treatments

for pathological aging. Over the past decades, multiple treatments for AD have been explored [51-53], but these primarily target symptoms rather than the underlying pathology. This highlights that early adoption of risk factor reduction, well before the onset of clinical symptoms, may be more effective in reducing the pathologic trajectory and limit future dementia cases [54]. Indeed, the 2024 Lancet Standing Commission on Dementia Prevention, Intervention and Care theorized that nearly half of all future dementia cases can be prevented by addressing established modifiable risk factors, even in individuals carrying genetic risk factors such as the APOE $\epsilon 4$ -allele [2].

A number of these modifiable risk factors include cardiovascular and metabolic conditions such as obesity, hypertension, hypercholesterolemia, and diabetes, which are major established contributors to aging-related cognitive decline and dementia [2, 55-57]. Because these risks are present for many individuals long before clinical symptoms of cognitive decline become apparent, their treatment has increasingly become the focus of preventive treatment strategies, providing opportunities for early intervention. Notably, these risk factors are strongly influenced by lifestyle patterns, which has prompted growing interest in lifestyle-based intervention studies [56-59]. Given the multifactorial etiology of aging-related cognitive decline and dementia, multidomain lifestyle-based interventions are particularly warranted, as they can directly target multiple risk factors and may help preserve cognitive functioning in aging [58-60].

So far, several multidomain lifestyle intervention studies have been conducted aimed at improving cognitive functioning in aging in older adults. Most notably, two large-scale RCTs, the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER [61]) and the United States Study to Protect Brain Health Through Lifestyle Intervention to Reduce Risk (US POINTER [62]), have both reported small but significant positive intervention effects on global or domain-specific cognitive outcomes. However, other similarly designed studies have found no significant intervention effects on cognitive functioning [63-66], or effects were only observed in specific at-risk subgroup [63, 64]. These latter findings suggest that participants with a greater potential for improvement (i.e., those with higher baseline risk for cognitive decline), benefitted more from the interventions. Importantly, the primary outcomes of these studies were cognitive measures, whereas effects of risk reduction could potentially be detected earlier using more sensitive measures, such as structural or functional MRI, which may reveal changes before effects on cognitive functions become measurable.

The field of lifestyle intervention studies incorporating measures from neuroimaging is still in an emerging phase. Several studies have previously reported positive

effects of, mostly single-domain, lifestyle interventions on neuroimaging markers associated with aging-related cognitive decline. For example, physical exercise interventions have been shown to improve functional brain measures including increased CBF, particularly in brain regions vulnerable to aging-related cognitive decline and dementia such as the frontal lobe and hippocampus [67, 68]. Similarly, adherence to healthy dietary patterns has been associated with greater gray and white matter integrity and reduced decline in total and hippocampal brain volume [59, 69-71]. However, the effects of multidomain lifestyle interventions on multiple measures of brain structure and function, particularly in populations at elevated risk of cognitive decline, remain largely unknown. Most previous studies focused on interventions incorporating one or two lifestyle domains, included either healthy older adults or patient populations (i.e., MCI or AD, in progressed stages of disease), or used only structural or a limited number of functional neuroimaging measures as primary outcomes. To address these gaps, a multimodal MRI approach is needed to comprehensively evaluate the effects of multidomain lifestyle interventions on multiple different measures of brain structure and function in older adults.

Thesis outline

With this thesis, our overall aim is to elucidate the biological mechanisms underlying cognitive improvement after a multidomain lifestyle intervention in older adults at risk for aging-related cognitive decline. We employ a multimodal neuroimaging protocol alongside a comprehensive set of lifestyle, (peripheral) vascular health, and cognitive functioning outcome measures to assess associations between these domains. With these associations, we aim to capture the multifactorial pathways through which the intervention may improve lifestyle, decrease cardiovascular risk, enhance brain health, and ultimately improve cognitive functioning. Together, this methodological approach may advance our understanding of how lifestyle interventions can mitigate cognitive decline and dementia risk, improve daily functioning, and guide the identification of effective, measurable targets for future interventions. This thesis and its aims are part of the Maintaining Optimal Cognitive function In Aging (MOCIA) research program [72].

In **Chapter 2**, the HELI (*‘Hersenfuncties na Leefstijlinterventie’*) multidomain lifestyle intervention study is introduced. This chapter provides a detailed description and rationale of the HELI study including the background, design, methodological approach, and selection of outcome measures. The methodology includes an outline of the description of study procedures, approaches to implement the intervention,

and data acquisition. The outcome measures specify the variables assessed across the domains of lifestyle, vascular health, brain health, and cognitive functioning. These markers are further contextualized within a multifactorial framework to formulate hypothesized peripheral and brain pathways that may ultimately influence cognitive functioning.

Chapter 3 explores this hypothesized multifactorial framework of cognitive aging by investigating cross-sectional associations between multiple measures within each domain of lifestyle, vascular health, MRI-based brain health, and cognitive functioning. While numerous studies have explored pairwise associations between these domains, few have integrated all aspects into a single comprehensive model to investigate the potential pathways linking them within the context of aging-related cognitive decline. Therefore, this chapter investigates these pathways in one integrated model, including markers from all domains, to determine whether associations between lifestyle, vascular health, and MRI-based brain health can ultimately explain variations in cognitive functioning in an at-risk population. By using this integrated approach within the same population, the analyses account for the multifactorial etiology and cardiovascular risk of aging-related cognitive decline and dementia. Examining these associations at both the domain and individual marker level may provide insights into between-domain pathways that could be influenced by multidomain lifestyle interventions.

In **Chapter 4**, the effects of the HELI multidomain lifestyle intervention on neuroimaging measures of brain functioning are investigated. Functional outcomes relevant to aging-related cognitive decline, including task-based BOLD fMRI and CBF perfusion imaging are examined. In addition, intervention-induced changes in measures of healthy lifestyle adherence, peripheral vascular health, and cognitive functioning are assessed and related to the neuroimaging outcomes. Together, this chapter presents a novel approach using a multimodal neuroimaging protocol incorporating multiple functional and structural MRI markers in older adults at risk of cognitive decline, with the aim of advancing insights into the brain mechanisms underlying multidomain lifestyle-induced cognitive benefits.

Chapter 5 summarizes and discusses the main findings, placing them in the broader context of clinical and practical relevance for the prevention of aging-related cognitive decline and dementia. The chapter also outlines future directions to further advance our understanding of and promote the maintenance of cognitive health in aging. In **Chapter 6**, a Dutch summary is provided of the primary findings and implications of this thesis.

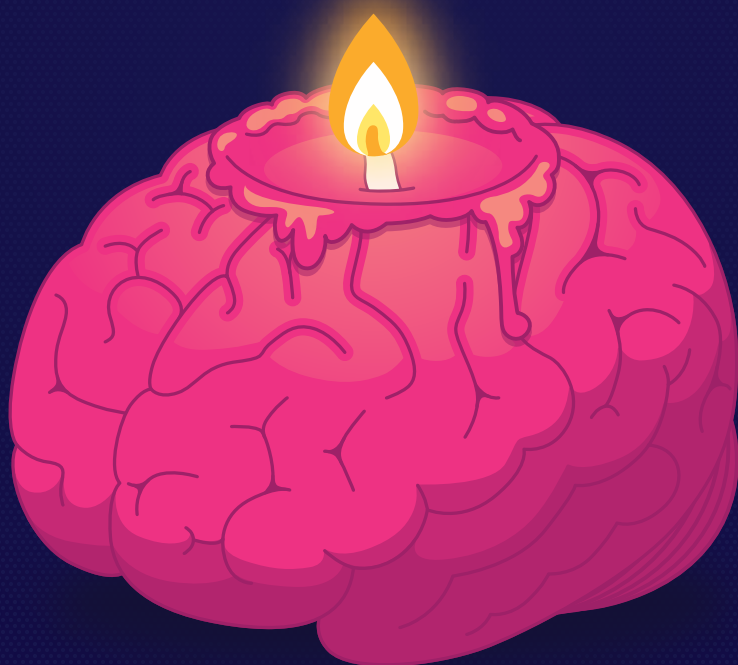
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PART II

Rationale and Design of the HELI study

Chapter 2

The Effects of a Multidomain Lifestyle Intervention on Brain Function and Its Relation With Immunometabolic Markers and Intestinal Health in Older Adults at Risk of Cognitive Decline: Study Design and Baseline Characteristics of the HELI Randomized Controlled Trial

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Abstract

Background

Studies of multidomain lifestyle interventions show mixed results on preventing or delaying cognitive decline in ageing. A better understanding of central and peripheral mechanisms underlying these interventions could help explain these mixed findings.

Objective

The HELI (Hersenfuncties na LeefstijlInterventie) study aims to investigate the brain and peripheral mechanisms of a multidomain lifestyle intervention in older adults at risk of cognitive decline.

Methods

The HELI study is a 6-month multicenter, randomized, controlled multidomain lifestyle intervention trial powered to include 104 Dutch older adults at risk of cognitive decline. Individuals were deemed at risk when scoring ≥ 2 points on a lifestyle-modifiable risk factor scale (e.g., overweight, physical inactivity, hypertension, hypercholesterolemia). The intervention consisted of 5 domains (diet, physical activity, stress management and mindfulness, cognitive training, and sleep) and participants were randomized to one of two groups: (1) a high-intensity coaching group with weekly supervised online and on-site group meetings, exercises and lifestyle-specific course materials, and (2) a low-intensity coaching group receiving general lifestyle health information sent through e-mail every two weeks. The primary study outcomes are changes between baseline and 6-month follow-up in (1) brain activation in dorsolateral prefrontal cortex (dlPFC) and hippocampus and task accuracy during a functional magnetic resonance imaging (fMRI) working memory task (2) arterial spin labeling-quantified cerebral blood flow in dlPFC and hippocampus, (3) systemic inflammation from blood plasma (interleukin-6, TNF- α , hs-CRP) and (4) microbiota profile from faeces (gut microbiome diversity (Shannon and phylogenetic diversity) and richness (Chao1)). In addition, we will investigate intervention-induced gut-immune-brain links by assessing relations between effects in primary brain and gut outcomes. Secondary study outcomes include (1) structural and neurochemical MRI, (2) anthropometric measurements, (3) neuropsychological test battery scores, (4) lifestyle-related questionnaire and smartwatch measures, and peripheral measures from (5) faecal, (6) blood, and (7) breath analyses.

Results

This work was supported by a Crossover grant (MOCIA 17611) of the Dutch Research Council (NWO), granted in December 2019. The MOCIA program is a public-private partnership. Between April 2022 and October 2023 we successfully included 102 older Dutch adults (mean age: 66.6, SD: 4.3 years; 67/102, 65.7% female) with ≥ 2 lifestyle-modifiable risk factors of cognitive ageing (median risk: 3, IQR 2-3). The most common self-reported lifestyle-modifiable risk factors at baseline were overweight or obesity (76/102, 74.5%), followed by hypertension (58/102, 56.9%), hypercholesterolemia (57/102, 55.9%), and physical inactivity (57/102, 55.9%).

Conclusions

The HELI study aims to enhance our understanding of working mechanisms of multidomain lifestyle interventions through its comprehensive characterization of central and peripheral markers. We intend to achieve this aim by assessing lifestyle intervention-induced changes in functional and structural MRI brain measures, as well as peripheral measures of the gut-immune-brain axis involved in cognitive ageing.

Introduction

Background

Multidomain lifestyle interventions have great potential to delay cognitive ageing, but the effects of previous trials are small and mixed. Furthermore, previous studies mainly focused on cognitive functioning as primary outcome. Consequently, underlying neurocognitive, central mechanisms and involved peripheral pathways remain poorly understood. The HELI (Hersenfuncties na Leefstijl) Interventie study is a 6-month multidomain lifestyle intervention in older adults at risk of cognitive decline based on modifiable lifestyle factors, focusing on involved central and peripheral mechanisms by using a broad range of neuroimaging, blood- and gut-related outcome measures. Below, we explain the rationale of our objectives and hypotheses.

Lifestyle interventions in Cognitive Aging

In our ageing population, the incidence of cognitive decline and incurable neurodegenerative diseases like Alzheimer dementia (AD) and other types of dementia is increasing drastically [1]. A substantial part of the risk factors for dementia, such as obesity, hypertension, type II diabetes and physical inactivity [2-6], is modifiable by changes in lifestyle. Indeed, observational research shows a relationship between a healthy lifestyle, such as a healthy diet, regular physical and cognitive exercise, not smoking, and maintaining social contacts, with a lower dementia risk [7, 8]. As the signs of neurodegenerative diseases - such as amyloid beta pathophysiology and tauopathy in AD - begin decades before the first symptoms become apparent [9, 10], paired with the fact that long-term healthy lifestyles show inverse relations with dementia risk, interventions addressing cognitive decline need to start at an early stage [11]. The World Health Organization (WHO) has therefore designated preventive multidomain lifestyle interventions as having the greatest potential to reduce the risk of cognitive decline and dementia within their 2019 guidelines [12].

Previous multidomain lifestyle intervention trials in ageing individuals show small but significant effects on both cognitive functioning and dementia risk, as concluded by a meta-analysis from 2022 [13]. Especially when compared to single-domain interventions, multidomain lifestyle programs showed stronger effects [14, 15]. However, previous studies are heterogeneous in population characteristics, intervention design, components and duration, and outcome measures. Moreover, when zooming in on individual studies and considering the latest published trials [16, 17], the reported effects can be considered mixed. The 24-month FINGER study [18] was the first large, longitudinal randomized controlled trial (RCT) in older adults at risk of cognitive decline ($n = 1260$), and found subtle but robust positive

effects of a multidomain lifestyle intervention on cognitive functioning. Other lifestyle interventions such as the preDIVA-study (n=2994) [3], MAPT (n=1680) [19], J-MINT (n=531) [16], and AgeWell.de (n=1030) [17] did not find differences in cognition or dementia risk. However, specific subgroups within these trials with higher dementia risk based on modifiable cardiovascular lifestyle factors [3, 19] did show improvements in cognitive functioning. Altogether, these findings point to specific at-risk older adults who are most likely to benefit from multidomain lifestyle interventions.

Crucially, these previous lifestyle intervention studies all had behavioral cognitive tests or dementia incidence as primary outcome(s). We still have a limited understanding via which brain mechanisms, and potential underlying peripheral pathways, these multidomain lifestyle interventions might exert their effects on cognition. To elucidate underlying brain mechanisms, different neuroimaging techniques are essential to analyze brain health and function *in vivo* in a longitudinal, intervention setting. Moreover, to understand involved peripheral pathways, we need extensive measures of inflammatory, metabolomic, and microbiome markers in blood and feces.

Among the previous lifestyle intervention studies, only FINGER (n=132, 2 years) and MAPT (n=68, 6 months) used (structural) neuroimaging in a subsample of participants. Within FINGER, no structural (i.e., anatomical) differences were found between intervention groups [20], whereas within MAPT, the lifestyle intervention group showed decreased deformation of periventricular areas and temporal lobes, and decreased whole-brain atrophy compared to placebo [19]. Additionally, PET scans within MAPT also demonstrated elevated levels of cerebral glucose metabolism within, e.g., the hippocampus and the adjacent parahippocampal gyrus [21]. These limited and heterogeneous neuroimaging findings highlight the poor understanding we currently have of underlying neurocognitive mechanisms of lifestyle changes. In addition, some of the previous studies did assess blood, cardiovascular- [3], inflammatory- [19], or metabolomic markers [18, 19], which confirmed that these pathways are influenced by lifestyle changes. However, none of the preceding lifestyle interventions have combined extensive blood and fecal measures to fully understand underlying peripheral(-central) mechanisms.

Therefore, within the HELI study, we will combine a broad neuroimaging protocol, including task-related functional MRI (fMRI) and quantitative imaging of cerebral blood flow (CBF) as primary central outcomes, with peripheral measures in blood and feces, including blood inflammatory profile and fecal microbiota composition as primary peripheral outcomes. Below, we will explain the rationale of our chosen primary and secondary outcome measures.

Aging Brain and Lifestyle

The longitudinal process of ageing impacts multiple facets of the brain, causing changes in both brain structure and function and, subsequently, in cognition. Age-related structural brain changes are characterized by changes in gray and white matter volume, leading to loss of overall brain volume [22]. Importantly, certain brain structures such as the hippocampus and (dorsolateral) prefrontal cortex (dlPFC) appear to shrink at a faster rate compared to other brain regions, possibly explained by their increased vulnerability to vascular risk factors such as hypertension and hypercholesterolemia [23–25]. These findings indicate a possible link between lifestyle-modifiable cardiovascular risk factors and ageing-related cognitive decline. In addition, the large interindividual differences reported in age-related structural and functional brain deterioration have been proposed to be partially explained by differences in adherence to healthy lifestyle patterns [26].

Earlier studies in pathological aging, specifically in mild cognitive impairment (MCI) and AD, have broadly established key patterns of functional brain deterioration, even in prodromal stages of disease before structural deterioration becomes apparent [27]. These findings indicate that measures of brain function may be more sensitive to interventions targeting age-related cognitive decline, and are more strongly modifiable by nature as opposed to brain structure. The most notable cognitive changes associated with normal and pathological ageing can be found in the more complex cognitive domains such as executive functioning and working memory [28]. Working memory is a fundamental cognitive process, supporting higher-order cognitive functions such as planning, problem-solving and decision-making. Although the overall effects of age on working memory, which shows gradual decreases with advancing age, have been well-established, multiple studies have also identified increased activity in regions of the prefrontal cortex (PFC) in older adults who show comparable working memory performance to young adults [29, 30]. These studies indicate that in ageing, differences in functional activation patterns, measured with fMRI, may already show a –perhaps compensatory– reorganization before detriments in cognitive performance are observed [28, 31]. However, as current literature remains scarce, it remains unclear how this potential functional reorganization is affected by a multidomain lifestyle intervention, especially in at-risk older adults.

Another brain function measure which is a potential cause underlying age-related decline in cognitive functioning, is decreased (local) CBF [32–35]. Multiple studies have reported the predictive value of quantified CBF on cognitive performance in both normal and pathological cognitive ageing [32–35]. Moreover, increased local CBF in specific brain regions such as the hippocampus and PFC, has been directly linked

to increased cognitive performance [32]. Although previous studies have found short-term positive effects on CBF after lifestyle improvements such as improved diet or increased physical exercise, it remains unclear whether these effects retain after a multidomain lifestyle intervention and how the increase in regional CBF translates to cognitive functioning in ageing [36].

Other promising brain measures in cognitive ageing research are the quantification of local iron deposition and neuroinflammation. The accumulation of iron across the lifespan (especially in the deep gray matter), although conventionally found in healthy ageing adults [37-39], has been directly linked with increased local oxidative stress leading to neurodegeneration [40-43] and decreased cognitive functioning in older adults with amyloid- β pathology [44]. Similarly, and in addition to intracranial iron deposition, neuroinflammation contributes to neurodegenerative processes in the normally ageing brain [45], for example, by affecting the cerebrovascular endothelium leading to neurovascular dysfunction [46]. These impairments could eventually contribute to decreased brain functioning and cognitive decline [47]. We now know that increased regional neuroinflammation –for example, measured by quantifying intracranial myo-inositol concentrations with magnetic resonance spectroscopy (MRS) – is strongly associated with memory dysfunction in normal ageing [48, 49], in MCI and in AD [49, 50]. These findings emphasize the effect of local neurodegenerative effects on specific cognitive domains associated with cognitive ageing and dementia.

We currently know that age-related structural and functional brain changes consequently cause cognitive decline, and that brain perfusion, neuroinflammation and iron accumulation are possible underlying mechanisms. A small number of lifestyle intervention studies, most of which target one specific domain, have reported positive associations between improved diet, exercise and cognitive training and neuroimaging measures of cerebral perfusion [51-53] and functional connectivity [54]. However, it is currently unknown to what extent a multidomain lifestyle intervention influences the involved mechanisms of age-related changes in brain activation. Applying a broad neuroimaging protocol could provide important insights into the effects of multimodal lifestyle improvements on the underlying brain mechanisms of age-associated cognitive decline and provide direct mechanistic targets for future interventions.

Gut-Brain Axis and Peripheral Mechanisms

Growing evidence indicates an important role for intestinal health and other peripheral factors, like inflammation, metabolic, and vascular health, in the development of

cognitive decline [55, 56]. Furthermore, a substantial part of lifestyle effects on brain functioning is expected to arise via mechanisms in the periphery. An overview of these hypothesized pathways and the link with brain health can be found in Figure 1. These pathways are expected to be influenced by multidomain lifestyle interventions at different levels. Below, we will describe the rationale behind these pathways.

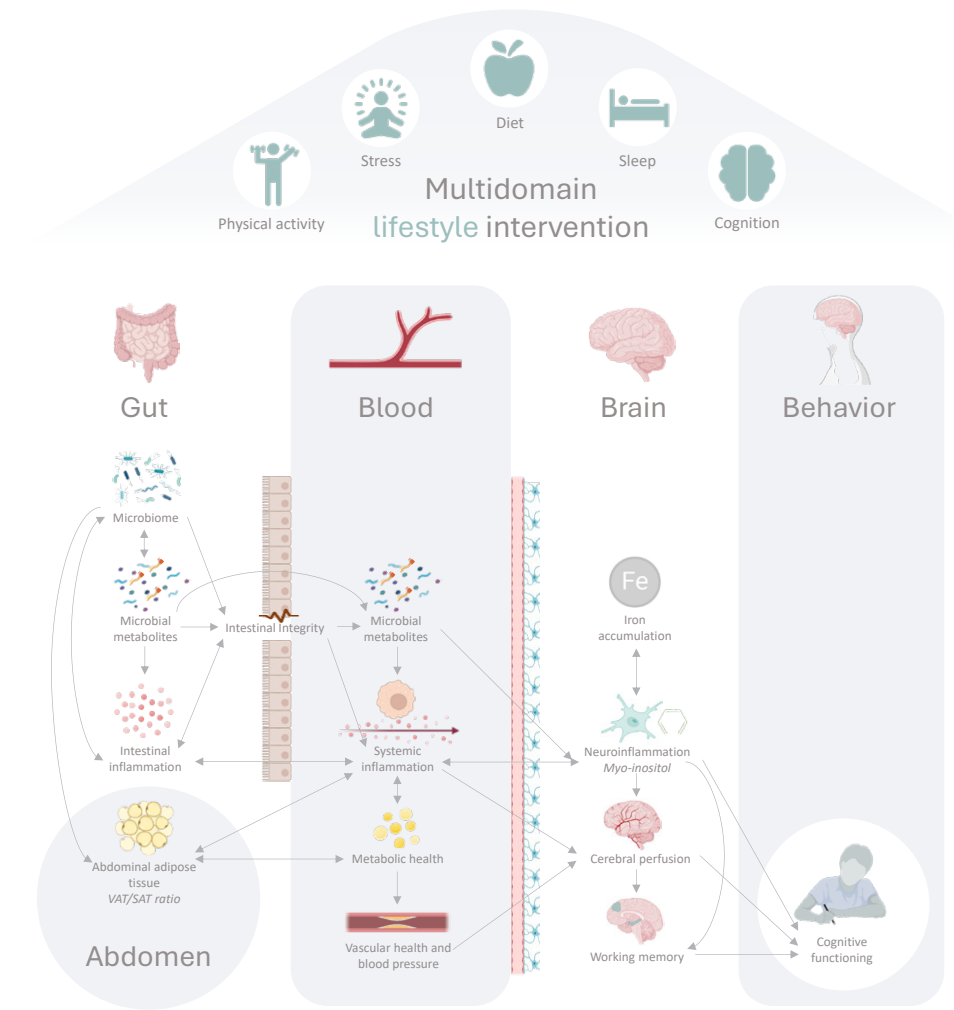


Figure 1. Hypothesized peripheral pathways. Hypothesized peripheral pathways from gut to brain affecting cognitive functioning, based on the existing evidence mentioned in the introduction. A multidomain lifestyle intervention is expected to affect these proposed pathways at different levels. Graphic is focused on HELI study outcomes. Not each presented pathway (arrow) will be tested in the HELI study.

The gut microbiome, in particular, has an increasingly recognized impact on brain functioning and behavior via different routes captured in the gut-brain axis. These include the modulation of immune signaling to the brain, affecting the hypothalamic–pituitary–adrenal axis, and transmission via the vagus nerve [57]. Microbiota-derived bioactive metabolites, such as short-chain fatty acids (SCFAs), tryptophan metabolites, (endo)toxins, and secondary bile acids, could mediate the effects of the gut microbiome on brain health [58]. They reflect microbiome function [59] and have demonstrated health effects on the host (e.g., [anti]inflammatory and metabolic processes) [60, 61], also in ageing [62].

We know that ageing is associated with changes in microbial composition and diversity and gut health [63]. Older adults more frequently show gut microbiome dysbiosis (imbalance) compared to younger individuals, potentially due to prolonged exposure to an unhealthy lifestyle [64, 65]. Particularly, microbial diversity and uniqueness seem to correlate with ageing in Western societies [66]. A lower faecal microbial diversity predicted poorer cognitive function in healthy older adults [67]. Microbial metabolites are also affected by ageing, as older adults show reduced faecal total SCFA levels [68]. SCFAs, especially butyrate, are known for their beneficial anti-inflammatory effects [69, 70], and have been linked to improved cognition in mice [71, 72]. Importantly, these microbial changes become more pronounced in the case of pathological cognitive ageing like AD. Compared to healthy aged-matched individuals, patients with AD show a decreased microbial diversity and stability over time [73], differences in abundance of individual taxa [56, 74] and more often have small-intestinal bacterial overgrowth (SIBO) [75]. In addition, a further decrease in levels of faecal SCFAs and tryptophan metabolites was found, which correlated with cognitive decline [76]. However, as these findings mainly come from cross-sectional studies, causal evidence from intervention studies to provide a stronger link between these gut-brain mechanisms, is still limited.

Amongst the proposed gut-brain pathways, inflammation is assumed to play a pivotal role in brain health. Dysbiosis of the gut microbiome can potentially impair the integrity of the intestinal barrier [77], thereby provoking a local inflammatory reaction that could eventually lead to systemic inflammation [78]. In addition, microbiota dysbiosis can also lead to the dysregulation of abdominal adipose tissue, affecting immune and insulin pathways, promoting systemic inflammation even more [79, 80]. Low-grade, systemic inflammation is commonly seen in ageing [81], and is characterized by a mild and sustained increase in immune mediators in the systemic circulation. For example, the acute-phase response marker C-reactive protein (CRP) and its major regulators interleukin (IL)-6 and tumor necrosis factor

(TNF)- α are increased [81, 82]. In turn, low-grade, systemic inflammation due to impaired intestinal health can lead to elevated levels of neuroinflammation [83] and is linked with neurodegenerative processes in the normally ageing brain.

Importantly, a direct causal role for the gut-immune-brain axis in ageing has been demonstrated in mice using faecal microbial transplantation (FMT) [84-87]. These findings emphasize that microbial modulation in humans might be of significant therapeutic benefit in preventing age-related inflammation and cognitive decline [88]. Altogether, the gut microbiome and its interaction with the host can be considered a crucial modulator of (un)healthy ageing, determining the rate of physical and cognitive decline [89].

There are numerous studies showing that gut microbiome composition and immune-metabolic health effects on the host are modified by diet and nutritional components [90-92], including the Mediterranean-type diets [93, 94]. In addition, other lifestyle components such as physical activity [95, 96], stress [97] and sleep [98, 99] are known to affect the microbiota composition and intestinal health, as well as immune-, vascular-, and metabolic health [100-107]. Importantly, we hypothesize that multiple lifestyle domains can synergistically affect peripheral markers, and should therefore be targeted simultaneously. However, it is still largely unclear how gut microbiome, immune and metabolic factors link to brain health in ageing, and which mechanisms are involved. By investigating how lifestyle-induced changes in these peripheral factors relate to changes in brain health, we can make stronger conclusions about the role of these peripheral-brain links in cognitive ageing. Moreover, elucidating peripheral-brain mechanisms might also explain individual differences seen in lifestyle interventions for healthy cognitive ageing.

Study Objectives and Hypothesis

We designed the HELI study (derived from the Dutch “HErsenfuncties na LeefstijlInterventie”, meaning “Brain function after lifestyle intervention”) to better understand the neurocognitive effects of a multidomain lifestyle intervention and underlying peripheral (central) mechanisms. The HELI study is a 6-month multidomain lifestyle intervention in older adults at risk of cognitive decline (based on lifestyle-modifiable cardiovascular risk factors) with a high- and low intensity intervention arm. We focus on 5 different lifestyle domains within the intervention period: diet, physical activity, stress management and mindfulness, cognitive training, and sleep. We will assess effects on multiple neuroimaging brain measures (primary: working memory task-related fMRI responses and performance, and quantitative imaging of CBF), peripheral measures related to the gut-immune-

brain axis (primary: blood inflammatory profile and fecal microbiota composition), as well as their connections. We propose that, compared to the low-intensity group, the high-intensity group will show greater improvement in central neuroimaging outcomes (e.g., fMRI, CBF) as well as peripheral outcomes (e.g., blood inflammatory profile and fecal microbiota composition). Moreover, we hypothesize that individual changes in central outcomes correlate with changes in peripheral outcomes, as summarized in Figure 1.

Methods: Trial design

The HELI study is a multicentre randomized controlled trial, aiming to investigate the effects of a 6-month multidomain lifestyle intervention on brain functioning and its relation with immunometabolic markers in ageing. Powered to include 104 total participants, HELI has two parallel-arm treatment groups: (1) a supervised “high-intensity” coaching intervention group with weekly group meetings, lifestyle-related information and exercises, and (2) a non-supervised “low-intensity” coaching intervention group, receiving information leaflets every two weeks. Because the participants of the high-intensity intervention arm were supervised in group context, we recruited and subsequently randomized participants in 4 different inclusion waves (between May 2022 and October 2023), consisting of 19-32 participants. Participants partook in outcome measure visits at baseline (T_0) and at 6 months follow-up (T_1) (see Figure 2).

The HELI study has been reviewed and accepted according to the Medical Research Involving Human Subjects Act (“Wet Medisch-wetenschappelijk Onderzoek met mensen” in Dutch) by an accredited Medical Research Ethics Committee (MREC), the MREC Oost-Nederland on December 2nd 2021 (ToetsingOnline filenumber NL78263.091.21, ClinicalTrials.gov ID NCT05777863).

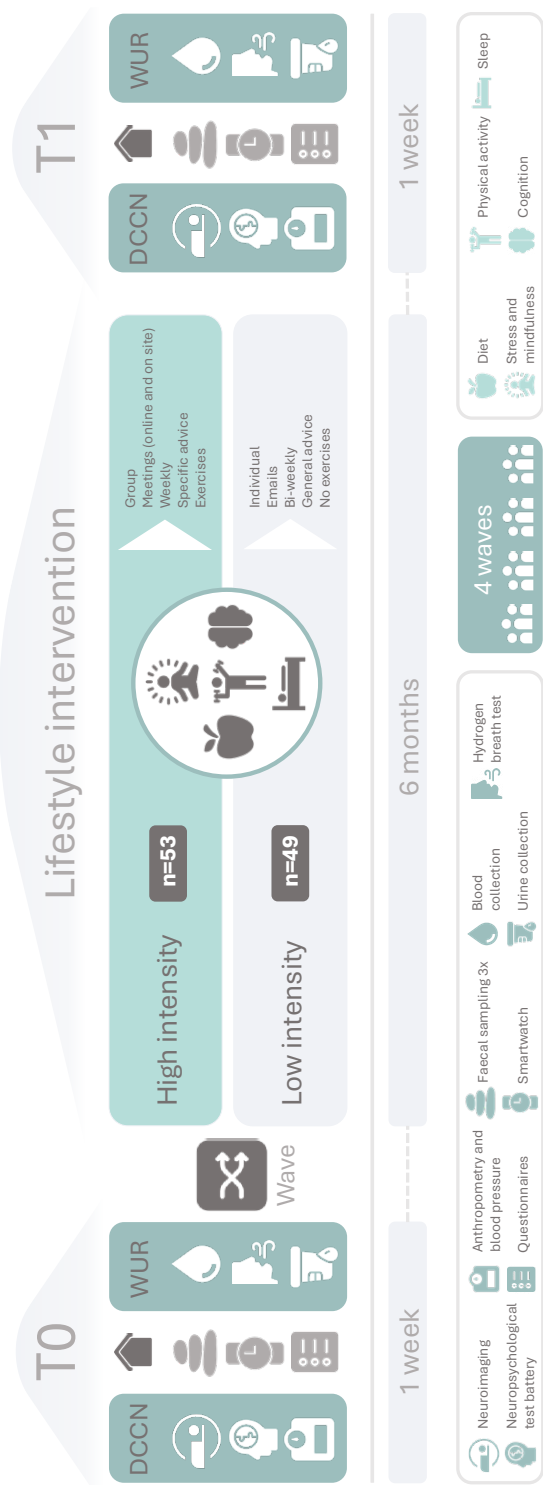


Figure 2. Overview study design. Schematic overview of study visits, assessments, and intervention groups. Before (T0) and after (T1) the intervention period of 6 months, participants visited the Donders Centre for Cognitive Neuroimaging (DCCN) and Wageningen University and Research (WUR) study centres with one week in between. Assessments included neuroimaging, neuropsychological tests, and anthropometrics (DCCN), faecal sampling, questionnaires, and wearing a smartwatch (at home), blood collection, urine collection and a hydrogen breath test (WUR). Participants received a 6-month multi-domain lifestyle intervention consisting of 5 domains: diet, physical activity, stress management & mindfulness, sleep and cognitive training. Each of the four inclusion waves was randomized over the two intervention arms (high intensity lifestyle intervention or low intensity lifestyle intervention) after T0 visits had been completed. The high intensity arm followed the intervention in group context, had weekly group meetings with supervisor, and received specific advice and exercises. The low intensity arm followed the intervention individually, received bi-weekly e-mails containing general advice and no exercises.

Methods: Participants, Interventions, and Outcomes

Study Setting

The HELI intervention is based on the FINGER-NL lifestyle intervention, a multicenter, randomized, controlled, multidomain lifestyle intervention effectiveness trial in The Netherlands. The rationale and study design of FINGER-NL has been published previously [108]. Both HELI and FINGER-NL are part of the overarching Maintaining Optimal Cognitive function In Ageing (MOCIA) research program [109], and follow an inclusion procedure with similar lifestyle modifiable risk factor inclusion criteria as the FINGER study [18, 110]. The HELI study is conducted at two separate research centers in The Netherlands, namely the Donders Institute for Brain, Cognition and Behavior, Center for Cognitive Neuroimaging (DCCN; Radboud University, Nijmegen, the Netherlands), and the Division of Human Nutrition and Health at Wageningen University and Research (WUR, Wageningen, the Netherlands). Study visits were performed at both centers, but inclusion, screening, randomization, guidance of the intervention, and monitoring was performed by the DCCN.

Recruitment

To achieve adequate participant enrolment and reach the target sample size of 104 included participants, we have used an array of recruitment methods. Our primary sources of enrollments came from: (1) recruitment advertising on Facebook; (2) WUR and DCCN research center participant databases; (3) newsletters of Dutch brain research institutions and associations (e.g., Netherlands Brain Foundation and Alzheimer Association Netherlands); (4) messages on WUR and DCCN research center websites and social media channels; (5) articles and recruitment dissemination via flyers, and in local newspapers, radio station, and television broadcast; and (6) word of mouth.

Eligibility Criteria

Inclusion Criteria

Individuals had to meet the following inclusion criteria to be eligible to participate in this study:

- (1) Aged between 60-75 (at moment of signing written informed consent);
- (2) Fluency in Dutch (speaking, reading and writing);
- (3) Willing to travel to the partaking research centers in Nijmegen and Wageningen (to ensure study center visits are plausible without excessive travel burden);
- (4) Score ≥ 2 points on the self-reported “Modifiable cardiovascular risk factor scale” (see Table 1 below), indicating a presently increased risk for cognitive decline

based on risk factors which are modifiable by lifestyle changes (adapted from Cardiovascular Risk Factors, Aging, and Incidence of Dementia [CAIDE] [111]).

Table 1. Modifiable cardiovascular risk factor scale.

Risk factor	Point(s)
Body Mass Index (BMI) ≥ 25 kg/m ² (overweight or obesity)	1
Physical inactivity (below the 2020 WHO guidelines [112]: <300 minutes of moderate intensity aerobic physical activity, or <150 minutes of vigorous intensity aerobic physical activity per week, spread out over several days)	1
Hypertension (systolic blood pressure ≥ 140 mmHg, and diastolic blood pressure ≥ 90 mmHg)	1 (2 points are assigned if hypertension is not being actively treated with antihypertensive medication, given the increased cardiovascular burden)
Hypercholesterolemia (total cholesterol > 5 mmol/L, or LDL-cholesterol > 3 mmol/L)	1
Diabetes type-II	1
Mild cardiovascular disease (e.g., intermittent claudication, varicose veins; In contrast, moderate or severe cardiovascular disease such as stroke, angina pectoris, heart failure, myocardial infarction or revascularization surgery in the last 12 months before pre-screening are exclusion criteria as described below under section “Exclusion criteria”)	1

Exclusion Criteria

Individuals who met any of the following criteria were excluded from participation:

Practicalities and Participation

Practical and participation-related factors, of one (or more) of the following:

- (1) Concurrent participation in other intervention trials;
- (2) No access to technological infrastructure (i.e., computers, smartphones, mobile applications, and internet access) because the intervention is primarily online (e.g., online support groups and mobile apps);
- (4) Cognitive impairment as determined by a score lower than 23 points on the Telephone Interview for Cognitive Status (TICS-M1) [113], performed during pre-screening before inclusion.

Clinical Diagnoses and Medical History

Clinical diagnosis by a certified doctor or general practitioner, of one (or more) of the following:

- (1) Cerebrovascular event (e.g., transient ischemic attack, cerebral hemorrhage, stroke);
- (2) Neurological disease (e.g., mild cognitive impairment, multiple sclerosis, Parkinson's disease, epilepsy);
- (3) Current malignant disease(s), with or without current treatment;
- (4) Current psychiatric disorder(s) (e.g., depression, psychosis, bipolar episodes);
- (5) Symptomatic, moderate to severe cardiovascular disease (e.g., stroke, angina pectoris, heart failure, myocardial infarction);
- (6) Revascularization surgery in the last 12 months at moment of pre-screening;
- (7) Inflammatory bowel disease (characterized by diarrhea);
- (8) Visual impairment, which is uncorrectable with visual aids (e.g., total or partial blindness);
- (9) Hearing or communicative impairment, which is uncorrectable with hearing aids;

Magnetic Resonance Imaging Safety-Specific Criteria

Contraindications for magnetic resonance imaging (MRI), of one (or more) of the following:

- (1) Metal objects located in the upper body (exceptions: tooth-fillings and/or dental crowns);
- (2) Metal splinters in the body, in particular within the eyes (e.g., through labor work in the metal industry);
- (3) Jewelry items or piercing that cannot be taken off;
- (4) Undergone brain surgery in the past;
- (5) Active implants within the body (e.g., pacemaker, neurostimulator, internal insulin pump, internal hearing aids that cannot be removed);
- (6) Medical plasters or patches which cannot or may not be taken off (e.g., nicotine patch);
- (7) Self-reported claustrophobia.

Interventions: Description

In the HELI multidomain lifestyle intervention five distinct lifestyle domains were combined, namely (1) diet, (2) physical activity, (3) stress management and mindfulness, (4) cognitive training, and (5) sleep. The intervention period was 6 months (26 weeks) in total, which was subsequently followed by the follow-up outcome measure visits (see Figure 3 for an overview).

The high-intensity intervention group received a structured and personalized intervention program consisting of weekly group sessions, active guidance from an intervention supervisor, and lifestyle domain-related individual and group exercises. The low-intensity intervention group received access to general lifestyle-related health information in an individual context, provided through information leaflets sent by e-mail once every 2 weeks. To promote intervention adherence and blinding, we adopted the FINGER-NL [108] and U.S. POINTER approach [114] of framing both groups as lifestyle intervention recipients, instead of using the label “control group”. The groups differ in intervention structure, engagement, and intensity rather than treatment versus control.

During communication with participants, we refrained from using the terms “high-intensity” and “low-intensity” to promote group blinding as much as possible. Instead, the intervention arms were called “weekly group sessions” for the high-intensity intervention group, and “independently working on your lifestyle” for the low-intensity intervention group. An extensive description and overview of the multidomain lifestyle intervention groups is provided below and depicted in Figure 3.

High-Intensity group

Design

In order to achieve the domain-specific goals and ambitions, we provided participants within the high-intensity group with three key intervention elements: (1) weekly online or on-site group sessions to provide information, exercises and personalized advice, (2) an extensive information folder containing supporting lifestyle-related information, exercises and relevant practical information, and (3) access to an online dashboard with a personal intervention environment. Each weekly group session took approximately 90 minutes. We expected that participants required an average of 90 minutes to put additional work in the provided lifestyle advices, exercises and other practical aspects (e.g., reading online dashboard and intervention information folder; see Supplements 3.1), resulting in a weekly expected intervention effort of 180 minutes.

Group Sessions

The weekly group sessions were offered throughout the 6-month (26 weeks) intervention period. For 20 out of 26 weekly sessions, a specific lifestyle domain acted as the central theme for a given week (Figure 3). At week 1, after the baseline measurements of all participants in a wave and subsequent randomization had been concluded (T_0), the intervention started off with an on-site introductory session.

The weekly group sessions provided participants with detailed lifestyle-related information, guidelines, exercises and advice, as well as the opportunity for discussion with other group members and with the intervention supervisor. Weekly group sessions were guided by the intervention supervisor and were composed of the following recurring elements: social interaction, introductory question round, reiteration of previous lifestyle domains, interactive dissemination, exercises, and a final question round (see Supplementary Table 1 for further details).

Over the 6-month intervention period, each of the five lifestyle domains was addressed in separate weekly group sessions (see Figure 3). We provided three distinct types of weekly group sessions:

- “Information sessions” primarily focused on giving general instructions, advice and exercises on a particular domain. During information sessions, participants were encouraged to ask questions, and share experiences, problems or advice with the group.
- “Boost sessions” primarily focused on recalling and reiterating domains which had been discussed in previous information or booster sessions, whilst also providing smaller amounts of new information. The main goal of boost sessions was to keep participants engaged in the multiple different lifestyle domains throughout the 6-month intervention period, and remind them of instructions, advice and exercises from previous sessions. During boost sessions, more time was reserved for repeating previously discussed material and information, group discussions, and sharing experiences, advice or problems regarding one or more specific domains.
- “Exercise sessions” primarily focused on explaining and performing muscle-strengthening exercises (full-body work-out, i.e., including all major muscle groups) in combination with balancing and stretching exercises in a group setting at an exercise center under the supervision of a physical exercise instructor specialized in instructing and guiding older adults. The exercise instructor provided instructions for conducting these exercises and for preventing strains or injuries.

Domains that were expected to require more instructions and guidance from the start (i.e., diet and physical exercise) have two information sessions and two boost sessions or one information session and two exercise sessions, whilst domains that required less initial group and personal guidance and instruction (i.e., stress management and mindfulness) had one information session and three boost sessions. The lifestyle domains of cognitive training and sleep differed from the information and boost session design, as these domains were composed of a psychoeducational program

for the domain of cognition (spanning four consecutive weeks) and a sleep course module for the domain of sleep (spanning five consecutive weeks), further explained below. A more detailed explanation of the ambitions, goals and content of the five lifestyle domains within the HELI lifestyle intervention is provided below and in Supplements 3.1.

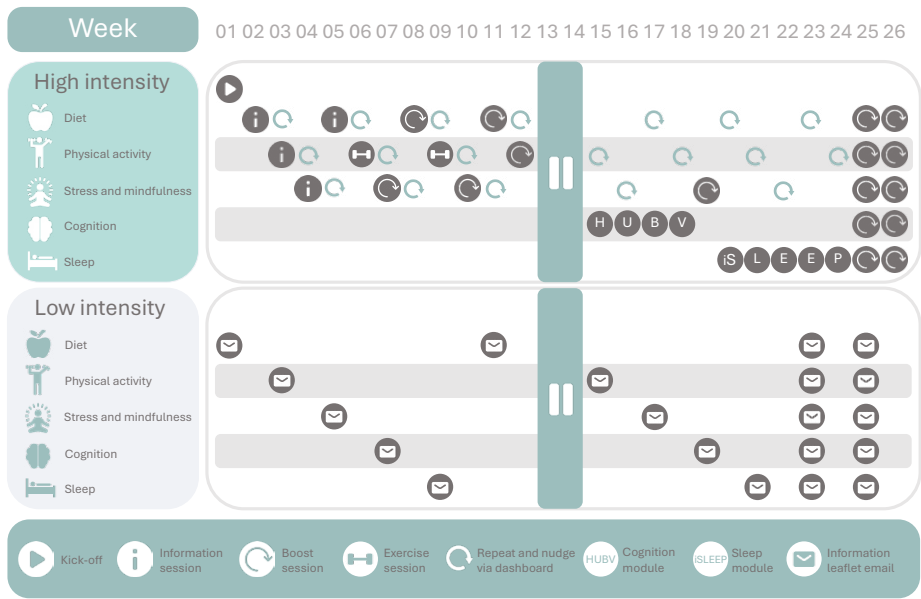


Figure 3. Intervention overview. High intensity: In the first week of the intervention, a kick-off session at location was used to give a general introduction and information about the study and the intervention set-up. The lifestyle domains of nutrition, physical activity, stress/mindfulness were structured with 1-2 information sessions and 1-2 boost sessions, whilst the domains of cognition and sleep were made up of 4/5 consecutive exercise weeks based on the validated modules “Houd uw brein vitaal” (HUBV) and “iSLEEP”, respectively. In addition, the domain of physical activity also included 2 exercise sessions at location. From week 25 onward, the sessions were used to recall all previously discussed domains whilst nearing the end of the intervention to keep participants engaged and to set future goals. Additionally, during the entirety of the intervention study period, previous domains were shortly repeated during the weekly meetings and participants received small domain-specific reminder messages through the online dashboard as a reminder to stay engaged with previous domains. Low intensity: this group only received general information and advice on a healthy lifestyle in the form of information leaflets shared by e-mail. Approximately halfway the intervention, a two-week break linked to summer or winter holidays was included for both the high and low intensity group.

Ambition and goals of lifestyle domains

1. Diet

Ambition and goals

The goal of the diet domain was to support participants to adhere to the Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet, a diet specifically designed to slow down aging-related cognitive decline [115], and the Dutch guidelines for a healthy diet [116] which is associated with protective effects on multiple major (chronic) diseases (e.g., coronary heart disease, stroke, diabetes, heart failure, and dementia). Specifically, we use the MIND-NL diet, which is adapted to the Dutch context and serving sizes [117]. Briefly, the MIND-NL diet specific food recommendations comprise (1) green leafy vegetables, (2) other vegetables, (3) berries and strawberries, (4) whole-grain products, (5) unsalted nuts, (6) (unfried) fish, (7) (unfried) poultry, (8) beans and legumes, and (9) olive oil. MIND-NL diet specific food restrictions comprise (1) full-fat cheese, (2) butter and stick margarine, (3) red and processed meat, (4) take-out, fried foods and snacks, (5) cookies, pastries and sweets; and (6) wine and alcoholic beverages [115]. The Dutch guidelines for a healthy diet consist of adhering to a healthy diet of the five major healthy food groups (in Dutch: “de Schijf van Vijf”): (1) fruit and vegetables, (2) (unsaturated) oil and spreads, (3) dairy, nuts, fish, legumes, meat, egg, or alternatives, (4) (whole-grain) bread, grain products and potatoes; and (5) hydration (water, tea, and coffee). As an additional component to this domain, to comply with Dutch national recommendations for older adults [118, 119], participants are advised to take additional vitamin D3 supplements throughout the intervention period (see Supplement 3.1 for age and sex-specific advised doses). For more details on the exercises, information and goals of the diet domain, see Supplements 3.1.

2. Physical activity

Ambition and goals

The goal of the physical activity domain was to support participants to adhere to the official 2020 WHO Physical Activity guidelines [112]. These guidelines recommend engaging in physical activity of moderate intensity (e.g., swimming, running, and cycling) for at least 300 minutes per week (spread out over several days), or 150 minutes of vigorous intensity aerobic physical activity (e.g., cycling at a fast pace and playing single tennis), or an equivalent combination of moderate- and vigorous-intensity physical activity (spread out over several days). Moreover, the guidelines recommend performing muscle-strengthening activities at moderate (or greater intensity) that involve all major muscle groups at least twice a week, combined with balance exercises. Lastly, amount of sedentary behavior (e.g., sitting and lying down) must be limited according to these guidelines.

3. Stress management and mindfulness

Ambition and goals

The goal of the stress management and mindfulness domain was to support participants in dealing with daily stress and becoming aware of their own thoughts and feelings, and to support participants in making brain-healthy choices regarding stress management. Participants received a selection of exercises from an evidence-based, self-guided online mindfulness training (provided through the VGZ Mindfulness Coach app [120]), as well as information regarding the potential negative effects of (chronic) stress on the body and brain, as well as practical advice on how to combat stress and improve relaxation.

4. Cognitive training

Ambition and goals

The goal of the cognitive training domain was to inform participants about functional and structural changes in the ageing brain, to support participants in learning about strategies that could aid in coping cognitive changes which are a part of normal ageing (e.g., informing participants about memory strategies and share tips to improve attention capabilities), and how to ultimately apply these strategies in daily life. The cognitive strategy training was based on the Dutch “Houd uw brein vitaal!” (HUBV; “Keep your brain fit!”) psychoeducational programme [121]. The HUBV program consists of two main parts: lifestyle & memory (complaints), and effective strategy training (see Supplements 3.1 for a more extensive description).

5. Sleep

Ambition and goals

The goal of the sleep domain was to support participants in improving sleep quality (and if applicable, reduce insomnia), and to inform participants about the possible health risks associated with poor sleep in ageing. Participants received a five-week sleep counselling module using a validated, guided, online Cognitive Behavioral Therapy for Insomnia (CBT-I), provided via i-Sleep (which was made available on paper to participants) [122, 123]. During the program, participants received information about the different stages of sleep, a healthy sleep pattern, how this pattern might change as we get older, and practical advice on how to improve sleep quality. The course consisted of the following modules: psycho-education, sleep hygiene, behavioral interventions (e.g., stimulus control and sleep restriction), relaxation, and dealing with worrying or sleep-obstructing thoughts. Specific recommendations and restrictions were also provided to improve sleep quality and quantity, such as proper diet, physical exercise, and refraining from smoking or using alcohol before bedtime.

Low-intensity group

Participants randomized to the low-intensity intervention group received general lifestyle-related health information, addressing the aforementioned five lifestyle domains. Participants received this general information through information leaflets (1-2 A4), through email, once every two weeks. The information leaflets discussed the lifestyle domains, but exclusively offered general health information without providing participants any individual or personal guidance based on their personal circumstances. For example: participants were generally told that a healthy diet promotes a healthy brain, and which kind of food components are promoted by the guidelines for a healthy diet, but participants were not guided or advised how their own personal diet should therefore be adjusted to fit these diet recommendations and guidelines.

The information of these leaflets was also handed to the high-intensity intervention group, to ensure both groups were provided with the same general health advice.

Outcomes

All outcome measures were measured at baseline (T_0) and after participation in the 6-month multidomain lifestyle intervention (T_1), during visits at both research centers. Blood drawing and breath tests were performed in a fasted state. Some outcome measures, such as lifestyle domain or adherence-related questionnaires, were also collected throughout the intervention period. For a detailed overview of all outcomes and the collection schedule, see Supplements 4.

Primary outcomes

The primary outcomes are the changes between baseline (T_0) and follow-up after 6 months (T_1) in:

- a) Brain activity during working memory in dlPFC [124] and hippocampus (Harvard-Oxford atlas), using fMRI blood-oxygen-level-dependent (BOLD) activity and task accuracy during a numerical N-back task [125];
- b) Cerebral perfusion levels, measured by MRI arterial spin labelling (ASL) in dlPFC and hippocampus;
- c) Peripheral immunometabolic biomarker levels in blood plasma (inflammation markers IL-6, TNF- α , hs-CRP) and faeces microbiota diversity (Shannon- and phylogenetic diversity) and richness (Chao1).

In addition, we will investigate intervention-induced gut-immune-brain links by assessing the relation between the effects found in the abovementioned primary peripheral markers and brain outcome measures.

Secondary outcomes

The secondary outcomes are the changes between baseline (T_0) and follow-up after 6 months (T_1) in:

- a) Structural MRI (e.g., gray/white matter volume, ventricular enlargement, abdominal adipose tissue T1 assessment), neurochemical assessment of neuroinflammation in dlPFC and hippocampus (using MRS) and whole brain analyses of primary neuroimaging outcomes (BOLD activity during working memory, cerebral perfusion);
- b) Anthropometric measurements (e.g., BMI, waist-to-hip ratio) and blood pressure;
- c) Cognitive test performance scores of cognitive domains predominantly affected by cognitive ageing (executive functioning, working memory and processing speed) as measured by a neuropsychological test battery (NTB) containing: (1) Rey Auditory Verbal Learning Test (RAVLT) [126], (2) Verbal Fluency Test (VFT) [127], (3) Trail Making Test (TMT) part A&B [128], (4) Digit Symbol Substitution Test (DSST) [129], and (5) Digit Span Test (DST) [129];
- d) Lifestyle domain-related questionnaire scores for (1) diet (MIND-adjusted Eetscore [130]), (2) physical activity (Short Questionnaire to Assess Health-enhancing Physical Activity (SQUASH) [131], LASA Sedentary Behavior Questionnaire (SBQ) [132] and SARC-F Sarcopenia Questionnaire [133]), (3) stress management and mindfulness (Perceived Stress Scale (PSS) [134], Hospital Anxiety and Depression Scale (HADS) [135], Five Facet Mindfulness Questionnaire (FFMQ) [136]), (4) cognitive training (Cognitive Failures Questionnaire (CFQ) [137] and Metamemory In Adulthood Questionnaire (MIA) [138]), (5) sleep (Pittsburgh Sleep Quality Index (PSQI) [139]).
- e) Physiological parameters measured on the wrist using a smartwatch over a 7-day period (day and night), including galvanic skin response, skin temperature and heart rate parameters (measured by photoplethysmography sensing), and contextual motion parameters (measured by an integrated 3-axis accelerometer and 3-axis gyroscope);
- f) Fecal analysis, including intestinal transit time [140], stool water content, pH, microbiota composition (both relative- and absolute abundances), metabolite profiles, and inflammatory biomarkers;
- g) Blood analysis, including intestinal integrity markers, inflammatory markers, cardio-metabolic and oxidative stress markers, nutritional status, (early) AD markers, and dietary- and microbiota-derived metabolites;
- h) Breath analysis to measure SIBO [141, 142].

Other study parameters

Other study outcomes include:

- a) Baseline demographics (age, sex, education level, ethnicity etc.) and medication history;
- b) Baseline intracranial myelin and iron deposition measured by quantitative susceptibility mapping (QSM) MRI, and by relaxation rates (R_1 and R_2^* – longitudinal and apparent transverse relaxation rates respectively);
- c) Baseline IQ based on the Dutch Adult Reading Test (DART) [143];
- d) Intervention adherence (in the high-intensity group) during the intervention period: group meeting attendance, and completion of provided exercises;
- e) Changes between baseline (T_0) and follow-up after 6 months (T_1) of the Montreal Cognitive Assessment (MoCA) [144];
- f) Urine analysis of microbiota-derived bioactive compounds (changes between baseline (T_0) and follow-up after 6 months (T_1);
- g) Changes between baseline (T_0) and follow-up after 6 months (T_1) in other health or lifestyle-related questionnaire scores, including (1) (psychosocial) complaints in daily life (Dutch Four Dimensional Complaint Questionnaire; 4DKL [145]), (2) quality of life (5-level EuroQol-5D (EQ-5D-5L [146]), (3) modifiable dementia risk score (“Lifestyle for BRAin health” (LIBRA) [147]), (4) social contact and perceived social support (Lubben Social Network Scale [148]), (5) cognitive coping strategies (Cognitive Emotions Regulation Questionnaire [149]), (6) apathetic symptoms (Starkstein Apathy Scale [150]), (7) gastrointestinal symptoms rating scale (GSRS) [151], and (8) stool consistency (Bristol Stool Scale [152]).

Sample size

The expected effect size for this study was determined based on other, mostly single-domain, lifestyle intervention studies with similar outcomes as our primary outcomes in the brain (e.g., cerebral perfusion, working memory assessments) and in the periphery (e.g., immunometabolic biomarkers, gut microbiome diversity). These studies generally found medium-to-large effect sizes [52, 53, 95, 106, 107, 153] (See Supplementary Table 4). Therefore, we deemed a slightly-above-medium effect size of 0.30 (Cohen’s $f(V)$) suitable for our primary outcomes from baseline (T_0 = week 0, before start intervention) to follow-up (T_1 = week 26, after end intervention). The sample size calculation was performed using GPower (version 3.1.9.6). Using a MANOVA (with repeated measures and within-between interaction) F-test with a power of 80% and a two-tailed α -error probability of 0.05, we calculated that a total of 90 study participants would be required. However, based on our experience with previous (f)MRI and intervention studies, we expect a potential loss of data of 15% due to poor (f)MRI data quality (e.g., motion artifacts)

and potential intervention drop-outs. Therefore, to reach sufficient power we would require a total sample size of 104 ($90 \text{ participants} \times 15\% \text{ potential data loss} = 103.5 = 104$) participants in this study.

Methods: Randomisation and blinding

Participants were randomized per inclusion wave, using stratified (2,4) block-randomization to provide a 1:1 allocation between our two intervention arms. Randomization was stratified by the factors sex (male vs. female), risk factor profile (medium risk 2-3 points vs. high risk ≥ 4 points) and a combined factor of education level + age (70+ years or low education vs. 60-69 years and high education, specified as university of applied science associate degree (Dutch: HBO associate degree) or higher). As with our sample size, (2,4) block-randomization was only possible with up to three strata, age and education were combined in one stratification factor as they are both important covariates of cognitive functioning and ageing-related cognitive decline [154].

An independent researcher from the DCCN was authorized to manually perform the randomization. This authorized researcher had no affiliation with the project and therefore had no bias in the allocation procedure.

Given the nature of the intervention arms, the participants and intervention supervisor were unable to remain blinded for the assignment to the intervention arms. Researchers conducting the follow-up outcome measure visits remained blinded and unaware of the intervention arm allocation. Only in case of unforeseen circumstances would deviations be made (e.g., an unblinded researcher conducting the MRI protocol). Neuropsychological tests and task trainings (i.e., n-back task) were conducted by a blinded researcher in all cases. Participants were asked to refrain from speaking about the intervention contents and experience during the follow-up outcome measure visits to prevent accidental unblinding.

Requirement for emergency unblinding was not applicable to this study, as participants were unblinded to the group allocation and the nature of the study setting.

Methods: Statistics and analysis

We will test for change between T_0 and T_1 in the primary and secondary outcome variables, both within-group and between-group using linear mixed-effect models with

random effects for intercept (individuals). Intervention group and time will be included as fixed effects, as well as the interaction term between intervention group and time to model the difference in change between T_0 and T_1 as a function of group allocation.

In addition, we will test for correlations in change (T_0 minus T_1) between (primary) brain outcomes and (primary) peripheral outcomes. We will select an appropriate correlation test for our data, based on whether assumptions are met. We will include both the high- and low intensity group if there is enough variance in change across both groups; else we will only include the high intensity group.

The level of significance will be set at 0.05 (two-sided). False Discovery Rate (FDR) correction will be used to account for multiple comparisons of secondary and exploratory outcomes. Family-Wise Error Rate (FWE) correction will be used for secondary whole-brain analyses.

As part of exploratory analyses to assess the domain-specific effects on our primary outcome measures, we will take the pre- and post-effects of the primary outcome measures and use domain-related questionnaire scores and smartwatch-derived physiological data as a measure of improvement for each respective domain as predictors in linear mixed-effect models.

We will perform an intention-to-treat analysis. Participants who withdrew from the study during the 6-month intervention period, but had not withdrawn consent, were invited to the T_1 (after week 26) follow-up outcome assessments. This means that we will use all available collected data, including collected (follow-up) data of participants who dropped out of intervention. Missing data or data with insufficient quality, however, will not be used in the analysis.

Results

This work was supported by a Crossover grant (MOCIA 17611) of the Dutch Research Council (NWO), granted in December 2019. Recruitment started in April 2022 and concluded in October 2023. Over this period a total of 279 people showed interest in participating and enrolled, of which 269 participated in the first round of telephone screening and 235 participated in the second round of telephone screening (see Figure 4). We were able to initially include our goal sample size of $N = 104$ participants, but two last-minute cancellations resulted in a final included sample size of $N = 102$ older Dutch adults. This sample size was subsequently randomized in the two parallel

intervention arms, with n = 49 participants in the “low-intensity” intervention group, and n = 53 participants in the “high-intensity” intervention group.

The baseline measurements at the DCCN and WUR centers were successfully completed by October 2023. The baseline mean age of our sample population was 66.6 (SD 4.3) years, of which 65.7% (n = 67) was female (see Table 2). The mean total years of education was 16.2 (SD 4.6) years. At baseline, 9.8% (n = 10) of participants had a low level of education (ISCED 0-2), 34.3% (n = 35) had a medium level of education (ISCED 3-4), and 55.9% (n = 57) had a high level of education (ISCED 5-8). The median risk, measured by points on the self-reported lifestyle-modifiable risk factor scale, was 3.0 (IQR: 2-3) points. The most common self-reported lifestyle-modifiable risk factors of cognitive ageing at baseline were overweight or obesity (74.5%), hypertension (56.9%), hypercholesterolemia (55.9%), and physical inactivity (55.9%). See Table 2 for an overview of the HELI study baseline characteristics.

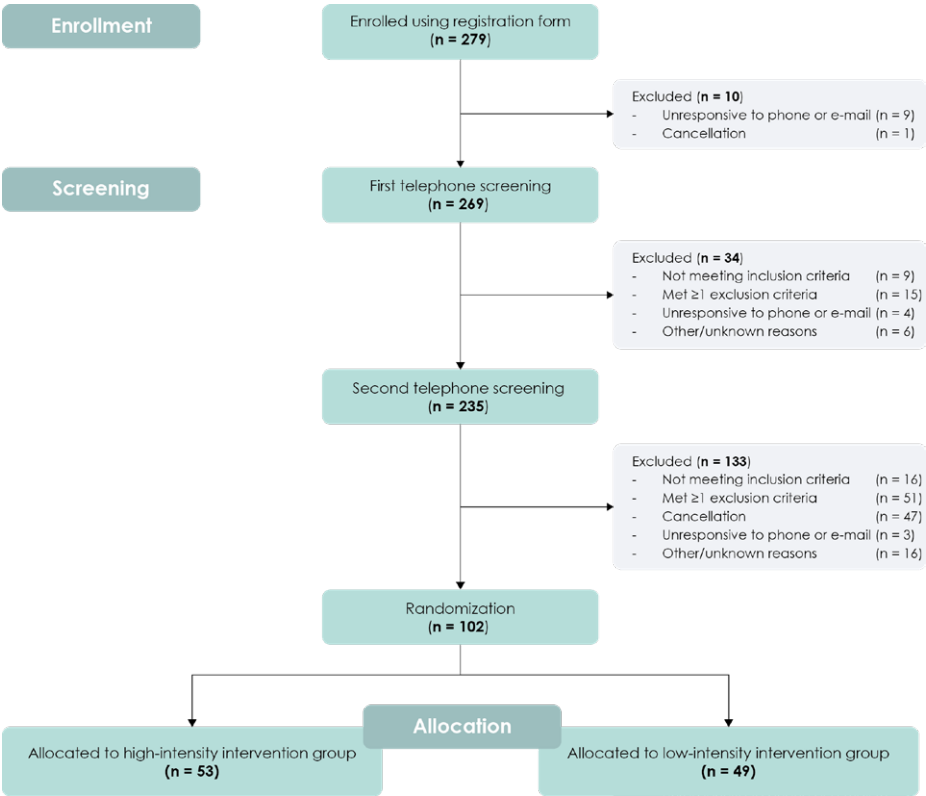


Figure 4. CONSORT study flowchart. Flowchart of HELI study participant enrollment, screening (inclusion and exclusion), randomization and allocation.

Table 2. Baseline characteristics of HELI study (N = 102)

Characteristic		
Participant demographics		
Age (years), mean (SD)	66.6	(4.3)
Sex (female), n (%)	67	(65.7)
Educational level, n (%) ^a		
Low	10	(9.8)
Medium	35	(34.3)
High	57	(55.9)
TICS-M1 score, mean (SD)	29.0	(3.6)
Self-reported inclusion factors		
Overweight and obese (Body Mass Index ≥ 25), n (%)	76	(74.5)
Obese (Body Mass Index ≥ 30), n (%)	35	(34.3)
Physically inactive, n (%) ^b	57	(55.9)
Hypertension, n (%) ^c	58	(56.9)
Hypertension medication usage, n (%)	84	(82.4)
Hypercholesterolemia, n (%) ^d	57	(55.9)
Diabetes type-II, n (%)	20	(19.6)
Mild cardiovascular disease, n (%) ^e	11	(10.8)
Risk severity		
Risk factor score, median (IQR)	3.0	(2.0, 3.0)

Abbreviations: SD standard deviation

^a Based on the International Standard Classification of Education (ISCED 2011) guidelines (low, medium, high education categorization)

^b Based on non-adherence to the WHO Guidelines on physical activity (300 or more minutes of moderate aerobic activity or 150 minutes of vigorous aerobic activity per week)

^c Based on systolic blood pressure of ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg

^d Based on total cholesterol > 5 mmol/L or LDL-cholesterol > 3 mmol/L

^e Based on mild cardiovascular disease not mentioned in exclusion criteria (e.g., varicose veins, arteriosclerosis)

Discussion

Principal findings

We described the design of the HELI study, an RCT focusing on the mechanisms of a 6-month multidomain lifestyle intervention in Dutch older adults at risk of cognitive decline, by assessing functional and structural MRI-related brain measures as well as peripheral measures related to the gut-immune-brain axis. The study design is based on the design of the FINGER-NL trial [108] and optimized for assessment of mechanisms instead of effectivity.

The HELI study is characterized by its unique multimodal approach, which enables us to obtain mechanistic insights of a multidomain lifestyle intervention. We are using a broad range of neuroimaging techniques (see Supplementary Table 3 for the MRI sequence protocol) and neurocognitive tests to acquire a comprehensive profile of brain health. In addition, we also measure an extensive set of peripheral intestinal- and immunometabolic markers, allowing us to investigate gut-immune-brain interactions and discover mediators of lifestyle effects on brain health. Combined with questionnaire data on lifestyle compliance, these brain, blood, and faecal markers have the potential to provide an elaborate overview on the pathways involved in lifestyle effects on cognitive ageing and to explain individual differences in effectivity.

Compared to previous 24-month multidomain lifestyle interventions in cognitive ageing [3, 18, 19, 155] and the FINGER-NL trial [108], the HELI study intervention of 6 months is significantly shorter. However, our multidomain lifestyle intervention was carefully modified from the 2-year FINGER-NL intervention into a more condensed and intensified 6-month program. Especially the high-intensity intervention group was characterized by more frequent meetings and group contact, requiring higher weekly effort. Within the field of functional neuroimaging, the duration of HELI (26 weeks) is relatively long compared to other (single-domain) lifestyle intervention studies (6-9 weeks) which reported significant central mechanistic intervention effects [52, 54]. The main focus of our study is on the underlying mechanisms of lifestyle adaptations on cognitive ageing, rather than on trial effectivity. Therefore, we consciously chose to use multiple specific brain and peripheral parameters as primary outcomes, instead of cognitive functioning. We expect these parameters to be more sensitive to lifestyle changes, and therefore to be affected in an earlier stage than cognitive functioning.

We ultimately managed to include a total of 102 participants within our study. Our study population shares a similar baseline lifestyle modifiable risk factor profile

(CAIDE) compared with the FINGER study, based on the total number of participants with hypertension, hypercholesterolemia, and diabetes (see Supplementary Table 5). Other previous multidomain lifestyle intervention studies in older adults, such as the pre-DIVA, MAPT and J-MINT studies, used inclusion criteria different from the CAIDE-related lifestyle modifiable risk factors, or included participants solely based on age. Compared with previous studies, our study population is relatively highly educated, and we were unable to include a comparable number of participants with a lower education level. However, many of these earlier studies completed participant inclusion over a decade ago, and education levels have generally increased over time, which may reflect cohort effects. Because education level is often used as a covariate in task-related neuroimaging outcomes and neuropsychological test data, this is an important limitation to consider during data interpretation and comparison of results with other studies. For a more detailed description of the baseline characteristic comparison between the HELI study and previous multidomain lifestyle intervention studies, see Supplementary Table 5.

To summarize, the most important strength of the HELI study is its unique combination of multimodal neuroimaging and peripheral phenotyping with a multidomain lifestyle intervention. Moreover, the study has several additional strengths. First, the design has two active arms which promotes blinding and intervention adherence, while preserving between-group comparisons of lifestyle interventions. Second, the group approach within the high-intensity group is a strength, as it encourages peer support and intervention adherence. Finally, the online-onsite combination establishes a future-oriented intervention framework that ensures both quality and broad applicability. Limitations of the study comprise the limited number of objective domain-specific adherence measures, and the selection bias towards more highly educated individuals, potentially due to the digital intervention format.

Conclusion

The HELI study offers a novel mechanistic approach, by integrating multimodal neuroimaging and peripheral phenotyping with a multidomain lifestyle intervention in older adults at risk of cognitive decline. Results of the HELI study can help explain the previously observed small effect sizes of multidomain lifestyle interventions to prevent cognitive decline in ageing, paving the way for personalized prevention strategies in the future.

Acknowledgments

Figure 1 was created with elements from BioRender [156], and adapted from BioRender, Remie L. (2025) [157]. Figures 1, 2 and 3 were created with elements from the Noun Project [158].

Funding Statement

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Conflicts of interest

Research programs of WF have been funded by ZonMW, NWO, EU-JPND, EU-IHI, Alzheimer Nederland, Hersenstichting CardioVascular Onderzoek Nederland, Health~Holland, Topsector Life Sciences & Health, stichting Dioraphte, Gieskes-Strijbis fonds, stichting Equilibrio, Edwin Bouw fonds, Pasman stichting, stichting Alzheimer & Neuropsychiatrie Foundation, Philips, Biogen MA Inc, Novartis-NL, Life-MI, AVID Radiopharmaceuticals, Roche BV, Eli-Lilly-NL, Fujifilm, Eisai, Combinostics. WF holds the Pasman chair. WF is recipient of ABOARD, which is a public-private partnership receiving funding from ZonMW (#73305095007) and Health~Holland, Topsector Life Sciences & Health (PPP-allowance; #LSHM20106). WF is recipient of TAP-dementia, receiving funding from ZonMw (#10510032120003).

WF has been an invited speaker at Biogen MA Inc, Danone, Eisai, WebMD Neurology (Medscape), NovoNordisk, Springer Healthcare, European Brain Council. All funding is paid to her institution. WF is consultant to Oxford Health Policy Forum CIC, Roche, Biogen MA Inc, and Eisai. All funding is paid to her institution. WF participated in advisory boards of Biogen MA Inc, Roche, and Eli Lilly. WF is member of the steering committee of EVOKE/EVOKE+ (NovoNordisk). All funding is paid to her institution. WF is member of the steering committee of PAVE, and Think Brain Health. WF was associate editor of Alzheimer, Research & Therapy in 2020/2021. WF is associate editor at Brain.

All other authors declare no conflict of interest.

Glossary

4DKL	Four Dimensional Complaint Questionnaire
AD	Alzheimer's disease
ASL	arterial spin labelling
BMI	body mass index
BOLD	blood-oxygen-level-dependent
CAIDE	Cardiovascular Risk Factors, Aging and Incidence of Dementia
CBF	cerebral blood flow
CBT-I	Cognitive Behavioral Therapy for Insomnia
CFQ	Cognitive Failures Questionnaire
CRP	C-reactive protein
DART	Dutch Adult Reading Test
DCCN	Donders Centre for Cognitive Neuroimaging
(dl)PFC	(dorsolateral) prefrontal portex
DSST	Digit Symbol Substitution Test
DST	Digit Span Test
EQ-5D-5L	5-level EuroQol-5D
FDR	False Discovery Rate
FFMQ	Five Facet Mindfulness Questionnaire
FINGER	Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability
fMRI	functional magnetic resonance imaging
FMT	fecal microbial transplantation
FWE	Family-Wise Error Rate
GSRS	Gastrointestinal Symptoms Rating Scale
HADS	Hospital Anxiety and Depression Scale
HELI	Hersenfuncties na LeefstijlInterventie
HUBV	Houd Uw Brein Vitaal
IL	interleukin
ISCED	International Standard Classification of Education
J-MINT	Japan-Multimodal Intervention Trial
LIBRA	Lifestyle for BRAin health
MAPT	Multidomain Alzheimer Prevention Trial

MCI	mild cognitive impairment
MIA	Metamemory In Adulthood
MIND	Mediterranean-DASH Intervention for Neurodegenerative Delay
MoCA	Montreal Cognitive Assessment
MOCIA	Maintaining Optimal Cognitive functioning In Aging
MREC	medical research ethical committee
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NTB	neuropsychological test battery
PET	positron emission tomography
PreDIVA	Prevention of Dementia by Intensive Vascular Care
PSQI	Pittsburgh Sleep Quality Index
PSS	Perceived Stress Scale
QSM	quantitative susceptibility mapping
RAVLT	Rey Auditory Verbal Learning Test
RCT	randomized controlled trial
SARC-F	Sarcopenia-F
SBQ	Sedentary Behavior Questionnaire
SCFA(s)	short-chain-fatty-acid(s)
SIBO	small-intestinal-bacterial-overgrowth
SQUASH	Short Questionnaire to Assess Health-enhancing Physical Activity
TMT	Trail Making Test
TNF	tumor necrosis factor
VFT	Verbal Fluency Test
WHO	World Health Organization
WUR	Wageningen University and Research

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Supplementary Materials

Recruitment: Screening and inclusion

Individuals willing to participate in the HELI study were directed to our online registration form in LimeSurvey (LimeSurvey GmbH, Hamburg, Germany. URL <http://www.limesurvey.org>). Within the online registration form, an initial pre-screening was included. Only individuals that passed the inclusion- and exclusion criteria pre-screening questions were redirected to the contact form in LimeSurvey, which shared their contact details with the researchers. No data from the pre-screening questions were saved within the survey.

Individuals who registered for the HELI study via the online registration- and contact form were subsequently contacted by phone by one of the study investigators, within 1 week. During this initial phone call, further study details were provided and potential participants had the ability to ask questions. After the phone call, the participant information folder containing extensive information about the study requirements and procedures, and a separate screening informed consent were sent to their provided home address by postal service. After 1-2 weeks, when the potential participant had read the study information, asked remaining questions and returned the signed screening informed consent, the screening informed consent was signed by an involved researcher and a telephonic screening was performed. During this screening by phone, all inclusion- and exclusion criteria were checked with the potential participant to ensure the participant was eligible for participation. In addition, a TICS-M1 (Modified Telephone Interview for Cognitive Status) [1] was performed to rule out participants with possible undiagnosed cognitive impairment. Subsequently, eligible participants were invited for baseline test assessments at the study centres.

A written informed consent for all study procedures was obtained during the first study visit, before randomization or any other study-specific procedures were performed.

Responsibility for intervention group guidance

For each inclusion wave a primary intervention supervisor was appointed. This supervisor role is carried out by one research team member, and their guidance responsibilities depended on the intervention arm (high-intensity, low-intensity or both). The intervention supervisors (MRvL, LBR, MGJ) remained in close contact with the lifestyle-domain experts of FINGER-NL, who assisted in designing the intervention content, to be able to get direct input (e.g. assistance in answering complex lifestyle-related questions). For the high-intensity intervention arm, the

intervention supervisor was responsible for preparing the schedule, organizing and presenting the weekly (online) group meetings, promoting group contact and peer-discussions, supporting and monitoring intervention adherence, discussing intervention-related problems, and addressing lifestyle-related questions from the group. For the low-intensity intervention arm, the intervention supervisor was responsible for sharing the health information periodically by e-mail and addressing lifestyle-related questions from participants by e-mail.

Intervention

High-intensity intervention

This first session was dedicated to introducing the participants to the intervention supervisor, presenting the concept of the intervention, handing over intervention material (e.g. information booklets), introducing the online dashboard, and registering the accounts necessary to gain access and participate in the online meetings and online dashboard. The online group sessions were hosted on a HELI-specific Microsoft Teams environment, with access to the online group sessions, slides and notes from previous sessions, a Q&A digital bulletin board, and a discussion channel to share personal experiences and advice.

The duration of each element depended on factors such as the needs of a particular group, total group size, or group session topic, but the intervention supervisor made sure that all necessary information for a particular session was communicated to the group. The elements of the group sessions were as follows:

- a) Before the start of the intervention session: as participants are joining the (online) session, the session focused on stimulating social interaction among the participants (e.g. discussing wellbeing).
- b) Introductory question round: participants were able to ask questions regarding all aspects of the intervention, such as practicalities or questions regarding previously covered lifestyle advice, exercises, use of the online dashboard, or questionnaires. Participants were encouraged to share advice and tips with one another.
- c) Reiteration of previous domains: participants received reminders of previously discussed lifestyle domain advice or exercises, or the supervisor started a short discussion about the current progress. The aim of this part of the group session was to remind participants of the advice and guidelines of the multiple different domains, and thereby maintain or improve adherence to all facets of the intervention throughout the entirety of the 6-month intervention period.

- d) Interactive information dissemination session; participants received lifestyle-related advice through an informal lecture-style presentation. Participants were then able to discuss this information with the supervisor and peers.
- e) Exercises; participants received domain-specific exercises to work on during the week after the group session. Exercises were subsequently discussed in the introductory question round of the next group session.
- f) Final question round and closing; participants were able to ask final questions, before the session was concluded.

Supplementary Table 1. Weekly high-intervention group session overview with domain-related session topics

Week	Domain	Session topics	Domains reiterated
1	-	Intervention introduction, introducing intervention supervisor and group members, registering accounts for applications, receiving IIF.	-
2	Diet	MIND diet introduction, MIND recommendations and restrictions, healthy diet patterns.	-
3	Physical activity	Guidelines physical activity, effect of physical activity on brain health, advice on moving more, taking into account safety whilst exercising.	Diet
4	Stress management & mindfulness	Information on what stress is, and how stress can affect brain health. Importance of relaxation on a healthy stress balance, introduction of mindfulness.	Diet + Physical activity
5	Diet	Information about reading food packaging labels, practicing making healthy diet choices by comparing different types of labels of similar products.	Physical activity + Stress management & mindfulness
6	Physical activity	Physical exercise session at exercise-centre (under supervision of physical exercise instructor), performing muscle-strengthening and balance exercises that can also be done at home.	-
7	Stress management & mindfulness	Discussion about how participants experience stress, and how participants handle stress and relaxation. A number of different mindfulness exercises and techniques are also discussed.	Diet + Physical activity
8	Diet	Examples of MIND-oriented breakfast, lunch, dinner and snack recipes. Exchange of advice, tips and encountered problems in adhering to the specific MIND recommendations and restrictions.	Physical activity + Stress management & mindfulness
9	Physical activity	Physical exercise session at exercise-centre (under supervision of physical exercise instructor), performing muscle-strengthening and balance exercises that can also be done at home.	-

Supplementary Table 1. Continued

Week	Domain	Session topics	Domains reiterated
10	Stress management & mindfulness	How does stress affect the way we eat, or exercise? Reiteration of some mindfulness exercises.	Diet + Physical activity
11	Diet	Interactive MIND quiz with questions regarding the MIND recommendations and restriction guidelines.	Physical activity + Stress management & mindfulness
12	Physical activity	Interactive physical exercise quiz with questions regarding the physical exercise (in the context of ageing, and the effects of exercising on body and brain health).	Diet + Stress management & mindfulness
13	-	Two-week break linked to summer or winter holidays	-
14	-	Two-week break linked to summer or winter holidays	-
15	Cognitive training	Information about memory, and memory complaints. Discussion about experiences with memory complaints. Exercise introduction of keeping a memory diary.	Diet + Physical activity + Stress management & mindfulness
16	Cognitive training	Information about external- and internal memory strategies to prevent complaints and decrease forgetfulness in daily life. Memory diary and cognitive training exercises are also discussed.	Diet + Physical activity + Stress management & mindfulness
17	Cognitive training	Information about the effect of attention on forgetfulness and memory, finding ways to stimulate the brain, thinking about the future. Cognitive training exercises are also discussed.	Diet + Physical activity + Stress management & mindfulness
18	Cognitive training	Prospects and goals for the near and distant future with regard to cognitive training are discussed. Memory diary and cognitive training exercises are also discussed.	Diet + Physical activity + Stress management & mindfulness
19	Stress management & mindfulness	Recap of mindfulness exercises. Prospects and goals for the near and distant future with regard to stress management and mindfulness are discussed.	Diet + Physical activity
20	Sleep	Information about the process of sleep (sleep cycles), the importance of sleep and factors that could influence sleep quality and quantity are discussed.	Diet + Physical activity + Stress management & mindfulness + Cognitive training
21	Sleep	Information about sleep patterns, and how to create a bedroom for improved sleep quality. The sleep diary and exercises are also discussed.	Diet + Physical activity + Stress management & mindfulness + Cognitive training

Supplementary Table 1. Continued

Week	Domain	Session topics	Domains reiterated
22	Sleep	Information about worrying thoughts, or fears that could impact sleep quality. The sleep diary and exercises are also discussed.	Diet + Physical activity + Stress management & mindfulness + Cognitive training
23	Sleep	Information about changing non-helping (negative) thoughts, which could impact sleep quality. The sleep diary and exercises are also discussed.	Diet + Physical activity + Stress management & mindfulness + Cognitive training
24	Sleep	Prospects and goals for the near and distant future with regard to cognitive training are discussed. The sleep diary and exercises are also discussed.	Diet + Physical activity + Stress management & mindfulness + Cognitive training
25	-	Reiteration of all discussed domains. Room for open discussion, and remaining questions. Future goals of adhering to the lifestyle changes are discussed.	-
26	-	Reiteration of all discussed domains. Room for open discussion, and remaining questions. Future prospects of adhering to the lifestyle changes are discussed.	-

Intervention Information Folder

During the first group session, which is hosted in-person by the intervention supervisor, all participants of the high-intensity intervention group received an Intervention Information Folder (IIF). The IIF contained information about, among other things, the study team and important contact information, time schedule, tutorials, domain-specific information and frequently asked (practical) questions.

Online dashboard

Participants within the high-intensity intervention arm were granted access to a personal intervention environment through an online dashboard. This online dashboard was part of the Online Personal Health Environment, of Ivido B.V. (certificates ISO 27001 and NEN 7510; CE-mark risk class I). We used this online dashboard to improve adherence to the intervention, by offering features such as reminders and invitations of online group sessions, or additional lifestyle domain-specific information and exercises. To keep participants engaged and periodically return to the online environment, new notifications were published on a weekly basis on the dashboard environment. After accepting the personal invitation to the HELI-specific dashboard environment, participants were instructed to periodically log-in to the dashboard (e.g. once every 2-3 days) to keep up to date with newly released notifications and features such as lifestyle-related exercises or advice.

1. Diet

Concise description

Participants were introduced and guided through the MIND diet as well as the Dutch guidelines for a healthy diet. Within the IFF a MIND-diet guide can be found which provides MIND-oriented food recipes for breakfast, lunch, dinner, and healthy snacks. For participants to create their own recipes, whilst adhering to the MIND-diet, information is also provided about reading labels on food packaging.

Apart from providing information, the intervention supervisor also encourages adherence to the MIND diet and Dutch guidelines for a healthy diet by sharing tips and recipes during the sessions and through the online dashboard and IIF. Additionally, the intervention supervisor assists in defining SMART (Specific, Measurable, Achievable, Relevant, Time-bound) formulated goals with respect to dietary change together with (individual) participants. Participants were also advised to take additional vitamin D3 supplements throughout the intervention period. The advised amount of vitamin D3 supplementation in our participant group of 60-75 years old was based on age, sex, exposure to sunlight and skin complexion:

- Men and women over 70 years of age: 20µg/day;
- Women under 70 years of age: 10µg/day;
- Men under 70 years of age, lacking exposure to direct sunlight: 10µg/day;
- Men under 70 years of age, with darker skin complexion: 10µg/day.

2. Physical activity

Concise description

During the group meetings (see Table 3), information is provided about the importance of being physically active and limiting sedentary behaviour for preserving brain health. Additionally, muscle-strengthening exercises (full-body work-out, i.e. including all major muscle groups) in combination with balancing and stretching exercises are explained and performed in a group setting during two group sessions, at an exercise centre (CleverMove, Wageningen, The Netherlands; <https://www.clever-move.nl>) under the supervision of a physical exercise instructor specialized in instructing and guiding older adults. The exercise instructor provides instructions for conducting these exercises and for preventing straining or injuries. These exercises, together with keeping up with a more active lifestyle (i.e. taking the bike or walking to do groceries instead of going by car, taking the stairs instead of an elevator, etc.) form the basis of the physical activity domain. Physical activity goals are set at the start of the intervention during the first physical activity domain group sessions and can differ between individuals based on individual level of fitness, regular habits and personal preferences. The exercises for both physical activity

and muscle strengthening gradually increase in frequency, intensity and duration over time.

Participants were also offered a mobile application to promote and keep track of daily walking ('Ommetje', Netherlands Brain Foundation), online exercise instructions with different difficulty grades suitable for older adults ('Cordaan Senioren Fitness Gym', Youtube; 'Heel Nederland Thuis in Beweging', MAX Vandaag), and a buddy system to jointly exercise.

3. Stress management & mindfulness

Concise description

Stress management & mindfulness counselling consists of an evidence-based, self-guided online mindfulness training (provided through the VGZ Mindfulness Coach app) [2]. A selection of exercises from the mobile VGZ Mindfulness Coach app are included in the online dashboard (which simultaneously acts as an active reminder to periodically follow the exercises), such as a 3-minute breathing exercise, body scan, mindfulness theory and visualization and counting of breath. These exercises consist of self-guided (audio) training, with 6 individual online sessions per week with an approximated time investment of 15 minutes each. Once introduced in week 4 of the intervention (see Table 3), the 3-minute breathing exercises from the VGZ Mindfulness Coach app are a regular component to start off the weekly group session throughout the entirety of the rest of the intervention period. Additionally, participants receive information regarding the potential negative effects of (chronic) stress on the body and brain, as well as practical advice on how to combat stress and improve relaxation.

4. Cognitive training

Concise description

The cognitive strategy training is based on the Dutch 'Houd uw brein vitaal!' (HUBV; 'Keep your brain fit!') psychoeducational programme [3]. The HUBV programme consists of two main parts: lifestyle & memory (complaints), and effective strategy training (see Table 3). Within the lifestyle & memory (complaints) part, participants receive information about forgetfulness and memory-strategies to incorporate into their lifestyle patterns. Participants are also instructed to keep a memory diary for a number of days. Within the effective strategy training, participants receive information about the importance of attention, information processing, action planning and goal management in order to prevent forgetful behaviour and set goals. Exercises are provided to participants to put this information to practice and incorporate effective strategies into their lifestyle patterns. The HUBV module is concluded with formulating (near and distant) prospects and goals.

5. Sleep

Concise description

The domain of sleep is addressed in five different group meetings, consisting of five consecutive sessions according to a specialized sleep-therapy programme. The sleep counselling uses a validated, guided, online Cognitive Behavioural Therapy for Insomnia (CBT-I), provided via i-Sleep (<https://www.i-sleep.nl>; which is made available on paper within the IIF) [4]. For the current study, a version of i-Sleep was created with small textual changes to enhance suitability for the study group (i.e. with a focus on older adults, not solely individuals with insomnia) without changing the actual programme contents. Each of the five individual and consecutive i-Sleep module group sessions provide theory and exercises. Exercises include keeping a sleep diary, and setting and tracking specific bedtimes. Participants are instructed to complete one session of the module (containing information and exercises) every week.

Throughout the entire 5-week programme, participants are asked to keep track of their sleep patterns by means of a daily sleep diary. In week 1, participants receive information about their sleep patterns and habits, and exercises are centred around keeping a sleep diary and answering questions about their current sleep management goals and their current lifestyle patterns. In week 2 and 3, emphasis is put on informing participants about how to improve current sleep patterns (e.g. via sleep restriction, by decreasing worrying thoughts or fear, and decreasing restlessness by finding relaxing activities). Participants are asked to remain logging the sleep diary, and exercises are offered to reflect on possible pitfalls or dangers that could stand in the way of improving current sleep patterns. In week 4, participants are informed about changing non-helping thoughts and their effects on sleep quality, and subsequently receive exercises to practice handling these kinds of thought patterns. In week 5, a summarization of week 1 through 4 is given and goal management is discussed to formulate future plans (and to longitudinally and effectively adhere to new or adjusted sleep patterns if applicable). During the programme, participants additionally receive information about the different stages of sleep, a healthy sleep pattern, how this pattern might change as we get older, and practical advice on how to improve sleep quality (e.g. setting specific times for sleeping, preparing the bedroom, finding relaxation before sleeping by shutting off electronic devices, etc.). Specific recommendations and restrictions are provided to improve sleep quality and quantity, such as proper diet, physical exercise, and refraining from smoking or using alcohol before bedtime.

Intervention: Modifications

All participants in the high-intensity intervention arm received the same lifestyle-related information and exercises. However, the intervention supervisor pursued a

personalized approach where each participant was encouraged and guided to more closely adhere to the lifestyle advice of domains which they are personally most interested in.

The low-intensity intervention arm had no personalized approach design. Participants received no additional personal supervision with regard to the information relayed in the biweekly general health information leaflets.

Intervention: Adherence

Improving adherence in order to limit drop-out rate, we utilized efforts to keep participants involved throughout their entire study participation (from baseline outcome measurement scheduling to intervention participation and subsequent follow-up outcome measurement scheduling). The high-intensity intervention group received weekly tips and reminders through the online dashboard, participated in the weekly group meetings and filled in monthly adherence questionnaires as well as a more extensive adherence questionnaire halfway through the intervention, thereby being reminded of the importance to stay on track of the intervention and granting the possibility for the intervention supervisor to identify adherence problems of individual participants throughout the intervention period. The low-intensity intervention group received bi-weekly information leaflets and completed the extensive adherence questionnaire halfway through the intervention. To improve adherence to the outcome visit measurements and prevent last-minute cancellations or no-shows, participants were sent appointment reminders through e-mail and/or by post.

Participant timeline

Participants underwent assessments at baseline (T_0) and at follow-up (T_1) at the two associated research centres with approximately one week in between visits (*Figure 3*). In the week between visits, participants were asked to collect faecal samples, wear a smartwatch and complete questionnaires (as described below).

At T_0 and T_1 participants visited the DCCN in Nijmegen for the following study aspects and assessments:

- **Signing informed consent forms.** At T_0 the full written informed consent form was signed in attendance of an involved research team member. Additionally, an MRI-screening and informed consent form was signed to check MRI-safety issues before actual scanning commences, both at T_0 and T_1 .
- **Information and practicing task.** Participants received information about the course of the day and practice the working memory n-back task.

- **MRI-scanning.** Participants underwent MRI-scanning of the brain and abdomen. Only after participants had been deemed metal free and safe to enter the MRI-room, the scan took place. The preparation and scans together had a duration of approximately 1.5 hours.
- **Anthropometrics and blood pressure assessment.** Length and weight were measured before MRI scanning. Blood pressure and heartrate were measured twice (or three times in case of large differences between first two measurements) on the non-dominant arm using an electronic arm blood pressure monitor (Omron X3). Participants had no coffee or tea before blood pressure were measured, to avoid influences of caffeine. Hip- and waist circumference were measured using a tape measure.
- **Transit time analysis.** Participants were asked to eat two muffins containing blue colouring agents (E133, E122) within 10 minutes, in accordance with the ‘blue poo’ method [5]. Participants received an information leaflet and gastrointestinal questionnaires to fill in at home.
- **Neuropsychological test battery (NTB).** Participants underwent a cognitive assessment for executive function, working memory and processing speed. The full assessment was performed in a neutral room and took approximately 45 minutes.
- **Exchange of materials and information.** Participants received the materials required to collect and store three faecal samples in their freezer, ingredients for a standardized meal to be consumed on the evening before the WUR study centre visit, a Chill+ smartwatch provided by IMEC Oneplanet (Wageningen, the Netherlands), and corresponding instructions. The smartwatch measured skin temperature, galvanic skin response, and movement. Participants were asked to wear this smartwatch for 7 consecutive days (1 week) during day and night and were instructed to charge the Chill+-smartwatch for at least an hour every day, preferably on the same time point. Additionally, participants received an invitation by e-mail via Castor EDC and Eetscore to fill in lifestyle domain-related questionnaires. Participants could fill in these questionnaires at their own pace at home.

In total, the DCCN study centre visit took approximately 4 hours from start to finish.

After this week, at T_0 and T_1 , participants visited the WUR whilst fasted since 20:00 the evening before, for the following study aspects and assessments:

- **Collect faecal samples.** In addition, the smartwatch and completed questionnaires were returned.
- **Blood drawing.** Blood was drawn via a finger-prick for direct measurement of CRP and white blood cell counts, and intravenously from the arm. In total, 25 mL

blood was collected: 6 mL in a serum tube, 6 mL in a lithium heparin plasma tube, 2 mL in a sodium fluoride plasma tube, and 13 mL in two K₂EDTA tubes, of which 3 mL for whole blood collection and analyses, and the 10 mL for plasma collection.

- **Urine collection.** Urine was collected during the visit on location.
- **SIBO detection.** A hydrogen breath-analysis after consumption of 75 gram glucose was performed to measure potential presence of SIBO. Diabetes patients were exempted from the SIBO breath test.

In total, the WUR study centre visit took approximately 3 hours from start to finish.

In Supplementary Table 2 below, a summary of the complete participant timeline can be found, including enrollment, assessments, outcomes and interventions.

Supplementary Table 2. Continued

Study phase	Enrollment	Baseline visits	Randomization	Intervention period						Follow-up visits	
Duration per wave	Continuous	2-3 months	1 week	6 months (26 weeks)						1-1.5 months	
Time point	E	T ₀	R	m ₁	m ₂	m ₃	T _{1/2}	m ₄	m ₅	m ₆	T ₁
Blood		X									X
Urine		X									X
Breath		X									X
Questionnaires		X		X	X	X	X	X	X	X	X
Smartwatch		X									X
Outcomes											
Primary											
dIPFC and hippocampus brain activity during working memory		X									X
Working memory performance		X									X
dIPFC and hippocampus cerebral perfusion levels		X									X
Blood IL-6, TNF- α , hs-CRP levels		X									X
Faecal microbiota diversity		X									X
Secondary											
Body mass index		X									X
Waist circumference		X									X

Supplementary Table 2. Continued

Study phase	Enrollment	Baseline visits	Randomization	Intervention period						Follow-up visits	
Duration per wave	Continuous	2-3 months	1 week	6 months (26 weeks)						1-1.5 months	
Time point	E	T ₀	R	m ₁	m ₂	m ₃	T _{1/2}	m ₄	m ₅	m ₆	T ₁
Metabolic profile in blood		X									X
Inflammation profile in blood		X									X
Intestinal integrity profile in blood		X									X
Brain health profile in blood		X									X
White blood cell count in blood		X									X
Small intestinal bacterial overgrowth		X									X
Other											
Baseline demographics questionnaire		X									
Intracranial iron deposition and myelin		X									X
Urine microbiota-derived bioactive compounds		X									
Gastro-intestinal questionnaires		X									X
Other questionnaires		X									X

Abbreviations: *dIPFC* dorsolateral prefrontal cortex; *HI* supervised High-Intensity coaching intervention group; *hs-CRP* high-sensitivity C-reactive protein; *IL-6* interleukin-6; *LI* unsupervised Low-Intensity coaching intervention group; *TICS* Telephone Interview for Cognitive Status; *TNF-α* tumor necrosis factor alpha

Supplementary table 3. HELL MRI sequence protocol.

Scan type	Sequence	TRT1/T12 (ms)	TE (ms)	Voxel size (mm)	Flip angle (°)	FOV(AP/RL/FH; mm)	Acceleration	Acquisition Time (min:sec)	Comments/ other
T1-weighted	MP2RAGE	6000 700/2400	2.34	1.0 × 1.0 × 1.0	6/6	216/176/256 (sagittal slab positioning)	iPAT 3	7:32	Used for anatomical reference and R1 maps
	VIBE	7	TE ₁ /TE ₂ = 2.46/3.69	1.2 × 1.2 × 3.0	9	274/380/108 (perpendicular between L4/ L5 spinal segments)	CAIPI 6 (2y,3z,2 shiftz)	0:10	Abdominal T1 for visceral/ subcutaneous adiposity distribution
B1 map	turboFLASH	10000	2.23	3.3 × 3.3 × 2.5 (2.5 slice spacing)	Sat: 80 Exc: 8	212/212/210	-	0:20	Used for B1
MRS	PRESS	2000	35	2.0 × 2.0 × 2.0	90x180x180	20/20/20 (in dlPFC and hippocampus)	-	2:18 (64 averages)	With water suppression
	PRESS	2000	35	2.0 × 2.0 × 2.0	90x180x180	20/20/20 (in dlPFC and hippocampus)	-	0:42 (16 averages)	No water suppression, for reference
WM task- based fMRI	EPI	1500	33.40	2.0 × 2.0 × 2.0	75	213/213/136 (transversal slab positioning)	Multi band 4	16:26	N-back (0b, 1b, 2b) working memory task

Supplementary Table 3. Continued

Scan type	Sequence	TRT1/TI2 (ms)	TE (ms)	Voxel size (mm)	Flip angle (°)	FOV(AP/RL/FH; mm)	Acceleration	Acquisition Time (min:sec)	Comments/ other
Perfusion imaging	ASL	4000 Saturation mode 90 And WET background Suppression	16.28	1.8 × 1.8 × 3.5	Exc: 90 Ref: 120	224/146/80 (transversal slab positioning)	TurboFactor12 EPI Factor 21 CAIPI 4 (2y,2z,1 shiftz) XA platform	4:20	SubBolus Dur: 400ms RF FA 25 RFgap = PLD 100ms 8-PLD time- encoded pCASL
	Mo	3000 Saturation mode 90	16.28	1.8 × 1.8 × 3.5	120	224/146/80 (transversal slab positioning)	TurboFactor12 EPIFactor 21 CAIPI 4 (2y,2z,1 shiftz) XA platform	0:39	Calibration image for time- encoded pCASL (500ms /1500ms /2500ms)
R2*/QSM	Multi-echo GRE	40	TE/ΔTE/TE ₀ = 3.54/5.32/35.46	0.9 × 0.9 × 0.9	15	230/188/266 (sagittal slab positioning)	CAIPI6 (1y,6z,2 shiftz)	8:34	Magnitude and phase saved, used for R2* and QSM computation

Abbreviations: AP anterior-posterior; ASL arterial spin labelling; CAIPI controlled aliasing in parallel imaging; dIPFC dorsolateral prefrontal cortex; EPI echo planar imaging; FH foot-head; fMRI functional magnetic resonance imaging; FOV field of view; GRE Gradient Echo Imaging; iPAT integrated parallel acquisition technique; Mo equilibrium magnetization; MP2RAGE Magnetization Prepared 2 Rapid Acquisition Gradient Echoes; MRS magnetic resonance spectroscopy; pCASL pseudocontinuous arterial spin labelling; PLD post-labelling delay; PRESS Point RESolved Spectroscopy; QSM quantitative susceptibility mapping; RF FA radiofrequency flip angle; RL right-left; TE echo time; TI inversion time; TR repetition time; VIBE Volumetric Interpolated Breath-hold Examination; WM working memory

Sample size

Supplementary Table 4. Overview of effects and effect sizes of previous relevant lifestyle (intervention) studies

Paper	Lifestyle domain(s)	Intervention duration	Active control	Observed effect(s)	Effect size
Brain function (MRI & cognition)					
Kleinloog <i>et al.</i> [6] Frontiers aging Neuroscience Dec. 2019	Exercise	2 months 3x 50 min per week	×	↗frontal lobe CBF	0.393 – 0.552 (<i>d</i>)
Thomas <i>et al.</i> [7] J. Alzheimer's Disease: JAD May. 2020	Exercise	1 year 3x 30 min per week	✓	↗hippocampal CBF	0.58 (<i>d</i>)
Li <i>et al.</i> [8] Frontiers Aging Neuroscience Mar. 2014	Exercise and Cognition (+Social)	6 weeks 3x 1h cognitive and exercise group sessions per week	✓	↗RS functional connectivity between medial PFC and medial temporal lobe	–0.036 to 0.201 (<i>z(r)</i>)
Valls-Pedret <i>et al.</i> [9] J. Alzheimer's Disease: JAD Jan. 2012	Diet	1 year Weekly meal plan	✓	↗(working) memory & MMSE in Med. diet components	0.094 – 0.149 (β)
Hardman <i>et al.</i> [10] Curr. Dev. in Nutrition: CDN Apr. 2020	Diet and/or Exercise	6 months Increasing walking times		↗Working memory (only in combined training)	0.06 (η^2)
Oswald <i>et al.</i> [11] European Journal of Ageing Nov. 2006	Cognition and/or Exercise	30 weeks 1x 45 min cognition or 90 min cognition and exercise per week	✓	↗composite cognitive function (in combined training)	0.75 (<i>d</i>)

Supplementary Table 4. Continued

Paper	Lifestyle domain(s)	Intervention duration	Active control	Observed effect(s)	Effect size
Joubert & Chainay [12] Clinical Interv. in Aging Jul. 2018	Cognition and/or Exercise	N/A: Systematic review	×	Both cognitive and physical training preferentially impact and enhance specific brain structures and function	Globally: η^2 0.001 – 0.88 in cognitive training; 0.01 – 0.97 in physical training; 0.14 – 0.34 in combined
Inflammation					
Ruangthai <i>et al.</i> [13] Arch. Of Gerontol. and Geriatr. Oct. 2020	Exercise	3 months 3x 1 hr combined land or combined water- based exercise	×	↓hs-CRP levels in both combined land or combined water-based exercise compared to control	0.7 – 1.1 (ES given as a standardized calculation of differences in group means ^a)
Sanada <i>et al.</i> [14] Int. J. of Molecular Sciences Apr. 2020	Mindfulness	N/A Systematic Review & meta-analysis	×	↓IL-6, TNF- α , and hsCRP after mindfulness-based intervention	0.6 – 0.66 (<i>d</i>)
Corrêa <i>et al.</i> [15] Scientific Reports Jul. 2020	Exercise	3 months 3x 50 min weekly resistance training	×	↓TNF- α levels, also associated with increased total sleep time affected by exercise	-0.23 (<i>r</i>)
Microbiome					
Grosicki <i>et al.</i> [16] Sleep Medicine Sep. 2020	Sleep	1 month Self-reported sleep quality	×	↑Gut microbial diversity in high self-reported sleep quality	-0.394 – -0.566 (<i>r</i>) and 0.474 – 0.553 (<i>r</i>)
Li <i>et al.</i> [17] Nature and Science of Sleep Nov. 2020	Sleep	1 month self-reported sleep quality assessment	×	↑microbiome richness and diversity associated with superior sleep quality	0.631 – 0.721 (<i>r</i>)

Supplementary Table 4. Continued

Paper	Lifestyle domain(s)	Intervention duration	Active control	Observed effect(s)	Effect size
Morita <i>et al.</i> [18] Nutrients Apr. 2019	Exercise	3 month Weekly 1h group training	✓	↑microbiome (<i>Bacteroides</i>) correlation following exercise	0.371 – 0.431 (<i>r</i>)
Microbiome-cognition links					
Bagga <i>et al.</i> [19] Gut Microbes Nov. 2018	Dietary supplement	1 month use of multispecies probiotics	✓	↑emotional memory, correlated with microbiome (<i>Bacteroides</i>)	0.3 – 0.4 (<i>r</i>)
Bloemendaal <i>et al.</i> [20] Translational Psychiatry Apr. 2021	Dietary supplement	1 month use of multispecies probiotics	✓	↑stress-related working memory performance, correlated with (<i>Ruminococcaceae</i> UCG003)	0.565 (<i>r</i>)

r 0.1 (small), 0.3 (medium), 0.5 (large)
d 0.2 (small), 0.5 (medium), 0.8 (large)
 η^2 ≥ 0.01 (small), ≥ 0.06 (medium), ≥ 0.14 (large)
* The standardized differences in means ES of 0.6 – 1.1 approximately translates to a Cohen's *d* of 0.3 – 0.5

Results

Supplementary Table 5. Baseline characteristic comparison of HELI study with previous multidomain lifestyle intervention studies

Study reference	Age (years)	Sex (females)	Education	Risk factors used for inclusion criteria and comparison of reported lifestyle-related risks
HELI study (present study)	66.6 (± 4.3)	67/102 (65.7%)	16.2 (± 4.6) total years 10/102 (9.8%) low level (ISCED 0-2) 37/102 (36.3%) medium level (ISCED 3-4) 55/102 (53.9%) high level (ISCED 5-8)	Inclusion based on 2 or more lifestyle modifiable risk factors of CAIDE dementia risk score: Overweight: 76/102 (74.5%) Physical inactivity: 57/102 (55.9%) Hypertension: 58/102 (56.9%) Hypercholesterolemia: 57/102 (55.9%) Diabetes: 20/102 (19.6%) Mild cardiovascular disease: 11/102 (10.8%)
FINGER-study Kivipelto <i>et al.</i> [21]	69.3 (± 4.7)	551/1190 (46.4%)	10.0 (± 3.4) total years	Inclusion based on CAIDE dementia risk score of 6 or higher. Physical inactivity: 343/1180 (29.1%) Hypertension: 779/1177 (66.2%) Hypercholesterolemia: 803/1180 (68.1%) Diabetes: 150/1180 (12.7%)
Pre-DIVA Richard <i>et al.</i> [22]	74.4 (± 2.3)	552/1004 (55.0%)	271/1004 (27%) ≤7 total years 615/1004 (61.3%) 7-12 total years 117/1004 (11.7%) ≥12 total years	Inclusion based on age and previous admission to general practitioner. Overweight: 743/1004 (74.0%) Physical inactivity: 94/1004 (9.4%) Mean SBP ± SD: 157 (21) Mean DBP ± SD: 80 (11) Total cholesterol >6.5mmol/L: 224/1004 (22.3%) Diabetes: 204/1004 (20.3%)

Supplementary Table 5. Continued

Study reference	Age (years)	Sex (females)	Education	Risk factors used for inclusion criteria and comparison of reported lifestyle-related risks
MAPT study Vellas <i>et al.</i> [23]	75.3 (± 4.4)	1088/1680 (64.8%)	85/1680 (5.2%) no diploma	Inclusion based on age (≥70 years) with spontaneous memory complaints, limitation in one instrumental activity of daily living, and slow gait. Mean BMI ± SD: 26.1 (4.1) Mean SBP ± SD: 141.0 (19.7) Mean DBP ± SD: 78.8 (11.5)
			286/1680 (17.4%) primary school certificate	
			553/1680 (33.7%) secondary education without high-school diploma	
			719/1680 (43.8%) high-school diploma or higher	
J-MINT Sakurai <i>et al.</i> [24]	74.5 (± 4.9)	226/433 (52.2%)	12.6 (± 2.5) total years	Inclusion based age (65–85 years) with cognitive decline adjusted for age and education. No lifestyle-related risk factors reported.
AgeWell.de trial Zülke <i>et al.</i> [25]	69.0 (± 4.9)	611/1176 (52.0%)	13/1030 (1.3%) ≤7 total years	Inclusion based on age (60–77 years) and a CAIDE dementia risk score of 9 or higher. Obese: 676/1176 (57.5%) Physical inactivity: 301/1176 (25.6%) Hypertension: 1000/1176 (85.0%) Hypercholesterolemia: 633/1176 (53.8%)
			456/1030 (44.3%) 7–9 total years	
			561/1030 (54.5%) ≥10 total years	
			227/1030 (22.1%) CASMIN 1 (i.e. ISCED 0–2)	
			546/1030 (53.0%) CASMIN 2 (i.e. ISCED 3–4)	
			256/1030 (24.9%) CASMIN 3 (i.e. ISCED 5–8)	

Abbreviations: BMI Body Mass Index; CAIDE Cardiovascular Risk Factors, Aging and Incidence of Dementia [26]; CASMIN Comparative Analysis of Social Mobility in Industrial Nations [27]; DBP diastolic blood pressure; ISCED International Standard Classification of Education [28]; SBP systolic blood pressure; SD standard deviation; Values are described as mean (±SD), or as n/total number of participants (%). The column with the comparison of lifestyle-related risks only shows data of lifestyle-related risk which has also been collected by the HELI study.

Data

Data collection plan

Researchers were trained properly before they were allowed to perform screenings and measurements independently (e.g. TICS, neuropsychological testing, anthropometrics, breathing test). The neuroimaging assessments were performed by MRI-certified researchers only. Blood was collected by nurses and directly processed by trained lab technicians. If possible, measurements (e.g. blood pressure, heart rate) were performed in duplicate.

Data management

Castor EDC was used as an electronic case report form (CRF) and allowed for careful monitoring data entrance. At the DCCN, a paper version of the CRF was used during the test session, and test session information was filled in on Castor EDC directly afterwards. At the WUR, test session information was filled in directly on Castor EDC, without use of a paper version of the CRF.

Neuroimaging data collected at the DCCN was directly transferred and stored securely on the Radboud Data Repository (RDR; <https://data.ru.nl>). Processing of all data on computers is protected by a site firewall. Restricted access to study data on the central storage system is implemented by means of specific user groups. Participant metadata is stored in password-protected databases. Details about data management procedures can be found on the website of the RDR.

Blood marker, microbiota composition and metabolite data analysed at the WUR is safely stored, archived, and registered in the Wageningen University data storage system according to the regulations of the WUR, and implemented within the Division of Human Nutrition and Health.

Monitoring

Data monitoring: formal committee

This study has a low risk for participants, and the participants are of a low-risk group. Therefore, a data monitoring committee (DMC) is not appointed for this study.

Data monitoring: interim analysis

All research conducted at the DCCN meets with the assessment of negligible-minimal risk- assessment as defined in the Dutch Federation Academic Medical Centers (NFU) indicating onsite minimal monitoring. The Clinical Research Coordinator is assigned

as designated on-site monitor. The CRC/monitor will assess the HELI study at least once during the data acquisition phase. Outcomes will be documented and reported to the principal investigator. In case of a critical finding the director will be notified by the CRC. He may decide to put the study on hold. Resuming the study requires the director's formal approval. Besides the annual reporting of monitor visits the Donders Management Team will also be notified of the occurrences of serious protocol breaches (violations). In all cases of study termination, appropriate steps will be taken to ensure the safety of study participants.

A study monitoring interim visit was conducted to ensure that the investigator and site were compliant with the study protocol, that data will be of high quality and integrity and that the facilities and staffing were adequate to continue best practices. The monitoring procedures on site concern all investigator-driven interventional research conducted at the DCCN.

The monitoring standard operating procedure (SOP) applies to all site personnel involved in the implementation, coordination and conduct of investigator-initiated interventional research. The principal investigator scheduled and arranged the monitoring visits as requested by the monitor. A monitor visit consisted of an occasional study sample taken at random during the initiation phase of the study. Monitoring visits were scheduled at mutually convenient times and every attempt was made to accommodate the monitoring visit. Prior to each visit, the principal investigator designee confirmed with the monitor what materials were to be reviewed so that appropriate documentation and files were made readily available.

These materials included:

- All available study-specific Informed Consent forms;
- Subject source documents and corresponding case report forms (CRFs) and/or access to the data management tool CastorEDC;
- Safety reports and/or Adverse event documentation;
- Study Site Master File/ Regulatory Binder;
- Applicable SOP's.

Harms

Adverse events (AE) are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to (the investigational product / trial procedure/ the experimental intervention). All AEs reported spontaneously by the subject or observed by the investigator or his staff will be recorded. A serious adverse event (SAE) is any untoward medical occurrence or effect that:

- Results in death;
- Is life threatening (at the time of the event);
- Requires hospitalization or prolongation of existing inpatients' hospitalization;
- Results in persistent or significant disability or incapacity;
- Is a congenital anomaly or birth defect; or
- Any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The staff will report all SAEs to the principal investigator as soon as possible after they have been observed. The investigator will report these to the sponsor without undue delay after obtaining knowledge of the events. The sponsor will report the SAEs to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

Auditing

Apart from a standard monitor initiation, interim and closing-out visits, no specific scheduled auditing visits are planned for this study.

Ethics and dissemination

Research ethics approval

This research study falls under the Medical Research Involving Human Subjects Act (WMO). The protocol has been reviewed and approved by the MREC Oost-Nederland of the Central Committee on Research Involving Human Subjects (CCMO), under approval number NL78263.091.21.

Protocol amendments

All substantial amendments will be notified to the METC that gave a favorable opinion. A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- The safety or physical or mental integrity of the subjects of the trial;
- The scientific value of the trial;
- The conduct or management of the trial; or
- The quality or safety of any intervention used in the trial.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

Consent or assent

Written informed consent are only signed and collected by research team members directly involved with the present study.

Confidentiality

Handling of personal data will comply EU privacy law, the General Data Protection Regulation (GDPR) and the Dutch Act on Implementation (in Dutch: Uitvoeringswet AVG, UAVG).

The Donders Institute has established a data management infrastructure (<https://data.donders.ru.nl>) to which all relevant data will be stored online in a central storage space during data collection. Research data will be copied to a central storage system. Processing of all data on computers is protected by a site firewall. Restricted access to study data on the central storage system is implemented by means of specific Unix user groups. Participant metadata is stored in password-protected databases. A second copy of the data is stored on the Radboud Data Repository (RDR) located on the Radboud University campus. Raw data will be archived according to institutional standards. For data processing, raw data will be uploaded to network attached storage that, in compliance with institutional guidelines, only researchers directly involved in the processing of data from the specific project will be able to access.

The investigator will ensure that the subject's anonymity will be maintained. In all documents, subjects will be identified by an identification code only – i.e., not by their names or any other feature by which subjects can be identified. The investigator will keep a separate Subject Identification Code List (Key-file), which matches identifying codes with the subjects' names. Key-files will be maintained by the investigator in a strict confidential way and preserved for a certain period of time meeting with demonstrable legitimate interest (according to GDPR). Subject anonymity will be maintained throughout all archiving steps. The identification code is shared with our multicenter partner WUR, so the data from the gathered at the Donders Institute and at Wageningen University is placed under the same identification code of an individual.

Declaration of interests

This work was supported by a Crossover grant (MOCIA 17611) of the Dutch Research Council (NWO). The MOCIA programme is a public-private partnership (see <https://mocia.nl/scientific/>). There are no competing interests to be declared.

Data access

Access to data is managed by the researchers involved with the study and responsible for the data, and only directly involved research team members will be able to give access rights to other individuals when necessary or requested.

Ancillary and post-trial care

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

Dissemination policy: trial results

Trial results will be published in open-access peer-reviewed journals.

Dissemination policy: authorship

Authorship eligibility guidelines and any intended use of professional writers

Authorshop guidelines are compliant with the Radboud University (Medical Center) Guidelines for publication and authorship. These guidelines are intended to provide a description of the conditions regarding eligibility for authorship, and to determine which order of authorship is the most appropriate.

Conditions for authorship

- a. Each author must have made a substantial contribution to the creative concept, the design or execution of the research described in the article and/or to the analysis or interpretation of the data.
- b. Each author must have made a substantial contribution to the drawing up or critical assessment of at least part of the intellectual concepts in the article.
- c. Without prejudice to the specific responsibility for his/her own contribution, each author should have given his/her approval for the final version of the entire manuscript.
- d. One of the authors must take responsibility for the article in its entirety (in principle the author to which any related correspondence is to be addressed).
- e. Neither the routine provision of data or material nor the facilitation of financial resources justifies co-authorship. A reference in the acknowledgements section to the contribution in question will usually suffice.

Basic principles for determining order of authorship

- a. Where possible, agreements regarding authorship should be made beforehand.
- b. The first author is the researcher who has made the greatest contribution to the project, including its execution: for example, a PhD student or postdoctoral fellow. The second author is the author that has made the second most important contribution.
- c. The last author has developed the key creative concept of the research and has supervised the research. The last author is generally the research group leader. In this respect, the penultimate author has played the second most important role. The head of department is not by default the last author because of his/her position.
- d. A shared first or last authorship is only possible in the event of equal contributions.

Dissemination policy: reproducible research

Papers will only be submitted to, and published in, open-access journals. The data used will be shared on a Data Sharing Collection of the RDR of the Radboud University, Nijmegen, The Netherlands.

Informed consents***Informed consent HELI study: English version***

Belonging to the HELI study.

- I have read the information letter. I was able to ask questions. My questions have been answered well enough. I had enough time to decide if I want to participate.
- I know that participating is voluntary. I also know that I can decide not to take part in the study at any time, or to stop participating. I do not have to explain why.
- I am willing to participate in all elements of this study which have been described in this information letter (such as visiting the research centers, partaking in the lifestyle intervention).
- I consent to the researcher to inform my general practitioner about my participation in this study.
- I consent to the researcher to disclose information to my general practitioner about possible incidental findings from this study which could have a clinical significance for my health.
- I consent to the collection, use and storage of my data and biomaterial. The researchers will only use this data to answer the research question of this study as explained in the information letter.
- I consent to the storage of my data and biomaterial, for a minimal period of 15 years.

- I am aware that for the purpose of monitoring this research study some designated people are able to look into my data. These designated people have been mentioned in the information letter. I consent to these designated people to access my data for monitoring purposes.
- Would you please tick 'Yes' or 'No' in the table below?

I consent to my data and biomaterial to be stored, and to be possibly used for other research, as mentioned in the information letter.	Yes ☐	No ☐
I consent to my (leftover) data and biomaterial to be stored, and to be possibly used for other future research, as mentioned in the information in the information letter. The biomaterial will be stored for this purpose for a minimum of 15 years.	Yes ☐	No ☐
I consent to be approached after this study to participate in future studies.	Yes ☐	No ☐
I consent to the researchers informing me which treatment I received/ which group I was in.	Yes ☐	No ☐

- I want to participate in this research study.

My name is (participant):

Signature Date __ / __ / __

.....

To be filled in by the researcher:

I declare that I have fully informed this participant with regard to the said research study.

Will information become known during the research study which could impact or influence the consent of the participant? Then I will inform the participant in time.

Name researcher (or his/her representative):

Signature Date __ / __ / __

.....

The participant receives a complete information letter, together with a signed version of the informed consent form.

10.2 Informed consent HELI study: Dutch version (original)

Behorende bij de HELI studie.

- Ik heb de informatiebrief gelezen. Ook kon ik vragen stellen. Mijn vragen zijn goed genoeg beantwoord. Ik had genoeg tijd om te beslissen of ik meedoe.
- Ik weet dat meedoen vrijwillig is. Ook weet ik dat ik op ieder moment kan beslissen om toch niet mee te doen met het onderzoek. Of om ermee te stoppen. Ik hoef dan niet te zeggen waarom ik wil stoppen.
- Ik ben bereid mee te doen aan alle onderdelen van dit onderzoek die in deze informatiebrief beschreven staan (o.a. de bezoeken aan onderzoekscentra, leefstijlinterventie).
- Ik geef de onderzoeker toestemming om mijn huisarts te laten weten dat ik meedoe aan dit onderzoek.
- Ik geef de onderzoeker toestemming om mijn huisarts informatie te geven over onverwachte bevindingen uit het onderzoek die van belang zijn voor mijn gezondheid.
- Ik geef de onderzoekers toestemming om mijn gegevens en lichaamsmateriaal te verzamelen en gebruiken. De onderzoekers doen dit alleen om de onderzoeksvraag van dit onderzoek te beantwoorden.
- Ik geef de onderzoekers toestemming dat mijn gegevens 15 jaar bewaard mogen worden op de onderzoekscentra.
- Ik weet dat voor de controle van het onderzoek sommige mensen al mijn gegevens kunnen inzien. Die mensen staan in deze informatiebrief. Ik geef deze mensen toestemming om mijn gegevens in te zien voor deze controle.
- Wilt u in de tabel hieronder ja of nee aankruisen?

Ik geef toestemming om mijn gegevens te bewaren om dit te gebruiken voor ander onderzoek, zoals in de informatiebrief staat.	Ja ☐	Nee ☐
Ik geef toestemming om mijn (overgebleven) lichaamsmateriaal te bewaren om dit te gebruiken voor ander toekomstig onderzoek, zoals in de informatiebrief staat. Het lichaamsmateriaal wordt daarvoor nog minimaal 15 jaar bewaard.	Ja ☐	Nee ☐
Ik geef toestemming om mij eventueel te vragen of ik wil meedoen met ander onderzoek.	Ja ☐	Nee ☐
Ik geef de onderzoekers toestemming om na het onderzoek te laten weten welke behandeling ik heb gehad/ in welke groep ik zat.	Ja ☐	Nee ☐

- Ik wil meedoen aan dit onderzoek.

Mijn naam is (proefpersoon):

Handtekening..... Datum: __ / __ / __

.....

In te vullen door de onderzoeker: Ik verklaar dat ik deze proefpersoon volledig heb geïnformeerd over het genoemde onderzoek.

Wordt er tijdens het onderzoek informatie bekend die de toestemming van de proefpersoon kan beïnvloeden? Dan laat ik dit op tijd weten aan deze proefpersoon.

Naam onderzoeker (of diens vertegenwoordiger):

Handtekening..... Datum: __ / __ / __

.....

De proefpersoon krijgt een volledige informatiebrief mee, samen met een getekende versie van het toestemmingsformulier.

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PART III

Pathways Linking Lifestyle to Cognition via Vascular and Brain Health

Chapter 3

Integrating Lifestyle, Vascular, Brain and Cognitive Markers: an Exploratory Multimodal Approach to Brain Aging

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Abstract

Background

Lifestyle modifiable risk factors are promising intervention targets to delay cognitive decline and dementia in older adults. While previous studies have reported associations between lifestyle, vascular health, (MRI-based) brain health and cognition, few have considered all domains simultaneously. In this exploratory cross-sectional study, we examined pathways linking lifestyle to cognition via vascular and brain health, and identified key variables contributing to shared variance between these domains.

Methods

We included 176 Dutch adults, aged 60–75 years old with modifiable risk of cognitive decline. Composite domain scores for lifestyle, vascular health, (MRI-based) brain health, and cognitive functioning were calculated. We used Structural Equation Modelling (SEM) to test direct and indirect pathways between composite domain scores, Canonical Correlation Analysis (CCA) to identify individual variables driving shared variance between domains, and Pearson's correlations to explore pairwise associations.

Results

SEM showed associations between lifestyle and vascular health ($\beta = 0.23, p = 0.002$), and vascular and brain health ($\beta = 0.18, p = 0.01$). No direct pathways were found between lifestyle and brain health, or between vascular health and cognitive functioning. CCA revealed vascular markers, specifically BMI (-0.837), blood pressure (-0.837), and blood lipids (-0.408 to -0.466), as the strongest drivers of shared variance with cerebral perfusion (-0.318 to -0.364).

Discussion

Our results indicate stepwise links between lifestyle, vascular health, and brain health. Integrating multimodal markers of all domains highlights the key role of vascular health in understanding lifestyle-related effects on brain health.

Introduction

The worldwide aging population causes a rapid growth in the prevalence of age-related cognitive decline and dementia. The World Health Organization (WHO) underlines the importance of targeting modifiable risk factors to reduce the risk of cognitive decline and dementia [1]. Risk factors for cognitive decline are multifaceted, arising from a complex interplay between environmental, genetic, and lifestyle-related factors [2]. Lifestyle-related risk factors, such as poor diet, physical inactivity, stress, and disturbed sleep, have been identified as promising, modifiable targets for preventive interventions.

Unhealthy lifestyle patterns contribute to cardiovascular risk factors including obesity, hypertension, hypercholesterolemia and type-II diabetes [3], and evidence shows that adopting a healthier diet and increasing physical activity can help reduce these risks [4]. Cardiovascular disease risk, in turn, is a well-established predictor of increased age-related cognitive decline and dementia [5], likely due to its effects on various distinct brain health markers, such as brain activity, perfusion and volume [6, 7]. Risk factors of cardiovascular disease negatively affect specific higher-order cognitive functions, such as working memory and episodic memory. They also impact the structure and function of associated brain regions, such as the hippocampus and dorsolateral prefrontal cortex (dlPFC) [6]. Together, this indicates a link between lifestyle, cardiovascular disease risk factors and cognitive outcomes, as well as an intermediate role for brain structure and function [6, 8-11].

While numerous studies have investigated bilateral associations between pairs of domains, such as lifestyle and cardiovascular health, or brain health and cognitive functioning, few have integrated all of these aspects into a comprehensive model to investigate potential pathways linking lifestyle, cardiovascular health, brain health, and cognitive functioning in populations at risk for cognitive decline. The current study aimed to address this knowledge gap by investigating an integrated model that includes multiple markers related to the domains of lifestyle, vascular health, (MRI-based) brain health, and cognitive functioning in 176 older adults aged 60–75 years old with an elevated risk of cognitive decline. We hypothesized a most probable stepwise pathway in which healthier lifestyle patterns are associated with improved vascular health, which in turn benefits brain health (measured with MRI) that ultimately relates to stronger cognitive functioning. We additionally hypothesized that more direct pathways could be involved, potentially bypassing mechanisms such as lifestyle factors directly affecting markers of brain health or cognitive functioning. In addition, we investigated which individual variables most strongly accounted for the associations observed between the different domains. These analyses provide

an overview of the possible relations between outcome measures related to lifestyle, vascular health, brain health, and cognitive functioning, by integrating multiple involved pathways into a more extensive and cohesive model.

Materials and methods

Participants

For this study, baseline data was sourced from two studies, the HELI (n = 102) and COMBI (n = 74) studies, which are part of the MOCIA research program [12] and recruited according to similar inclusion and exclusion criteria (explained in detail previously ([13]; in press). Participants were aged between 60–75 years at moment of study inclusion. Inclusion criteria were based on the presence of at least two lifestyle-modifiable factors of the Cardiovascular Risk Factors, Aging, and Incidence of Dementia (CAIDE [14]) score: (1) body mass index (BMI) ≥ 25 , (2) physical inactivity, (3) hypertension, (4) hypercholesterolemia, (5) diabetes type-II, and (6) mild cardiovascular disease. Exclusion criteria included history of cerebrovascular events, neurological disease, current malignant disease, and current psychiatric disorders. To exclude participants with possible undiagnosed (mild) cognitive impairment, a Telephone Interview for Cognitive Status (TICS-M1 [15]) was performed during prescreening with a cutoff score of ≥ 23 (of a total of 39) as an additional requirement for inclusion. Data acquisition took place between May 2022 and March 2024 at the Donders Center for Cognitive Neuroimaging of the Radboud University (RU-DCCN; Nijmegen, The Netherlands) and at the Division of Human Nutrition and Health of Wageningen University and Research (WUR-HNH; Wageningen, The Netherlands). All participants provided informed written consent.

Demographics

Participants completed questionnaires on demographics including age, sex, and education level. Education level was stratified in levels of low, medium and high education based on the International Standard Classification of Education (ISCED 2011 [16]).

Lifestyle markers

All participants completed validated questionnaires on the lifestyle domains of diet, physical activity, stress and mindfulness, and sleep.

Diet score

A Dutch Healthy Diet (DHD) index score was calculated as a measure of adherence to the Dutch dietary guidelines 2015 [17]. The DHD-index score was derived from results

of the Dutch Mediterranean-Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay (MIND-NL) food frequency questionnaire (FFQ) [18, 19] in HELI participants, and from a separate extensive FFQ in COMBI participants [20]. From both FFQs, a DHD-index score was calculated based on questions with regard to consumption of the following food groups: (1) vegetables, (2) fruit, (3) whole-wheat products, (4) legumes, (5) nuts, (6) dairy, (7) fish, (8) tea, (9) spreadable and cooking fats, (10) red meat, (11) processed meat, (12) sugary drinks, (13) alcohol, (14) salt, and (15) other unhealthy food choices (e.g. take-away). Each food group was graded from 0-10 points (based on a 2-day average intake [21]), resulting in a maximum of 150 points for all food groups combined. A higher DHD-index score indicates stronger adherence to the Dutch dietary guidelines 2015 [17]. One participant failed to complete the FFQ, resulting in n=1 excluded DHD-index dataset.

Physical activity score

We assessed physical activity by using the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH), which is based on the Dutch guidelines for physical activity. Participants scored their physical activity by amount of minutes per day, and days per week of specific activities in the last months. Activities included: (1) walking to work, (2) cycling to work, (3) cycling to work with electric bike, (4) light activity at work, (5) intense activity at work, (6) light home chores, (7) intense home chores, (8) cleaning home, (9) walking in free time, (10) cycling in free time, (11) cycling in free time with electric bike, (12) gardening, and (13) sport activities. For each activity an activity score was calculated based on the amounts of minutes per day and days per week spent on the activity, multiplied by a factor of metabolic equivalent of task (MET) to correct for the physical intensity of each activity. A total score was calculated of all activities multiplied by the corresponding MET factors, with a higher SQUASH-score indicating more physical activity on a weekly basis.

In addition, we used the LASA Sedentary Behavior Questionnaire (LASA-SBQ), a self-report questionnaire specifically designed to assess sedentary behavior in older adults [22]. Participants scored the average time in minutes per day spent on 10 different sedentary behavior items during weekdays and weekends. The sedentary behavior items consisted of: (1) napping on chair or couch, (2) reading while seated or lying down, (3) listening to music while seated or lying down, (4) watching television, video or DVD, (5) performing a hobby while seated (e.g. knitting, playing music), (6) talking (in-person or on the phone) while seated, (7) sitting behind a computer, (8) performing sitting activities (e.g. writing a letter, administrative tasks), (9) commuting by car, bus or train, (10) visiting activities while seated (e.g. church, theater, cinema). A higher score indicates a higher amount of sedentary behavior.

In total, data from 6 participants were excluded from analysis of these physical activity questionnaires due to clear input errors (e.g., indicating activities on more than 7 days per week, more hours of activity per day than waking hours, etc.).

Psychological wellbeing scores

We assessed scores of psychological wellbeing in three different components: stress, mood, and mindfulness.

We used the 10-item Perceived Stress Scale (PSS) [23] to determine perceived stress levels based on a Likert scale between 0 ('never') and 4 ('very often'), with total scores indicating low perceived stress (0–13), moderate perceived stress (14–26) and high perceived stress (27–40).

We used the Hospital Anxiety and Depression Scale (HADS) [24] to assess the anxiety and depression-related complaints from the past four weeks. The HADS questionnaire consists of two subscales: an anxiety-scale and a depression-scale, each with 7 questions with multiple-choice answers based on Likert scales. We summed both subscale scores into a total score. A higher score indicates a greater frequency of anxiety- or depression-related complaints.

Because of the significant positive impact of mindfulness on stress, stress perception, reduced anxiety and depression, we have additionally used the Five Facet Mindfulness Questionnaire (FFMQ) to assess multiple components of mindfulness. The FFMQ consists of five subscales: observing, describing, acting with awareness, non-judgmental (of inner experience), and non-reactivity (to inner experience). Each subscale has between 7–8 questions with multiple-choice answers based on a Likert scale between 1 ('never or very rarely true') and 5 ('very often or always true'). We summed all subscale scores into a total score. A higher total score indicates greater level of mindfulness.

Sleep quality score

The Pittsburgh Sleep Quality Index (PSQI) [25] was used to assess sleep quality. The PSQI measures sleep quality and sleep disturbance over the past month, and consists of seven components: subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. Each component is scored between 0–3 points with a maximum of 21 points. We summed all subscale scores into a total score. A higher score indicates worse sleep quality.

Cardiovascular health markers

BMI and blood pressure

Length and weight were measured to calculate BMI (kg/m^2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an Omron X3 Comfort digital blood pressure monitor. Blood pressure was measured two times, and three times if the first two measurements showed a significant deviation between each measurement. Multiple blood pressure measurements were averaged, and the averaged SBP and DBP values were then used to calculate mean arterial pressure ($\text{MAP} = \text{DBP} + \frac{1}{3} \times (\text{SBP} - \text{DBP})$ [26]).

Blood cardio-metabolic markers

Blood tubes (Vacutainers, BD) containing sodium fluoride were used to analyze glucose. Blood tubes containing lithium heparin were used to analyze insulin, total-high-density lipoprotein (HDL) cholesterol, total-low-density lipoprotein (LDL) cholesterol, and triglycerides. Blood tubes were inverted ten times, and afterwards centrifuged ($3000 \times g$) for 8 min at 20°C . Plasma was collected and stored at -80°C until further analysis. The blood cardio-metabolic marker analyses were performed at the Clinical Chemistry and Hematology Laboratory of Gelderse Vallei Hospital (Ede, The Netherlands) using standard clinical laboratory assays. In short, glucose was analyzed in $500 \mu\text{L}$ plasma using the enzymatic assay called glucose hexokinase 3 on an Atellica™ CH analyzer with a measuring interval of $0.2\text{--}38.9 \text{ mmol/L}$. Insulin was analyzed with the IMMULITE® 2000 Insulin solid-phase, enzyme-labeled chemiluminescent immunometric assay. We calculated a Homeostatic Model Assessment for Insulin Resistance ($\text{HOMA-IR} = (\text{fasted insulin in } \mu\text{U/mL} \times \text{fasted glucose in mmol/L}) / 22.5$) value for each participant as a measure of insulin resistance. Total HDL- and LDL-cholesterol, and triglycerides, were analyzed in $500 \mu\text{L}$ plasma using the Atellica™ CH analyzer with a measuring interval of $0.52\text{--}3.34 \text{ mmol/L}$ for HDL-cholesterol, $0.13\text{--}25.90 \text{ mmol/L}$ for LDL-cholesterol, and $0.11\text{--}6.22 \text{ mmol/L}$ for triglycerides. Blood drawing was performed in the morning, and participants were instructed to remain fasted from 20.00 PM the day before the outcome measure visit. Blood drawing was not successful for every participant, as in four cases an insufficient amount of blood could be collected for the analyses, and in one case, venipuncture was unsuccessful.

Brain health (neuroimaging) markers

We acquired multiple measures of brain structure and function from neuroimaging. All participants were scanned with a 3T MAGNETOM Skyra MR scanner (Siemens AG, Healthcare Sector, Erlangen, Germany) using a standard 32-channel head coil

at the RU-DCCN. Total scanning time was approximately 90 minutes, including preparation and handling time. To reduce head movement during scanning, the participants' heads were fixed using tightly fitted head cushions. A detailed overview of all sequence parameters has been provided previously ([13]; in press). We used the dorsolateral prefrontal cortex and hippocampus as specific regions of interest (ROIs).

Structural brain imaging, parcellation and volumetrics

We acquired structural images using a T1-weighted MP2RAGE sequence (TR = 6000 ms, TE = 2.34 ms, TI1/2 = 700/2400 ms, flip angle = 6°, voxel size = 1.0 mm isotropic). The uniform, second inversion (UNI x INV2 [27]) MP2RAGE images were processed by performing cortical reconstruction and volumetric segmentation using the 'aparc+aseg' segmentation pipeline from the Freesurfer image analysis suite, which is documented and freely available for download online (<http://surfer.nmr.mgh.harvard.edu/>). Hippocampal volumes were extracted from the parcellated Aseg Atlas ('aseg_stats'), and the rostral middle frontal volumes were extracted from the Desikan-Killiany atlas ('aparc_volume') as a proxy for dorsolateral prefrontal cortex volume. All extracted brain volume measures are in mm³ and shown as a ratio of total intracranial volume (ICV).

fMRI brain activity

We performed task-based functional magnetic resonance imaging (fMRI) to assess working memory (WM)-related neural recruitment and performance. We acquired fMRI images with a blood-oxygen-level-dependent (BOLD) contrast using a whole-brain T2*-weighted gradient-echo single-echo echo planar imaging (EPI) sequence (TR = 1500 ms, TE = 33.40 ms, flip angle = 75°, voxel size = 2.0mm isotropic), incorporating multiband acceleration with a factor of 4. To assess WM-related neural recruitment and performance, we conducted an N-back task during fMRI [28]. During the N-back task, participants were asked to actively monitor and remember a series of numerical visual stimuli (numbers from 1 – 9) during a specific timeframe, and to give a response once the presented stimulus was identical to the stimulus presented an 'n' number (0, 1, or 2) of trials before. We performed a 15-minute, three-condition block design with the following conditions: 0-back (control condition; respond when stimulus showed the number 1), 1-back (respond when shown stimulus is identical to stimulus shown one number back), and 2-back (respond when shown stimulus is identical to stimulus shown two numbers back). Participants received the task instructions outside of the scanner and were able to perform a practice trial whilst an involved researcher observed to verify that the participant understood the instructions.

We used fMRIPrep (23.2.0; RRID:SCR_016216 [29]) to preprocess the anatomical and fMRI data, and subsequently performed first level and ROI analyses. Realignment

and coregistration of the functional scans to the native T1-weighted anatomical scan was performed before the images were susceptibility distortion-corrected and normalized to standard MNI152 space. An extensive description of all fMRIprep preprocessing steps is provided in the appendix (Appendix A: Supplementary Material). We used Statistical Parametric Mapping 12 (SPM12; <https://www.fil.ion.ucl.ac.uk/spm/software/spm12/>) in MATLAB R2024a (Mathworks Inc.; <https://nl.mathworks.com/products/matlab.html>) to perform spatial smoothing of the preprocessed BOLD time series using a 6 mm full-width half-maximum (FWHM) Gaussian smoothing kernel. The data quality of subject-specific fMRI datasets and task performance was evaluated before including the data in the group analysis. Participants were excluded if they appeared to have misunderstood the N-back task during scanning (failed task execution), as indicated by either less than 30% target accuracy of the 0-back (control) condition and/or more than 35% false positive responses to non-targets in any of the 0-, 1- or 2-back conditions. Furthermore, we evaluated the quality of the complete fMRI dataset by examining reports and motion parameters provided by fMRIprep. For datasets with possible questionable data quality, indicated by a framewise displacement mean >0.5mm or max >4mm), the first-level main task (2-back – 0-back) contrast ($p < 0.05$ FWE-corrected) was visually inspected for fronto-parietal activation patterns consistent with WM. Following these quality control assessments, $n = 5$ participants were excluded because of failed task execution, and $n = 3$ participants were excluded because of questionable fMRI data quality. Additionally, $n = 5$ participants had no available fMRI data due to issues with the MRI-scanner during acquisition, leading to a total of $n = 13$ excluded fMRI datasets.

For the first-level analyses, we performed a general linear model (GLM) to model the block-related BOLD responses. The model included three condition-specific regressors based on the three N-back task conditions (0-back, 1-back, 2-back). In addition to these condition-specific regressors, we also included a number of confound regressors: framewise displacement, six realignment parameters, six anatomical principal component noise regressors (aCompCor), six temporal principal component noise regressors (tCompCor), all cosine regressors, and all independent components labeled as noise by ICA-AROMA. ICA-AROMA components were taken from fMRIprep version 23.0.2 (RRID:SCR_002502) and incorporated into the model, as opposed to using AROMA-denoised data. To avoid removing task-related variance shared with the noise regressors, ICA-AROMA components showing >0.05 covariation with the task design were excluded from the model. Each experimental condition in the block design was modeled as a separate regressor by applying the hemodynamic response function provided by SPM. To account for low-frequency

drift, cosine regressors were included instead of applying a high-pass filter. The primary WM contrast was defined as 2-back minus 0-back, and first level statistical parametric contrast maps were generated for each individual participant.

To analyse neural recruitment in the dlPFC and hippocampus, we created independent ROI masks of these brain regions. The dlPFC mask was created based on the dlPFC-specific regions associated with WM activation from a large-scale 1091-study meta-analysis (Neurosynth; <https://neurosynth.org/analyses/terms/working%20memory/>), which were overlaid with peak dlPFC voxels associated with aging-related N-back effects from a 96-study meta-analysis [30]. The hippocampus mask was taken from the Harvard-Oxford subcortical atlas [31]. The mean ROI β -values of all task-based regressors retrieved from SPM12 were taken and used for the group analyses.

Cerebral blood flow

Arterial spin labelling allows for an absolute quantification of cerebral blood flow (CBF) without the use of an intravenous contrasting agent, but instead labels arterial blood water in the carotid arteries using an inversion pulse before the blood travels to and through the brain. We used a vessel scout sequence (TR = 47.90 ms, TE = 8.15 ms, flip angle = 9° , voxel size = $1.2 \times 1.2 \times 6.0$ mm) to visualize the carotid arteries of each participant and manually placed the tagging plane perpendicular to the feeding arteries according to anatomical landmarks. We subsequently performed a Hadamard-encoded multi-PLD pseudocontinuous ASL (pCASL) sequence to acquire perfusion maps and acquired an equilibrium magnetization (M_0) calibration image for each participant. A necessary MRI scanner software update and subsequent sequence update in May 2023, required a change of our pCASL sequence. From March 2022 to May 2023, we used a pCASL sequence (TR = 4000 ms, TE = 16.28 ms, excitation flip angle = 120° , refocusing flip angle = 90° , voxel size = $1.8 \times 1.8 \times 3.5$ mm, sub-bolus duration = 400 ms, PLD = 100 ms, repeats = 2) with water suppression enhanced through T1 effects (WET) background suppression, accompanied by an M_0 calibration protocol (TR = 500/1000/1500 ms, TE = 16.28 ms, flip angle = 120° , voxel size = $1.8 \times 1.8 \times 3.5$ mm) to be used as reference. From May 2023 to March 2024, we used the pCASL and M_0 sequences with the same contrast parameters and total acquisition time as the original sequence but with additional undersampling during the 3D-GRASE readout present in both pCASL and M_0 calibration protocols (originally 2-fold and later 2x2 with CAIPI displacement) to reduce sensitivity to subject motion. Both sequences were customer developed by the Fraunhofer Institute for Digital Medicine MEVIS, with the first being implemented in the Siemens IDEA proprietary environment and the latter being implemented in the vendor-agnostic gammaSTAR. We used the FSL BASIL 'asl_gui' toolset [32] to process the

acquired perfusion maps and calibration images and create quantified CBF images. Perfusion images were Hadamard-decoded and motion corrected, and an ASL-mask was created to only capture voxels containing ASL signals. Using BASIL, we analyzed the 14 volumes (7 PLDs $\times$ 2 repeats) of each pre-subtracted perfusion image grouped by repeats, with a constant sub-bolus duration of 0.4 seconds (0.5 – 2.9 s). To add anatomical reference and registration to the quantified perfusion output in native space, we used the FSL_ANAT pipeline on the T1-weighted MP2RAGE structural image and supplied BASIL with the 'T1.anat' output directory. For calibration, we specified the M_0 image as a proton-density calibration image (sequence TR = 1000, calibration gain = 10). The perfusion maps of all participants were visually inspected to assess data quality, and outliers of quantified perfusion were cross-checked with perfusion map quality assessment to rule out possible acquisition errors or excessive motion artifacts. Following these quality control assessments, $n = 1$ participant had an acquisition error (little to no signal), and $n = 4$ participants had excessive motion artifacts. Additionally, $n = 10$ participants had no available ASL data due to issues with the MRI-scanner during acquisition, leading to a total of $n = 15$ excluded ASL datasets.

To measure CBF in whole-brain grey matter (GM) and white matter (WM) and our ROIs of the dlPFC and hippocampus, we extracted and averaged the quantified CBF values of each voxel overlaid with a bilateral Freesurfer ASEG structural parcellation-derived GM/WM and hippocampus mask, and with the (native-space transformed) dlPFC-mask that was created for the fMRI analysis.

Neuroinflammation

Brain myo-inositol is used as a quantifiable measure of activated microglia, used as a neuroimaging spectroscopy marker of local increased neuroinflammation [33]. We used the total quantified myo-inositol, with total water as a reference, within a voxel placed in the left dlPFC. To locally quantify mI within this voxel, we used Point RESolved Spectroscopy (PRESS) ^1H -MRS sequence (TR = 2000 ms, TE = 35 ms, flip angle = 90° , voxel size = 20 mm isotropic) together with chemically selective water suppression (CHESS) to suppress water proton signals. We were unable to acquire hippocampal MRS data due to insufficient spectrum quality, and thus only MRS data from the voxel within the left dlPFC was used in analysis. The voxel was manually placed in our ROIs by using a T1-weighted image to navigate. During voxel placement, we prevented the inclusion of non-brain regions within the voxel, such as cerebrospinal fluid (CSF) or the skull, as much as possible. Prior to acquisition, we performed a shimming step within the selected voxel to reduce magnetic field inhomogeneities. During shimming, we used a FWHM value of <30 as a measure of shimming success according to the Siemens MRS guidelines [34]. If time allowed, we ran the shimming procedure two times and

used the lowest FWHM measurement as an indicative of spectrum quality to decide the final MRS acquisition sequence used for further preprocessing and analysis.

Output from the unsuppressed and suppressed MRS sequences were pre-processed using Osprey 2.4.0 [35], an open-source software package for pre-processing, including linear-combination modelling, tissue correction and ultimately spectrum analysis and metabolite quantification. For each participant, individual metabolite transients were frequency-corrected using lineshape reference and tissue-corrected with anatomical MP2RAGE data. The metabolite transients were then averaged to create the spectrum for further analysis. When required, eddy-current correction [36] was performed using water-unsuppressed lineshape reference data. The processed spectra were subsequently analyzed using Osprey's linear combination modeling with default parameters. The metabolite spectra were fitted over a 0.5–4.0 ppm frequency range with 0.4 ppm knot spacing. Water reference data was separately fitted over a range of 2.0–7.4 ppm. The spectroscopy data was coregistered to the native-space T1-weighted anatomical scan for each participant, and tissue segmentation was performed using SPM12 to estimate fractional tissue volumes within the region of interest. Metabolite concentrations were then corrected for GM/WM/CSF tissue composition and relaxation times and scaled to water concentration, which ultimately generated molal concentration estimates for myo-inositol. The quantified myo-inositol values were corrected for the relative residual amplitude (relResA), a measure of spectrum quality. Manual inspections of the pre-aligned, post-aligned, averaged, and fitted spectra were performed to ensure sufficient data quality. Additionally, creatine signal-to-noise (SNR), FWHM and relResA were considered as data quality metrics. Based on the manual inspection of the spectra, and of the data quality metrics, a total of $n = 7$ MRS datasets were excluded.

Cognitive functioning markers

We administered a neuropsychological test battery incorporating a number of tests to assess the cognitive domains that are predominantly affected in cognitive aging, namely executive function, processing speed, episodic memory, and working memory. All cognitive functioning markers were corrected for education level.

Executive function tests

The Trail Making Test (TMT) and Verbal Fluency Test (VFT) were used as measures of executive function.

For the TMT, participants were provided a worksheet and were asked to connect encircled numbers by drawing a line, using a pencil. The TMT consisted of two parts:

TMT-A and TMT-B. In TMT-A, participants were asked to draw a line between encircled numbers, from 1–25, in ascending order (i.e., 1 – 2 – 3 – 4 – etc.) as fast as possible. In TMT-B, participants were asked to draw a line between numbers 1–13 as fast as possible, but this time each number alternated with letters from the English alphabet in ascending order (i.e., 1 – A – 2 – B – 3 – C – etc.). For both TMT-A and TMT-B completion time were measured, and we calculated a TMT-B/TMT-A ratio score to account for TMT-A completion time and thereby obtain a more pure measure of set-shifting.

For the VFT, participants were asked to name as many different animals as possible within 60 seconds. The VFT was scored as the number of correct uniquely named animals within 60 seconds.

Processing speed tests

The Digit Symbol Substitution Test (DSST) was used as a measure of processing speed. For the DSST, participants were provided a worksheet containing nine different symbols, each corresponding to a number between 1–9. On the same worksheet, rows of numbers between 1–9 were provided. The participants were asked to go through the numbers one-by-one, and match as many symbols to their corresponding number within 90 seconds. The DSST was scored as the amount of correctly entered symbols within 90 seconds.

Working memory tests

The Digit Span Test (DST) and N-back task performance were used as measures of working memory.

For the DST, participants were verbally presented with sequences of digits and were instructed to recall the sequence. The DST consisted of two parts: DST-forward and DST-backward. For the DST-forward, participants were asked to repeat the presented digit sequence in the exact same order. For the DST-backward, participants were asked to repeat the presented digit sequence in reverse order. The digit sequences consisted of spans of increasing lengths, starting at a digit span of two. Each span consisted of two distinct digit sequences. The maximum span length was 8 for both versions of the DST, and span length was only increased if one or two of the digit sequences in a specific span were correctly recalled. The DST was scored as the number of correctly recalled digit sequences, and we calculated a sum-score of the forward and backward DST scores.

Performance on the N-back task was assessed using d-prime (d'), calculated as $d' = z(H) - z(F)$, where $z(H)$ represents the z-score of the hit rate and $z(F)$ the z-score of

the false alarm rate. This signal detection theory measure considers both hit rate and false alarm rate. We calculated a contrast d' -score by subtracting the 0-back d' -score from the 2-back d' -score to measure WM-specific performance.

2.6.4 Episodic memory test

The Rey Auditory Verbal Learning Test (RAVLT) was used as a measure of episodic memory. For the RAVLT, participants were verbally presented with a list of 15 different words and asked to recall as many words as possible immediately after the presentation (immediate recall). This process was repeated four times, in order for the participant to learn the different words (encoding phase). After 15 minutes, participants were again asked to recall as many of the 15 words as possible (delayed recall). We used the delayed recall outcome of the RAVLT, as we expect this measure to be more strongly affected by aging as opposed to the immediate recall outcome. The RAVLT delayed recall was scored as the number of correctly recalled words.

Statistical analysis

Statistical methods and analyses were performed in R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). We performed data quality control assessments to prevent data input errors (for questionnaires) and removed data points with poor data quality (e.g. data acquisition or processing errors). We performed an outlier analysis based on the interquartile range (IQR) method for all included variables. Specifically, values falling below the lower bound of or above the higher bound of) were flagged as potential outliers. IQR-outliers were subsequently cross-checked against data quality control assessments. Data points that exceeded the lower- or upper IQR bound, and failed data quality control assessments or showed reported protocol deviations during acquisition, were considered invalid and were removed from the dataset. Before analysis, data normality and linearity were assessed using QQ- and scatterplots. All variables were standardized by scaling to z-scores prior to group analyses.

Baseline characteristics were calculated using mean and standard deviations (SD), or counts and percentages. Individual outcome measures were z-standardized and grouped into four outcome domains: (1) lifestyle, (2) vascular health, (3) brain health, and (4) cognitive functioning (see Table 1). For interpretation purposes, we transformed the dataset such that higher z-scores indicate better/healthier outcomes. We achieved this by applying a -1 multiplication to a number of z-standardized outcome variables for the domains of lifestyle (LASA-SBQ, PSS, HADS, PSQI), vascular health (BMI, MAP, HOMA-IR, blood LDL-cholesterol, blood triglycerides), brain health (myo-inositol dIPFC), and cognitive functioning (TMT-B/TMT-A ratio

score). Composite domain scores were calculated by summing the z-scores of all individual variables. When multiple measurements assessed the same marker (e.g., several physical activity or stress-related questionnaires in the lifestyle domain), their z-scores were first averaged.

Table 1. Outcome domains and individual variables used in domain composite scores.

Lifestyle	Vascular health	Brain health	Cognitive function
DHD-index	BMI	fMRI dlPFC	TMT
SQUASH	MAP	fMRI hippocampus	VFT
LASA-SBQ	HOMA-IR	CBF dlPFC	DSST
PSS	HDL-cholesterol	CBF hippocampus	DST
FFMQ	LDL-cholesterol	Volume dlPFC	N-back performance
HADS	Triglycerides	Volume hippocampus	RAVLT delayed recall
PSQI		Myo-inositol dlPFC	

Abbreviations: BMI = body mass index; CBF = cerebral blood flow; DHD = Dutch Healthy Diet; dlPFC = dorsolateral prefrontal cortex; FFMQ = Five Facet Mindfulness Questionnaire; fMRI = functional magnetic resonance imaging; HADS = Hospital Anxiety and Depression Scale; HDL = high-density lipoprotein; HOMA-IR = homeostatic model assessment of insulin resistance; LDL = low-density lipoprotein; MAP = mean arterial pressure; PSQI = Pittsburgh Sleep Quality Index; PSS = Perceived Stress Scale; LASA-SBQ = LASA Sedentary Behavior Questionnaire; SQUASH = Short Questionnaire to Assess Health-enhancing physical activity

To explore predictive relationships between the four outcome domains, we first performed simple linear regression model analyses with lifestyle as a predictor of vascular health, vascular health as a predictor of brain health, and brain health as a predictor of cognitive functioning (i.e., the hypothesized pathway). We then performed structural equation modelling (SEM) to evaluate a range of models incorporating the four outcome domains to examine each potential direct and indirect pathway within one integrated model. Additionally, we performed SEM analyses including sex as a covariate to assess its effect on the relationships between the outcome domains. We used the 'lavaan' and 'lavaanplot' R-packages to analyze the SEM model pathways and model fits. Individual model fits were assessed using a Chi-squared (χ^2) test ($p > 0.05$ indicating good model fit), Comparative Fit Index (CFI), and the Root Mean Square Error of Approximation (RMSEA). A higher CFI-score or lower RMSEA-score indicated a better model fit. Model fit comparison was then performed by using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC), with lower AIC and BIC values indicating a better model fit. The model with the lowest AIC and BIC score was highlighted as the strongest model.

To investigate which variables within each outcome domain explain the strongest covariance between domains and potentially act as primary driving forces of associations between sets within our integrated model, we performed canonical correlation analyses (CCA). We defined the CCA variable sets X and Y based on the pathways between the outcome domains of the strongest model indicated by the SEM analyses (e.g., if the SEM analysis revealed a significant association between the lifestyle and vascular health domains, we defined the lifestyle-domain as set X and the vascular health-domain as set Y within the CCA). Prior to CCA, each variable was adjusted for sex by fitting a linear regression model with sex as the predictor and using the residuals as the corrected outcomes. For each CCA model, we first computed the correlation matrices of set X and Y. The CCAs were then performed using the 'CCA' R-package, including all variables of set X and Y. With CCA, canonical dimensions are computed by comparing different weighted combinations (canonical coefficients) of variables in set X and Y which show the strongest paired linear correlation. Canonical dimensions are comprised of canonical variates U and V, with corresponding canonical loadings of each individual variable. The canonical loading indicates the strength of correlation of an individual variable with canonical variate U or V, with a higher loading indicating a stronger association. A Wilk's Lambda (λ) test of significance was performed on the computed canonical dimensions of each CCA, and the most significant canonical dimensions were further explored.

After conducting CCAs, we performed pairwise Pearson's correlation analyses among all individual variables to explore specific associations between variables, beyond the broader domain-level relationships. We repeated the simple linear modeling of the total domain scores and the Pearson's correlation analysis of all individual variables separately for men and women.

The significance threshold was set at $p < 0.05$ for all analyses.

Results

Baseline demographics

Baseline characteristics of the total study sample ($N = 176$) are shown in Table 2. The most common inclusion risk factors were BMI ≥ 25 , hypercholesterolemia, hypertension, and physical inactivity. Most of the participants had a medium-to-high educational background ($n = 161$) (Table 2).

Table 2. Baseline characteristics

Characteristic	Available datasets (<i>n</i>)		
<i>Demographics</i>			
Age (years), mean (SD)	66.08	(4.10)	176
Sex (female), n (%)	113	(63.07%)	176
BMI ≥25, n (%)	136	(77.27%)	176
Physical inactivity ^a , n (%)	95	(53.98%)	176
Hypertension ^b , n (%)	98	(55.68%)	176
Hypercholesterolemia ^c , n (%)	104	(59.09%)	176
Diabetes type-2, n (%)	28	(15.91%)	176
Mild cardiovascular disease ^d , n (%)	20	(11.36%)	176
Smoking status (current smoker), n (%)	3	(1.70%)	176
Medication use, n (%)			
Antihypertensive drugs	61	(34.66%)	176
Diabetes medication	18	(10.23%)	176
Cholesterol-lowering drugs	34	(19.32%)	176
Educational level ^e , n (%)			176
Low	15	(8.52%)	
Medium	64	(36.36%)	
High	97	(55.11%)	
<i>Lifestyle domain</i>			
DHD diet, mean (SD)	97.78	(18.15)	175
SQUASH physical activity, mean (SD)	6924.08	(3739.51)	169
LASA-SBQ sedentary behavior, mean (SD)	325.60	(146.09)	176
PSS stress perception, mean (SD)	11.97	(4.20)	176
FFMQ mindfulness, mean (SD)	2.98	(0.26)	176
HADS anxiety and depression, mean (SD)	6.97	(5.05)	176
PSQI sleep quality, mean (SD)	5.90	(3.48)	175
<i>Vascular health domain</i>			
BMI (kg/m²), mean (SD)	29.44	(5.66)	176
MAP (mmHg), mean (SD)	107.55	(12.86)	175
HOMA-IR, mean (SD)	3.72	(2.93)	160

Table 2. Continued

Characteristic	Available datasets (n)		
Blood lipid profile (mmol/L), mean (SD)			
HDL-cholesterol	1.50	(0.40)	173
LDL-cholesterol	3.45	(1.04)	173
Triglycerides	1.63	(0.67)	173
Brain health domain			
WM (N-back) fMRI activation in ROIs (au), mean (SD)			
dIPFC	0.37	(0.15)	162
Hippocampus	-0.04	(0.07)	162
CBF in ROIs (ml/100g/min), mean (SD)			
dIPFC	40.80	(13.49)	163
Hippocampus	42.81	(11.07)	163
Brain ROI volume ratio with ICV (mm³), mean (SD)			
dIPFC	1.60 x 10 ⁻²	1.50 x 10 ⁻³	172
Hippocampus	4.90 x 10 ⁻³	5.00 x 10 ⁻⁴	172
Myo-inositol in DLPFC (mmol/L), mean (SD)	10.09	(1.38)	167
Cognitive functioning domain			
Trail Making Test B/A ratio, mean (SD)	2.20	(0.63)	176
Verbal Fluency Test, mean (SD)	25.62	(5.62)	176
Digit Symbol Substitution Test, mean (SD)	52.28	(9.96)	176
Digit Span total, mean (SD)	15.65	(3.27)	176
N-back task performance (2b-ob d'), mean (SD)	-1.79	(0.99)	172
RAVLT delayed recall, mean (SD)	7.92	(3.12)	176

Abbreviations: ASL = Arterial Spin Labelling; *au* = arbitrary unit; BMI = body mass index; DHD = Dutch Healthy Diet; dIPFC = dorsolateral prefrontal cortex; fMRI = functional magnetic resonance imaging; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; ICV = Intracranial volume; MAP = mean arterial pressure; PSQI = Pittsburgh Sleep Quality Index; RAVLT = Rey Auditory Verbal Learning Test; ROIs = regions of interest; LASA-SBQ = LASA Sedentary Behavior Questionnaire; SD = standard deviation; SQUASH = Short Questionnaire to Assess Health-enhancing physical activity.

^a Based on non-adherence to the WHO Guidelines on physical activity (300 or more minutes of moderate aerobic activity or 150 minutes of vigorous aerobic activity per week)

^b Based on systolic blood pressure of ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg

^c Based on total cholesterol > 5 mmol/L or LDL-cholesterol > 3 mmol/L

^d Based on mild cardiovascular disease not mentioned in exclusion criteria (e.g. varicose veins, arteriosclerosis)

^e Based on the International Standard Classification of Education (ISCED 2011) guidelines (low, medium, high education categorization)

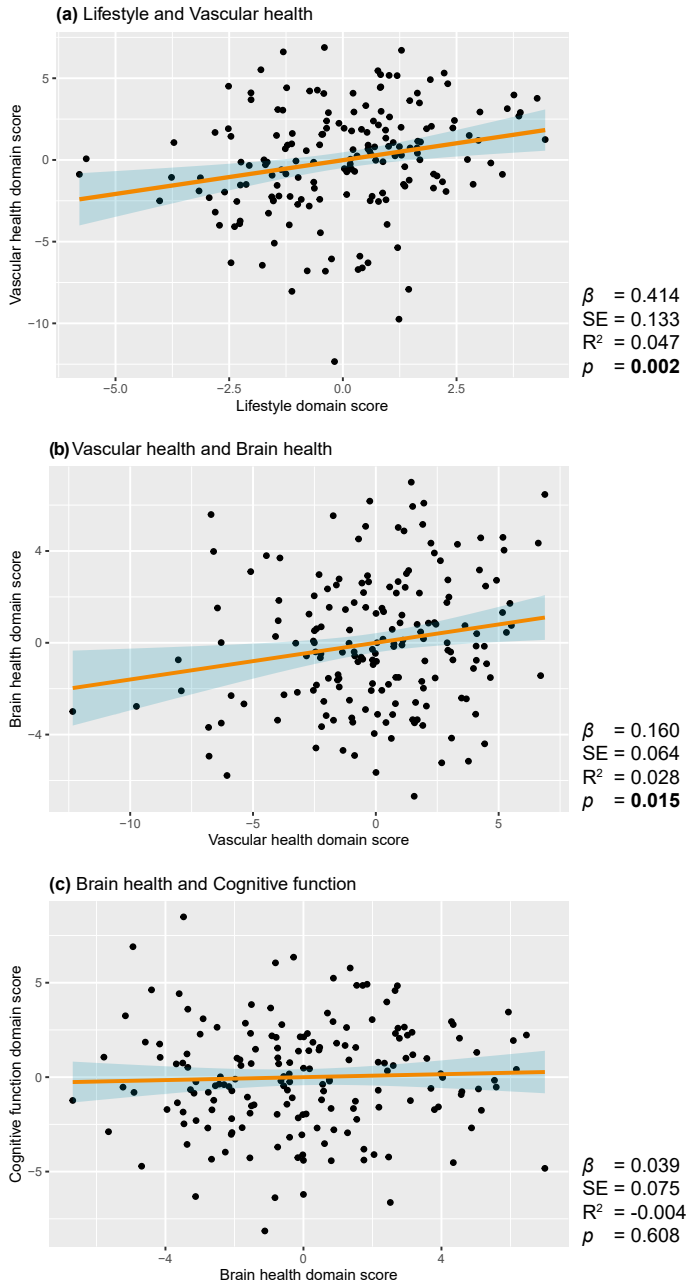


Figure 1. Associations between z-summed total domain composite scores.

(a-c) Linear regression plots showing relationships between domain composite scores of (a) lifestyle and vascular health, (b) vascular health and brain health, and (c) brain health and cognitive functioning. Shaded areas indicate 95% confidence interval. The 95% confidence interval is indicated by shaded areas. R^2 is reported as adjusted R^2 .

Correlational relationships between domains of lifestyle, vascular health, brain health, and cognitive functioning

Simple linear regression modelling between the four total z-sum domain scores revealed two significant relationships. The domain of lifestyle weakly predicted vascular health ($\beta = 0.41$, $SE = 0.13$, $p = 0.002$), and the model explained 4.7% of variance ($R^2 = 0.047$) (Figure 1). The domain of vascular health weakly predicted brain health ($\beta = 0.16$, $SE = 0.06$, $p = 0.01$), and the model explained 3% of variance ($R^2 = 0.03$). There was no predictive value of the domain of brain health on cognitive functioning.

Direct and indirect pathways between lifestyle, vascular risk factors, brain health and cognitive functioning

To assess any possible indirect and direct pathways between the four total domain scores, we constructed eight different SEM models (Figure 2). Models 1 – 7 did not significantly differ from the observed data (χ^2 -test $p > 0.350$), indicating good model fit. Model 1 (pathway between lifestyle and cognitive functioning through vascular and brain health, without other direct pathways) showed the strongest SEM model fit based on lowest comparative model fit indices AIC and BIC (Table 3).

Table 3. Model fit indices and model fit tests of all constructed SEM models.

Models	Model fit indices				Model fit test	
	CFI	RMSEA	AIC	BIC	χ^2 (df)	p-value
1	1.00	0.00	2692.93	2711.95	1.16 (3)	0.76
2	1.00	0.00	2693.89	2716.08	0.12 (2)	0.94
3	1.00	0.00	2694.83	2717.02	1.06 (2)	0.59
4	1.00	0.00	2694.93	2717.12	1.16 (2)	0.56
5	1.00	0.00	2695.79	2721.15	0.02 (1)	0.89
6	1.00	0.00	2695.88	2721.25	0.12 (1)	0.73
7	1.00	0.02	2696.81	2722.17	1.04 (1)	0.31
8	1.00	0.00	2697.77	2726.30	0.00 (0)	NA*
1 + Sex	1.00	0.00	3577.03	3611.90	1.36 (3)	0.71

Model 1 examined the hypothesized stepwise domain pathway order of lifestyle affecting cognitive functioning through vascular health and brain health whilst models 2–8 examined additional direct pathways between the domains of lifestyle and brain health or cognitive functioning, or vascular health and cognitive functioning. Model 1, showing strongest fit, was additionally analysed with sex (female) as a covariate.

Abbreviations: AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; CFI = Comparative Fit Index; RMSEA = Root Mean Square of Approximation.

* no p-value is provided because of model saturation ($\chi^2 = 0.00$, $df = 0$).

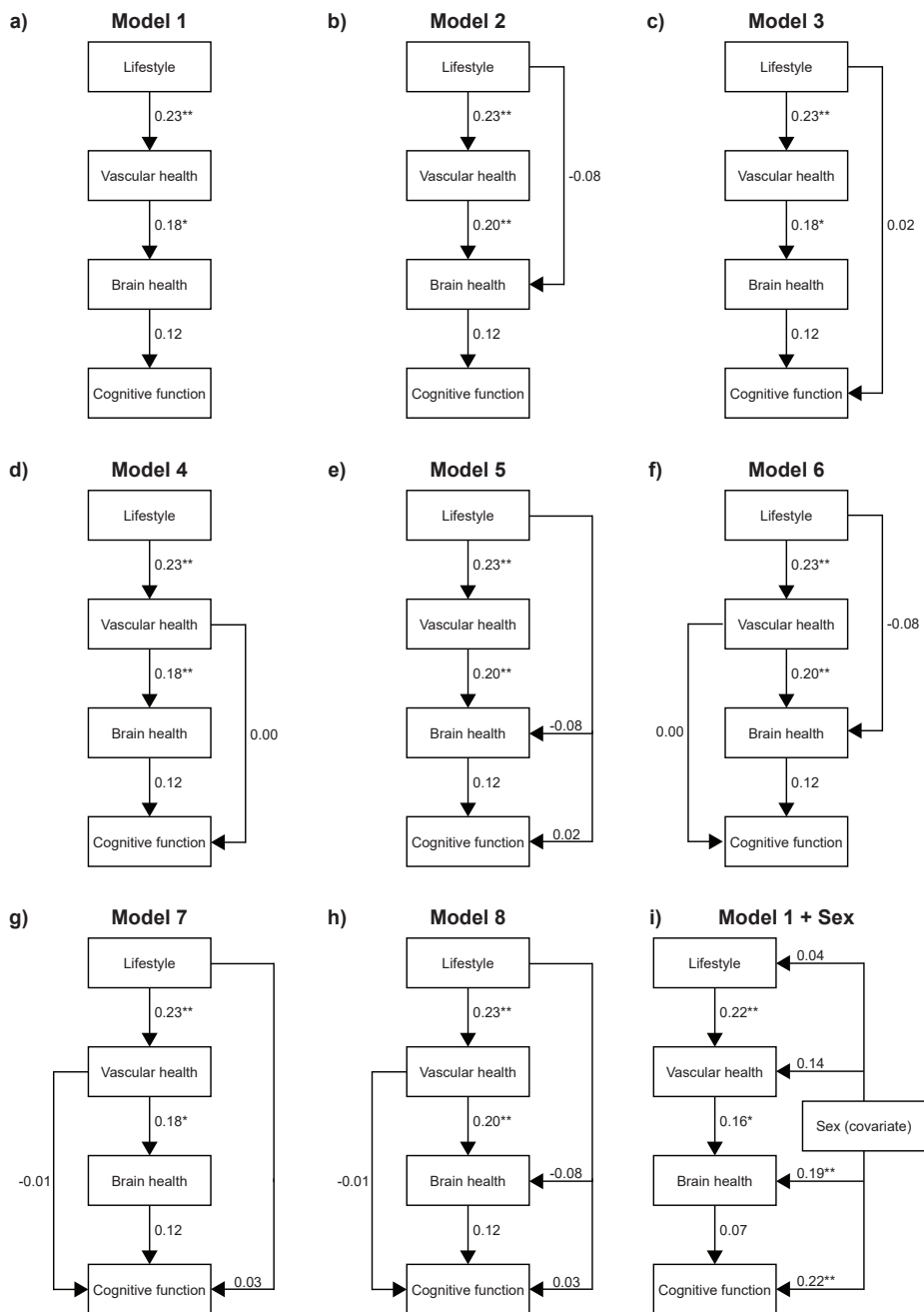


Figure 2. SEM models with all four domain scores.

(a-h) SEM models containing the four domain scores with possible direct and indirect pathway combinations.

(i) Model containing pathways of strongest fitting SEM model (Model 1), with addition of Sex as a covariate.

* = $p < 0.05$, ** = $p < 0.01$

In Model 1, significant shared variance was observed between the sets of lifestyle and vascular health ($\beta = 0.23$, $p = 0.002$) and between vascular health and brain health ($\beta = 0.18$, $p = 0.01$). There was no significant shared variance between the sets of brain health and cognitive functioning. Adding sex (female) as a covariate to Model 1 (Figure 2i) showed that sex significantly explains variance within the sets of brain health ($\beta = 0.19$, $p < 0.01$) and cognitive functioning ($\beta = 0.22$, $p < 0.01$). The AIC and BIC comparative model fit indices for this model were higher than Model 1 (without added covariate), indicating poorer model fit.

Shared variance between individual outcomes of lifestyle, vascular health, brain health, and cognitive functioning

To investigate which variables within each outcome domain explain the strongest covariance between domains, we constructed four different CCA models based on the associations found between the outcome sets in SEM model 1. We found no significant canonical correlations between the outcome sets of lifestyle and vascular health, or brain health and cognitive functioning (Appendix A: Supplementary Tables 1A–B, 2A–B). The CCA analysis between the domains of vascular health and brain health showed a trend-significant canonical correlation (Table 4). The first canonical dimension was trend-significant ($\lambda = 0.63$, $F(42, 548) = 1.336$, $p = 0.081$), indicating that around 18.6% ($r = 0.431$) of variance within the brain health set can be explained by variables from the vascular health set within this dimension. The other five dimensions showed no significant canonical correlations, and therefore only canonical dimension 1 was analyzed further.

Table 4. Tests of canonical dimensions of CCA model of vascular health and brain health domains.

Dimension	Canonical coefficient	Approximate F-statistic	Degrees of freedom	Wilk's (λ)test-statistic	p-value
1	0.431	1.336	42, 548	0.633	0.081
2	0.324	1.019	30, 470	0.777	0.441
3	0.273	0.854	20, 392	0.868	0.646
4	0.195	0.640	12, 315	0.938	0.807
5	0.154	0.501	6, 240	0.975	0.807
6	0.032	0.061	2, 121	0.999	0.941

Canonical dimensions 1–6: Linear combinations of variables (dimensions) that maximize shared variance between two variable sets. Dimension 1 captures the strongest shared variance, followed by dimension 2, and so on.

Table 5. Canonical loadings and standardized canonical coefficients of dimension 1 between vascular health and brain health markers

Variables	Canonical loadings	Standardized canonical coefficients
<i>Vascular health domain</i>		
BMI	-0.370	-0.033
MAP	-0.837	-0.757
HOMA-IR	-0.373	-0.350
HDL-cholesterol	0.108	-0.263
LDL-cholesterol	-0.408	-0.225
Triglycerides	-0.466	-0.289
<i>Brain health domain</i>		
WM fMRI activation dlPFC	0.028	-0.044
WM fMRI activation hippocampus	-0.044	0.033
CBF dlPFC	-0.318	-0.130
CBF hippocampus	-0.364	-0.827
Volume dlPFC	0.215	0.517
Volume hippocampus	0.027	-0.007
Myo-inositol dlPFC	-0.022	-0.010

Abbreviations: BMI = body mass index; CBF = cerebral blood flow; dlPFC = dorsolateral prefrontal cortex; fMRI = functional magnetic resonance imaging; HDL = high-density lipoprotein; HOMA-IR = homeostatic model assessment of insulin resistance; LDL = low-density lipoprotein; MAP = mean arterial pressure; WM = working memory

All variables were transformed so that higher scores indicate healthier outcomes. Positive canonical loadings correspond to healthier scores, whereas negative canonical loadings correspond to less healthy scores.

Table 5 shows the canonical loadings and standardized canonical correlation coefficients of the variables within canonical dimension 1 (for dimensions 2–5, see Appendix A: Supplementary Table 2B). Within the vascular health domain variables, MAP showed the highest canonical loading magnitude (-0.837; strong effect size), indicating that MAP (blood pressure) has the strongest association with the canonical variate of dimension 1 (U1) compared with other variables. BMI, HOMA-IR, LDL-cholesterol and triglycerides all show similar canonical loading magnitude of small-to-moderate effect sizes (-0.370 – -0.466). HDL-cholesterol shows the lowest association with canonical variate U1. For the brain health domain variables, CBF in the DLPFC and hippocampus showed the strongest canonical loading magnitude (-0.318 – -0.364; moderate effect size), indicating that the measures of local CBF

(perfusion) have the strongest association with the canonical variate of dimension 1 (Y_1) compared with other variables. DLPFC volume showed a small canonical loading magnitude, and WM fMRI activation in DLPFC and hippocampus and myo-inositol in DLPFC showed negligible associations with canonical variate Y_1 (Table 5). Participants with a poorer vascular health status (indicated by lower MAP, BMI, HOMA-IR, LDL-cholesterol and triglycerides scores) showed worse perfusion (indicated by lower CBF scores) in the DLPFC and hippocampus, as well as a larger DLPFC volume.

Correlational relationships between individual lifestyle, vascular health, brain health and cognitive functioning markers

To explore potential specific associations between individual variables of each domain, beyond the broader domain-level relationships, we performed a pairwise Pearson's correlation analysis on all included variables (Figure 3). We observed a cluster of small correlations between better diet score and BMI ($r = 0.22$), HOMA-IR ($r = 0.16$), HDL-cholesterol ($r = 0.23$) and triglycerides ($r = 0.20$). These correlations indicate that lower BMI, lower insulin resistance, higher HDL-cholesterol, and lower triglycerides are associated with higher dietary score. A cluster of small associations was also observed between CBF in the dLPFC and hippocampus and vascular health domain variables. Specifically, CBF in the dLPFC was associated with BMI ($r = 0.21$), MAP ($r = 0.26$), and HOMA-IR ($r = 0.17$). Similarly, CBF in the hippocampus was associated with BMI ($r = 0.20$) and MAP ($r = 0.30$). In addition, a weak negative correlation between hippocampal CBF and physical activity score ($r = -0.16$) was observed. These correlations indicate that lower BMI, lower MAP, and lower HOMA-IR are associated with higher CBF in hippocampus and/or dLPFC, and that lower physical activity is associated with higher hippocampal CBF. For WM fMRI activation in the hippocampus, we observed a weak negative correlation with diet score ($r = -0.17$), indicating that better diet scores were associated with lower WM task-related brain activation in the hippocampus. For the variables of the cognitive functioning domain, we found that healthy diet score positively correlated with TMT performance ($r = 0.15$) and negatively with VFT performance ($r = -0.18$). Lower LDL-cholesterol levels correlated with higher executive function measured by VFT ($r = 0.16$). A small correlation between WM fMRI activation in dLPFC and executive function measured by TMT was found ($r = 0.16$). Higher WM fMRI neural recruitment in the hippocampus was positively associated with episodic memory performance in the RAVLT delayed recall measure ($r = 0.16$).



Figure 3. Pearson's pairwise correlation matrix of all included variables. Only significant correlations ($p < 0.05$) are shown. All variables have been transformed so a higher score indicates a better/healthier outcome. *Abbreviations:* BMI = body mass index; CBF = cerebral blood flow; DHD = Dutch Healthy Diet; dlPFC = dorsolateral prefrontal cortex; DSST = Digit Symbol Substitution Test; DST = Digit Span Test; FFMQ = Five Faceted Mindfulness Questionnaire; fMRI = functional magnetic resonance imaging; HADS = Hospital and Anxiety Depression Scale; HDL = high-density lipoproteins; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; LDL = low-density lipoproteins; MAP = mean arterial pressure; PSQI = Pittsburgh sleep quality index; PSS = Perceived Stress Scale; SBQ = Sedentary Behavior Questionnaire; SQUASH = Short Questionnaire to Assess Health-enhancing physical activity; RAVLT = Rey Auditory Verbal Learning Test; TMT = Trail Making Test; VFT = Verbal Fluency Test; WM = working memory.

Exploratory analyses for males and females

Because of the observed significant effects of sex (female) as a covariate in SEM model with the strongest model fit, we performed post-hoc analyses in subsets to further explore differences in associations between the total domain scores, as well as the individual outcome measures between men and women. In women, simple linear

regression models showed a significant association between the composite domains of lifestyle and vascular health ($\beta = 0.55$, $SE = 0.17$, $p = 0.002$), and a trend-significant association between the composite domains of vascular health and brain health ($\beta = 0.15$, $SE = 0.08$, $p = 0.058$), as opposed to no significant correlations in men ($p > 0.34$) (Appendix A: Supplementary Figure 1). In the pairwise Pearson's correlation analyses, women showed more positive associations between diet score and vascular health measures of BMI ($r = 0.27$), HOMA-IR ($r = 0.26$) and triglycerides ($r = 0.21$) compared to men. Compared to women, men showed more positive associations between vascular health measures BMI and HOMA-IR in measures of CBF within the dLPFC ($r = 0.30 - 0.31$) and hippocampus ($r = 0.33$). The complete results of the total domain simple linear regression models, as well as the individual outcome measure Pearson's correlation analysis, are presented in Appendix A: Supplementary Figures 1-3.

Discussion

In this cross-sectional exploratory study in older adults, we investigated a model integrating multiple facets of lifestyle, vascular health, brain health and cognitive functioning to identify possible correlational pathways and to isolate the strongest variables driving the shared variance between these domains. We hypothesized that we would find a pathway in a sequential order of the domain of lifestyle correlating with vascular health, which in turn correlates with brain health, which ultimately is associated with cognitive functioning. We found significant weak correlations between the lifestyle and vascular health domain scores, and between vascular health and brain, but observed no significant association between the domains of brain health and cognitive functioning. Except for the latter non-significant brain-cognition association, these findings are in line with our primary hypothesis of sequential effects. The integration of any additional direct pathways between the domains (e.g., from vascular health to cognitive functioning) did not improve the model. We furthermore found that the CCA models confirmed the established link between vascular and brain health, with CBF in the dLPFC and hippocampus emerging as the strongest contributors to shared variance within this population. These results were replicated in bivariate correlations between individual outcomes of vascular and brain health.

Our observed sequential links between lifestyle patterns, vascular health, brain health, and, subsequently, cognitive health, are largely in line with previous studies that have found bivariate links between lifestyle and vascular health, vascular health and brain health, or brain health and cognitive functioning in aging [3, 37-41].

Although we were unable to find a statistically significant association between the collective set of brain health markers and cognitive functioning, the observed coefficients did point in our hypothesized direction (i.e. healthier outcomes of brain structure and function are collectively associated with higher cognitive functioning). This final step, the translation from neuroimaging brain health markers to cognitive functioning measured by neuropsychological tests, is notoriously difficult [42]. Nevertheless, multiple studies have reported associations between measures of brain health and cognitive performance in aging, such as increased fMRI responses [43], CBF [11, 44], global and local brain volumes [9, 45, 46], and neuroinflammation [47]. Our observed inconsistent findings compared to previous studies may be explained by differences in study populations, as these prior studies generally included populations significantly older [9, 44, 45] or younger than 60-75 years [43, 46], or individuals with diagnosed mild cognitive impairment or Alzheimer's Disease [11, 47]. Additionally, many of these studies consisted of longitudinal data or much larger sample sizes which, compared to the cross-sectional design and limited sample size of the current study, may have limited our ability to reduce between-individual variability and detect subtle associations.

Although direct associations between healthy lifestyle patterns on brain health markers or cognitive functioning have been reported previously [48, 49], we did not observe similar associations. Lifestyle markers showed minimal association with markers of brain health or cognitive functioning, both when examined individually and when combined into a composite domain score, except for mixed associations between dietary score and measures of executive functioning performance indicating task-specific effects on executive function performance. Additionally, our SEM model analysis indicated that the addition of any direct pathways between lifestyle and brain health, lifestyle and cognitive functioning, or vascular health and cognitive functioning, directly lowered the model's fit. In our population of older at-risk adults, the impact of lifestyle on brain health (and potentially cognitive functioning) was strongest when mediated through vascular health. This finding is in line with previous studies, which indicate strong correlations between cardiovascular risk and specific brain regions involved in higher-order cognitive functions, such as the dlPFC and hippocampus, which support executive functioning, working memory, and episodic memory [6, 8]. The mixed findings between our analysis and previously reported protective factors of lifestyle on cognition, could be due to methodological differences. Our cross-sectional analysis approach is not able to capture long-term or causal effects, as opposed to longitudinal intervention studies [48, 50, 51]. However, not all longitudinal lifestyle intervention studies found direct effects of positive lifestyle changes on measures of cognitive functioning, which suggests that the

relationship between lifestyle and cognition is still not fully understood [52-54] and might benefit from the inclusion of vascular factors as mediator.

The observed variance of multiple vascular health markers such as BMI, blood pressure (MAP), and blood-lipid markers with brain health markers was predominantly expressed in shared variance with CBF within the dlPFC and hippocampus. This relatively strong association between cardiovascular health and our CBF measures is in line with the sensitivity of the dlPFC and hippocampus structures to cardiovascular burden [6, 8, 55]; however, we did not include whole-brain CBF measures, which could display similar associations. We also found that participants with unhealthier scores of BMI, MAP, HOMA-IR, LDL-cholesterol and triglycerides had higher dlPFC volumes. This finding was unexpected, as the (dl) PFC has been reported to be negatively affected by such cardiovascular risk factors in an earlier longitudinal follow-up study in healthy and at-risk adults [56]. Although current literature does not yet offer an explanation for this finding, it is possible that our cross-sectional analysis was not able to capture the long-term detrimental effects of cardiovascular risk on dlPFC. This notion is supported by the lack of significant bivariate correlations between these vascular and volumetric outcomes, suggesting that volumetric outcomes may be less robust than the vascular-CBF links which were consistently significant across all our analyses.

We did not find any significant bivariate associations within the models relating lifestyle and vascular health markers, and brain health and cognitive functioning markers. The associations found when using total domain scores were largely lost within the CCA models where all variables of each set were individually weighted. This finding potentially points to an additive effect where the whole effect of all variables within the different sets (i.e., the z-score summed sets) was greater than the sum of its parts (i.e., the individual markers). Within the context of lifestyle effects on cognitive decline, previous studies, including a meta-analysis, have shown that lifestyle interventions targeting multiple different lifestyle domains have broader positive effects on cardiovascular risk factors than single-domain interventions [57, 58]. This suggests a potential additive effect: targeting multiple mechanisms underlying cognitive aging may improve overall intervention effectiveness. The latent composite domain score used in the current study, based on the sums of z-scaled markers, can provide a more robust and simplified way to compare multiple different outcome measures compared to single metrics and more closely follows the multifactorial etiology of aging-related cognitive decline and dementia. Our approach could especially be useful when grouping measures that assess the same underlying domain. Therefore, the present analysis performed on this latent domain

variable could provide significant results, which were lost when looking at the individual components used to calculate the composite score.

Our study has several limitations. We performed exploratory data analyses with a cross-sectional approach. Therefore, even though our analyses revealed significant relations between a number of lifestyle, vascular health and brain health markers, the possibility of an unknown variable influencing these associations cannot be ruled out completely. Additionally, although evidence of the directionality of the hypothesized pathways and associations is robust, we are unable to formulate conclusions of possible causal relations between the observed associations. Another point of caution concerns our created brain health score, where we used higher dlPFC and hippocampal fMRI activation during the working memory N-back task as indicators of better brain health. Although the dlPFC and hippocampus are strongly associated with working memory-related tasks [59, 60], both brain regions show distinct task-related activation patterns which at times are non-parallel and may indicate different cognitive processes [61]. Therefore, one could argue that a higher neural recruitment in the dlPFC and hippocampus does not necessarily indicate a healthier brain outcome. Multiple studies have reported conflicting evidence of differences in hyper- or hypoactivation between healthy younger and older adults [62, 63], as well as in patients with mild cognitive impairment or dementia [64]. The possibility of compensatory mechanisms and individual differences influencing brain activation should be considered, especially considering the older age of our study population and their inclusion based on multiple cardiovascular risk factors. To add to this, we found one association which could possibly point to reduced compensatory need, where participants with lower WM fMRI activation in the hippocampus adhered to healthier dietary patterns. Another limitation of our study is that we only observed small associations between variables or domain composite scores. Therefore, interpretation of these results should be cautious and nuanced. One possible explanation for the lack of strong associations is the heterogeneity in risk profiles within our study sample. Because participants were at-risk of cognitive decline, comparisons between individuals with, for example, low versus high blood pressure do not necessarily represent a contrast between unhealthy and healthy individuals, as participants with lower blood pressure by definition had other risk factors. This lack of a healthy control group and the heterogeneity of individual risk factor profiles may have attenuated the observed associations. Lastly, it should be noted that we only analyzed simple linear relationships between individual markers and total domain scores, leading to possible oversimplified conclusions. With our current analysis, we were unable to examine potential non-linear, quadratic, or moderation effects among our included outcome variables. Thereby, more complex associations

were not tested, and we only examined the possible direct and indirect pathways of our hypothesized sequential pathway order. As part of further exploratory analyses, other sequential orders (e.g., lifestyle-brain health associations mediated through cognitive functioning or vascular health markers) could be considered.

In conclusion, our study has extended previous studies by integrating multiple outcome measures of lifestyle, vascular health, brain health, and cognitive functioning into one integrated model to investigate the potential partial- or full mediation pathway of lifestyle ultimately affecting brain health and cognitive functioning. Our findings indicate a possible additive effect of multiple facets of lifestyle through vascular health on brain health. Future lifestyle intervention studies on such vascular, brain and cognitive markers, will offer more insight into their causal relationships in aging.

Glossary

aCompCor	anatomical principal component noise regressors
AIC	Akaike Information Criterion
ASL	arterial spin labelling
BIC	Bayesian Information Criterion
BMI	body mass index
BOLD	blood-oxygen-level-dependent
CAIDE	Cardiovascular Risk Factors, Aging, and Incidence of Dementia
CBF	cerebral blood flow
CCA	canonical correlation analysis
CFI	Comparative Fit Index
CHES	chemically selective water suppression
CSF	cerebrospinal fluid
DBP	diastolic blood pressure
DHD	Dutch Healthy Diet
dlPFC	dorsolateral prefrontal cortex
DSST	Digit Symbol Substitution Test
DST	Digit Span Test
EPI	echo planar imaging
FFMQ	Five Facet Mindfulness Questionnaire
FFQ	Food Frequency Questionnaire
fMRI	functional magnetic resonance imaging
FWHM	full width half maximum

GLM	general linear model
HADS	Hospital Anxiety and Depression Scale
HDL	high-density lipoprotein
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
ICV	intracranial volume
IQR	interquartile range
ISCED	International Standard Classification of Education
LDL	low-density lipoprotein
MAP	mean arterial pressure
MET	metabolic equivalent of task
MIND-NL	Dutch Mediterranean-Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay
pCASL	pseudocontinuous arterial spin labelling
PRESS	Point RESolved Spectroscopy
PSQI	Pittsburgh Sleep Quality Index
PSS	Perceived Stress Scale
RAVLT	Rey Auditory Verbal Learning Test
relResA	relative residual amplitude
RMSEA	Root Mean Square Error of Approximation
ROIs	regions of interest
RU-DCCN	Radboud University Donders Center for Cognitive Neuroimaging
SBP	systolic blood pressure
LASA-SBQ	LASA Sedentary Behavior Questionnaire
SD	standard deviations
SEM	structural equation modelling
SNR	signal-to-noise ratio
SQUASH	Short Questionnaire to Assess Health-enhancing physical activity
tCompCor	temporal principal component noise regressors
TICS-M1	Telephone Interview for Cognitive Status (Modified version)
TMT	Trail Making Test
VFT	Verbal Fluency Test
WHO	World Health Organization
WM	working memory
WUR-HNH	Wageningen University department of Human Nutrition and Health

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Mark R. van Loenen: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Project Administration. **Lianne B. Remie:** Validation, Investigation, Data Curation, Writing – Original Draft, , Writing – Review & Editing, Project Administration. **Mara P.H. van Trijp:** Investigation, Data Curation, Writing – Review & Editing, Project Administration. **Mechteld M. Grootte Bromhaar:** Investigation, Data Curation, Project Administration. **José P. Marques:** Resources, Data Curation, Writing – Review & Editing, Funding Acquisition. **Jurgen A.H.R. Claassen:** Writing – Review & Editing, Funding Acquisition. **Wilma T. Steegenga:** Conceptualization, Methodology, Supervision, Project Administration, Funding Acquisition. **Joukje M. Oosterman:** Conceptualization, Methodology, Validation, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition. **Esther Aarts:** Conceptualization, Methodology, Validation, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition.

Statement on Conflicts of Interest

All authors declare that there is no conflict of interest.

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Appendix A. Supplementary Material

Supplementary table 1A. Tests of canonical dimensions of CCA model of lifestyle and vascular health domains.

Dimension	Rc	Rc ²	Wilks' λ	F-statistic	df	p-value
1	0.353	0.125	0.739	1.011	42, 636.658	0.454
2	0.277	0.077	0.844	0.788	30, 546	0.784
3	0.207	0.043	0.914	0.623	20, 455.327	0.897
4	0.177	0.031	0.955	0.532	12, 365.405	0.894
5	0.113	0.013	0.986	0.323	6, 278	0.925
6	0.031	0.001	0.999	0.066	2, 140	0.936

Canonical dimensions 1–6: Linear combinations of variables (dimensions) that maximize shared variance between two variable sets. Dimension 1 captures the strongest shared variance, followed by dimension 2, and so on. Canonical correlations (Rc), squared canonical correlations (Rc²), Wilks' λ , F-statistic, degrees of freedom (df), and p-values for each canonical dimension between lifestyle and vascular health domains.

Supplementary table 1B. Canonical loadings of all dimensions of CCA model of lifestyle and vascular health domains.

Variable	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5	Dimension 6
Set X (Lifestyle)						
DHD	-0.598	0.064	-0.330	0.339	-0.294	0.570
SQUASH	-0.487	-0.259	-0.318	-0.687	-0.064	-0.300
LASA	-0.475	0.709	0.390	-0.294	0.103	-0.022
PSS	-0.003	0.448	-0.133	0.203	-0.355	-0.605
HADS	-0.078	0.095	0.161	0.197	0.055	-0.187
FFMQ	-0.471	-0.388	0.232	0.405	0.281	-0.446
PSQI	0.159	0.279	-0.630	0.187	0.605	-0.090
Set Y (Vascular health)						
BMI	-0.770	0.053	0.383	-0.192	-0.469	0.032
MAP	-0.166	0.098	0.472	-0.686	0.349	0.383
HOMA-IR	-0.695	-0.530	-0.116	0.229	0.189	0.366
HDL-cholesterol	-0.459	0.315	-0.582	-0.001	-0.251	0.536
LDL-cholesterol	-0.260	0.633	0.285	0.362	0.538	-0.171

Supplementary table 1B. Continued

Variable	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5	Dimension 6
Triglycerides	-0.706	0.073	-0.453	-0.208	0.455	-0.200

Canonical loadings (correlation between each variable and the canonical variate) of each canonical dimension for the domains of lifestyle (set X) and vascular health (set Y). Data was Z-scored, and select variables were transformed so higher Z-scores indicate healthier outcomes.

Abbreviations: BMI = body mass index; DHD = Dutch Healthy Diet; FFMQ = Five Faceted Mindfulness Questionnaire; HADS = Hospital and Anxiety Depression Scale; HDL = high-density lipoproteins; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; LDL = low-density lipoproteins; MAP = mean arterial pressure; PSQI = Pittsburgh sleep quality index; PSS = Perceived Stress Scale; SBQ = Sedentary Behavior Questionnaire; SQUASH = Short Questionnaire to Assess Health-enhancing physical activity.

Supplementary table 2A. Tests of canonical dimensions of CCA model of vascular health and brain health domains.

Dimension	Rc	Rc ²	Wilks' λ	F-statistic	df	p-value
1	0.431	0.186	0.633	1.336	42, 547.540	0.081
2	0.324	0.105	0.777	1.019	30, 470	0.441
3	0.273	0.075	0.868	0.854	20, 392.312	0.646
4	0.195	0.038	0.938	0.640	12, 315.136	0.807
5	0.154	0.024	0.975	0.501	6, 240	0.807
6	0.032	0.001	0.999	0.061	2, 121	0.941

Canonical dimensions 1–6: Linear combinations of variables (dimensions) that maximize shared variance between two variable sets. Dimension 1 captures the strongest shared variance, followed by dimension 2, and so on. Canonical correlations (Rc), squared canonical correlations (Rc²), Wilks' λ, F-statistic, degrees of freedom (df), and p-values for each canonical dimension between vascular health and brain health domains.

Supplementary table 2B. Canonical loadings of all dimensions of CCA model of vascular health and brain health domains.

Variable	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5	Dimension 6
Set X (Vascular health)						
BMI	-0.370	0.659	0.255	0.477	-0.149	0.336
MAP	-0.837	-0.204	0.007	0.308	-0.319	-0.247
HOMA-IR	-0.373	0.612	0.123	0.055	0.653	-0.206
HDL-cholesterol	0.108	-0.012	0.864	0.355	0.338	-0.041
LDL-cholesterol	-0.408	-0.380	-0.102	-0.070	0.345	0.745
Triglycerides	-0.466	0.125	0.653	-0.522	0.193	0.178

Supplementary table 2B. Continued

Variable	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5	Dimension 6
Set Y (Brain health)						
WM fMRI activation dlPFC	0.065	0.231	-0.270	0.231	-0.425	-0.118
WM fMRI activation hippocampus	-0.101	-0.266	-0.424	-0.076	0.109	0.743
CBF dlPFC	-0.050	0.821	0.066	-0.424	0.250	0.274
CBF hippocampus	-0.738	0.010	0.142	0.518	0.390	0.101
Volume dlPFC	-0.844	0.039	-0.086	0.420	-0.011	0.195
Volume hippocampus	0.499	0.111	-0.080	0.740	0.023	0.262
Myo-inositol dlPFC	0.064	0.000	0.777	0.175	-0.332	0.488

Canonical loadings (correlation between each variable and the canonical variate) of each canonical dimension for the domains of vascular health (set X) and brain health (set Y). Data was Z-scored, and select variables were transformed so higher Z-scores indicate healthier outcomes. Positive canonical loadings correspond to healthier scores, whereas negative canonical loadings correspond to less healthy scores.

Abbreviations: BMI = body mass index; CBF = cerebral blood flow; dlPFC = dorsolateral prefrontal cortex; fMRI = functional magnetic resonance imaging; HADS = Hospital and Anxiety Depression Scale; HDL = high-density lipoproteins; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; LDL = low-density lipoproteins; MAP = mean arterial pressure; WM = working memory.

Supplementary table 3A. Tests of canonical dimensions of CCA model of brain health and cognitive functioning domains.

Dimension	Rc	Rc ²	Wilks'λ	F-statistic	df	p-value
1	0.305	0.093	0.795	0.730	42, 613.206	0.897
2	0.260	0.068	0.877	0.585	30, 526	0.963
3	0.201	0.040	0.940	0.410	20, 438.744	0.990
4	0.120	0.014	0.980	0.224	12, 352.176	0.997
5	0.074	0.005	0.994	0.127	6, 268	0.993
6	0.014	0.000	1.000	0.012	2, 135	0.988

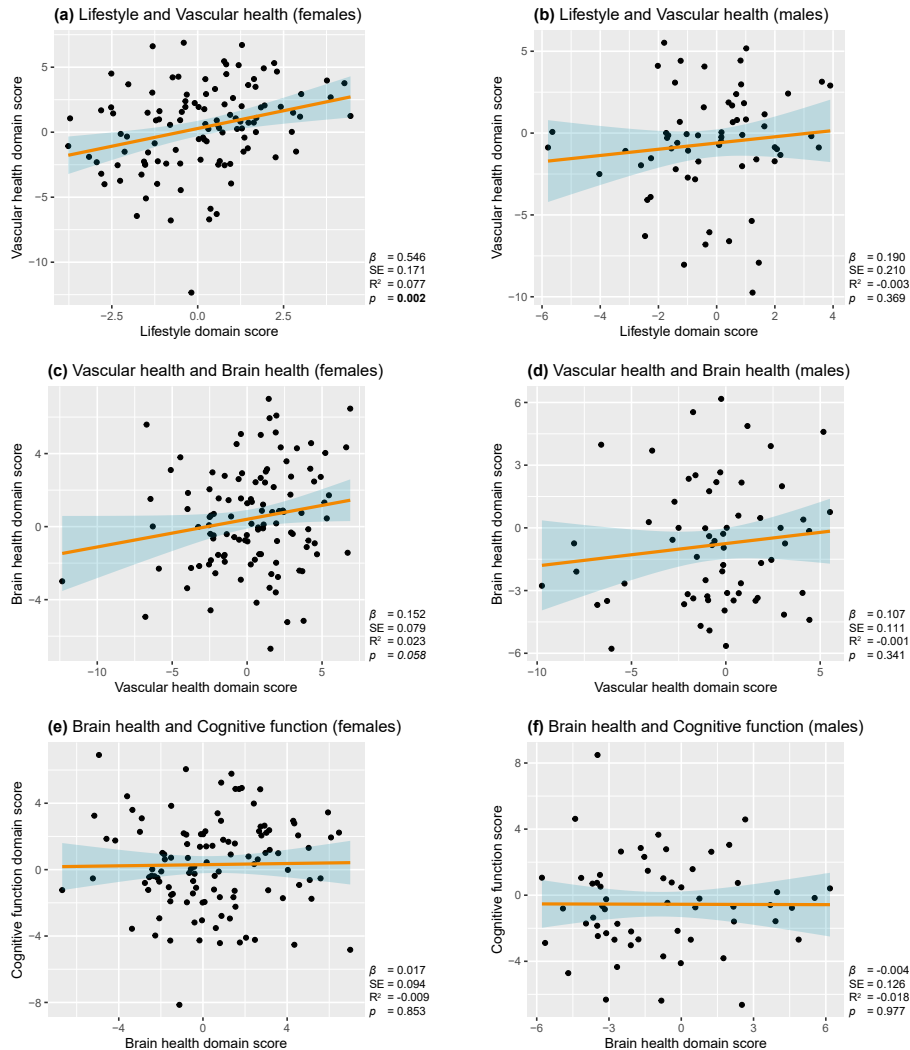
Canonical dimensions 1–6: Linear combinations of variables (dimensions) that maximize shared variance between two variable sets. Dimension 1 captures the strongest shared variance, followed by dimension 2, and so on. Canonical correlations (Rc), squared canonical correlations (Rc²), Wilks'λ, F-statistic, degrees of freedom (df), and p-values for each canonical dimension between brain health and cognitive functioning domains.

Supplementary table 3B. Canonical loadings of all dimensions of CCA model of brain health and cognitive functioning domains.

Variable	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5	Dimension 6
Set X (Brain health)						
WM fMRI activation dlPFC	-0.360	0.522	0.217	-0.459	0.263	0.439
WM fMRI activation hippocampus	0.310	-0.193	0.711	-0.352	-0.253	0.415
CBFdlLPFC	0.396	0.568	0.416	0.316	-0.172	-0.467
CBF hippocampus	-0.021	0.332	0.679	0.384	-0.245	-0.468
Volume dlPFC	-0.055	0.004	-0.140	-0.664	-0.329	-0.649
Volume hippocampus	0.104	-0.071	-0.070	0.047	-0.388	-0.019
Myo-inositol dlPFC	-0.111	0.234	-0.307	0.244	-0.709	0.438
Set Y (Cognitive functioning)						
TMT Executive function	0.317	-0.276	-0.125	0.872	-0.215	-0.029
VFT Executive function	0.572	-0.269	-0.070	-0.135	0.657	-0.381
DSST Processing speed	-0.575	0.650	-0.081	0.444	0.102	-0.179
DST Working memory	0.320	0.788	0.185	-0.030	-0.071	0.486
N-back Working memory	-0.333	0.007	-0.148	0.040	0.547	0.753
RAVLT Episodic memory	0.194	-0.219	0.940	0.133	0.098	-0.061

Canonical loadings (correlation between each variable and the canonical variate) of each canonical dimension for the domains of brain health (set X) and cognitive functioning (set Y). Data was Z-scored, and select variables were transformed so higher Z-scores indicate healthier outcomes. Positive canonical loadings correspond to healthier scores, whereas negative canonical loadings correspond to less healthy scores.

Abbreviations: CBF = cerebral blood flow; dlPFC = dorsolateral prefrontal cortex; DSST = Digit Symbol Substitution Test; DST = Digit Span Test; fMRI = functional magnetic resonance imaging; RAVLT = Rey Auditory Verbal Learning Test; TMT = Trail Making Test; VFT = Verbal Fluency Test; WM = working memory.



Supplementary figure 1. Associations between z-summed total domain scores in Female vs. Male subsets.

Linear regression plots showing relationships between domain scores of lifestyle and vascular health, vascular health and brain health, and brain health and cognitive functioning in Female (panels a, c, and e) and Male (panels b, d, and f) subsets. The 95% confidence interval is indicated by shaded areas. R^2 is reported as adjusted R^2 .



Supplementary figure 2. Female subset correlation matrix of all included lifestyle, vascular, brain and cognitive functioning outcome measures. Only significant correlations ($p < 0.05$) are shown. Select variables were transformed so higher Z-scores indicate healthier outcomes.

Abbreviations: BMI = body mass index; CBF = cerebral blood flow; DHD = Dutch Healthy Diet; dlPFC = dorsolateral prefrontal cortex; DSST = Digit Symbol Substitution Test; DST = Digit Span Test; FFMQ = Five Faceted Mindfulness Questionnaire; fMRI = functional magnetic resonance imaging; HADS = Hospital and Anxiety Depression Scale; HDL = high-density lipoproteins; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; LDL = low-density lipoproteins; MAP = mean arterial pressure; PSQI = Pittsburgh sleep quality index; PSS = Perceived Stress Scale; SBQ = Sedentary Behavior Questionnaire; SQUASH = Short Questionnaire to Assess Health-enhancing physical activity; RAVLT = Rey Auditory Verbal Learning Test; TMT = Trail Making Test; VFT = Verbal Fluency Test; WM = working memory.



Supplementary figure 3. Male subset correlation matrix of all included lifestyle, vascular, brain and cognitive functioning outcome measures. Only significant correlations ($p < 0.05$) are shown. Select variables were transformed so higher Z-scores indicate healthier outcomes.

Abbreviations: BMI = body mass index; CBF = cerebral blood flow; DHD = Dutch Healthy Diet; dlPFC = dorsolateral prefrontal cortex; DSST = Digit Symbol Substitution Test; DST = Digit Span Test; FFMQ = Five Faceted Mindfulness Questionnaire; fMRI = functional magnetic resonance imaging; HADS = Hospital and Anxiety Depression Scale; HDL = high-density lipoproteins; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; LDL = low-density lipoproteins; MAP = mean arterial pressure; PSQI = Pittsburgh sleep quality index; PSS = Perceived Stress Scale; SBQ = Sedentary Behavior Questionnaire; SQUASH = Short Questionnaire to Assess Health-enhancing physical activity; RAVLT = Rey Auditory Verbal Learning Test; TMT = Trail Making Test; VFT = Verbal Fluency Test; WM = working memory.



PART IV

Assessing Brain Responses to Lifestyle Interventions

Chapter 4

The Effects of a Multidomain Lifestyle Intervention on Neuroimaging Measures of Brain Functioning in Older Adults

In preparation:

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Abstract

Introduction

Multidomain lifestyle interventions may slow down ageing-related cognitive decline, but the brain mechanisms underlying these effects remain unclear. Using neuroimaging, we investigated brain changes associated with a 6-month multidomain lifestyle intervention in Dutch older adults at risk for accelerated age-related cognitive decline.

Methods

Participants aged 60–75 years with ≥ 2 lifestyle-modifiable cardiovascular risk factors (e.g., BMI ≥ 25 , physical inactivity, hypertension, hypercholesterolemia) were recruited and randomized to a high-intensity intervention group or active control group. The multidomain lifestyle intervention included five lifestyle domains (diet, physical activity, stress management & mindfulness, cognitive training, and sleep). Primary outcomes were changes in working memory task-related functional magnetic resonance imaging (fMRI) BOLD response and cerebral blood flow (CBF) in the dorsolateral prefrontal cortex (dlPFC) and hippocampus, and working memory N-back task performance. Secondary outcomes included lifestyle questionnaire scores, cardiovascular health outcomes (e.g., BMI, blood pressure, blood lipids), and cognitive test scores. Intervention-induced effects between baseline and 6-month follow-up were investigated with linear mixed models with Group, Time, and a Group $\times$ Time interaction as fixed effects, and within-subject repeated measures as random effect.

Results

Of 102 participants at baseline, 86 completed the 6-month follow-up assessment. No group differences were observed for fMRI outcomes or dlPFC CBF. A significant Group $\times$ Time interaction effect was found for hippocampal CBF ($\beta = -5.58$, 95%CI = -9.65 to -1.52), unstandardized $\beta = -0.25$, $p = 0.04$), indicating reduced hippocampal CBF in the intervention group compared to the active control group. This hippocampus-specific reduction in CBF was not associated with other brain health outcomes or cognitive test scores. Both groups showed comparable improvements in self-reported Dutch healthy diet adherence, perceived stress, and cognitive test scores of processing speed, episodic memory, and working memory. Self-reported MIND-NL dietary pattern adherence and sleep quality improved more in the intervention group, however sleep quality showed significant group differences at baseline.

Discussion

The multidomain lifestyle intervention showed a significant effect for hippocampal CBF, although the direction of the effect was unexpected. These hippocampus-specific CBF reductions in the intervention group were not linked to functional brain-health markers or cognitive test scores, suggesting limited physiological or functional relevance. Both groups improved in self-reported lifestyle questionnaire scores and blood pressure. The observed effect sizes of neuroimaging and cognitive results might have been stronger with a control group that did not modify lifestyle over time. Finally, the observed cognitive test score improvements across both groups most likely reflect learning effects over time.

Background

Due to population aging, the number of people affected by accelerated age-related cognitive decline and dementia has been steadily increasing over the past decades. In 2019, an estimated 57.4 million people were living with dementia, and this number is expected to nearly double every two decades, representing a growing public health challenge worldwide [1, 2]. Livingston and colleagues (2024) identified fourteen modifiable risk factors potentially accounting for 45% of global dementia cases [2, 3], highlighting the opportunities for dementia prevention. These risk factors include lifestyle-related factors such as obesity, physical inactivity, hypertension, hypercholesterolemia and type-II diabetes [4-6]. In line, the 2019 WHO guidelines for global risk reduction of accelerated cognitive decline, mild cognitive impairment (MCI) and dementia such as Alzheimer's disease (AD), emphasize preventive multidomain lifestyle interventions as holding the greatest potential to combat late-onset AD (LOAD) [7]. Such interventions target multiple different domains of lifestyle simultaneously, including diet, physical activity, stress management and other health behaviors, in order to address the diverse established risk factors contributing to dementia.

In recent years, multiple studies have investigated the effectivity of multidomain lifestyle interventions in improving cognitive functioning and dementia risk, with the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) trial being the most well-known example [8]. The FINGER trial demonstrated small improvements in cognitive outcomes after a 2-year intervention period, and this finding was recently replicated in the US Study to Protect Brain Health Through Lifestyle Intervention to Reduce Risk (US-POINTER) trial in the United States [9]. Moreover, similar patterns were observed in specific at-risk subgroups in related trials such as the Multidomain Alzheimer Preventive Trial (MAPT [10]) in France, and the Prevention of Dementia by Intensive Vascular Care (PreDIVA [11]) trial in The Netherlands. A 2022 meta-analysis further supported these findings, by reporting that multiple multidomain lifestyle interventions in aging populations improved composite cognitive test scores and reduced dementia risk factors, especially in subgroups with increased risk of aging-related cognitive decline and dementia [12]. However, several multidomain lifestyle intervention trials have reported no effects on cognition [13, 14], and even among the studies demonstrating cognitive benefits, effect sizes are small. Collectively, these studies have reported varying results. One way to better understand this variability, may be to look at the possible underlying brain mechanisms that drive the reported cognitive benefits of multidomain lifestyle interventions.

Previous research has shown that higher-order cognitive domains, such as executive functioning and working memory, become predominantly affected by cognitive decline [15]. Consistent with these findings, neuroimaging studies have reported age-related changes in functional magnetic resonance imaging (fMRI) activation and cerebral blood flow (CBF) in the dorsolateral prefrontal cortex (dlPFC) and hippocampus, regions that support these cognitive functions [16-18]. Importantly, changes in brain function such as neural activity and CBF are often detectable before structural brain deterioration becomes apparent, even in prodromal stages of dementia [19]. This suggests that functional brain measures may serve as more sensitive and potentially early modifiable intervention targets, given that structural deterioration is largely irreversible. To date, a few lifestyle intervention studies examined neuroimaging outcomes, showing increased regional CBF and fMRI activation in dlPFC and hippocampus following the intervention [20-25]. Although these diet and physical activity-centered intervention studies have reported promising effects of lifestyle interventions on these markers of brain function, they were limited by small sample sizes ($n = 17$ to 61), heterogeneous imaging outcomes, substantial variation in study populations (ranging from healthy adults to people with MCI or dementia), and typically assessed only one or two neuroimaging modalities, which limit the direct comparison of findings across studies. As a result, it remains unclear whether multidomain lifestyle interventions show effects on the same neurobiological mechanisms, and how these mechanisms are correlated.

The overall aim of the HELI study is to investigate the neurobiological brain mechanisms of a 6-month multidomain lifestyle intervention and their underlying peripheral (central) mechanisms in a cohort of older adults at risk of accelerated ageing-related cognitive decline [26]. For this specific study, we tested whether intervention-related changes in lifestyle were associated with improvements in primary outcomes of brain function in two regions of interest (ROIs), namely the dlPFC and hippocampus, and whether these brain changes related to cognitive performance. We hypothesized that the multidomain lifestyle intervention would affect mechanisms most closely related with age-related cognitive decline, such as region-specific neurovascular coupling and CBF. Therefore, our primary objectives were to evaluate intervention effects on (1) ROI-specific CBF levels, (2) ROI-specific fMRI neural recruitment during a N-back working memory task, and (3) N-back task accuracy. Secondary objectives were to (1) investigate intervention effects on additional neuroimaging markers of brain health, such as indices of neuroinflammation and brain volumetrics, and (2) to examine whether changes in the brain health markers were associated with measures of lifestyle domain adherence, cardiovascular health, and cognitive functioning. With this mechanism-oriented study, we aim to clarify

why previous multidomain lifestyle intervention studies have shown small and mixed effects on cognitive benefits, and to improve our understanding of the subtle effects and individual differences underlying these outcomes.

Methods

Study design

The HELI study is a Dutch multicenter, single-blind randomized controlled trial. The study protocol, including a comprehensive description of the study rationale, outcome measures, and baseline demographics, has been published elsewhere [26]. This study has been approved by an accredited Dutch national research ethics committee, the Medical Research Ethics Committee (MREC) Oost-Nederland, and is registered under file number NL78263.091.21. The MREC approval of the study is in accordance with the Dutch Medical Research Involving Human Subjects Act, which complies with the 1964 Declaration of Helsinki (and subsequent amendments). The HELI study is registered with ClinicalTrials.gov under Trial ID NCT05777863. The study has been conducted at the Donders Institute for Brain, Cognition and Behavior, Center for Cognitive Neuroimaging (DCCN; Radboud University, Nijmegen, The Netherlands), and at the Division of Human Nutrition and Health at Wageningen University and Research (WUR; Wageningen, The Netherlands).

Participants

The HELI study included Dutch participants aged 60–75 years at baseline, with elevated risk of cognitive decline based on lifestyle-modifiable cardiovascular risk factors, as defined by the Cardiovascular Risk Factors, Aging and Incidence of Dementia (CAIDE) risk index [27]. Participants were considered at risk if they self-reported two or more of the following: overweight (body mass index (BMI) ≥ 25), physical inactivity (according to 2020 WHO guidelines [28]), hypertension (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg), hypercholesterolemia (total cholesterol > 5 mmol/L and/or LDL-cholesterol > 3 mmol/L), diabetes type-II, or mild cardiovascular disease (e.g., intermittent claudication, varicose veins). Hypertension contributed one risk point, with an additional point assigned if participants were not using antihypertensive medication. Participants were excluded if they provided self-reported indication of the following: cognitive impairment or dementia, not fluent in spoken and written Dutch, contraindications for MRI, and clinical conditions that could interfere with intervention participation or study outcomes. The modified Telephone Interview for Cognitive Status (TICS-M1) was additionally performed to exclude participants with undiagnosed cognitive impairment or dementia. The

full inclusion and exclusion criteria, and full prescreening procedure, have been described in detail elsewhere ([26]).

All participants were fully informed about the purpose, procedures, and potential risks of the study. Participation was voluntary, and participants could withdraw from the study at any time. All participants provided written informed consent to participate in the study.

Sample size

Our expected effect size was based on previous, primarily single-domain, lifestyle intervention studies incorporating similar brain outcome measures. Overall, these studies generally reported medium (to large) effect sizes. We therefore selected a slightly above medium effect-size of 0.30 (Cohen's $f(V)$) to detect intervention-induced changes from baseline to 6-month follow-up. Sample size was calculated in GPower 3.1.9.6 for a repeated-measures MANOVA with within-between interaction, 80% power, and a two-tailed α -error probability of 0.05, resulting in a required sample of 90 participants. Allowing for approximately 15% data loss due to acquisition issues or drop-outs, we aimed to recruit 104 total participants to ensure sufficient statistical power.

Randomization and masking

We utilized a stratified (2,4) block-randomization based on sex (male / female), inclusion risk factor profile (medium risk: 2–3 points / high risk ≥ 4 points), and a combined factor of education level and age (≥ 70 years or low education/60–69 years and high education). Education level was classified in low, medium or high, according to the 2011 International Standard Classification of Education (ISCED) [29]. An independent researcher allocated participants to the two intervention arms: a low-intensity general health advice group (active control) and a high-intensity supervised group coaching intervention group. This study was single-blind, as participants were aware of the intervention arm assignment. Due to ethical considerations, intervention content had to be disclosed before formal inclusion, making blinding of the intervention impossible. Outcome measure visits and data acquisition were conducted by researchers blinded to intervention allocation, and all data preprocessing and analyses were performed using dummy participant IDs. A more extensive description of study randomization and blinding has been described previously ([26]).

Intervention

An extensive description of the intervention group design, lifestyle domain-specific contents and rationale, has been provided previously ([26]). The 6-month HELI multidomain lifestyle intervention was based on the FINGER-NL [30] intervention design, which is part of the World-Wide FINGERS initiative [31]. The intervention

targeted five different lifestyle domains: (1) diet, (2) physical activity, (3) stress management and mindfulness, (4) cognitive training, and (5) sleep. The active control group received general lifestyle-related health information on each of the lifestyle domains, through e-mail once every two weeks. The intervention group followed a structured and supervised intervention program with weekly group meetings, exercises and course-program materials. The weekly intervention group meetings were predominantly held online. Participants received lifestyle-related information, advice and exercises about each of the five incorporated lifestyle domains.

Diet

For healthy and balanced dietary patterns and behaviors, participants received information and support based on the Mediterranean Dietary Approaches to Stop Hypertension (DASH) intervention for Neurodegenerative Delay (MIND) diet [32] and the Dutch guidelines for a healthy diet [33, 34]. Participants were also advised to adhere to the Dutch national recommendations for vitamin D3 intake in older adults (provided in detail previously [26]).

Physical activity

As part of the domain of physical activity, participants were encouraged and guided to adhere to the 2020 WHO Physical Activity guidelines [28]. On-site physical exercise group sessions were combined with information and home exercises coupling whole-body muscle-strengthening and balance exercises. Participants were instructed and assisted to limit sedentary behavior patterns.

Stress management and mindfulness

For stress management and mindfulness, participants were provided with a range of exercises from an evidence-based, self-directed online mindfulness program. Participants also received educational material on the impact of (chronic) stress and mental health, along with practical strategies for managing stress and relaxation.

Cognitive training

The cognitive training domain was based on a Dutch psychoeducational program [35] including information and exercises to teach strategies for managing age-related changes in cognition, such as memory and attention. Participants were encouraged to integrate these cognitive strategies in daily life.

Sleep

Participants received access to a five-week validated, guided sleep counselling course program [36]. Information and exercises were provided on sleep stages, age-related changes in sleep patterns, and practical strategies to enhance sleep quality.

Lifestyle-related measures

At baseline and follow-up, participants filled in questionnaires to assess adherence to lifestyle guidelines or physical wellbeing about the lifestyle domains of diet, physical activity, stress management and mindfulness, and sleep. Lifestyle-related questionnaires included the *Eetscore* food frequency questionnaire to assess adherence to the 2015 Dutch dietary guidelines (Dutch Healthy Diet; DHD) index [37] and to the Dutch MIND (MIND-NL [32, 33]) score; the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH [38]) as a measure of adherence to the Dutch guidelines for physical activity; the LASA Sedentary Behaviour Questionnaire (SBQ [39]) to quantify weekly sedentary behaviour; the Perceived Stress Scale (PSS [40]) to measure situational stress perception; the Hospital Anxiety and Depression Scale (HADS [41]) to assess depressive and anxiety symptoms; the Five Facet Mindfulness Questionnaire (FFMQ [42]) to assess multiple subscales of mindfulness (e.g., non-judgmental thoughts, acting with awareness); and the Pittsburgh Sleep Quality Index (PSQI [43]) to assess multiple components of sleep quality. For the HADS, FFMQ and PSQI, we calculated a total score by summing the individual scores of their respective subscales or components.

Vascular health measures

We used BMI, waist-hip ratio, blood pressure, and blood cardio-metabolic markers as measures of vascular health. Height and weight were measured to calculate BMI (kg/m^2). Waist and hip circumference were measured using a tape measure. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice with an Omron X3 Comfort digital blood pressure monitor, with a third measurement obtained if the first two showed substantial variation. All available SBP and DBP measurements were subsequently averaged. The averaged blood pressure values were then used to calculate mean arterial pressure (MAP) as [44]. Blood samples were collected at baseline and follow-up using BD Vacutainer tubes to analyze glucose, insulin, total-HDL and LDL-cholesterol, and triglycerides, using methods explained in detail previously [45].

Neuroimaging measures: acquisition, preprocessing and analysis

Acquisition

All MRI scans were obtained with a Siemens (Erlangen, Germany) 3T MAGNETOM Skyra MR scanner at the Donders Centre for Cognitive Neuroimaging, Radboud University, in Nijmegen, The Netherlands. First baseline MRI scans started on May 10th 2022, and final follow-up scans were concluded on May 23rd 2024. The neuroimaging scanning protocol included a (1) structural T1-weighted Magnetization Prepared

2 Rapid Acquisition Gradient Echoes (MP2RAGE) sequence (TR/TE = 6000/2.34 ms, TI1/TI2 = 700/2400 ms, voxel size = 1.0 mm isotropic, flip angle = 6/6°); (2) N-back task-based fMRI (TR/TE = 1500/33.40 ms, voxel size = 20 mm isotropic, flip angle = 75°) EPI sequence; (3) pseudocontinuous arterial spin labelling (pCASL) (TR/TE = 4000/16.28 ms, voxel size = 1.8×1.8×3.5 mm, flip angle exc/ref = 90/120°); and a (4) Point Resolved Spectroscopy (PRESS) Magnetic Resonance Spectroscopy (MRS) sequence (TR/TE = 2000/35 ms, voxel size = 20 mm isotropic, flip angle = 90°×180°×180°). A detailed overview of all MRI sequence parameters has been provided previously [26].

For the fMRI, we used an N-back task to investigate working memory-related neural recruitment and task performance. Participants viewed digits (1-9) and responded when the stimulus matched one presented n trials earlier ($n = 0, 1$ or 2). The 0-back served as a control condition, only requiring attention (respond to “1”). The block-design task lasted 15 minutes, and participants practiced beforehand to ensure understanding. Due to a necessary MRI scanner update in 2023 during data acquisition, the pCASL sequence was changed. Although the sequence parameters remained identical, the sequence version (pre- vs post-update) was included as a covariate in the statistical models including CBF measures to account for potential sequence-specific effects. An extensive overview of all sequence parameters, including parameters for calibration images and B1 maps, has been provided previously ([46]; in press).

Preprocessing and analysis

Image preprocessing of the T1-weighted MP2RAGE images included cortical reconstruction and volumetric segmentation using FreeSurfer's *aparc+aseg* pipeline (<http://surfer.nmr.mgh.harvard.edu/>). From the resulting parcellations, whole-brain grey matter (GM), white matter (WM), and hippocampal masks were derived from the ASEG atlas (*aseg_stats*), while the rostral middle frontal region from the Desikan-Kiliany atlas (*aparc_volume*) was used as a proxy for the dlPFC. Volumes of whole-brain GM, WM, dlPFC and hippocampus were subsequently derived from the ASEG parcellations. All volumetric measures were calculated to mm³.

Preprocessing of the fMRI images was performed with *fMRIPrep* (23.2.0; RRID:SCR_016216, see Supplementary Appendix 1), including realignment, distortion correction, coregistration to native anatomical scans, and normalization to MNI152 space. Prior to analysis, preprocessed fMRI images were spatially smoothed with a 6-mm full-width at half-maximum (FWHM) Gaussian kernel using Statistical Parametric Mapping 12 (SPM12; <https://www.fil.ion.ucl.ac.uk/spm/software/spm12/>), implemented in MATLAB R2024a (Mathworks inc.; <https://nl.mathworks.com/>

products/matlab.html). Using generalized linear models in SPM12, first-level analyses were performed with regressors modelling the three task conditions. Confound regressors per session included motion parameters, framewise displacement, six *aCompCor* and *tCompCor* components, every cosine regressor, and ICA-AROMA noise components (excluding those correlating >0.05 with the task design). The main working memory contrast of interest was 2-back minus 0-back. For the ROI analyses, the dlPFC mask was created by combining working memory-related brain activation from a Neurosynth [47, 48] map with peak dlPFC voxels in a prior meta-analysis on aging-related N-back effects [49]. The hippocampus mask was derived from the Harvard-Oxford subcortical atlas [50]. We used the mean β -values within our ROIs for the 2-back minus 0-back (2b-0b) contrast, derived from SPM12, as part of the group analyses.

The Hadamard time-encoded pCASL perfusion images were preprocessed using the FSL Linear Image Registration Tool (FLIRT) and subsequently decoded using a Hadamard decoding scheme to acquire motion-corrected decoded perfusion images. We used the FSL BASIL '*asl_gui*' toolbox to generate quantified whole-brain CBF maps. We used BASIL to analyze 14 pre-subtracted perfusion volumes (7 post-labelling delays with 2 repeats) using a 0.4 second bolus duration (0.5 – 2.9 s) and a 3D GRASE readout. For anatomical reference and registration of the CBF maps, the T1-weighted MP2RAGE scan was processed with *fsl_anat*, and the corresponding output was supplied to BASIL. Calibration was performed with a proton-density Mo calibration image (TR/TE = 3000/16.28 ms, flip angle = 120° , voxel size = $1.8 \times 1.8 \times 3.5$ mm), and an ASL-specific mask was provided to only assess voxels containing perfusion signals. GM, WM, and hippocampus masks were derived from FreeSurfer ASEG parcellations, while the standard space dlPFC mask, initially created for the fMRI analysis, was transformed to native space. Quantified CBF values were subsequently extracted from the voxels of each respective mask. Averaged CBF values were then calculated across all voxel CBF values within each mask.

We used intracranial myo-inositol concentrations (with total water as a reference), measured with MRS, as a marker of neuroinflammation [51]. Myo-inositol concentrations were quantified within a 20mm isotropic voxel positioned in the left dlPFC. Spectra were acquired with a PRESS sequence in combination with chemically selective water suppression (CHESS). Voxel placement was manually guided by the participant's T1-weighted image, whilst avoiding non-brain tissue such as CSF, meninges or the skull. Before acquisition, shimming was performed within the voxel to minimize local field inhomogeneity. Preprocessing of both water-suppressed and unsuppressed spectra was conducted in Osprey 2.4.0 software. By preprocessing metabolite and lineshape reference, as well as native anatomical T1-weighted

MP2RAGE images, averaged spectra were created. If necessary, water-unsuppressed lineshape reference data was used to perform eddy-current correction [52]. Metabolite spectra were modeled over 0.5–4.0 ppm (with 0.4 ppm knot spacing). Water reference spectra were fitted separately over 2.0–7.4 ppm. After coregistration of the spectroscopy data to the T1-weighted image, we performed tissue segmentation in SPM12 to estimate total GM, WM and CSF fractions within the voxel. Total metabolite estimates were corrected for tissue composition, relaxation times, and scaled to total water concentration. Final myo-inositol estimates were additionally adjusted for the spectral quality measure of relative residual amplitude (relResA).

Cognitive functioning measures

We used a neuropsychological test battery to assess cognitive domains predominantly affected by cognitive decline. This included the Trail Making Test (TMT [53]) part A and B (TMT-A/B) and Verbal Fluency Test (VFT [54]) for executive functioning; the Digit Symbol Substitution Test (DSST [55]) for processing speed; the forward and backward-summed score of the Digit Span Test (DST [55]) and N-back task performance (during fMRI) for working memory; and the delayed-recall Rey Auditory Verbal Learning Test (RAVLT [56]) for episodic memory. To account for TMT-A completion time, we calculated a TMT-B/TMT-A ratio score. We used the VFT ‘Animals’ at baseline, and VFT ‘Professions’ at follow-up. For the DST, we calculated a sum-score of the forward and backward DST scores. N-back task performance was measured with the d-prime (d'), calculated as $d' = \frac{Z(H) - Z(F)}{\sqrt{0.5}}$ where H is the hit rate, and F is the false alarm rate.

Outcomes

The primary outcomes were changes from baseline to 6-month follow-up in (1) cerebral perfusion in our defined ROIs (dlPFC and hippocampus), (2) ROI-specific neural recruitment during a N-back working memory task, and (3) N-back task accuracy (performance as assessed with d'). We used quantified CBF in ml/100g tissue/min as the measure of cerebral perfusion levels, blood-oxygen-level-dependent (BOLD) activity as the measure of neural recruitment, and 2b-ob d' performance scores as the measure for task accuracy.

Secondary outcomes included changes from baseline to 6-month follow-up in lifestyle-questionnaire measures of diet (*Etscore* questionnaire), physical activity and sedentary behavior (SQUASH and SBQ), stress management and mindfulness (PSS, HADS, FFMQ), and sleep quality (PSQI). Additional secondary outcomes comprised (cardio)vascular health indices, other brain health outcomes, and cognitive functioning outcomes. The (cardio)vascular health indices comprised of

BMI, MAP, and blood-derived cardiometabolic markers including HOMA-IR, HDL- and LDL-cholesterol, and triglycerides. Other brain health outcomes included dlPFC myo-inositol concentrations, and whole-brain GM and WM volumes. Cognitive functioning outcomes included all individual neuropsychological test outcomes, including the TMT, VFT, DSST, DST, RAVLT.

Data quality assessments

Prior to analysis, we performed data cleaning and quality control assessments of all measures whilst blinded for intervention group allocation. Participants who clearly failed to perform the N-back task correctly, defined as having either a target accuracy <30% on the 0-back condition or a non-target accuracy <65% on any of the 0-, 1-, or 2-back conditions, were removed from further analysis. fMRI data quality was subsequently assessed using the fMRIprep reports and motion metrics. Datasets with mean framewise displacement >0.5mm or maximum displacement >3mm underwent additional inspection. The first-level 2-back contrast map (thresholded at $p = 0.001$ uncorrected) was visually examined for canonical fronto-parietal activation patterns associated with working memory. Datasets lacking any discernible fronto-parietal activity were excluded from analysis. For MRS, spectra at different processing stages (including pre-aligned, post-aligned, averaged, and fitted) were visually examined to assess overall quality. We evaluated lipid peak absence (1-2 ppm), sufficient water suppression (3-4 ppm), metabolite-of-interest peak presence (myo-inositol at 3.03 ppm), chemical shift drift absence (indicative of movement-related artifacts), and shimming effectivity as measures of sufficient data quality. Additionally, Osprey's quality metrics, such as the creatine signal-to-noise ratio and FWHM were reviewed. Datasets that did not meet acceptable quality standards based on these metrics, were excluded from further analysis. Questionnaire data were manually checked for implausible entries (e.g., >7 days per week, more reported hours than possible wake time). We performed simple outlier analyses for all variables using the interquartile range (IQR) method, where values below or above were flagged as potential outliers. Flagged data points that also failed quality control assessments were considered invalid and excluded from the dataset. Prior to analysis, assumption checks of data normality and linearity were performed with QQ- and scatterplots.

Statistical analysis

Intervention effects on primary outcomes (changes in neural recruitment and CBF within dlPFC and hippocampal ROIs, and N-back task performance) and secondary outcomes (lifestyle questionnaire scores, vascular health indices, brain health outcomes, and cognitive functioning) from baseline to 6-month follow-up were investigated using linear mixed models (LMMs). Although statistical analyses have

been preregistered to include MANOVA, LMMs were used to allow the inclusion of participants with missing data at baseline or follow-up. In all models, Group, Time and their interaction (Group $\times$ Time) were included as fixed effects, and within-subject repeated measures were modeled as a random effect. Covariates depended on the dependent variable: pCASL sequence version (pre- vs. post-MRI scanner update) for CBF; relResA for dlPFC neuroinflammation outcomes; and total intracranial volume (ICV) for whole-brain GM and WM volumes. False discovery rate (FDR) correction was separately applied for primary and secondary LMM results to adjust for multiple comparisons. All analyses followed an intention-to-treat approach, which included participants regardless of intervention adherence, protocol deviations or study drop-out.

To further interpret unexpected findings and to investigate associations between individual and composite delta scores of primary and secondary outcome measures, we performed exploratory post-hoc subgroup, correlation, regression and canonical correlation analysis (CCA) models. To preserve statistical power, the correlation, regression and CCA exploratory analyses were performed across the total study sample (i.e., without between-groups comparisons). For interpretability, all outcomes were normalized to Z-scores. Several outcomes were multiplied by -1 to ensure that higher Z-scores consistently reflected healthier outcomes. These included lifestyle questionnaires scores (SBQ, PSS, HADS, PSQI), vascular health indices (BMI, waist-hip ratio, MAP, HOMA-IR, blood LDL-cholesterol, blood triglycerides), a neuroimaging outcome (dlPFC neuroinflammation), and a cognitive functioning outcome (TMT-B/TMT-A ratio). For the subgroup analyses, we performed median splits to re-run the hippocampal CBF LMM in two subgroups: (1) participants with the lowest baseline hippocampal CBF (representing those with the greatest potential for improvement over time), and (2) participants with the largest decrease in MAP from baseline to follow-up (representing those with the strongest decrease of SBP and DBP over time).

For the correlation analysis, we used pairwise Pearson's correlation to explore relationships between delta (Δ , as $T_1 - T_0$) scores of all included outcome measures across the entire study sample, and for the intervention and active control group separately. To correct for baseline differences, the Δ -score of each variable was adjusted for baseline value by using residuals from linear models with the baseline score as a predictor. For the regression analyses, we used simple linear models to test if primary outcome Δ -scores are associated with Δ -composite scores of lifestyle questionnaire outcomes, or vascular health outcomes. Composite scores were calculated as the sum of Δ -scores across domain-specific variables. When multiple

variables measured the same outcome (e.g., several diet-specific questionnaire scores), their Δ -scores were first averaged before inclusion in the sum. Additionally, we investigated associations between multiple individual lifestyle and vascular health outcome measures and our primary outcomes. Specifically, multiple linear regression (MLR) models were performed for each primary fMRI or ASL-based outcome Δ -score as the dependent variable, with either all lifestyle questionnaire Δ -scores or all vascular health Δ -scores as independent variables in one model. Finally, to identify which outcome measures contributed most to the shared variance between primary outcome Δ -scores and lifestyle/vascular health Δ -scores, we performed CCA.

All statistical analyses were performed using R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). We used the R-package 'lme4' version 1.1-35.4 [57] to perform the LMMs. Reported p-values throughout the manuscript are FDR-corrected unless specified otherwise, and significance was set at $p < 0.05$ for all analyses.

Results

Participant flowchart and characteristics

Between April 5th 2022 and September 1st 2023, 279 participants were screened for eligibility. Of these screened participants, $N = 102$ participants were randomized to either the active control group ($n = 49$) or the intervention group ($n = 53$) (Figure 1). Baseline outcome assessments started on May 10th, 2022, and the final follow-up assessment was completed on May 23rd, 2024. In total, 16 participants fully discontinued their study participation and did not complete any follow-up assessments (active control group: $n = 10$; intervention group: $n = 6$), resulting in a total of 86 participants completing the 6-month follow-up outcome assessments (Table 1). Additionally, four participants in the intervention group discontinued the intervention but completed the follow-up assessments. Their data was therefore included in the intention-to-treat analyses. The control and intervention group had similar baseline characteristics.

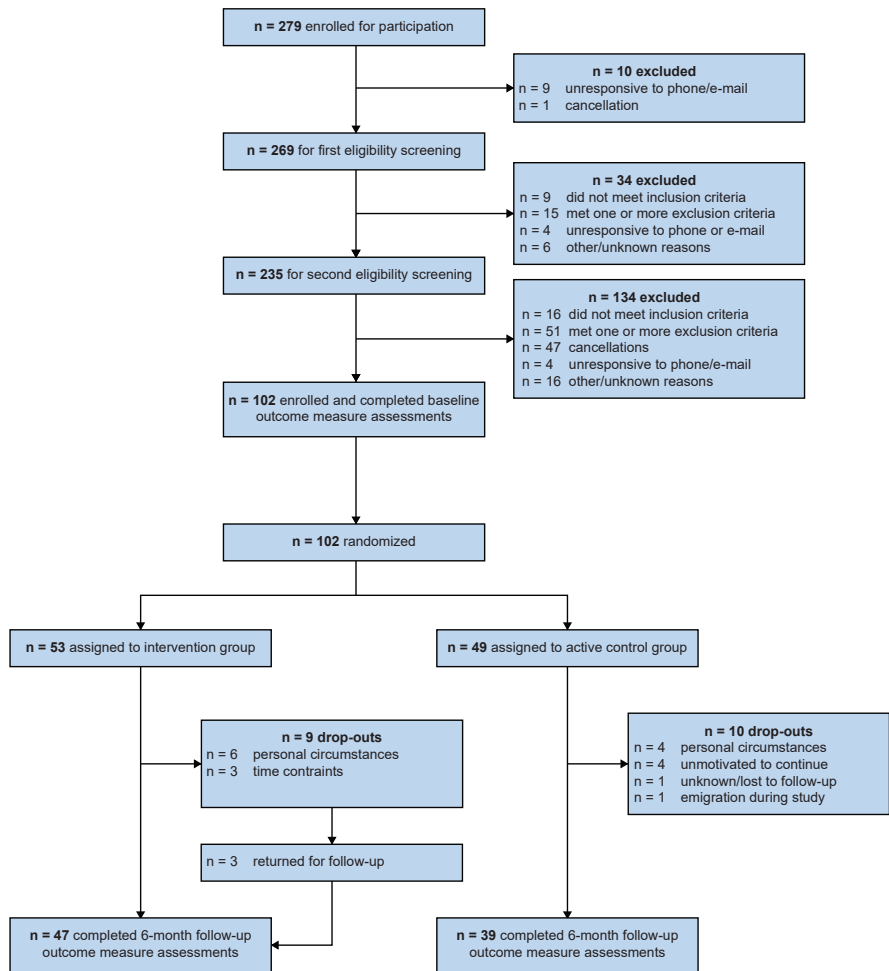


Figure 1. Flowchart of study enrollment, inclusion, exclusion, randomization and outcome measure visits.

Table 1. Baseline characteristics of complete HELI study dataset (N = 102).

	Active control group (<i>n</i> = 49)	Intervention group (<i>n</i> = 53)
Demographics		
Age, years	66.84 (4.26)	66.28 (4.36)
Sex		
Female	32 (65%)	35 (66%)
Male	17 (35%)	18 (34%)
Education level		
Low	5 (10%)	5 (9%)
Medium	16 (33%)	19 (36%)
High	28 (57%)	29 (55%)
MoCA	27.43 (1.87)	26.79 (2.21)
Cardiovascular risk factors		
BMI ≥ 25	34 (69%)	42 (79%)
Physical inactivity	30 (61%)	27 (51%)
Hypertension	26 (53%)	32 (60%)
Untreated hypertension	9 (18%)	9 (17%)
Hypercholesterolemia	26 (53%)	31 (58%)
Diabetes type-2	10 (20%)	10 (19%)
Mild cardiovascular disease	9 (18%)	2 (4%)
Risk factor score, median (IQR)	3 (2.0, 4.0)	3 (2.0, 3.0)
Lifestyle questionnaires		
Diet		
DHD-index	100.88 (16.42)	100.38 (17.81)
MIND-NL	8.69 (1.86)	8.39 (2.20)
Physical activity		
SQUASH	6986.15 (3530.70)	7204.98 (3890.49)
SBQ	376.91 (170.08)	404.88 (191.51)
Stress and mindfulness		
PSS	12.53 (3.85)	12.30 (4.53)
HADS	7.31 (4.84)	7.51 (5.43)
FFMQ	2.99 (2.35)	2.97 (2.88)
Sleep		
PSQI	5.45 (3.16)	6.83 (3.77)

Table 1. Continued

	Active control group (<i>n</i> = 49)	Intervention group (<i>n</i> = 53)
Vascular health indices		
BMI	28.93 (4.73)	30.98 (5.87)
Waist-hip ratio	0.94 (0.09)	0.97 (0.11)
MAP	106.61 (13.10)	110.39 (14.09)
HOMA-IR	4.16 (3.11)	4.25 (3.80)
Blood cardiometabolic markers (mmol/L)		
HDL-cholesterol	1.44 (0.42)	1.46 (0.41)
LDL-cholesterol	3.52 (1.07)	3.33 (0.88)
Triglycerides	1.57 (0.77)	1.74 (0.67)
Brain health indices		
Working memory fMRI BOLD response in ROIs		
dlPFC	0.37 (0.15)	0.35 (0.13)
Hippocampus	-0.04 (0.08)	-0.02 (0.06)
CBF (ml/100g tissue/min)		
dlPFC	37.57 (11.41)	39.31 (12.78)
Hippocampus	40.85 (10.64)	42.48 (10.69)
Brain ROI volume (mm ³)		
dlPFC	23721.02 (2931.02)	24526.11 (2826.72)
Hippocampus	7717.82 (2931.02)	7866.43 (764.71)
Myo-inositol in dlPFC (mmol/L)	10.35 (1.46)	9.95 (1.27)
Neuropsychological tests		
TMT (TMT-B/TMT-A ratio)	2.17 (0.55)	2.31 (0.82)
VFT	25.96 (4.89)	26.00 (6.06)
DSST	51.31 (10.60)	52.57 (9.70)
DST	14.92 (3.10)	15.45 (3.69)
RAVLT delayed recall	7.51 (3.12)	7.77 (3.24)
N-back performance (2b-ob d')	2.36 (0.62)	2.31 (0.87)

Data are presented as mean ($\pm$ SD) for continuous variables, and *n* (%) for categorical variables, unless specified otherwise.

Abbreviations: BMI = body mass index; BOLD = blood-oxygen-level-dependent; DHD = Dutch Healthy Diet; dlPFC = dorsolateral prefrontal cortex; DSST = Digit Symbol Substitution Test; DST = Digit Span Test; FFMQ = Five Faceted Mindfulness Questionnaire; fMRI = functional magnetic resonance imaging; HADS = Hospital and Anxiety Depression Scale; HDL = high-density lipoproteins; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; LDL = low-density lipoproteins; MAP = mean arterial pressure; PSQI = Pittsburgh sleep quality index; PSS = Perceived Stress Scale; ROIs = regions of interest; SBQ = Sedentary Behavior Questionnaire; SQUASH = Short Questionnaire to Assess Health-enhancing physical activity; RAVLT = Rey Auditory Verbal Learning Test; TMT = Trail Making Test; VFT = Verbal Fluency Test.

Linear mixed model intervention effects

Intervention effects on primary outcomes

We observed a significant Group $\times$ Time interaction effect in hippocampal CBF, in the absence of group differences at baseline (active control group 40.85 ± 10.52 ml/100g/min, intervention group 42.94 ± 10.94 ml/100g/min, $p = 0.34$). The intervention group showed a hippocampal CBF decrease compared to the active control group from baseline to follow-up ($\beta = -5.58$, standardized $\beta = -0.25$, 95%CI = -9.65 to -1.52, $p = 0.04$) (Figure 2b, Supplementary Table 1). LMM analyses of working memory task-related fMRI BOLD response in the dlPFC and hippocampus, as well as CBF in the dlPFC, revealed no significant Time or Group $\times$ Time interaction effect. The covariate of pCASL sequence version was significant in both CBF LMMs, and indicated that the post-update sequence resulted in higher quantified CBF measurements (Supplementary Table 1). A significant Time effect was observed for the N-back working memory task during fMRI, indicating that both groups significantly improved accuracy between baseline and follow-up (Figure 2e).

Intervention effects on secondary outcomes

From the secondary LMM analyses, a significant Group $\times$ Time interaction was observed in MIND-NL diet adherence and sleep quality, with the intervention group showing a greater increase in MIND-NL score ($\beta = 1.79$, 95%CI = 0.92 to 2.67, $p = 0.002$) (Figure 3b) and greater decrease in PSQI score compared to the control group ($\beta = -1.76$, 95%CI = -2.79 to -0.72, $p = 0.012$) (Figure 3d, Supplementary Table 2). Significant effects of Time were found across both groups in DHD-index score ($\beta = 8.08$, 95%CI = 4.83 to 11.33, $p < 0.001$), MIND-NL score ($\beta = 1.16$, 95%CI = 0.65 to 1.67, $p < 0.001$), perceived stress score ($\beta = -1.844$, 95%CI = -2.70 to -0.99, $p = 0.001$), MAP ($\beta = -3.65$, 95%CI = -5.55 to -1.74, $p = 0.003$), DSST score ($\beta = 2.19$, 95%CI = 1.00 to 3.39, $p = 0.005$), and RAVLT delay score ($\beta = 2.23$, 95%CI = 1.66 to 2.80, $p < 0.001$). In all instances of significant changes over the intervention period, changes were associated with better outcomes (MAP, DSST, RAVLT) or self-reported improvements (DHD, MIND-NL). No significant Time or Group $\times$ Time interaction effects were found for other measures of lifestyle (SQUASH, SBQ, HADS, FFMQ), vascular health (BMI, waist-hip ratio, HOMA-IR, HDL-cholesterol, LDL-cholesterol, triglycerides), brain health (myo-inositol in dlPFC, dlPFC volume, hippocampal volume), or cognitive functioning (TMT, VFT, DST) (Supplementary Table 2).

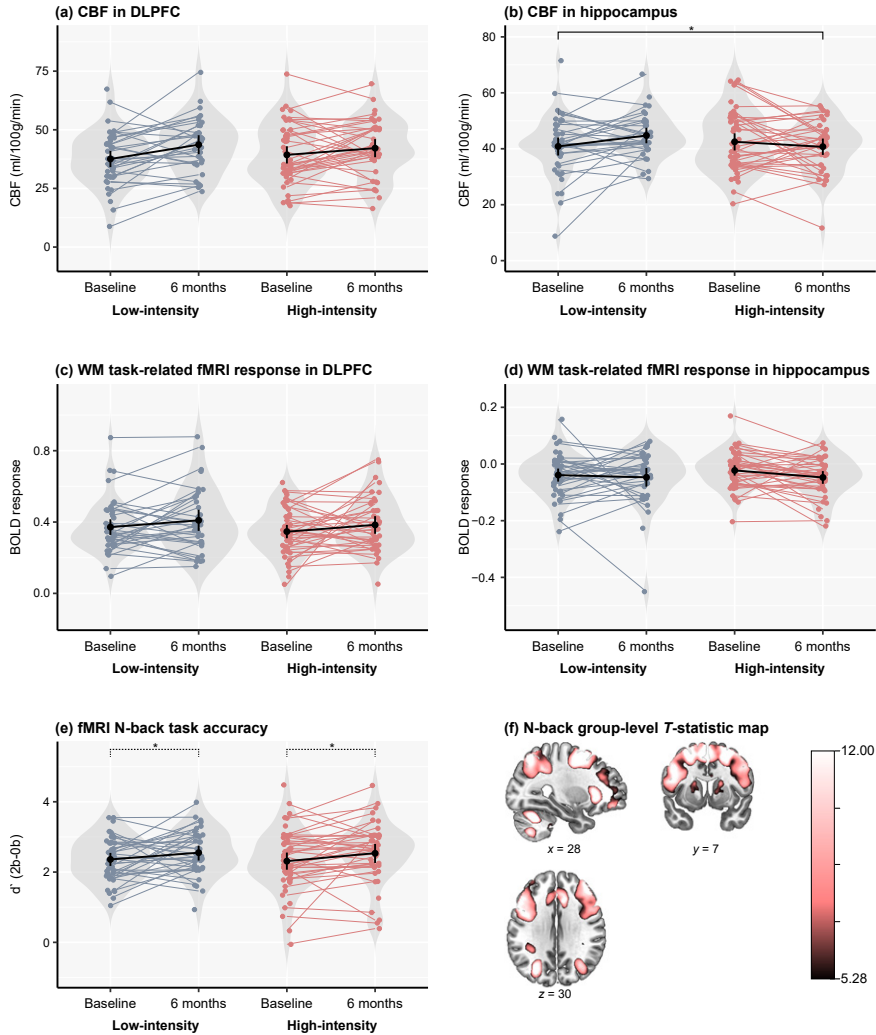


Figure 2. Changes in primary outcome measures from baseline to 6 month follow-up. (a-e) Results from intention-to-treat repeated-measures linear mixed models of (a) CBF in dlPFC ($n = 98$), (b) CBF in hippocampus ($n = 98$), (c) working memory task-related fMRI BOLD response in dlPFC ($n = 99$), (d) working memory task-related fMRI BOLD response in hippocampus ($n = 99$), and (e) fMRI N-back task accuracy ($n = 100$). (f) The 2b-0b contrast across groups and timepoints (baseline and follow-up). Activation shown is $p < 0.05$ after FWE-correction. Data represent individual measurements (colored dots and connected lines), and group means $\pm$ 95% CI. Dashed line indicates a significant *Time* interaction. Solid line indicates a significant *Group* $\times$ *Time* interaction effect. (f) SPM group-level T-statistic map for the N-back contrast. * = $p < 0.05$

Abbreviations: BOLD = blood-oxygen-dependent-level; CBF = cerebral blood flow; CI = confidence interval; dlPFC = dorsolateral prefrontal cortex; FWE = family-wise error.

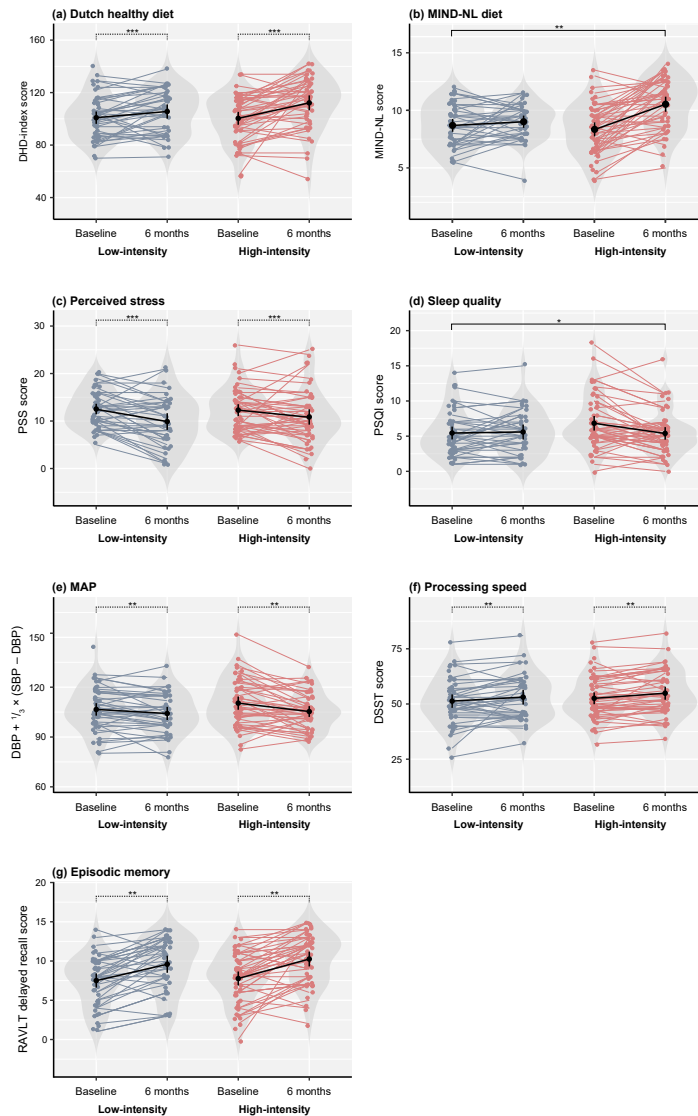


Figure 3. Significant changes in secondary outcome measures from baseline to 6 month follow-up. (a-g) Results from intention-to-treat repeated-measures linear mixed models of (a) DHD-index ($n = 102$), (b) MIND-NL diet score ($n = 101$), (c) perceived stress ($n = 102$), (d) sleep quality ($n = 102$), (e) blood pressure ($n = 101$), (f) processing speed ($n = 102$), and (g) episodic memory ($n = 102$). Data represent individual measurements (colored dots and connected lines), and group means $\pm$ 95% CI. Dashed line indicates a significant *Time* interaction. Solid line indicates a significant *Group* $\times$ *Time* interaction effect. Significance indicators: * = $p < 0.05$, ** = $p < 0.01$, *** = $p \leq 0.001$.

Abbreviations: BMI = body mass index; CI = confidence interval; DBP = diastolic blood pressure; DHD = Dutch Healthy Diet; LDL = low-density lipoprotein cholesterol; MAP = mean arterial pressure; MIND-NL = Dutch Mediterranean-DASH Intervention for Neurodegenerative Delay; PSS = Perceived Stress Scale; PSQI = Pittsburgh Sleep Quality Index; SBP = systolic blood pressure.

Exploratory post-hoc analyses

Because of the unexpected decrease of hippocampal CBF in the intervention group, we performed exploratory subgroup, correlation, regression, and CCA models.

Subgroup analyses

Subgroup analyses were performed to explore whether participants with the lowest baseline hippocampal CBF ($n = 47$) or those with the largest reduction in MAP between baseline and follow-up ($n = 41$) would show an increase instead of a decrease in hippocampal CBF. However, no significant Time, or Group $\times$ Time interaction effects were found in either subgroup analysis (Supplementary Table 3).

Correlations between outcome Δ -scores

Pairwise Pearson's correlations were performed between Δ -scores of all study variables in the total study sample (Supplementary Figure 1). A cluster of correlations was observed between lifestyle questionnaire outcomes and vascular health outcomes. However, most did not remain significant after FDR-correction. After correction, higher DHD-index score was significantly correlated with higher MIND-NL score, and lower BMI, waist-hip ratio, and LDL-cholesterol.

When examined separately within the control and intervention group, no significant FDR-corrected correlations were observed between lifestyle, vascular health, brain health, or cognitive functioning outcomes (Supplementary Figures 2 and 3).

Next, regression analyses were performed to investigate associations between primary outcomes and either composite or individual Δ -scores of lifestyle and vascular health outcomes. Simple linear regression analyses revealed no significant associations between changes in primary outcomes and composite lifestyle or vascular health Δ -scores (Supplementary Table 4). Similarly, multiple linear regression models showed no significant relationships between primary outcome measures and lifestyle or vascular health Δ -scores (Supplementary Table 5).

CCA analyses

CCA models were used to explore shared variance between sets of variables (X , Y) and identify individual variables contributing most to this shared variance. Three CCA models were constructed: (1) lifestyle outcome Δ -scores as set X and vascular health outcome Δ -scores as set Y , (2) vascular health outcome Δ -scores as set X and brain health outcome Δ -scores as set Y , and (3) brain health outcome Δ -scores as set X and cognitive functioning outcome Δ -scores as set Y . No significant canonical dimensions

were found in the lifestyle-vascular health model (Supplementary Table 6A), the vascular health-brain health model (Supplementary Table 7A), or the brain health-cognitive functioning model (Supplementary Table 8A). Canonical loadings for each model are shown in Supplementary Tables 6-8B.

Discussion

With this study, we aimed to investigate the brain mechanisms that could underlie the effects of multidomain lifestyle interventions on cognition reported in previous trials.

We observed a significant reduction in hippocampal CBF at follow-up in the intervention group compared with the active control group. In contrast, no intervention-related improvements were observed in N-back task-related fMRI BOLD response in the dlPFC or hippocampus, nor in CBF in the dlPFC. Both groups showed – independent of the intervention – significant improvement in N-back working memory task accuracy, processing speed (DSST), and episodic memory (RAVLT delayed recall) over time. Other secondary outcomes showed stronger adherence to the MIND-NL diet and greater self-reported improvement in sleep quality in the intervention group compared to the active control group. In contrast, other self-reported lifestyle outcomes (DHD-index, PSS) and blood pressure (MAP) improved in both groups over time, independent of the intervention. Exploratory analyses did not reveal additional intervention effects.

Current literature on the effects of lifestyle interventions on measures of brain function is limited. Previous studies, often targeting only one or two lifestyle domains, have shown beneficial effects of improved diet, physical activity, and cognitive training, on neural recruitment [58] and CBF [20-22]. Contrary to these findings and our hypotheses, we observed no significant effects on working memory-related fMRI BOLD response in either ROI or CBF increase in the dlPFC, but we did find a statistically significant decrease in hippocampal CBF in the intervention group compared to the active controls.

The role of CBF in cognitive performance has been previously investigated in both normal and pathological aging, where higher CBF was linked to better cognitive functioning [18, 59, 60]. In the Agewell.de trial, which included a similar at-risk population of older adults (for a comparison of HELI with previous multidomain lifestyle interventions, see [26]), a potentially beneficial effect was reported: mean GM-CBF was increased in the intervention group and this was associated with

intervention-induced reductions in systolic blood pressure [61]. This raises the question why our results indicate a drop in hippocampal CBF within our intervention group. Although cerebral hypoperfusion in Alzheimer's Disease (AD) has been widely reported [62], a smaller number of AD studies have also indicated hippocampus-specific hyperperfusion [63, 64], possibly hinting at compensatory mechanisms. However, De Jong *et al.* [65] showed that blood pressure-lowering medication directly increased hippocampal CBF in AD patients, suggesting a link between cardiovascular risk reduction and hippocampus-specific CBF increase in AD. This finding aligns more closely with the studies proposing that cerebral hypoperfusion is a mechanism underlying AD, and is influenced by cardiovascular risk factors. In healthy adults, higher CBF has also been associated with better cognitive functioning outcomes, although the strongest observed effects were in frontal regions rather than the hippocampus specifically [18]. To further explore the unexpected finding in hippocampal CBF, we performed exploratory post-hoc subgroup analyses in participants with the lowest baseline hippocampal CBF (i.e., strongest potential for positive change), and in participants with the largest pre-post decrease in MAP (i.e., strongest blood pressure reduction). Although neither analysis was statistically significant, both effect sizes showed similar patterns of decreased hippocampal CBF in the intervention group compared to the active controls. However, the observed effect size of hippocampal CBF decrease was small, and CBF values in our ROIs remained within the normal range for both groups over time at follow-up. It is therefore possible that the observed decrease, as well as the null finding in dlPFC CBF, reflect normal fluctuations in CBF influenced by external factors. Although efforts were made during data acquisition to minimize variability due to such factors at baseline and follow-up outcome measure visits, influences such as stress, sleepiness, breathing rate, heart rate, or temperature may still have affected CBF quantification [66]. Moreover, the absence of significant associations between the change in CBF over time and other outcome measures, including fMRI BOLD response and cognitive functioning, further supports this interpretation and may indicate that the observed hippocampus-specific CBF decrease has no physiological or functional relevance.

Furthermore, we found no evidence of improvement in ROI-specific neural activity during the N-back working memory fMRI task. Although no prior studies have investigated multidomain lifestyle effects on working memory-related BOLD responses, research has established gradual ageing-related declines in brain activation associated with working and episodic memory [49, 67, 68], with more pronounced reductions seen in pathological aging compared to normal aging (for a review, see [15]). In our study, both the intervention and active control group showed

no measurable difference in dlPFC or hippocampal working memory task-related fMRI BOLD responses over a period of six months. Although working memory-related fMRI responses in the dlPFC and hippocampus are established sensitive markers of age-related functional brain changes, we were unable to conclude that a 6-month multidomain lifestyle intervention had a significant effect on these measures. It is possible that our intervention duration was not sufficiently long enough to capture the true associations between changes in lifestyle patterns, reduced cardiovascular risk, and subsequent changes in functional brain mechanisms, as longer multidomain lifestyle intervention trials such as Agewell.de have shown effects on whole-brain GM CBF over four years [61] rather than within six months. Nevertheless, unimodal intervention studies focusing primarily on diet or physical exercise have reported significant increases in brain activation within (pre)frontal ROIs, using intervention durations between 12 weeks and 6 months [24, 25]. These findings suggest that perhaps unimodal programs of diet or physical activity may be particularly influential in modulating fMRI-based brain activation measures, and the inclusion of additional domains such as stress management, cognitive training, and sleep quality in a multidomain intervention may have diluted the specific effects of diet and physical activity.

Our results indicate significant improvements in both groups in neuropsychological test scores, specifically in processing speed and episodic memory, as well as in working memory. These cognitive domains are particularly vulnerable to normal and pathological aging, likely due to structural and functional changes in their associated brain regions [15]. The FINGER [8] and US-POINTER [9] trials showed greater improvements in executive functioning, processing speed, and episodic memory in the intervention groups compared to controls. We only replicated the general time effects across both groups in the same domains, instead of finding significant differences between the intervention and control group on these cognitive outcomes. With the interpretation of longitudinal within-subject cognitive outcome assessments, it is likely that these findings reflect learning effects, thereby explaining the observed improvements in cognitive functioning over time without differences between groups [69].

Beyond the neuroimaging outcomes and cognitive functioning outcomes, we also assessed potential intervention-induced effects on lifestyle and vascular health. At six-month follow-up, we demonstrated that both groups improved in diet quality scores (DHD-index), while the intervention group additionally showed significantly improved MIND-NL diet adherence and sleep quality compared to active controls. We also found significant improvements in blood pressure (MAP) across both groups,

independent of the intervention. These findings suggest the multidomain lifestyle advice, even in the active control group, may have been beneficial for multiple domains of lifestyle and for blood pressure, which is a well-established cardiovascular risk factor for cognitive decline [2]. This finding is in line with results from the AgeWell.de trial, where the odds of hypertension were reduced across the total study sample, and beneficial time effects on healthy dietary patterns were observed [14]. In the JMINT trial, the intervention group showed greater reductions compared to the control group in anthropometric measurements, including decreased body fat percentage and free-fat mass after 6 and 18 months. Interestingly, changes in BMI and systolic blood pressure were only observed after 18 months [13]. Additionally, the FINGER trial reported that the intervention group showed greater improvements compared to the control group in BMI reduction, intake of fish and vegetables, and physical activity after two years [8]. However, direct comparisons are challenging, as the scoring methods and definitions of these lifestyle factors differ across studies. For example, HELI used composite indices of dietary health (DHD-index and MIND-NL), whereas the FINGER trial used simpler categorical measures such as fish intake (at least twice per week) and daily vegetable consumption. These components are also captured within the DHD and MIND-NL indices, which could be further explored in future studies. Similarly, HELI assessed physical activity using the more comprehensive SQUASH questionnaire, while the FINGER trial used a cut-off of two physical activities per week as a measure of success. Secondary outcomes of the US-POINTER trial, including measures of lifestyle and cardiovascular health, have not yet been published, and the MAPT trial did not report comparable measures [9, 10]. Differences between our findings and those from the AgeWell.de, JMINT, and FINGER trials may be explained by differences in assessment of lifestyle and vascular factors, and differences in study duration. This notion is supported by findings from the JMINT trial, which showed significant effects at 18-month follow-up, whereas no significant effects were observed at 6-month follow-up [13]. Furthermore, study design differences may contribute, as the FINGER, JMINT, and AgeWell.de trials are all effectivity studies with cognitive functioning as primary endpoints and follow-up durations up to two years, whereas the HELI study is a mechanistic study with a significantly shorter follow-up due to the sensitivity of the included primary outcomes.

In exploratory analyses, intervention-induced improvements in DHD-index and MIND-NL scores were significantly correlated with lower BMI, waist-hip ratio and LDL-cholesterol across the total study population (Supplementary Figure 1). These findings indicate that positive changes in dietary lifestyle were associated with improvements in vascular health-related outcomes in our total cohort. We

found no further correlations between our primary, lifestyle, or vascular health outcomes (Supplementary Tables 4-5), nor did CCA analysis reveal shared variance between outcomes of lifestyle, vascular health, and brain outcomes (Supplementary Tables 6A-7A). However, because participants were included based on elevated lifestyle-modifiable cardiovascular risk factors of cognitive decline, some vascular measures likely showed limited variability, which could have contributed to the observed null effects and may not appropriately reflect patterns in broader populations. These null findings may also reflect limited statistical power due to the relatively small sample sizes. In accordance with this, in a larger exploratory observational analysis paper combining the HELI participant dataset at baseline with another similar intervention cohort, some of these relationships were observed [45], supporting the possibility that the current analyses were underpowered rather than reflecting a true absence of associations between these variables. However, it is also possible that the observational between-subject differences found in this exploratory baseline study were larger compared to within-subject intervention changes, and the outcome measures we have analyzed simply do not show considerable change over an intervention period of 6 months. Similarly, one prior diet-oriented lifestyle intervention study in cognitively healthy older adults with familial history of dementia found that stronger adherence to the MIND diet did not lead to significant differences in cognitive functioning or MRI brain volumetrics compared to a control diet with mild caloric restriction over three years [70]. These findings highlight the difficulty of linking lifestyle intervention-induced dietary changes on MRI-measures of brain integrity and cognition over longer periods of time.

The HELI study is the first to investigate an extensive array of brain and peripheral health outcome measures to assess the effects underlying a multidomain lifestyle intervention in an older population at risk of cognitive decline. A major strength of our study is the use of an extensive neuroimaging protocol, which includes multiple functional brain measures potentially associated with cognitive improvement reported in similar multidomain lifestyle intervention studies such as the FINGER and US-POINTER trials. Most previous work on the mechanisms underlying cognitive decline has focused on either healthy older adults or clinical populations such as MCI and AD. MCI populations specifically are often used as an intermediate stage between healthy and pathological aging [71]. In contrast, our study bridges the gap between healthy aging and MCI and dementia by including a still-healthy, but at-risk population, which is a population highlighted by the World Health Organization as a group most likely to benefit from preventive approaches aimed at delaying or preventing dementia [72].

Several limitations of the current study should be acknowledged. Firstly, because participants were selected based on specific cardiovascular risk factors for cognitive decline, it is possible that some individuals were already in (undiagnosed) prodromal stages of MCI or dementia. Although the TICS-M1 was used during prescreening, this instrument cannot completely guarantee to exclude early cognitive impairment. This possibility complicates the interpretation of our findings with regard to the mechanisms involved in the preclinical phase of cognitive decline, such as the decrease in hippocampal CBF. Secondly, we used an active control group as opposed to a wait-list or placebo-controlled group. A wait-list design was considered unfeasible, as participants enrolling in a lifestyle intervention study already show motivation to improve aspects of their lifestyle, possibly even without the active guidance used in the intervention group. Assigning participants to a wait-list condition could be discouraging and might even result in higher drop-out rates. Additionally, due to ethical considerations, it was not possible to assign participants to a control group that required them to deliberately refrain from adhering to healthier lifestyle behaviors. As a result, we implemented two intervention arms that differed in intensity and level of guidance. Yet, we observed improved lifestyle effects on self-reported questionnaires and in blood pressure in both groups. The observed effect sizes of our neuroimaging and cognitive results might have been larger if we had included a control group that did not change their lifestyle over time. Lastly, our study population had an unequal sex distribution (66% female, 34% male) and a relatively high education level (56% high educated), which may limit the generalizability of our findings to the broader population.

In conclusion, our findings indicate that older adults at-risk of accelerated cognitive decline in both the intervention and active control groups showed improvements over time on multiple domains of lifestyle and markers of vascular health, as well as on cognitive test scores of processing speed, episodic memory, and working memory. The intervention group, receiving a higher-intensity multidomain lifestyle intervention, unexpectedly showed decreased instead of increased hippocampal CBF relative to the control arm. However, because we found no correlations between this hippocampus-specific CBF decrease in the intervention group with outcomes of cognitive functioning or other markers of brain health, this effect is likely of limited clinical relevance. No intervention effects were observed for working memory-related fMRI BOLD responses in the dlPFC or hippocampus, nor for CBF in the dlPFC. Furthermore, we did not find significant correlations between changes in individual lifestyle or vascular health measures and functional brain markers, which emphasizes the complexity of associating behavioral and neural effects in multidomain lifestyle interventions. Overall, our results primarily reflect learning

effects on neuropsychological assessments, consistent with previous multidomain lifestyle intervention studies, but unlike the FINGER and US-POINTER trials which reported intervention effects, we found no evidence of intervention-related improvements in brain health outcomes with clear functional relevance. Future studies with larger samples and extended follow-up are necessary to clarify the brain mechanisms through which multidomain lifestyle interventions may show beneficial cognitive effects. Additionally, analyses incorporating genotype data, such as APOE4-carrier status, may reveal different brain health-related intervention effects that warrant further investigation.

Glossary

AD	Alzheimer's disease
BMI	body mass index
BOLD	blood-oxygen-level-dependent
CAIDE	cardiovascular Risk Factors, Aging and Incidence of Dementia
CBF	cerebral blood flow
CCA	canonical correlation analysis
CHESS	chemically selective water suppression
CI	confidence interval
DBP	diastolic blood pressure
DHD	Dutch Healthy Diet
dlPFC	dorsolateral prefrontal cortex
DSST	Digit Symbol Substitution Test
DST	Digit Span Test
FDR	false discovery rate
FFMQ	Five Facet Mindfulness Questionnaire
FINGER	Finnish Geriatric Intervention to Prevent Cognitive Impairment and Disability
FLIRT	FSL Linear Image Registration Tool
fMRI	functional magnetic resonance imaging
FWE	family-wise-error
FWHM	full-width at half-maximum
GM	gray matter
GRE	Gradient Echo Imaging
HADS	Hospital Anxiety and Depression Scale
HDL	high-density lipoprotein
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
IQR	interquartile range
ISCED	International Standard Classification of Education
LDL	low-density lipoprotein
LOAD	late-onset Alzheimer's disease
MAP	mean arterial pressure
MAPT	Multidomain Alzheimer Preventive Trial

MCI	mild cognitive impairment
MIND-NL	Mediterranean DASH intervention for Neurodegenerative Delay
MIND-NL	Dutch Mediterranean-Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay
LMMs	linear mixed models
MP2RAGE	Magnetization Prepared 2 Rapid Acquisition Gradient Echoes
MREC	Medical Research Ethics Committee
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
pCASL	pseudocontinuous arterial spin labelling
PreDIVA	Prevention of Dementia by Intensive Vascular Care
PRESS	Point Resolved Spectroscopy
PSQI	Pittsburgh Sleep Quality Index
PSS	Perceived Stress Scale
QSM	quantitative susceptibility mapping
RAVLT	Rey Auditory Verbal Learning Test
relResA	relative residual amplitude
ROIs	regions of interest
SBP	systolic blood pressure
SBQ	Sedentary Behaviour Questionnaire
SQUASH	Short Questionnaire to Assess Health-enhancing physical activity
TICS-M1	Telephone Interview for Cognitive Status (modified)
TMT	Trail Making Test
US-POINTER	US Study to Protect Brain Health Through Lifestyle Intervention to Reduce Risk
VFT	Verbal Fluency Test
WM	white matter

Author contributions

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Conflicts of interest statement

JAHRC: PI in the ABOARD consortium. Local PI in EVOKE trial (NovoNordisk); consultancy or speaker for Eisai, Lilly, BMS, Novo Nordisk; Editor for JCBFM; SAB member for Alzheimer Nederland; Dutch National Guidelines committee member for dementia.

All other authors declare no conflict of interest.

Statement on informed written consent

All participants provided written informed consent to participate in the study.

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Supplementary Material

Supplementary table 1. Mixed linear model results for all primary outcome measures from baseline to 6 month follow-up.

Outcome (n)	Effect	β (estimate)	SE	df	t-value	95% CI (upper, lower)	p-value, uncorrected	p-value, FDR-corrected
fMRI outcomes								
Working memory fMRI BOLD response in dlPFC (n = 99)	Intercept	0.374	0.022	148.928	16.687	0.330, 0.417	NA	NA
	Group	-0.030	0.031	147.434	-0.969	-0.091, 0.031	0.334	0.437
	Time	0.040	0.018	84.785	2.261	0.005, 0.075	0.026	0.089
	Group $\times$ Time	0.006	0.036	83.240	0.157	-0.064, 0.075	0.876	0.931
Working memory fMRI BOLD response in hippocampus (n = 99)	Intercept	-0.039	0.011	157.621	-3.430	-0.062, -0.017	0.001	NA
	Group	0.017	0.016	156.658	1.073	-0.014, 0.048	0.285	0.437
	Time	-0.015	0.010	89.922	-1.564	-0.035, 0.004	0.121	0.258
	Group $\times$ Time	-0.019	0.020	88.231	-0.986	-0.058, 0.019	0.327	0.437
Working memory N-back task accuracy (n = 100)	Intercept	2.338	0.113	138.27	20.613	2.116, 2.560	NA	NA
	Group	-0.041	0.157	137.26	-0.264	-0.348, 0.266	0.793	0.898
	Time	0.230	0.079	82.311	2.913	0.075, 0.385	0.005	0.026
	Group $\times$ Time	-0.005	0.158	81.202	-0.030	-0.314, 0.305	0.976	0.976

Supplementary table 1. Continued

Outcome (n)	Effect	β (estimate)	SE	df	t-value	95% CI (upper, lower)	p-value, uncorrected	p-value, FDR-corrected
pCASL outcomes								
CBF in dlPFC (n = 98)	Intercept	34.166	1.755	138.082	19.472	30.727, 37.604	NA	NA
	Sequence version	10.427	1.715	138.349	6.079	7.065, 13.789	<0.001	<0.001
	Group	2.259	2.281	126.668	0.991	-2.210, 6.729	0.324	0.437
	Time	-0.882	1.347	88.627	-0.655	-3.523, 1.759	0.514	0.625
	Group $\times$ Time	-3.602	2.128	75.789	-1.692	-7.774, 0.570	0.095	0.23
CBF in hippocampus (n = 98)	Intercept	38.552	1.523	143.599	25.316	35.568, 41.537	NA	NA
	Sequence version	7.045	1.578	151.603	4.465	3.953, 10.138	<0.001	<0.001
	Group	1.936	1.963	134.554	0.986	-1.912, 5.783	0.326	0.437
	Time	-2.591	1.287	91.438	-2.014	-5.113, -0.069	0.047	0.133
	Group $\times$ Time	-5.583	2.074	76.639	-2.691	-9.649, -1.517	0.009	0.037

Results shown are derived from mixed linear models with group, time (total), and their interaction (Group $\times$ Time) as fixed effects, and within-subject repeated measures as a random effect. The control group was used as the reference group. FDR-corrected significant ($p < 0.05$) Time, and Group $\times$ Time effects are shown in bold.
Abbreviations: BOLD = blood-oxygen-level-dependent; CBF = cerebral blood flow; CI = confidence interval; df = degrees of freedom; dlPFC = dorsolateral prefrontal cortex; FDR = False Discovery Rate; SE = standard error.

Supplementary table 2. Mixed linear model results for all secondary outcome measures from baseline to 6 month follow-up.

Outcome	Effect	β (estimate)	SE	df	t-value	95% CI (lower, upper)	p-value, uncorrected	p-value, FDR-corrected
Lifestyle questionnaires (n)								
DHD-index (n = 102)	Intercept	100.878	2.541	136.013	39.701	95.897, 105.858	NA	NA
	Group	-0.500	3.525	136.013	-0.142	-7.409, 6.409	0.887	0.97
	Time	8.076	1.657	88.383	4.873	4.828, 11.325	0.000	<0.001
	Group $\times$ Time	6.690	3.315	87.121	2.018	0.193, 13.186	0.047	0.24
MIND-NL (n = 101)	Intercept	8.695	0.295	150.972	29.489	8.117, 9.273	NA	NA
	Group	-0.346	0.404	149.339	-0.856	-1.137, 0.446	0.393	0.787
	Time	1.245	0.223	91.069	5.575	0.807, 1.683	0.000	<0.001
	Group $\times$ Time	1.794	0.447	87.812	4.016	0.918, 2.669	0.000	0.002
SQUASH (n = 102)	Intercept	7088	578.075	138.466	12.261	5954.972, 8221.027	NA	NA
	Group	107.541	809.327	140.964	0.133	-1478.739, 1693.822	0.894	0.970
	Time	399.204	397.578	86.881	1.004	-380.048, 1178.456	0.318	0.716
	Group $\times$ Time	-88.535	795.155	85.592	-0.111	-1647.039, 1469.969	0.912	0.970
SBQ (n = 102)	Intercept	376.907	25.944	140.217	14.527	326.056, 427.758	NA	NA
	Group	27.969	35.992	140.217	0.777	-42.575, 98.514	0.438	0.800
	Time	-9.317	17.634	91.456	-0.528	-43.880, 25.245	0.599	0.937
	Group $\times$ Time	-28.530	35.268	89.286	-0.809	-97.656, 40.596	0.421	0.797

Supplementary table 2. Continued

Outcome	Effect	β (estimate)	SE	df	t-value	95% CI (lower, upper)	p-value, uncorrected	p-value, FDR-corrected
PSS (n = 102)	Intercept	12.531	0.687	135.959	18.242	11.184, 13.877	NA	NA
	Group	-0.229	0.953	135.959	-0.240	-2.096, 1.639	0.811	0.970
	Time	-1.844	0.436	91.775	-4.232	-2.698, -0.990	0.000	0.001
	Group $\times$ Time	1.207	0.871	90.094	1.385	-0.501, 2.915	0.170	0.470
HADS (n = 102)	Intercept	7.306	0.709	136.182	10.311	5.917, 8.695	NA	NA
	Group	0.203	0.983	136.182	0.207	-1.723, 2.130	0.836	0.970
	Time	-0.782	0.449	92.069	-1.740	-1.663, 0.099	0.085	0.361
	Group $\times$ Time	-0.973	0.899	90.387	-1.082	-2.735, 0.789	0.282	0.716
FFMQ (n = 102)	Intercept	2.986	0.038	148.313	78.911	2.911, 3.06	NA	NA
	Group	-0.011	0.052	148.313	-0.204	-0.114, 0.092	0.839	0.970
	Time	-0.002	0.028	92.264	-0.060	-0.056, 0.053	0.952	0.970
	Group $\times$ Time	0.051	0.055	90.058	0.927	-0.057, 0.160	0.356	0.778
PSQI (n = 102)	Intercept	5.449	0.473	126.054	11.513	4.521, 6.377	NA	NA
	Group	1.381	0.657	126.054	2.104	0.094, 2.668	0.037	0.224
	Time	-0.544	0.265	88.947	-2.052	-1.063, -0.024	0.043	0.239
	Group $\times$ Time	-1.756	0.530	87.468	-3.313	-2.794, -0.717	0.001	0.012
Vascular health indices								
BMI (n = 102)	Intercept	28.927	0.771	102.895	37.533	27.416, 30.437	NA	NA
	Group	2.050	1.069	102.895	1.917	-0.046, 4.145	0.058	0.278
	Time	-0.253	0.155	84.472	-1.628	-0.557, 0.052	0.107	0.389
	Group $\times$ Time	-0.507	0.311	84.350	-1.634	-1.116, 0.101	0.106	0.389

Supplementary table 2. Continued

Outcome	Effect	β (estimate)	SE	df	t-value	95% CI (lower, upper)	p-value, uncorrected	p-value, FDR-corrected
Waist-hip ratio (n = 102)	Intercept	0.940	0.014	147.079	67.464	0.913, 0.967	NA	NA
	Group	0.028	0.019	147.079	1.432	-0.010, 0.066	0.154	0.448
	Time	-0.023	0.010	93.764	-2.367	-0.043, -0.004	0.020	0.148
	Group $\times$ Time	-0.031	0.020	92.280	-1.560	-0.070, 0.008	0.122	0.400
MAP (n = 101)	Intercept	106.605	1.826	121.926	58.394	103.027, 110.184	NA	NA
	Group	3.780	2.544	121.926	1.486	-1.207, 8.767	0.140	0.438
	Time	-3.645	0.973	86.887	-3.748	-5.551, -1.739	<0.001	0.004
	Group $\times$ Time	-3.099	1.945	86.062	-1.593	-6.912, 0.713	0.115	0.393
HOMA-IR (n = 93)	Intercept	0.096	0.008	110.554	11.976	0.080, 0.112	NA	NA
	Group	-0.005	0.011	111.181	-0.452	-0.027, 0.017	0.652	0.970
	Time	-0.002	0.004	71.560	-0.560	-0.011, 0.006	0.577	0.924
	Group $\times$ Time	0.004	0.009	70.490	0.490	-0.013, 0.021	0.626	0.959
HDL-cholesterol (n = 102)	Intercept	1.441	0.061	108.400	23.771	1.322, 1.560	NA	NA
	Group	0.023	0.084	108.075	0.279	-0.141, 0.188	0.781	0.970
	Time	-0.005	0.02	82.500	-0.274	-0.044, 0.033	0.784	0.970
	Group $\times$ Time	-0.011	0.039	82.013	-0.276	-0.088, 0.066	0.783	0.970
LDL-cholesterol (n = 102)	Intercept	3.526	0.138	122.209	25.605	3.256, 3.796	NA	NA
	Group	-0.193	0.191	121.480	-1.012	-0.567, 0.181	0.313	0.716
	Time	-0.159	0.072	85.410	-2.216	-0.300, -0.018	0.029	0.192
	Group $\times$ Time	-0.233	0.144	84.142	-1.625	-0.515, 0.048	0.108	0.389

Supplementary table 2. Continued

Outcome	Effect	β (estimate)	SE	df	t-value	95% CI (lower, upper)	p-value, uncorrected	p-value, FDR-corrected
Triglycerides (n = 102)	Intercept	1.590	0.101	117.364	15.734	1.392, 1.788	NA	NA
	Group	0.150	0.140	116.762	1.072	-0.124, 0.424	0.286	0.716
	Time	-0.067	0.046	84.738	-1.433	-0.158, 0.024	0.155	0.448
	Group $\times$ Time	-0.219	0.093	83.750	-2.361	-0.401, -0.037	0.021	0.148
Brain health indices								
Myo-inositol dIPFC (n = 98)	Intercept	10.23	0.343	157.971	29.783	9.557, 10.903	NA	NA
	relResA	0.131	0.249	86.385	0.526	-0.357, 0.618	0.302	0.716
	Group	-0.424	0.362	155.618	-1.169	-1.134, 0.286	0.841	0.970
	Time	0.044	0.094	152.906	0.469	-0.141, 0.229	0.684	0.970
dlPFC volume (n = 101)	Group $\times$ Time	-0.242	0.492	90.449	-0.493	-1.206, 0.721	0.551	0.902
	Intercept	3268.229	2437.844	102.066	1.341	-1509.945, 8046.403	NA	NA
	Total ICV	-2.442	113.262	82.189	-0.022	-224.435, 219.552	0.858	0.970
	Group	112.525	454.724	106.165	0.247	-778.734, 1003.785	0.933	0.970
Hippocampal volume (n = 101)	Time	0.013	0.002	101.991	8.465	-323.621, 329.972	0.887	0.970
	Group $\times$ Time	-11.235	226.627	81.991	-0.050	-455.423, 432.953	0.799	0.970
	Intercept	3534.131	726.689	116.891	4.863	2109.820, 4958.443	NA	NA
	Total ICV	-39.187	17.752	81.582	-2.208	-73.980, -4.394	0.727	0.970
	Group	-32.939	137.894	100.737	-0.239	-303.211, 237.332	0.968	0.970
	Time	0.003	0.000	117.218	5.864	-81.602, 20.964	0.413	0.797
	Group $\times$ Time	-17.737	35.553	81.614	-0.499	-87.421, 51.947	0.813	0.970

Supplementary table 2. Continued

Outcome	Effect	β (estimate)	SE	df	t-value	95% CI (lower, upper)	p-value, uncorrected	p-value, FDR-corrected
Neuropsychological tests								
TMT (n = 102)	Intercept	2.17	0.098	156.520	22.170	1.978, 2.361	NA	NA
	Group	0.141	0.136	156.520	1.035	-0.126, 0.407	0.302	0.716
	Time	-0.047	0.076	94.099	-0.618	-0.197, 0.102	0.538	0.900
	Group $\times$ Time	-0.131	0.152	92.349	-0.859	-0.430, 0.168	0.392	0.787
VFT (n = 102)	Intercept	25.959	0.786	131.579	33.030	24.419, 27.500	NA	NA
	Group	0.041	1.090	131.579	0.037	-2.096, 2.178	0.970	0.970
	Time	0.427	0.473	89.867	0.903	-0.500, 1.354	0.369	0.781
	Group $\times$ Time	-0.196	0.946	88.784	-0.207	-2.050, 1.658	0.836	0.970
DSST (n = 102)	Intercept	51.306	1.456	113.638	35.232	48.452, 54.160	NA	NA
	Group	1.260	2.020	113.638	0.624	-2.700, 5.219	0.534	0.900
	Time	2.194	0.609	86.346	3.605	1.001, 3.387	0.001	0.005
	Group $\times$ Time	-0.114	1.217	85.819	-0.093	-2.499, 2.272	0.926	0.97
DST (n = 102)	Intercept	14.918	0.502	121.347	29.725	13.935, 15.902	NA	NA
	Group	0.534	0.696	121.347	0.768	-0.830, 1.899	0.444	0.800
	Time	0.189	0.254	88.123	0.744	-0.308, 0.686	0.459	0.806
	Group $\times$ Time	-0.949	0.507	87.353	-1.871	-1.944, 0.045	0.065	0.291

Supplementary table 2. Continued

Outcome	Effect	β (estimate)	SE	df	t-value	95% CI (lower, upper)	p-value, uncorrected	p-value, FDR-corrected
RAVLT delayed recall (n = 102)	Intercept	7.510	0.460	135.505	16.327	6.609, 8.412	NA	NA
	Group	0.263	0.638	135.505	0.413	-0.987, 1.514	0.680	0.970
	Time	2.233	0.291	90.760	7.684	1.664, 2.803	<0.001	<0.001
	Group $\times$ Time	0.179	0.581	89.570	0.308	-0.960, 1.318	0.759	0.970

Results shown are derived from mixed linear models with group, time (total), and their interaction (Group $\times$ Time) as fixed effects, and within-subject repeated measures as a random effect. The control group was used as the reference group. FDR-corrected significant ($p < 0.05$) Time, and Group $\times$ Time effects are shown in bold.

Abbreviations: BMI = body mass index; CI = confidence interval; df = degrees of freedom; DHD = Dutch Healthy Diet; dlPFC = dorsolateral prefrontal cortex; DSST = Digit Symbol Substitution Test; DST = Digit Span Test; FDR = False Discovery Rate; FFMQ = Five Faceted Mindfulness Questionnaire; fMRI = functional magnetic resonance imaging; HADS = Hospital and Anxiety Depression Scale; HDL = high-density lipoproteins; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; LDL = low-density lipoproteins; MAP = mean arterial pressure; PSQI = Pittsburgh sleep quality index; PSS = Perceived Stress Scale; SE = standard error; SBQ = Sedentary Behavior Questionnaire; SQUASH = Short Questionnaire to Assess Health-enhancing physical activity; RAVLT = Rey Auditory Verbal Learning Test; TMT = Trail Making Test; VFT = Verbal Fluency Test.

Supplementary table 3. Mixed linear model results of exploratory median-split subset analyses of (trend) significant primary outcomes.

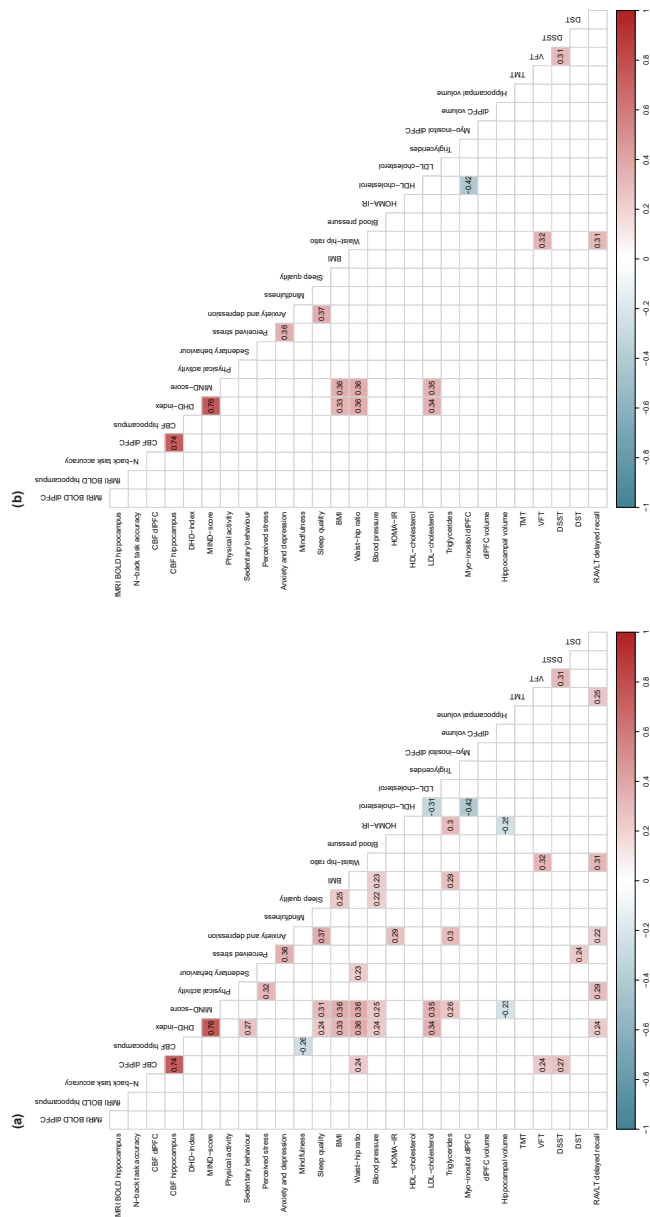
Outcome	Effect	β (estimate)	SE	df	t-value	95% CI (upper, lower)	p-value, uncorrected	p-value, FDR-corrected
Baseline CBF								
lower median split (n = 47)								
CBF in dlPFC	Intercept	27.743	1.749	64.982	15.864	24.315, 31.170	NA	NA
	Sequence version	8.916	2.234	64.238	3.991	4.538, 13.294	0.000	0.001
	Group	1.610	2.365	63.799	0.681	-3.025, 6.244	0.498	0.764
	Time	1.176	1.932	44.623	0.609	-2.610, 4.963	0.546	0.688
	Group $\times$ Time	-1.653	2.578	38.208	-0.641	-6.706, 3.401	0.525	0.764
CBF in hippocampus	Intercept	32.710	1.514	69.648	21.612	29.743, 35.676	NA	NA
	Sequence version	6.146	2.050	71.684	2.999	2.129, 10.163	0.004	0.015
	Group	0.550	2.043	68.852	0.269	-3.454, 4.554	0.788	0.901
	Time	1.072	1.821	48.504	0.589	-2.497, 4.642	0.559	0.688
	Group $\times$ Time	-4.687	2.485	40.422	-1.886	-9.557, 0.184	0.066	0.177
ΔMAP								
upper median split (n = 41)								
CBF in dlPFC	Intercept	36.512	3.566	58.052	10.240	29.523, 43.500	NA	NA
	Sequence version	11.538	2.716	56.931	4.249	6.216, 16.860	0.000	0.001
	Group	1.315	4.169	55.227	0.316	-6.855, 9.486	0.754	0.901
	Time	-2.423	2.184	36.063	-1.110	-6.702, 1.857	0.275	0.488

Supplementary table 3. Continued

Outcome	Effect	β (estimate)	SE	df	t-value	95% CI (upper, lower)	p-value, uncorrected	p-value, FDR-corrected
CBF in hippocampus	Group $\times$ Time	-4.953	3.440	33.545	-1.440	-11.696, 1.790	0.159	0.318
	Intercept	40.934	2.954	58.703	13.858	35.144, 46.723	NA	NA
	Sequence version	7.976	2.270	57.955	3.513	3.526, 12.426	0.001	0.005
	Group	0.325	3.451	55.974	0.094	-6.439, 7.089	0.925	0.986
	Time	-4.532	1.833	36.618	-2.473	-8.124, -0.940	0.018	0.058
	Group $\times$ Time	-6.282	2.893	34.043	-2.172	-11.952, -0.612	0.037	0.118

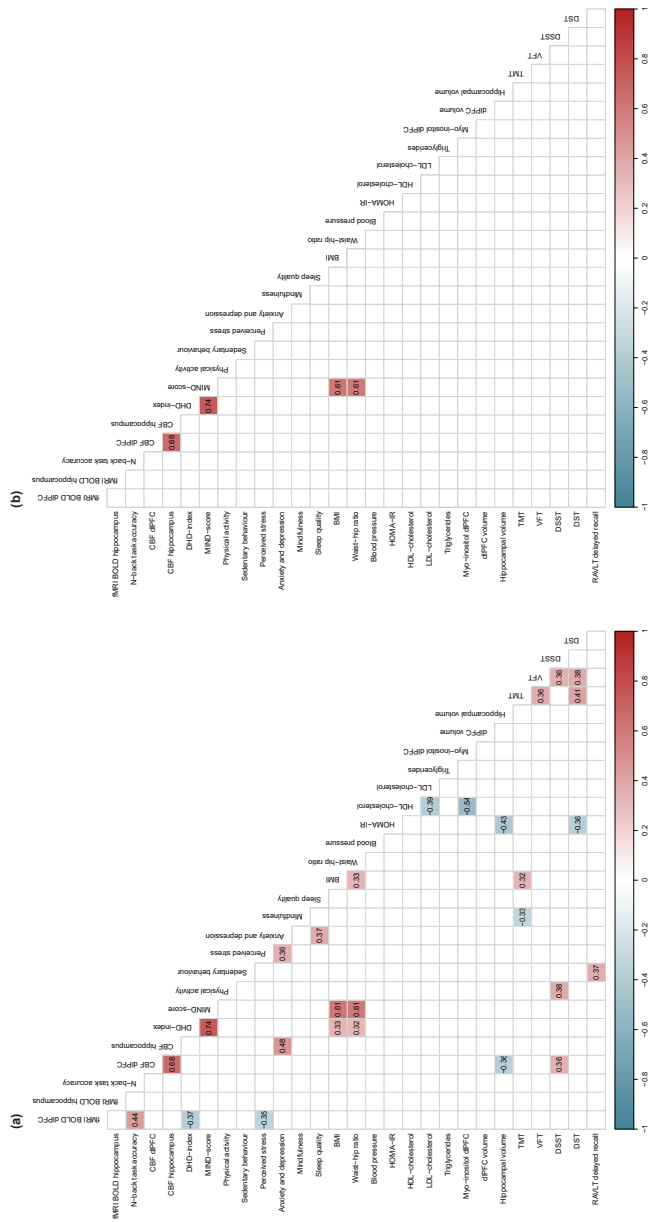
Results shown are derived from mixed linear models with group, time (total),, and their interaction (Group $\times$ Time) as fixed effects, and within-subject repeated measures as a random effect. The control group was used as the reference group.

Abbreviations: CBF = cerebral blood flow; CI = confidence interval; df = degrees of freedom; dlPFC = dorsolateral prefrontal cortex; MAP = mean arterial pressure; SE = standard error.



Supplementary figure 1. Whole-group pairwise Pearson's correlations of all outcome Δ -scores (n = 51–86). Panels show (a) uncorrected significance levels and (b) FDR-corrected significance levels ($p < 0.05$) across the total study population. Data was Z-scored, and select variables were transformed so higher Z-scores indicate healthier outcomes.

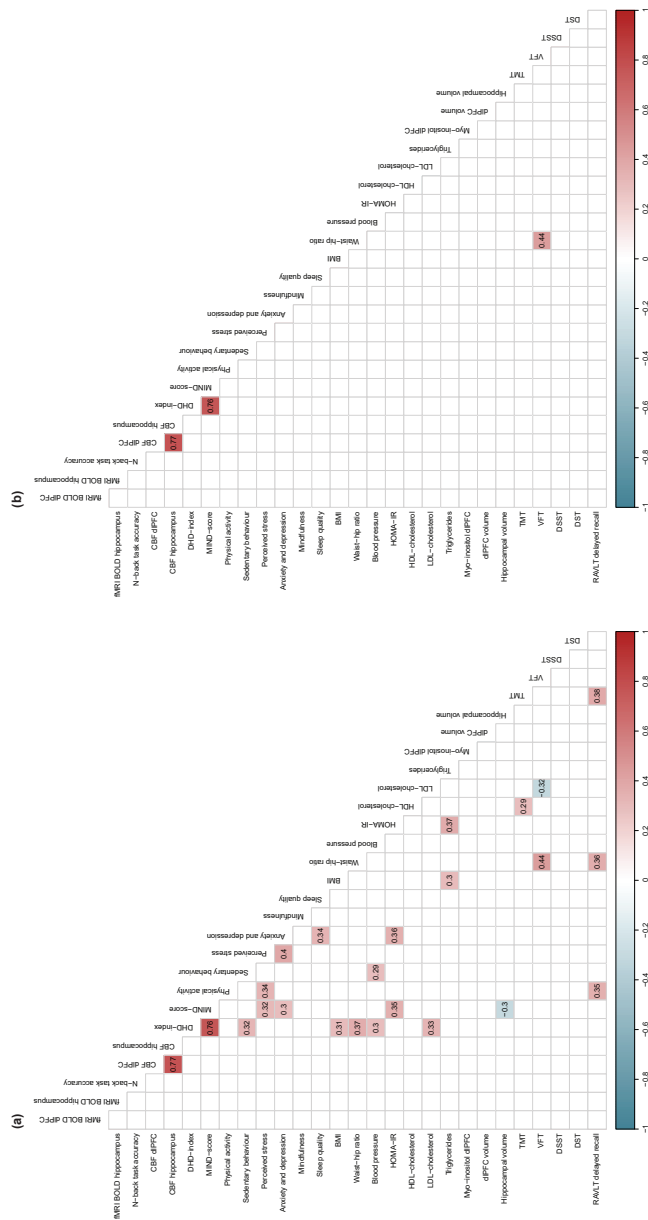
Abbreviations: BMI = body mass index; BOLD = blood-oxygen-level-dependent; DHD = Dutch Healthy Diet; dPFC = dorsolateral prefrontal cortex; DSST = Digit Symbol Substitution Test; DST = Digit Span Test; FFMQ = Five Faceted Mindfulness Questionnaire; fMRI = functional magnetic resonance imaging; HADS = Hospital and Anxiety Depression Scale; HDL = high-density lipoproteins; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; LDL = low-density lipoproteins; MAP = mean arterial pressure; PSQI = Pittsburgh sleep quality index; PSS = Perceived Stress Scale; SBQ = Sedentary Behavior Questionnaire; SQUASH = Short Questionnaire to Assess Health-enhancing physical activity; RAVLT = Rey Auditory Verbal Learning Test; TMT = Trail Making Test; VFT = Verbal Fluency Test.



Supplementary figure 2. Active control group pairwise Pearson's correlations of all outcome Δ -scores (n = 23–39).

Panels show (a) uncorrected significance levels and (b) FDR-corrected significance levels (p < 0.05) across the active control group. Data was Z-scored, and select variables were transformed so higher Z-scores indicate healthier outcomes.

Abbreviations: BMI = body mass index; BOLD = blood-oxygen-level-dependent; DHD = Dutch Healthy Diet; dPFC = dorsolateral prefrontal cortex; DSST = Digit Symbol Substitution Test; DST = Digit Span Test; FFMQ = Five Faceted Mindfulness Questionnaire; fMRI = functional magnetic resonance imaging; HADS = Hospital and Anxiety Depression Scale; HDL = high-density lipoproteins; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; LDL = low-density lipoproteins; MAP = mean arterial pressure; PSQI = Pittsburgh sleep quality index; PSS = Perceived Stress Scale; SBQ = Sedentary Behavior Questionnaire; SQUASH = Short Questionnaire to Assess Health-enhancing physical activity; RAVLT = Rey Auditory Verbal Learning Test; TMT = Trail Making Test; VFT = Verbal Fluency Test.



Supplementary figure 3. Intervention group pairwise Pearson's correlations of all outcome Δ -scores (n = 26–48).

Panels show (a) uncorrected significance levels and (b) FDR-corrected significance levels (p < 0.05) across the intervention group. Data was Z-scored, and select variables were transformed so higher Z-scores indicate healthier outcomes.

Abbreviations: BMI = body mass index; BOLD = blood-oxygen-level-dependent; DHD = Dutch Healthy Diet; dlPFC = dorsolateral prefrontal cortex; DSST = Digit Symbol Substitution Test; DST = Digit Span Test; FEMQ = Five Faceted Mindfulness Questionnaire; I = functional magnetic resonance imaging; HADS = Hospital and Anxiety Depression Scale; HDL = high-density lipoproteins; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; LDL = low-density lipoproteins; MAP = mean arterial pressure; PSQI = Pittsburgh sleep quality index; PSS = Perceived Stress Scale; SBQ = Sedentary Behavior Questionnaire; SQUASH = Short Questionnaire to Assess Health-enhancing physical activity; RAVLT = Rey Auditory Verbal Learning Test; TMT = Trail Making Test; VFT = Verbal Fluency Test.

Supplementary table 4. Results of exploratory simple linear regression analyses.

Outcome	Effect	β (estimate)	SE	t-value	95% CI (upper, lower)	p-value, uncorrected	p-value, FDR-corrected
Lifestyle Δ-composite score models (n)							
CBF in dlPFC (n = 68)	Intercept	0.013	0.123	0.105	-0.228, 0.254	NA	NA
	Lifestyle Δ -score	-0.033	0.058	-0.558	-0.147, 0.082	0.578	0.723
CBF in hippocampus (n = 68)							
	Intercept	0.040	0.120	0.334	-0.196, 0.276	NA	NA
	Lifestyle Δ -score	-0.076	0.057	-1.326	-0.187, 0.036	0.189	0.723
Working memory fMRI BOLD response in dlPFC (n = 71)							
	Intercept	0.017	0.120	0.143	-0.218, 0.253	NA	NA
	Lifestyle Δ -score	-0.034	0.057	-0.586	-0.146, 0.079	0.560	0.723
Working memory fMRI BOLD response in hippocampus (n = 71)							
	Intercept	0.005	0.121	0.039	-0.233, 0.243	NA	NA
	Lifestyle Δ -score	0.008	0.058	0.143	-0.105, 0.122	0.886	0.947
Working memory N-back task accuracy (n = 75)							
	Intercept	-0.019	0.116	-0.167	-0.246, 0.207	NA	NA
	Lifestyle Δ -score	0.004	0.056	0.067	-0.105, 0.113	0.947	0.947
Vascular health Δ-composite score models (n)							
CBF in dlPFC (n = 70)							
	Intercept	0.000	0.118	-0.001	-0.232, 0.231	NA	NA
	Lifestyle Δ -score	0.063	0.039	1.639	-0.012, 0.139	0.106	0.723
CBF in hippocampus (n = 70)							
	Intercept	0.000	0.120	0.000	-0.235, 0.235	NA	NA
	Lifestyle Δ -score	0.032	0.039	0.811	-0.045, 0.109	0.420	0.723

Supplementary table 4. Continued

Outcome	Effect	β (estimate)	SE	t-value	95% CI (upper, lower)	p-value, uncorrected	p-value, FDR-corrected
Working memory fMRI BOLD response in dlPFC (n = 73)	Intercept	-0.005	0.117	-0.045	-0.235, 0.224	0.964	NA
	Lifestyle Δ -score	0.040	0.038	1.039	-0.035, 0.114	0.302	0.723
Working memory fMRI BOLD response in hippocampus (n = 73)	Intercept	0.003	0.118	0.025	-0.228, 0.234	NA	NA
	Lifestyle Δ -score	-0.022	0.038	-0.567	-0.097, 0.053	0.572	0.723
Working memory N-back task accuracy (n = 78)	Intercept	-0.006	0.114	-0.055	-0.230, 0.217	NA	NA
	Lifestyle Δ -score	0.026	0.037	0.698	-0.047, 0.099	0.488	0.723

Results shown are derived from linear regression models with primary outcome measures as dependent variable, and lifestyle or vascular health composite Δ -score as dependent variable.
Abbreviations: BOLD = blood-oxygen-level-dependent; CBF = cerebral blood flow; CI = confidence interval; dlPFC = dorsolateral prefrontal cortex; FDR = False-Discovery-Rate; SE = standard error.

Supplementary table 5. Results of exploratory multiple linear regression analyses.

Outcome	Effect	β (estimate)	SE	t-value	95% CI (upper, lower)	p-value, uncorrected	p-value, FDR-corrected
Lifestyle Δ -score models (n)							
CBF in dlPFC (n = 59)	Intercept	0.024	0.143	0.166	-0.257, 0.304	NA	NA
	Δ DHD-index	0.141	0.222	0.636	-0.294, 0.576	0.528	0.865
	Δ MIND-NL	-0.144	0.221	-0.653	-0.578, 0.289	0.517	0.865
	Δ SQUASH	0.049	0.165	0.299	-0.274, 0.373	0.766	0.982
	Δ SBQ	-0.012	0.156	-0.079	-0.319, 0.294	0.937	0.982
	Δ PSS	0.133	0.168	0.793	-0.196, 0.463	0.431	0.865
	Δ HADS	-0.151	0.186	-0.809	-0.516, 0.214	0.422	0.865
	Δ FFMQ	-0.010	0.150	-0.068	-0.305, 0.284	0.946	0.982
	Δ PSQI	0.030	0.167	0.180	-0.298, 0.358	0.858	0.982
	Intercept	0.08	0.128	0.621	-0.172, 0.331	NA	NA
CBF in hippocampus (n = 59)	Δ DHD-index	-0.106	0.189	-0.559	-0.475, 0.264	0.578	0.905
	Δ MIND-NL	0.174	0.191	0.911	-0.200, 0.547	0.366	0.865
	Δ SQUASH	0.043	0.148	0.289	-0.248, 0.333	0.773	0.982
	Δ SBQ	-0.084	0.132	-0.638	-0.342, 0.174	0.526	0.865
	Δ PSS	0.022	0.141	0.154	-0.256, 0.299	0.878	0.982
	Δ HADS	-0.216	0.170	-1.272	-0.549, 0.117	0.209	0.865
	Δ FFMQ	-0.054	0.135	-0.401	-0.319, 0.211	0.690	0.982
	Δ PSQI	0.110	0.152	0.726	-0.187, 0.407	0.471	0.865
	Intercept	0.08	0.128	0.621	-0.172, 0.331	NA	NA
	Δ DHD-index	-0.106	0.189	-0.559	-0.475, 0.264	0.578	0.905

Supplementary table 5. Continued

Outcome	Effect	β (estimate)	SE	t-value	95% CI (upper, lower)	p-value, uncorrected	p-value, FDR-corrected
Working memory fMRI BOLD response in dlPFC (n = 63)	Intercept	0.025	0.119	0.210	-0.208, 0.258	NA	NA
	Δ DHD-index	0.141	0.169	0.833	-0.191, 0.472	0.409	0.865
	Δ MIND-NL	-0.100	0.180	-0.558	-0.452, 0.252	0.579	0.905
	Δ SQUASH	0.107	0.133	0.807	-0.153, 0.367	0.423	0.865
	Δ SBQ	-0.007	0.120	-0.056	-0.243, 0.229	0.956	0.982
	Δ PSS	0.051	0.128	0.400	-0.200, 0.302	0.691	0.982
	Δ HADS	0.213	0.155	1.377	-0.090, 0.517	0.175	0.865
	Δ FFMQ	-0.369	0.134	-2.754	-0.632, -0.106	0.008	0.614
	Δ PSQI	-0.197	0.137	-1.440	-0.465, 0.071	0.156	0.865
Working memory fMRI BOLD response in hippocampus (n = 63)	Intercept	0.029	0.127	0.227	-0.219, 0.277	NA	NA
	Δ DHD-index	0.150	0.180	0.832	-0.203, 0.503	0.409	0.865
	Δ MIND-NL	-0.215	0.191	-1.124	-0.590, 0.160	0.266	0.865
	Δ SQUASH	-0.010	0.141	-0.070	-0.287, 0.267	0.944	0.982
	Δ SBQ	-0.048	0.128	-0.373	-0.299, 0.204	0.711	0.982
	Δ PSS	-0.109	0.136	-0.798	-0.376, 0.159	0.429	0.865
	Δ HADS	0.323	0.165	1.957	0.000, 0.647	0.056	0.718
	Δ FFMQ	-0.313	0.143	-2.192	-0.593, -0.033	0.033	0.718
	Δ PSQI	-0.168	0.146	-1.151	-0.453, 0.118	0.255	0.865

Supplementary table 5. Continued

Outcome	Effect	β (estimate)	SE	t-value	95% CI (upper, lower)	p-value, uncorrected	p-value, FDR-corrected
Working memory N-back task accuracy (n = 65)	Intercept	0.062	0.131	0.473	-0.195, 0.319	NA	NA
	Δ DHD-index	-0.329	0.204	-1.617	-0.728, 0.070	0.112	0.865
	Δ MIND-NL	0.229	0.203	1.127	-0.169, 0.626	0.265	0.865
	Δ SQUASH	0.022	0.151	0.144	-0.275, 0.318	0.886	0.982
	Δ SBQ	-0.092	0.143	-0.643	-0.373, 0.189	0.523	0.865
	Δ PSS	-0.219	0.154	-1.418	-0.521, 0.084	0.162	0.865
	Δ HADS	0.010	0.171	0.059	-0.325, 0.345	0.953	0.982
	Δ FFMQ	0.009	0.138	0.064	-0.261, 0.279	0.949	0.982
	Δ PSQI	0.190	0.154	1.238	-0.111, 0.491	0.221	0.865
Vascular health Δ -score models (n)							
CBF in dlPFC (n = 54)	Intercept	-0.005	0.147	-0.036	-0.294, 0.283	NA	NA
	Δ BMI	-0.204	0.187	-1.092	-0.570, 0.162	0.280	0.865
	Δ Waist-hip ratio	0.102	0.15	0.683	-0.192, 0.396	0.498	0.865
	Δ MAP	0.234	0.158	1.476	-0.077, 0.544	0.147	0.865
	Δ HOMA-IR	0.296	0.163	1.814	-0.024, 0.617	0.076	0.816
	Δ HDL-cholesterol	-0.099	0.154	-0.64	-0.400, 0.203	0.525	0.865
	Δ LDL-cholesterol	-0.083	0.164	-0.507	-0.405, 0.239	0.614	0.941
	Δ Triglycerides	0.045	0.155	0.294	-0.258, 0.348	0.770	0.982

Supplementary table 5. Continued

Outcome	Effect	β (estimate)	SE	t-value	95% CI (upper, lower)	p-value, uncorrected	p-value, FDR-corrected
CBF in hippocampus (n = 54)	Intercept	0.073	0.153	0.476	-0.228, 0.374	NA	NA
	Δ BMI	-0.379	0.194	-1.949	-0.760, 0.002	0.057	0.718
	Δ Waist-hip ratio	0.124	0.156	0.792	-0.183, 0.430	0.433	0.865
	Δ MAP	0.163	0.165	0.99	-0.160, 0.486	0.327	0.865
	Δ HOMA-IR	0.209	0.17	1.228	-0.125, 0.543	0.226	0.865
	Δ HDL-cholesterol	0.106	0.16	0.661	-0.208, 0.420	0.512	0.865
	Δ LDL-cholesterol	-0.022	0.171	-0.127	-0.357, 0.314	0.899	0.982
	Δ Triglycerides	0.072	0.161	0.445	-0.244, 0.387	0.659	0.982
	Intercept	0.135	0.134	1.008	-0.128, 0.399	NA	NA
	Δ BMI	-0.018	0.17	-0.108	-0.351, 0.314	0.915	0.982
Working memory fMRI BOLD response in dlPFC (n = 55)	Δ Waist-hip ratio	0.145	0.136	1.066	-0.121, 0.411	0.292	0.865
	Δ MAP	-0.027	0.154	-0.172	-0.328, 0.275	0.864	0.982
	Δ HOMA-IR	0.029	0.151	0.192	-0.266, 0.324	0.849	0.982
	Δ HDL-cholesterol	0.402	0.175	2.294	0.059, 0.746	0.026	0.718
	Δ LDL-cholesterol	0.156	0.153	1.017	-0.145, 0.456	0.314	0.865
	Δ Triglycerides	0.181	0.144	1.251	-0.102, 0.464	0.217	0.865

Supplementary table 5. Continued

Outcome	Effect	β (estimate)	SE	t-value	95% CI (upper, lower)	p-value, uncorrected	p-value, FDR-corrected
Working memory fMRI BOLD response in hippocampus (n = 55)	Intercept	0.07	0.105	0.665	-0.136, 0.275	NA	NA
	Δ BMI	-0.027	0.132	-0.207	-0.287, 0.232	0.837	0.982
	Δ Waist-hip ratio	-0.115	0.106	-1.086	-0.323, 0.093	0.283	0.865
	Δ MAP	-0.248	0.12	-2.065	-0.483, -0.013	0.045	0.718
	Δ HOMA-IR	-0.001	0.117	-0.005	-0.231, 0.230	0.996	0.997
	Δ HDL-cholesterol	0.174	0.137	1.273	-0.094, 0.442	0.209	0.865
	Δ LDL-cholesterol	0.152	0.12	1.268	-0.083, 0.386	0.211	0.865
	Δ Triglycerides	0.02	0.113	0.182	-0.200, 0.241	0.857	0.982
Working memory N-back task accuracy (n = 60)	Intercept	0.018	0.134	0.135	-0.244, 0.281	NA	NA
	Δ BMI	-0.165	0.173	-0.955	-0.504, 0.174	0.344	0.865
	Δ Waist-hip ratio	0.089	0.14	0.632	-0.186, 0.363	0.530	0.865
	Δ MAP	0.2	0.148	1.353	-0.090, 0.489	0.182	0.865
	Δ HOMA-IR	0.131	0.154	0.848	-0.171, 0.433	0.400	0.865
	Δ HDL-cholesterol	-0.008	0.148	-0.055	-0.298, 0.282	0.956	0.982
	Δ LDL-cholesterol	0.001	0.153	0.004	-0.299, 0.300	0.997	0.997
	Δ Triglycerides	0.047	0.145	0.324	-0.237, 0.331	0.747	0.982

Results shown are derived from multiple linear regression models with primary outcomes as dependent variables, and all lifestyle or vascular health-related Δ -scores as independent variables.

Abbreviations: BMI = body mass index; BOLD = blood-oxygen-level-dependent; CI = confidence interval; df = degrees of freedom; dlPFC = dorsolateral prefrontal cortex; FDR = False Discovery Rate; fMRI = functional magnetic resonance imaging; HDL = high-density lipoproteins; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; LDL = low-density lipoproteins; MAP = mean arterial pressure; SE = standard error.

Supplementary table 6A. Canonical correlation dimension-level results of lifestyle and vascular health variable sets.

Dimension	Rc	Rc ²	Wilks' λ	F-statistic	df1, df2	p-value
1	0.729	0.532	0.187	1.017	56, 156.096	0.456
2	0.605	0.366	0.400	0.717	42, 139.474	0.894
3	0.413	0.171	0.631	0.496	30, 122	0.986
4	0.359	0.129	0.761	0.445	20, 103.765	0.980
5	0.293	0.086	0.874	0.369	12, 84.956	0.971
6	0.185	0.034	0.956	0.250	6, 66	0.957
7	0.102	0.010	0.990	0.178	2, 34	0.838

Canonical correlations (Rc), squared canonical correlations (Rc²), Wilks' λ, F-statistic, degrees of freedom (df), and p-values for each canonical dimension between lifestyle outcome Δ-scores (set X) and vascular health outcome Δ-scores (set Y).

Supplementary table 6B. Canonical correlation analysis between lifestyle and vascular health outcomes.

Variable	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5	Dimension 6	Dimension 7
Set X (Lifestyle)							
DHD-index	-0.840	-0.004	0.355	-0.188	-0.061	-0.246	0.144
MIND-NL	-0.757	0.385	0.394	-0.138	0.096	0.055	-0.201
SQUASH	-0.001	0.217	-0.139	0.329	0.024	-0.698	0.509
SBQ	-0.510	-0.234	-0.331	0.141	-0.169	0.191	0.343
PSS	0.019	-0.214	-0.075	-0.251	0.490	-0.716	-0.233
HADS	-0.179	0.600	-0.328	-0.600	0.155	-0.292	-0.068
FFMQ	-0.255	0.134	-0.103	-0.163	-0.787	-0.384	-0.258
PSQI	-0.460	0.274	-0.378	0.419	0.058	-0.236	-0.341
Set Y (Vascular health)							
BMI	-0.284	0.440	0.184	0.301	0.275	-0.725	-0.005
Waist-hip ratio	-0.586	0.182	-0.256	-0.154	0.374	-0.027	-0.628
MAP	-0.445	0.203	-0.371	0.047	-0.380	-0.239	0.648
HOMA-IR	0.177	0.430	0.264	-0.648	0.543	-0.013	0.022
HDL-cholesterol	0.438	0.332	-0.688	0.375	0.048	0.127	-0.257
LDL-cholesterol	-0.338	0.181	0.564	0.083	0.133	0.558	0.446
Triglycerides	-0.186	0.694	0.355	-0.072	-0.437	0.051	-0.399

Canonical loadings (correlation between each variable and the canonical variate) of each canonical dimension for lifestyle outcome Δ -scores (set X) and vascular health outcome Δ -scores (set Y). Data was Z-scored, and select variables were transformed so higher Z-scores indicate healthier outcomes.

Abbreviations: BMI = body mass index; DHD = Dutch Healthy Diet; FFMQ = Five Faceted Mindfulness Questionnaire; HADS = Hospital and Anxiety Depression Scale; HDL = high-density lipoproteins; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; LDL = low-density lipoproteins; MAP = mean arterial pressure; PSQI = Pittsburgh sleep quality index; PSS = Perceived Stress Scale; SBQ = Sedentary Behavior Questionnaire; SQUASH = Short Questionnaire to Assess Health-enhancing physical activity.

Supplementary table 7A. Canonical correlation dimension-level results of vascular health and brain health outcomes variable sets.

Dimension	Rc	Rc ²	Wilks'λ	F-statistic	df1, df2	p-value
1	0.729	0.532	0.231	0.864	49, 126.266	0.716
2	0.491	0.241	0.492	0.548	36, 112.544	0.980
3	0.406	0.165	0.648	0.486	25, 98.088	0.979
4	0.390	0.152	0.776	0.449	16, 83.124	0.964
5	0.253	0.064	0.916	0.279	9, 68.295	0.978
6	0.142	0.020	0.979	0.157	4, 58	0.959
7	0.036	0.001	0.999	0.038	1, 30	0.847

Canonical correlations (Rc), squared canonical correlations (Rc²), Wilks'λ, F-statistic, degrees of freedom (df), and p-values for each canonical dimension between vascular health outcome Δ-scores (set X) and brain health outcome Δ-scores (set Y).

Supplementary table 7B. Canonical correlation analysis between vascular health and brain health outcomes.

Variable	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5	Dimension 6	Dimension 7
Set X (Vascular health)							
BMI	0.008	-0.457	0.488	-0.403	-0.513	0.252	-0.252
Waist-hip ratio	-0.153	-0.455	0.099	-0.416	0.137	0.704	0.269
MAP	-0.080	0.360	-0.383	-0.326	-0.760	0.159	-0.084
HOMA-IR	0.227	-0.764	-0.174	0.549	-0.119	0.135	0.005
HDL-cholesterol	-0.821	0.004	0.291	0.184	-0.102	-0.108	0.430
LDL-cholesterol	0.596	0.436	0.032	0.425	-0.123	0.501	-0.086
Triglycerides	-0.404	-0.243	-0.103	0.153	0.108	0.612	-0.598
Set Y (Brain health)							
CBF in dlPFC	-0.023	-0.799	-0.367	0.385	0.021	0.144	-0.236
CBF in hippocampus	-0.206	-0.534	-0.295	0.542	-0.322	-0.208	-0.380
Working memory fMRI BOLD response in dlPFC	-0.573	0.155	0.086	0.637	-0.012	0.130	0.467
Working memory fMRI BOLD response in hippocampus	0.076	0.068	0.217	0.722	0.628	-0.124	0.103
Myo-inositol dlPFC	-0.084	-0.169	0.122	-0.460	0.398	-0.656	0.386
dlPFC volume	0.342	-0.413	0.292	0.216	-0.462	-0.209	0.569
Hippocampal volume	-0.413	-0.305	0.810	-0.169	-0.001	0.187	-0.129

Canonical loadings (correlation between each variable and the canonical variate) of each canonical dimension for vascular health outcome Δ -scores (set X) and brain health outcome Δ -scores (set Y). Data was Z-scored, and select variables were transformed so higher Z-scores indicate healthier outcomes. *Abbreviations:* BMI = body mass index; BOLD = blood-oxygen-level-dependent; CBF = cerebral blood flow; dlPFC = dorsolateral prefrontal cortex; HDL = high-density lipoproteins; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; LDL = low-density lipoproteins; MAP = mean arterial pressure.

Supplementary table 8A. Canonical correlation dimension-level results of brain health and cognitive functioning outcomes variable sets.

Dimension	Rc	Rc ²	Wilks' λ	F-statistic	df1, df2	p-value
1	0.466	0.217	0.602	0.622	42, 228.592	0.966
2	0.328	0.108	0.769	0.448	30, 198	0.995
3	0.256	0.066	0.862	0.383	20, 166.781	0.993
4	0.244	0.060	0.922	0.350	12, 135.225	0.978
5	0.130	0.017	0.981	0.168	6, 104	0.985
6	0.048	0.002	0.998	0.062	2, 53	0.940

Canonical correlations (Rc), squared canonical correlations (Rc²), Wilks' λ, F-statistic, degrees of freedom (df), and p-values for each canonical dimension between brain health outcome Δ-scores (set X) and cognitive functioning outcome Δ-scores (set Y).

Supplementary table 8B. Canonical correlation analysis between brain health and cognitive functioning outcomes.

Variable	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5	Dimension 6
Set X (Brain health)						
CBF in dlPFC	0.076	-0.562	-0.515	-0.232	-0.401	0.224
CBF in hippocampus	0.463	-0.662	-0.407	0.193	-0.329	-0.064
Working memory fMRI BOLD response in dlPFC	-0.485	-0.510	0.305	0.301	-0.332	0.035
Working memory fMRI BOLD response in hippocampus	-0.539	0.022	-0.627	0.283	0.012	-0.220
Myo-inositol dlPFC	0.335	0.383	-0.101	-0.391	-0.609	-0.024
dlPFC volume	0.110	-0.470	0.154	-0.626	0.113	-0.566
Hippocampal volume	0.216	-0.252	-0.058	-0.231	0.484	0.446
Set Y (Cognitive functioning)						
TMT	-0.638	-0.513	0.341	-0.270	0.291	0.236
VFT	0.057	-0.361	-0.191	-0.639	-0.615	-0.210
DSST	-0.287	-0.283	-0.257	0.412	-0.689	-0.356
DST	-0.522	0.372	-0.213	-0.380	0.254	-0.579
RAVLT delayed recall	-0.041	-0.608	-0.605	-0.071	0.491	0.128
Working memory N-back task accuracy	-0.475	0.269	-0.201	0.104	-0.186	0.785

Canonical loadings (correlation between each variable and the canonical variate) of each canonical dimension for brain health outcome Δ -scores (set X) and cognitive functioning outcome Δ -scores (set Y). Data was Z-scored, and select variables were transformed so higher Z-scores indicate healthier outcomes.

Abbreviations: BOLD = blood-oxygen-level-dependent; CBF = cerebral blood flow; dlPFC = dorsolateral prefrontal cortex; DSST = Digit Symbol Substitution Test; DST = Digit Span Test; RAVLT = Rey Auditory Verbal Learning Test; TMT = Trail Making Test; VFT = Verbal Fluency Test.

Supplementary table 9. Overview of all reported adverse events during the trial.

	Active control group (n = 49)	Intervention group (n = 53)
Total adverse events	7	13
Participants with ≥1 adverse event	8 (16%)	12 (23%)
Mild adverse events		
Minor skin rash/irritation	2 (10%)	3 (15%)
Mild stress related to outcome visit	0 (0%)	2 (10%)
Discomfort during MRI scan	2 (10%)	3 (15%)
Nauseous	1 (5%)	0 (0%)
Heartburn	0 (0%)	1 (5%)
Bladder problem	1 (5%)	0 (0%)
Moderate adverse events		
Gastrointestinal problems	0 (0%)	2 (10%)
Falling injury	0 (0%)	1 (5%)
Serious adverse events		
Hospital stay for eye surgery	1 (5%)	0 (0%)
Hospital stay for bowel surgery	0 (0%)	1 (5%)

No adverse events were considered related or possibly related to the interventions. The majority of total mild adverse events, and all of the adverse events (possibly) related to trial participation were short-lived or resolved immediately. No moderate or serious adverse events were considered related or possibly related to trial participation.
Data are presented as n (%).

SUPPLEMENTARY APPENDIX 1. Preprocessing with fMRIPrep

Results included in this manuscript come from preprocessing performed using *fMRIPrep* 23.2.0 (Esteban et al. (2019); Esteban et al. (2018); RRID:SCR_016216), which is based on *Nipype* 1.8.6 (K. Gorgolewski et al. (2011); K. J. Gorgolewski et al. (2018); RRID:SCR_002502).

Preprocessing of B_0 inhomogeneity mappings

A total of 2 fieldmaps were found available within the input BIDS structure for this particular subject. A B_0 nonuniformity map (or *fieldmap*) was estimated from the phase-drift map(s) measure with two consecutive GRE (gradient-recalled echo) acquisitions. The corresponding phase-map(s) were phase-unwrapped with *prelude* (FSL None).

Anatomical data preprocessing

A total of 2 T1-weighted (T1w) images were found within the input BIDS dataset. Each T1w image was corrected for intensity non-uniformity (INU) with *N4BiasFieldCorrection* (Tustison et al. 2010), distributed with ANTs 2.5.0 (Avants et al. 2008, RRID:SCR_004757). The T1w-reference was then skull-stripped with a *Nipype* implementation of the *antsBrainExtraction.sh* workflow (from ANTs), using OASIS3oANTs as target template. Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was performed on the brain-extracted T1w using *fast* (FSL (version unknown), RRID:SCR_002823, Zhang, Brady, and Smith 2001). An anatomical T1w-reference map was computed after registration of 2 T1w images (after INU-correction) using *mri_robust_template* (FreeSurfer 7.3.2, Reuter, Rosas, and Fischl 2010). Brain surfaces were reconstructed using *recon-all* (FreeSurfer 7.3.2, RRID:SCR_001847, Dale, Fischl, and Sereno 1999), and the brain mask estimated previously was refined with a custom variation of the method to reconcile ANTs-derived and FreeSurfer-derived segmentations of the cortical gray-matter of *Mindboggle* (RRID:SCR_002438, Klein et al. 2017). Volume-based spatial normalization to two standard spaces (MNI152NLin6Asym, MNI152NLin2009cAsym) was performed through nonlinear registration with *antsRegistration* (ANTs 2.5.0), using brain-extracted versions of both T1w reference and the T1w template. The following templates were selected for spatial normalization and accessed with *TemplateFlow* (23.1.0, Ciric et al. 2022): *FSL's MNI ICBM 152 non-linear 6th Generation Asymmetric Average Brain Stereotaxic Registration Model* (Evans et al. (2012), RRID:SCR_002823; *TemplateFlow* ID: MNI152NLin6Asym), *ICBM 152 Nonlinear Asymmetrical template version 2009c* (Fonov et al. (2009), RRID:SCR_008796; *TemplateFlow* ID: MNI152NLin2009cAsym).

Functional data preprocessing

For each of the 2 BOLD runs found per subject (across all tasks and sessions), the following preprocessing was performed. First, a reference volume was generated, using a custom methodology of *fMRIPrep*, for use in head motion correction. Head-motion parameters with respect to the BOLD reference (transformation matrices, and six corresponding rotation and translation parameters) are estimated before any spatiotemporal filtering using *mcflirt* (FSL, Jenkinson et al. 2002). The estimated *fieldmap* was then aligned with rigid-registration to the target EPI (echo-planar imaging) reference run. The field coefficients were mapped on to the reference EPI using the transform. The BOLD reference was then co-registered to the T1w reference using *bbregister* (FreeSurfer) which implements boundary-based registration (Greve and Fischl 2009). Co-registration was configured with six degrees of freedom. Several confounding time-series were calculated based on the *preprocessed BOLD*: framewise displacement (FD), DVARS and three region-wise global signals. FD was computed using two formulations following Power (absolute sum of relative motions, Power et al. (2014)) and Jenkinson (relative root mean square displacement between affines, Jenkinson et al. (2002)). FD and DVARS are calculated for each functional run, both using their implementations in *Nipype* (following the definitions by Power et al. 2014). The three global signals are extracted within the CSF, the WM, and the whole-brain masks. Additionally, a set of physiological regressors were extracted to allow for component-based noise correction (*CompCor*, Behzadi et al. 2007). Principal components are estimated after high-pass filtering the *preprocessed BOLD* time-series (using a discrete cosine filter with 128s cut-off) for the two *CompCor* variants: temporal (*tCompCor*) and anatomical (*aCompCor*). *tCompCor* components are then calculated from the top 2% variable voxels within the brain mask. For *aCompCor*, three probabilistic masks (CSF, WM and combined CSF+WM) are generated in anatomical space. The implementation differs from that of Behzadi et al. in that instead of eroding the masks by 2 pixels on BOLD space, a mask of pixels that likely contain a volume fraction of GM is subtracted from the *aCompCor* masks. This mask is obtained by dilating a GM mask extracted from the FreeSurfer's *aseg* segmentation, and it ensures components are not extracted from voxels containing a minimal fraction of GM. Finally, these masks are resampled into BOLD space and binarized by thresholding at 0.99 (as in the original implementation). Components are also calculated separately within the WM and CSF masks. For each *CompCor* decomposition, the k components with the largest singular values are retained, such that the retained components' time series are sufficient to explain 50 percent of variance across the nuisance mask (CSF, WM, combined, or temporal). The remaining components are dropped from consideration. The head-motion estimates calculated in the correction step were also placed within the corresponding confounds file.

The confound time series derived from head motion estimates and global signals were expanded with the inclusion of temporal derivatives and quadratic terms for each (Satterthwaite et al. 2013). Frames that exceeded a threshold of 0.5 mm FD or 1.5 standardized DVARS were annotated as motion outliers. Additional nuisance timeseries are calculated by means of principal components analysis of the signal found within a thin band (*crown*) of voxels around the edge of the brain, as proposed by (Patriat, Reynolds, and Birn 2017). All resamplings can be performed with a *single interpolation step* by composing all the pertinent transformations (i.e. head-motion transform matrices, susceptibility distortion correction when available, and co-registrations to anatomical and output spaces). Gridded (volumetric) resamplings were performed using *nitransforms*, configured with cubic B-spline interpolation.

Many internal operations of *fMRIPrep* use *Nilearn* 0.10.2 (Abraham et al. 2014, RRID:SCR_001362), mostly within the functional processing workflow. For more details of the pipeline, see <https://fmriprep.readthedocs.io/en/latest/workflows.html>.

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PART V

Discussion and Conclusion

Chapter 5

General Discussion

Accelerated aging-related cognitive decline and dementia are associated with a multifactorial and interrelated set of risk factors, many of which are lifestyle-modifiable. As a result, multidomain lifestyle interventions, targeting several of these risk factors simultaneously, have gained increasing attention as preventive strategies to reduce cognitive decline and dementia risk. However, their effects on cognitive functioning, the primary outcome in many multidomain lifestyle intervention trials in older adults due to its close relation to quality of life and independent living, have been mixed. While some studies reported significant intervention-induced benefits on cognitive functioning, others found no cognitive improvements or only found effects limited to specific at-risk subgroups (**Chapter 1**). This thesis aimed to improve our understanding of the relation between multifaceted lifestyle and cardiovascular risk factors and markers of brain health, as well as the biological mechanisms underlying multidomain lifestyle interventions and their potential beneficial effects on cognitive functioning. To this end, we applied a multimodal outcome measure protocol, acquiring a combination of multiple lifestyle and vascular health outcomes together with a broad set of functional and structural neuroimaging measures. In this conclusory chapter, I first summarize the main findings of the thesis and their implications. These findings are then discussed within a broader context of multimodal research, cohort selection, and the practical feasibility of preventive intervention designs aimed at delaying the onset of cognitive decline and dementia. Methodological considerations related to study design and outcome measures are subsequently discussed, followed by perspectives for future research to further evaluate the potential of preventive interventions targeting cognitive decline. The chapter closes with the overarching conclusions of the thesis.

Summary of main findings and conclusions

Chapter 2 described the design and rationale of the HELI study, a 6-month randomized controlled multidomain lifestyle intervention trial in Dutch older adults at risk of cognitive decline. The HELI study included 102 participants between 60–75 years old with two or more lifestyle-modifiable risk factors of cognitive decline. Most commonly self-reported risk factors were overweight or obesity, hypertension, hypercholesterolemia, and physical inactivity. Women were slightly overrepresented, and participants were relatively highly educated compared to other multidomain lifestyle intervention study cohorts. The HELI study is characterized by its mechanistic focus, integrating multimodal measures of lifestyle, vascular health, MRI-based brain health, and cognitive functioning. This comprehensive acquisition protocol enables the investigation of intervention-induced effects and links across multiple peripheral and brain pathways within a single cohort.

In **Chapter 3**, the methodological strength of our protocol multimodality was explored by examining cross-sectional associations between multiple measures of lifestyle, vascular health, (MRI-based) brain health, and cognitive functioning. Baseline HELI data were combined with data from the COMBI study, a gut-health intervention also part of the MOCIA research program with similar inclusion criteria and acquisition protocols, resulting in a total sample of 176 at-risk Dutch older adults aged 60–75 years old. Associations were found between composite scores of lifestyle and vascular health, and between vascular health and brain health, but brain health was not associated with cognitive functioning. Additionally, no direct links were observed between lifestyle and brain health or between vascular health and cognition. Individual outcome analyses showed that BMI, blood pressure, blood lipids, and cerebral perfusion contributed most to shared variance found between vascular and brain health measures. These findings indicate cross-sectional stepwise links between multiple measures of lifestyle, vascular health and brain health, supporting evidence for the multiplicity of pathways and mechanisms involved in cognitive decline. However, this effect was most strongly reflected in the association between several vascular health markers and cerebral perfusion within the dlPFC and hippocampus.

Chapter 4 investigated the HELI multidomain lifestyle intervention-induced effects on neuroimaging markers of brain structure and function. Primary outcomes included N-back task-related BOLD fMRI responses and cerebral blood flow (CBF) in the dlPFC and hippocampus, as well as N-back task performance. The high-intensity intervention group showed a small but significant reduction in hippocampal CBF at follow-up compared to active controls, with quantified values remaining within normal physiological ranges for the participant cohort demographics, potentially reflecting normal CBF fluctuations. No other between-group intervention-induced changes were observed for the other primary outcomes. Secondary analyses revealed improvements in Dutch healthy diet adherence, stress perception, BMI, blood pressure, and episodic memory performance across both intervention groups, indicating overall effectivity of both multidomain intervention arms on these measures related to lifestyle, vascular health and cognitive functioning. The high-intensity intervention group additionally showed improved MIND-NL diet adherence and sleep quality over time, although significant baseline differences in sleep quality between groups warrant cautious interpretation of this particular finding. Conclusively, our findings indicate that the multidomain lifestyle intervention had positive effects on cognitive performance, irrespective of group allocation, but we found no supporting evidence linking outcomes of lifestyle, vascular health or brain health to these cognitive benefits. The observed cognitive benefits may therefore primarily reflect learning effects over time.

Interpreting multimodal neuroimaging markers of cognitive decline

Cognitive decline and dementia are longitudinal and multifactorial processes, influenced by complex interactions between factors such as lifestyle behavior, cardiovascular health, and the structural and functional integrity of the brain [1, 2]. However, many previous intervention studies investigating lifestyle effects on brain health outcomes have placed focus on only one or two lifestyle domains, targeting isolated risks such as physical inactivity or poor diet [3-7], and are therefore limited in their ability to capture interrelated mechanisms spanning multiple peripheral and central systems (e.g., vascular, metabolic, and neural). To more fully capture these pathways and their interactions, multidomain lifestyle intervention study designs capable of assessing multiple mechanisms within a single framework are required [2, 8].

In **Chapter 3**, we reported findings that support this notion: cross-sectional associations between lifestyle, cardiovascular health, and brain health were stronger when domains were represented by composite scores integrating multiple facets of each domain, compared to associations between individual variables. This suggests that composite, multimodal assessments of distinct peripheral and brain mechanisms may better explain links between these domains, potentially capturing additive effects across mechanisms. Although not all outcomes showed significant associations, we showed that CBF was a key contributor to the associations found between composite scores of brain and cardiovascular health, with BMI and blood pressure showing particularly strong links to CBF. These findings are consistent with the established role of BMI and blood pressure as risk factors for CBF dysfunction, which in turn is implicated as a causal factor for cognitive decline in MCI, AD and vascular dementia [9-12].

However, in **Chapter 4**, we were unable to translate these cross-sectional findings into multimodal longitudinal intervention effects. While significant intervention-related effects were again limited to CBF, we observed an unexpected negative effect on hippocampal CBF in the high-intensity intervention group. This finding contrasts with previous lifestyle intervention studies which have generally reported CBF increases [3, 5, 10, 13], including hippocampus-specific effects [3, 14].

Moreover, we did not observe intervention-induced changes in working memory task-related fMRI BOLD responses during a working memory task, a cognitive ability strongly affected by cognitive decline and dementia [15]. This finding aligns with the

canonical correlation analyses from **Chapter 3**, where fMRI BOLD responses showed negligible shared variance with cardiovascular health markers, indicating no direct cross-sectional association between task-related BOLD responses and cardiovascular risk. Although effects of lifestyle change on task-related BOLD responses in aging remain largely unexplored, previous research using rs-fMRI has shown that lifestyle improvements can enhance resting-state activity and functional connectivity, particularly within and between the frontal and medial lobes [16-18]. These shifts in functional connectivity have been suggested to slow aging-related functional reorganization and improve network integration (i.e., efficiency of information transfer across different brain networks) in older adults by promoting neural plasticity [19], indicating a potential for lifestyle interventions to influence functional brain markers detectable by fMRI. Based on our findings, however, we were unable to identify lifestyle intervention-induced working memory task-related BOLD responses indicative of potential cognitive benefits. Although BOLD signal changes are strongly dependent on CBF [20], one possible explanation for the discrepancy in our BOLD fMRI and ASL findings is that ASL quantifies resting-state perfusion, whereas our fMRI outcome reflects specific task-evoked changes in CBF and blood oxygenation within our ROIs. Therefore, these two different imaging modalities likely captured different aspects of neurovascular coupling and brain function across distinct physiological states.

In addition to ROI-specific CBF and working memory task-based BOLD fMRI responses, we also assessed secondary neuroimaging outcomes, including structural MRI and an MRS-derived proxy marker of neuroinflammation, as part of our multimodal neuroimaging protocol. These modalities capture distinct yet related aspects of brain integrity: structural MRI is able to capture processes such as region-specific cortical thinning and atrophy, while MRS markers such as myo-inositol provide insight into neuroinflammatory mechanisms implicated in neurovascular dysfunction and cognitive decline [21, 22]. Structural deterioration is a well-established marker of cognitive decline and dementia, with aging-related patterns including frontal cortical thinning and hippocampal volume loss [23-26]. However, such structural deterioration is a gradual and longitudinal process [27], and the absence of detectable effects in our study likely reflects the relatively short follow-up time. Additionally, other multidomain lifestyle intervention studies that used brain integrity measures reported mixed findings [28-30], highlighting the limited understanding of lifestyle-modification effects on neuroimaging measures of structural brain integrity. Similarly, although MRS-based indices of neuroinflammation have been strongly associated with memory dysfunction across normal aging [31], MCI [32, 33] and dementia [32], we found no evidence that our

lifestyle intervention influenced these neuroinflammatory processes within our at-risk cohort. However, due to methodological and technical constraints, this neuroinflammation measure was limited to a broad region within the dlPFC, which may not have captured neuroinflammation-related effects on a broader whole-brain scale or in other brain regions relevant to aging-related dysfunction. Despite including a comprehensive multimodal set of neuroimaging outcomes previously associated with aging-related cognitive decline, we were unable to establish links between these markers of brain function and structure and cognitive functioning. Because our markers of functional and structural brain integrity have individually been extensively linked to cognitive decline [1, 23, 24, 34], we had expected to observe these associations, especially in the cross-sectional analyses of **Chapter 3**.

Previous multidomain lifestyle interventions found heterogeneous effects on cognitive functioning [29, 35-38], and even when improvements were observed, effect sizes were small and may have been partly attributable to test-retest learning [39]. Cognitive functioning remains a logical and clinically relevant endpoint due to its direct impact on quality of life and functional independence, and its status as a hallmark symptom of cognitive decline and dementia. However, the heterogeneous intervention effects and generally small effect sizes suggest that the influence of lifestyle interventions on slowing the onset of cognitive decline is not yet fully understood and these effects may only be small at most. This is further supported by the results of this thesis, in which we were unable to isolate clear and direct links between brain mechanisms hypothesized to underlie lifestyle-modification-induced cognitive benefits. Of note however, although the novelty of our multimodal approach did not reveal significant results across many of our hypothesized pathways or their links to cognition, our cross-sectional and intervention-effect analyses consistently highlighted CBF outcomes. These findings demonstrate the sensitivity of CBF measures to multidomain lifestyle interventions compared with other functional and structural brain outcomes. However, future research should investigate the directionality of these effects across different levels of cardiovascular risk and stages of cognitive decline in order to clarify the precise implications of cerebral perfusion-related changes.

At-risk cohort selection for preventive research

Clearly defined inclusion and exclusion criteria are essential for selecting study participant cohorts that are internally consistent, while still allowing study findings to be meaningfully interpreted in the broader population [40]. In cognitive decline

studies, cohorts are often divided into healthy older adults (i.e., those without apparent MCI, dementia, or subjective cognitive complaints) and patient populations (such as MCI or specific dementia diagnoses). Patient cohorts are informative because they typically show more advanced stages of aging-related brain deterioration and cognitive decline, allowing for more comprehensive detection of underlying mechanisms using methods such as MRI or neuropsychological assessments [1, 41, 42]. However, normal and pathological cognitive aging exist along a continuous scale, with many of the same mechanisms of deterioration occurring at different rates [43, 44]. In the context of preventive studies aimed at slowing these processes, healthy older adults may show small but difficult-to-detect changes in the mechanisms underlying cognitive decline due to insufficient progressive deterioration, while patients may have limited capacity for recovery because of the largely irreversible nature of neurodegenerative disease such as AD [45]. This makes it challenging to compare and generalize findings from patient-oriented studies to prodromal or healthy populations and vice-versa, especially considering that the potential benefit of preventive interventions on the delay of onset of cognitive aging are thought to be greatest when implemented in earlier stages of pathological aging [46, 47].

Using at-risk cohorts may offer a strategic middle ground between healthy older adults and patient populations. By including participants with elevated risk for progressive aging-related cognitive and brain deterioration, underlying mechanisms may be more strongly affected than in healthy adults [48]. At the same time, these mechanisms may remain in modifiable states before irreversible neurodegenerative processes push individuals toward the pathological phase of the continuous aging scale (i.e., MCI) [47, 48]. This is particularly important for intervention studies investigating changes over time, as the study population must have sufficient capability for improvement in order to allow detection of measurable intervention-induced effects. Indeed, several multidomain lifestyle intervention studies only observed significant cognitive benefit in specific subgroups with elevated dementia risk, underlining that participants with elevated baseline risks may have greater potential for intervention success [29, 49]. In **Chapter 4** we also conducted exploratory analyses within our at-risk cohort, focusing on subsets of participants with lowest baseline hippocampal CBF (i.e., highest CBF-specific risk) or participants with the largest blood pressure delta scores (i.e., strongest cardiovascular improvement strongly linked to CBF), but found no additional effects, possibly indicating that our inclusion criteria already captured participants with sufficient cardiovascular risk exposure. However, subgroup analyses based on apolipoprotein-E $\epsilon 4$ (APOE4) carrier status, education level, or on stratification factors previously associated with stronger lifestyle intervention effects such as untreated hypertension [49] or lower

baseline cognitive screening scores (e.g., Mini-Mental State Examination (MMSE), or Montreal Cognitive Assessment (MoCA)) [29], have not yet been explored within our specific cohort.

This then raises the question of why we were unable to replicate associations between the cardiovascular- and neuroimaging related outcome measures previously reported in healthy or patient (e.g., AD) cohorts within our at-risk population. One potential explanation is that heterogeneity within our at-risk subgroup was relatively high, as the lifestyle-modifiable cardiovascular risk profile was assessed through cross-sectional reports and did not reflect the duration or severity of risk exposure (e.g., poor diet, hypertension, hypercholesterolemia, diabetes type-2). For example, participants reporting current physical inactivity may have only recently adopted less favorable habits, whereas others may have experienced long-term exposure to adverse behaviors, contributing to substantial within-group heterogeneity despite similar assumed cardiovascular risk levels. Additionally, despite (pre)screening for MCI using the Telephone Interview for Cognitive Status (TICS-M1 [50]), the at-risk profile and high education level of our sample may have limited detection of subtle or early-stage cognitive impairment [51]. In comparison, healthy or patient cohorts are typically more well-defined due to the absence or presence of strict clinical diagnostic criteria according to international standards [52-54]. **Chapter 2** showed minor differences in cardiovascular risk assessment, risk factor distribution, and demographic variables (e.g., education level) when comparing baseline characteristics of the HELI study with other relevant multidomain lifestyle intervention studies targeting similar at-risk populations. Because inclusion criteria for “at-risk” cohorts are inherently dependent on study-specific definitions and outcome measures, discrepancies between studies likely contribute to the variability in reported findings. In contrast, results from well-established healthy or patient cohorts can result in more consistent results due to clearer and more uniform inclusion definitions, although these cohorts also struggle with considerable heterogeneity in aging progression or disease etiology [55-57].

The central role of cardiovascular burden in neurodegeneration supports the rationale for including a cohort with a multifaceted elevated cardiovascular risk, enabling our multidomain lifestyle intervention to target several modifiable risk factors simultaneously, which is in line with current guidelines for dementia prevention initiatives [46, 47]. The lack of expected significant intervention-induced changes, as reported in **Chapter 4**, illustrates the difficulty in isolating brain mechanisms specifically linked to cardiovascular-related cognitive benefits and may indicate the involvement of additional, undetected mechanisms outside our current hypotheses. One possible explanation may lie in the limited follow-up duration of 6 months,

which may have been too short to detect meaningful mechanistic improvements resulting from lifestyle intervention-induced cardiovascular changes. For example, a review by Palta and colleagues (2022) on the effect of blood pressure management on white matter disease progression and cognitive decline risk indicated that relatively long trial follow-ups are required to fully capture blood pressure management-related effects on these outcomes, with significant trial results typically observed only in studies with follow-ups exceeding three years [58]. Importantly, the current cardiovascular focus of this thesis does not account for other established risk factors for cognitive decline, such as inflammatory [59], genetic [60], or environmental risks [61]. While genetic and environmental risks are largely non-modifiable [47], potential immunometabolic-specific effects (such as inflammatory markers and faecal microbiota analyses; described in **Chapter 2**) may have gone undetected because our cohort was primarily selected based on cardiovascular risk factors.

Feasibility and practicality of multidomain lifestyle interventions

Considering the longitudinal etiology of aging-related cognitive decline, in which markers of brain deterioration emerge long before cognitive symptoms become apparent [62-64], early intervention is widely regarded as essential [46, 47]. The continued lack of success in curative treatments for dementia further underlines the need to act before irreversible neurodegenerative damage has occurred [65-67], which is consistent with the 2024 Lancet Standing Commission on Dementia Prevention, Intervention and Care which indicated that around 45% of global dementia cases are potentially preventable by addressing 14 modifiable risk factors throughout the life course [47]. However, the feasibility of such interventions warrant careful consideration. Feasibility can be viewed from multiple distinct, but complementary perspectives [68]. In the context of this thesis, I will particularly discuss two aspects: the scalability of implementing multidomain lifestyle interventions in the broader population (implementation), and the ability of individuals to adhere to relatively intensive lifestyle programs over extended periods of time (adherence) [68]. Both aspects have to be considered for these interventions to become practically implementable as preventive strategies against cognitive decline.

Multidomain lifestyle interventions fundamentally require considerable effort, not only from participants who must invest substantial time, motivation, and behavioral commitment, but also from local healthcare systems that have to provide the necessary supervision and support structure to facilitate such programs [69, 70].

Although multidomain lifestyle intervention trials are advancing our understanding of the modifiability of cognitive decline risk at earlier stages of aging, their intensive and resource-demanding designs raise questions regarding national and global scalability [69, 70]. In this context, the feasibility of implementing these complex intervention programs is likely greater in high-income countries or settings with well-developed healthcare and support infrastructures, whereas low- and middle-income regions may face substantial challenges despite experiencing a similar burden of dementia prevalence [46, 71, 72]. Moreover, while such interventions can be successfully implemented within controlled study populations of limited size, translating these programs into real-world public health contexts with larger target populations remains a major challenge. Current initiatives, such as the World-Wide FINGERS (WW-FINGERS) network [73], are actively assessing the adaptability, implementability, and effectivity of multidomain lifestyle intervention designs across different geographical, cultural and economic settings. However, this area of research is still relatively novel, and these initiatives are currently mostly conducted in medium- to high-income countries and settings, emphasizing the difficulty of implementing complex intervention designs in regions with less developed healthcare infrastructures or limited economic resources.

Another key component of feasibility is the capacity of participants to maintain adherence to the intervention program over longer periods of time [68]. Current multidomain lifestyle intervention studies are designed with adherence in mind, typically providing structured, domain-specific content in a stepwise manner to avoid overwhelming participants and to promote sustainable behavioral change [74]. These programs aim to encourage long-lasting improvements in daily lifestyle patterns by teaching participants about practical tools to support autonomous, health-conscious decision-making. Given the longitudinal etiology of cognitive decline, and the rationale for targeting modifiable risk factors early on in the aging process, maintaining these intervention-induced risk reductions likely requires long-term adherence to the adopted healthier lifestyle patterns [75]. However, because this field of research is still relatively novel and longitudinal follow-up results beyond trial participation are scarce, it is unclear to what extent participants continue to adhere to lifestyle modifications once formal supervision and study participation have concluded, and what is needed to support sufficient post-study adherence. In order to capture the long-term effects of lifestyle intervention-induced dementia risk reduction, more extensive follow-up periods and continued adherence monitoring would be required beyond the active intervention phase [47]. Moreover, another important adherence-related challenge relates to cohort selection bias. Individuals who voluntarily participate in lifestyle intervention trials may be more health-

conscious, motivated, and willing to modify their behavior compared to the general population, even in active control intervention groups. They may also be more highly educated, reflected in greater awareness of such studies being conducted, or being more economically able to participate, resulting in a study population that is less representative of the general population [76, 77]. This particular selection bias is evident in the HELI study population, which is highly educated relative to the Dutch national average [78]. To translate intervention-induced dementia risk reduction to a broader context of the general population, future interventions should therefore aim to include individuals across diverse demographic and socioeconomic groups. In this context, low-intensity interventions may also play a role, as they could provide more accessible and appealing programs to less motivated or resource-limited individuals. In support of this, as described in **Chapter 4**, even the active control intervention group achieved modest but significant improvements in diet quality, perceived stress, and blood pressure, which may indicate that such approaches can still provide meaningful risk reduction. Lower-intensity designs could also potentially reduce study drop-out rate, which is generally higher in more intensive multidomain lifestyle intervention programs [29, 35-37, 49, 79]. However, in our study, multiple participants that dropped out from the active control group reported motivational reasons related to insufficient guidance as drop-out reasons. In contrast, drop-out cases in the intervention group only cited personal circumstances and time constraints as reasons, suggesting that while the active control group may have required lower practical burden to adhere to the program, interpersonal communication and guidance was an important component to sustain participant motivation and adherence. Multidomain lifestyle interventions aimed at delaying the longitudinal process of cognitive decline are fundamentally characterized by the facilitation of prolonged modifications in daily lifestyle patterns as opposed to short-term compliance. Effectively achieving and adhering to these habitual changes therefore requires sustained effort and ongoing engagement, which may rely on continued supervisory support (e.g., defining individual goals, providing personalized feedback). Alternatively, digitally delivered or web-based interventions, which are also being explored within the MOCIA consortium, may offer scalable and cost-effective means of delivering lifestyle interventions for broader audiences, designed specifically to support long-term program engagement and adherence to achieve behavioral change. Together, these approaches could further support the development of multidomain lifestyle programs that are innovative, scalable, and practically feasible for a broad population that may ultimately provide beneficial effects on long-term dementia risk reduction.

Methodological considerations

The rationale underlying methodological study design directly influences the interpretation of intervention effects and is therefore a crucial consideration in mechanistic studies. Careful evaluation of methodological choices, along with their strengths and shortcomings, is therefore warranted.

The multimodality of the neuroimaging protocol is a core theme and strength of this thesis. As described in **Chapter 2**, the selection of multimodal neuroimaging outcome measures was based on current evidence regarding the modifiability of specific dementia risk-related factors and their associations with cerebrovascular mechanisms. These mechanisms have been prominently highlighted as key drivers behind the trajectory of aging-related cognitive decline and of subsequent cognitive symptoms. However, other markers have also shown promise as potential intervention targets, among which additional markers of cerebral small vessel disease (SVD) were of particular interest, such as white matter hyperintensities, lacunar infarctions, or cerebral microbleeds. SVD holds strong clinical relevance as a major contributor to vascular dementia [80], a relationship that has been further supported by recent longitudinal studies [81, 82]. Moreover, risk factors for SVD closely align with the lifestyle-modifiable dementia risk factors targeted by current multidomain lifestyle interventions [46, 47, 83]. Importantly, neuroimaging markers of SVD are detectable long before the onset of clinical symptoms of these neurodegenerative disorders [82], drawing a parallel with the preclinical processes observed in cognitive decline and dementia in cohorts without diagnosed SVD. We therefore considered the inclusion of white matter structural integrity neuroimaging markers derived from diffusion tensor imaging (DTI), such as fractional anisotropy (FA) and mean diffusivity (MD) images to assess microstructural damage to white matter tracts [84, 85], and fluid-attenuated-inverse-recovery (FLAIR) imaging to assess volumetric quantification of white matter hyperintensities (WMH) [86, 87]. However, we expected that functional markers, such as task-related fMRI and ASL perfusion imaging, would be more sensitive to lifestyle effects over a six-month intervention period, and would therefore be more easily detectable mechanistic changes compared to structural integrity markers of SVD. For example, previous exercise-based lifestyle interventions with a weekly intervention burden comparable to HELI have reported increased CBF in the frontal lobe after two months in a sedentary older male cohort [3], and in the hippocampus after one year in an MCI cohort [13]. Additionally, an intervention study combining exercise and cognitive training found increased resting-state functional connectivity between the medial PFC and medial temporal lobe after six weeks [18]. Furthermore, during the HELI design phase, ethical considerations were taken into

account as the final neuroimaging protocol already required up to 90 minutes of preparation and scanning per participant, and adding further MRI sequences would have substantially increased participant burden.

Another important consideration in outcome measure selection relates to our choice of subjective, self-reported lifestyle behavior assessments, as opposed to objective measures. In current literature, lifestyle domains such as diet or sleep quality are predominantly assessed using self-reported tools, including validated questionnaires or logbooks. These approaches are cost-effective and applicable for longitudinal follow-up studies, but are prone to bias, for example caused by socially desirable responding or selective recall effects [88, 89]. In the HELI study, we used validated questionnaires to assess change in adherence to lifestyle patterns targeted by the intervention. Notably, because we used within-individual repeated-measurements of these questionnaires, systematic bias will be applied across all measurements which may reduce a portion of introduced response bias [88, 89]. However, participants may have been more inclined to report positive lifestyle changes at follow-up, which could have introduced additional systematic bias that may have differed between the two intervention groups. Objective measures, such as wearables or other at-home objective monitoring tools, are currently being developed and show promise for capturing more comprehensive data of lifestyle behavior, particularly for physical activity and sleep quality [90, 91]. Extending such examples of objective monitoring over longitudinal periods remains challenging because of high cost and technological limitations. Additionally, specific lifestyle domains such as diet remain difficult to objectively capture [92]. As a result, longitudinal studies will continue to rely primarily on self-reported tools to assess lifestyle compliance, which complicates interpretation of comparisons with more objective outcomes, such as cardiovascular risk markers (e.g., blood pressure, blood lipids) or neuroimaging measures. The development and integration of more validated, objective lifestyle measures would be valuable to increase the capability to capture and interpret the associations between intervention-induced lifestyle modifications, cardiovascular risk reduction, and their potential beneficial effects on measures of cognitive performance.

Lastly, stratification of cohort selection based on lifestyle-related, modifiable risk factors for cognitive decline-related lifestyle modifiable risk factors must be considered [47]. As discussed in this chapter and in **Chapter 1** and **Chapter 4**, several methodological challenges arise from variability in risk factor inclusion criteria. For instance, as discussed earlier, different HELI participants may have received identical inclusion risk factor profile scores for, e.g., presence of hypertension or physical inactivity, despite distinct differences in exposure histories. Additionally, the

interpretation of multidomain intervention effects is complicated by heterogeneity of the multifactorial risk factor profiles within the included cohort. A lack of intervention-induced change in blood pressure or physical activity levels at follow-up may have fundamentally different implications for an individual with long-term established hypertension or sedentary behavioral patterns, compared to someone already within healthy, age-appropriate ranges. More selective inclusion criteria could reduce this heterogeneity and improve interpretation of intervention-induced effects, but this would also directly limit the feasibility of multidomain lifestyle intervention studies as these studies are specifically designed to target multiple, likely interrelated mechanisms simultaneously. Therefore, cohort selection has to be carefully considered in future multidomain lifestyle intervention research.

Directions for future research

Results from this thesis reinforce findings from previous single-modality studies, by showing that the effects of lifestyle on brain function are largely explained by variance in cardiovascular risk. However, in our multimodal analyses of intervention effects, we were unable to find evidence that our hypothesized pathways of functional brain integrity, particularly in the dlPFC and hippocampus, could explain the cognitive benefits of multidomain lifestyle interventions in older adults. Furthermore, we were unable to find associations between neuroimaging-based brain health markers and cognitive functioning. In this section, I provide potential directions for future directions, focusing on neuroimaging multimodality, cohort considerations, intervention adherence and effect on dementia risk in the long-term, and conclude with remarks on the practical aspects of intervention implementation intended for broader target populations. Together, future research may integrate these factors to provide novel insights into how lifestyle modification exactly impacts mechanisms of brain health, identify more clearly defined intervention targets, and provide tools to enlarge outreach and dissemination.

MRI is capable to capture structural and functional brain mechanisms underlying aging-related cognitive decline before cognitive symptoms appear, underlining the potential of neuroimaging as a sensitive method to follow brain aging trajectories in vivo. However, MRI is time- and resource intensive, and as discussed earlier, careful consideration of MRI-sequence selection is essential for the interpretation of mechanistic intervention study results. Although we did not observe clear intervention-induced effects on outcomes of functional or structural brain integrity that could be translated to cognitive functioning, our findings in **Chapter 3** and

Chapter 4 indicate a particular sensitivity of CBF-specific outcomes within the brain. Future studies could extend CBF analyses to broader regions of the brain implicated in cognitive decline, AD and vascular dementia, such as total frontal-, parietal- and temporal lobe CBF [93, 94]. Arterial transit time (ATT), a measure derived from ASL analysis, or cerebral oxygen extraction fraction (OEF) and metabolic rate of oxygen (CMRO₂), maps acquirable by QSM and ASL, could also serve as alternative hemodynamic measures to assess local perfusion abnormalities throughout the brain, or within specific regions of interest [95, 96]. Beyond CBF, task-related BOLD fMRI could examine cognitive domains affected in cognitive decline and dementia beyond working memory, such as episodic memory, attention or response inhibition [97]. Additionally, rs-fMRI could be used to explore effects on functional connectivity of specific brain networks such as the default mode network (DMN), which exhibits disrupted patterns in MCI and dementia [98, 99] and has been suggested as an effective early detectable marker of pathological aging [100].

A second direction for future research is the definition and selection of at-risk study populations. Considerable heterogeneity exists within risk factor profiles of study populations within the field of cognitive decline and dementia research. Although defining “at-risk” populations is challenging as they occupy a grey area between healthy aging and pathological aging, future studies would profit from more standardized and widely applied definitions of these populations across cognitive aging research. Reducing the variability in cohort demographics would allow for more meaningful comparisons of results across studies, and strengthen the interpretability of findings.

Another important consideration in interpreting the implications of cognitive benefits following multidomain lifestyle intervention studies, is that the long-term impact on actual dementia risk remains unknown. Similarly, it remains unclear to what extent participants are capable or willing to maintain adherence to lifestyle modifications made during the intervention over more extended periods of time. These knowledge gaps should be addressed in the future to assess feasibility of multidomain lifestyle interventions as a preventive approach to combat cognitive decline. This could be achieved by performing extended, longitudinal follow-up measurements to evaluate the persistence of intervention-related cognitive improvement, adherence to lifestyle modifications, and effects on dementia risk reduction. Although such follow-up studies require substantial resources, they are essential for evaluating the potential of multidomain lifestyle interventions to meaningfully delay cognitive decline and dementia onset.

Finally, current multidomain lifestyle intervention designs aim to simultaneously target several lifestyle factors associated with modifiable cardiovascular risk for

cognitive decline and dementia. While such comprehensive programs are ambitious, their intensity often results in relatively high dropout rates in longer intervention study designs. The feasibility and applicability of multidomain lifestyle interventions in a broader population context, may rely on the feasibility of adhering to programs for extended periods of time. It may therefore be more feasible to have programs focus on two or three core lifestyle domains most strongly associated with cardiovascular risk reduction or to individualize interventions based on the lifestyle domains in which participants have the greatest interest or potential for improvement. These approaches would help maintain an intensity at a level manageable for general target audiences and could also improve adherence, as the intervention program would be more personally tailored to each individuals' interests and potential for improvement. Future studies should investigate which domains show the greatest effect in decreasing cardiovascular risk and thereby promoting cognitive benefit, in order to tailor intervention designs that are both effective and scalable to large populations. Lower-intensity intervention designs may also facilitate scalability because of broader implementation possibilities, as they would require less supervision and healthcare system resources.

Conclusion

Aging-related cognitive decline and dementia are complex and multifactorial processes that pose major societal challenges. Multidomain lifestyle interventions show promise as preventive measures, aimed at slowing the onset of cognitive decline by addressing a multitude of lifestyle-modifiable cardiovascular risk factors.

This thesis applied a novel, multimodal neuroimaging approach that reinforced the role of cardiovascular factors as key targets for multidomain lifestyle interventions in combatting cognitive decline. At the same time, our findings underline the challenges in isolating markers of structural and functional brain integrity that are directly associated with cognitive benefits. Although this thesis focused primarily on mechanisms related to cardiovascular risk and neuroimaging measures of brain function, vascular factors alone are unlikely to be the only pathway linking peripheral health to the functional and structural integrity of the brain. Future research, including ongoing work within the HELI study and the broader MOCIA consortium, will be able to further explore the multifactorial modifiability of brain aging and their associated mechanisms within the periphery and brain.

Improving our understanding of the peripheral and brain mechanisms underlying multidomain lifestyle intervention-induced cognitive improvement must go hand in hand with consideration for real-world applicability. Striking this balance will be essential to achieving meaningful preventive impact on the rise of cognitive decline and dementia around the world.

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PART VI

Dutch Summary

Chapter 6

Nederlandse Samenvatting

Voor veel mensen vormen cognitieve achteruitgang en dementie beangstigende vooruitzichten van ouder worden. Over het algemeen leggen we ons erbij neer dat ons lichaam gedurende het leven tekenen van slijtage zal vertonen. We accepteren bijvoorbeeld dat onze gewrichten verstijven, onze spieren verzwakken, en dat we onszelf op latere leeftijd mogelijk met behulp van wandelstokken of rollators moeten ondersteunen om langere afstanden af te leggen. Dergelijke ouderdomsverwante uitdagingen zijn zaken die zichtbaar en meetbaar zijn, en waarop concreet actie kan worden ondernomen. Dit staat in contrast met de veroudering van de hersenen. Ons brein vormt het fundament van ons bewustzijn en onze persoonlijke identiteit, en herbergt de talloze herinneringen en ervaringen die bepalen hoe we de wereld om ons heen ervaren en begrijpen. Het stelt ons in staat om te leren, te lachen en lief te hebben, en is daarmee nauw verbonden met ons gevoel van persoonlijke identiteit. Waar een gewrichtsprothese of rollator onze bewegingsvrijheid kan ondersteunen of herstellen, bestaat er geen vervanging voor de zeer complexe neurale netwerken van de hersenen. Cognitieve achteruitgang bedreigt hierdoor niet alleen zelfstandigheid van leven, maar ook het gevoel van cognitieve autonomie en individualiteit.

Huidige verschuivingen in de demografie leiden tot een zogenaamde dubbele vergrijzing: niet alleen neemt het totale aantal ouderen toe, maar de gemiddelde leeftijd binnen deze groep stijgt gelijktijdig mee. Deze demografische ontwikkeling resulteert in een hogere incidentie van ouderdomsgerelateerde cognitieve achteruitgang en dementie, dat in de komende decennia zal leiden tot aanzienlijke maatschappelijke en economische druk. Niet alleen patiënten en (mantel)zorgers zullen deze druk moeten opvangen, maar ook de gezondheidszorg zal steeds meer middelen moeten inzetten voor het bieden van langdurige zorg. Om de stijging van dementiegevallen af te remmen en cognitief functioneren bij het ouder worden te ondersteunen, is het van cruciaal belang om beter inzicht te krijgen in de mechanismen die ten grondslag liggen aan cognitieve achteruitgang en dementie, en te begrijpen hoe deze processen mogelijk kunnen worden afgeremd of voorkomen.

Cognitieve achteruitgang en dementie worden gekenmerkt door multifactoriële, langdurige, en grotendeels onomkeerbare achteruitgang van de structuur en functie van de hersenen. Deze processen worden beïnvloed door een combinatie van risicofactoren, waaronder cardiovasculaire, metabole, genetische, en omgevingsfactoren. Met name cardiovasculaire en metabole risicofactoren, zoals hoge bloeddruk, fysieke inactiviteit, hoog cholesterolgehalte en diabetes, zijn van bijzonder belang omdat deze aantoonbaar beïnvloedbaar zijn door gezonde(re) leefstijlpatronen. Zogenaamde multidomein leefstijlinterventie studies, waarbij positieve veranderingen in meerdere leefstijldomeinen zoals voeding, beweging, stress en

slaap worden toegepast, worden om deze reden als een sterk potentiële kandidaat gezien door internationale gezondheidsorganisaties om de toekomstige stijging van dementiegevallen te beperken. Een aantal multidomein leefstijlinterventie studies hebben gunstige effecten op cognitief functioneren geconstateerd, maar het is nog onduidelijk via welke perifere (lichamelijke) en hersenmechanismen deze verbeteringen verklaard kunnen worden. Het is belangrijk om een beter begrip te krijgen van deze betrokken mechanismen, omdat de gevonden interventie-effecten in eerdere studies over het algemeen relatief klein zijn en er grote individuele verschillen bestaan in hoeveel mensen profiteren van dergelijke interventies. Inzicht in de betrokken mechanismen kan helpen om deze individuele verschillen beter te begrijpen, en het daadwerkelijke mechanistische effect onderliggend aan cognitieve verbetering te verduidelijken.

In dit proefschrift wordt de multifactoriële aard van cognitieve achteruitgang en dementie nader onderzocht aan de hand van een uitgebreid multimodaal onderzoeksprotocol, waarin uitkomstmaten van leefstijl, cardiovasculaire gezondheid, hersengezondheid en cognitief functioneren gelijktijdig worden geanalyseerd binnen één onderzoekscohort van ouderen met een verhoogd cardiovasculair risico op cognitieve achteruitgang en dementie. Een fundamenteel onderdeel van deze multimodale onderzoeksaanpak is het uitgebreide MRI-protocol, bestaande uit verschillende sequenties om zowel de functionele als structurele integriteit van de hersenen te onderzoeken. Deze multimodale MRI-uitkomstmaten worden in dit proefschrift toegepast om de betrokken hersenmechanismen te verkennen die de positieve effecten van een multidomein leefstijlinterventie op cognitief functioneren kunnen verklaren. Hierbij ligt de nadruk op cardiovasculair risico, omdat huidige kennis met betrekking tot de veronderstelde betrokken hersenmechanismen er het sterkst op wijzen dat deze een cardiovasculaire oorsprong hebben.

Hoofdstukoverzicht en bevindingen

In Hoofdstuk 2 werd de HELI-studie (*“Hersenvuncties na LeefstijlInterventie”*) geïntroduceerd, door middel van een uitgebreide beschrijving van de achtergrond, onderzoeksopzet, methodologie, en selectie van uitkomstmaten. De HELI-studie is een enkelblind, gerandomiseerd onderzoek met controlegroep, waarin een zes maanden durende multidomein leefstijlinterventie werd uitgevoerd onder Nederlandse ouderen met een verhoogd risico op ouderdomsgerelateerde cognitive achteruitgang. De HELI-studie onderscheidt zich door de multimodaliteit van de verzamelde onderzoeksgegevens, waarbij verschillende soorten metingen van

leefstijl, cardiovasculaire gezondheid, (op MRI-gebaseerde) hersengezondheid, en cognitief functioneren voor het eerst in één protocol zijn geïntegreerd. Binnen de multimodale MRI-metingen werden zowel functionele als structurele MRI-scans toegepast. Sequenties die functionele integriteit meten waren onder andere taakgerelateerd ‘blood-oxygen-dependent-level’ (BOLD) functionele MRI (fMRI) tijdens een N-back werkgeheugentaak, en cerebrale perfusiemetingen. Beide sequenties hebben zich hierbij specifiek gericht op hersengebieden die sterk betrokken zijn bij ouderdomsgerelateerde cognitieve achteruitgang en dementie, te weten de dorsolaterale prefrontale cortex (dlPFC) en hippocampus. Deze uitgebreide onderzoeksopzet en verscheidenheid van uitkomstmaten maakt het mogelijk om interventie-effecten, en mogelijke onderlinge verbanden, tussen de veronderstelde perifere en hersenmechanismen gelijktijdig te onderzoeken. De HELI-studie includeerde in totaal 102 deelnemers tussen 60 en 75 jaar oud, waarvan iedereen minstens twee leefstijl-gerelateerde risicofactoren voor cognitieve achteruitgang had, waaronder overgewicht of obesitas, fysieke inactiviteit, hoge bloeddruk, cholesterolverhoging, en diabetes type 2. De studiepopulatie bestond voor een iets groter deel uit vrouwen dan mannen, en was gemiddeld hoger opgeleid dan het Nederlandse gemiddelde en vergelijkbare cohorten uit eerdere multidomein leefstijlinterventie studies. De demografische gegevens bij aanvang van de HELI-studie waren vergelijkbaar met die van de populatie van de FINGER-studie (*“Finish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability”*), de eerste multidomein leefstijlinterventie studie waarbij verbetering op cognitief functioneren werd aangetoond. De FINGER-NL (Nederlands replicatieonderzoek van FINGER) en HELI studies bouwen voort op deze opzet, maar zijn uitgebreid met extra leefstijldomeinen zoals slaap, en stressmanagement en mindfulness, en hanteren een apart dieetprogramma gebaseerd op de richtlijnen Gezonde Voeding van de Gezondheidsraad en een Nederlands-aangepast mediterraan dieet (MIND-NL; een dieet dat risico op cognitieve achteruitgang verlaagt). Een heldere beschrijving van de onderzoeksopzet en baseline demografische gegevens draagt bij aan de vergelijkbaarheid van methodieken, cohorten en resultaten tussen studies, en verbetert daarmee de interpretatie van resultaten binnen het onderzoeksveld.

Hoofdstuk 3 onderzocht de multifactoriële aard van cognitieve achteruitgang door middel van associatieanalyse tussen uitkomstmaten binnen de domeinen leefstijl, cardiovasculaire gezondheid, (op MRI-gebaseerde) hersengezondheid en cognitief functioneren. Waar eerdere studies voornamelijk enkelvoudige verbanden tussen deze afzonderlijke domeinen hebben onderzocht en beschreven binnen de verouderingsliteratuur, werden in dit hoofdstuk al deze domeinen geïntegreerd in één omvattende dataset en gezamenlijke statistische analyses. Deze aanpak stelde

ons in staat om de mogelijk betrokken mechanismen te verkennen waarlangs leefstijl via cardiovasculaire gezondheid invloed uitoefent op de functionele en structurele integriteit van de hersenen, en daarmee op cognitief functioneren. Voor deze analyses werden baselinegegevens van de HELI-studie samengevoegd met data van de COMBI-studie, een interventieonderzoek binnen het MOCIA-consortium met vergelijkbare inclusiecriteria en uitkomstmaten. De uiteindelijke dataset bestond uit 176 Nederlandse ouderen tussen de 60 en 75 jaar oud met twee of meer leefstijl-gerelateerde risicofactoren voor cognitieve achteruitgang. We toonden aan dat er significante verbanden bestonden tussen samengestelde domeinscores van leefstijl en cardiovasculaire gezondheid, en tussen cardiovasculaire gezondheid en hersengezondheid. Er werden echter geen directe associaties gevonden tussen de samengestelde domeinscores van hersengezondheid en cognitief functioneren, en er was ook geen bewijs dat leefstijlfactoren een direct effect hadden op hersengezondheid zonder tussenkomst van cardiovasculaire factoren. Op het niveau van individuele uitkomstmaten bleek dat met name BMI, bloeddruk en bepaalde bloedlipiden het sterkst samenhangen met de doorbloeding van de hersenen. Deze bevindingen wijzen op een stapsgewijs verband tussen leefstijl, cardiovasculaire gezondheid en hersengezondheid, in lijn met resultaten van eerdere, enkelvoudige studies. De samenhang tussen deze domeinen bleek sterker binnen samengestelde domeinscores dan de individuele uitkomstmaten, wat duidt op een mogelijk additief effect van verschillende leefstijlfactoren. Alhoewel er meerdere MRI-uitkomstmaten betrokken werden die de functionele en structurele integriteit van de hersenen weerspiegelen, bleek met name de doorbloeding van de dlPFC en hippocampus duidelijk geassocieerd met een selectief aantal cardiovasculaire gezondheidsfactoren.

Waar in Hoofdstuk 3 correlationeel onderzoek werd belicht, waarbij uitkomstmaten op één tijdstip werden gemeten en daardoor geen causale verbanden kunnen worden vastgesteld, onderzocht Hoofdstuk 4 de longitudinale effecten van de HELI multidomein leefstijlinterventie op meerdere MRI-uitkomstmaten van de functie en structurele integriteit van de hersenen. De primaire focus lag hierbij op de uitkomstmaten van functionele hersenintegriteit binnen de dlPFC en hippocampus: BOLD fMRI respons tijdens een N-back werkgeheugen taak en cerebrale perfusie. Ook werd de prestatie op deze N-back taak geanalyseerd. Als onderdeel van secundaire analyses werden veranderingen in leefstijl, cardiovasculaire gezondheid en cognitief functioneren bekeken ter controle van interventie-effectiviteit en gerelateerd aan de MRI-uitkomstmaten. Binnen de primaire analyses liet de hoog-intensieve interventiegroep een verrassende, kleine maar significante afname in hippocampale perfusie zien bij follow-up ten opzichte van de actieve controlegroep. De gekwantificeerde perfusiewaarden bleven echter binnen fysiologische normaalgrenzen

voor deze populatie, wat mogelijk normale variaties in cerebrale perfusie weerspiegelt. Er werden geen significante verschillen tussen de interventiegroepen gevonden voor de N-back taak-gerelateerde BOLD fMRI respons of op taak-specifieke prestatie. Secundaire analyses toonden aan dat de interventie- en actieve controlegroep evenveel verbeterden in naleving van voedingspatronen volgens de Richtlijnen Gezonde Voeding van de Gezondheidsraad, mate van stresservaring, BMI, bloeddruk, en episodisch geheugen. De hoog-intensieve interventiegroep liet vergeleken met de actieve controlegroep daarnaast meer verbeteringen zien in naleving van het MIND-NL dieet en in slaapkwaliteit, hoewel significante baselineverschillen in slaapkwaliteit tussen de interventiegroepen om een voorzichtige interpretatie van deze bevinding vragen. De bevindingen van dit onderzoek suggereren dat de gevonden cognitieve verbetering van beide groepen grotendeels toe te schrijven valt aan leereffecten op de neuropsychologische testen, en dat er geen bewijs is voor interventie-gerelateerde verbetering in uitkomstmaten van hersengezondheid met een duidelijke klinische relevantie. Mogelijk heeft ons multimodale MRI-protocol de daadwerkelijk onderliggende hersenmechanismen niet volledig kunnen vastleggen, heeft de heterogeniteit binnen de HELI onderzoekspopulatie de interpretatie van de resultaten bemoeilijkt, of zijn de gevonden verbeteringen in cognitief functioneren in eerdere effectiviteitsstudies grotendeels verklaarbaar door leereffecten, zonder dat er sprake is van duidelijk meetbare mechanistische veranderingen in de hersenen. Een andere mogelijke uitleg is dat de actieve controlegroep op zelfstandige wijze vergelijkbare positieve leefstijlveranderingen heeft doorgemaakt vergeleken met de interventiegroep, waardoor de verschillen tussen beide groepen te klein zijn geworden om significante interventie-gerelateerde effecten te meten. Resultaten uit hoofdstuk 4 tonen aan dat beide groepen verbeteringen laten zien in zelf-gerapporteerde maten van gezonde leefstijlpatronen, bloeddruk, en cognitief functioneren. Het is daarom niet uitgesloten dat er mogelijk sterkere interventie-gerelateerde effecten gemeten hadden kunnen worden in een onderzoeksopzet waarbij een controlegroep betrokken wordt waarbij geen veranderingen in leefstijl plaatsvinden tussen baseline en follow-up.

Conclusie

Ouderdomsgerelateerde cognitieve achteruitgang en dementie zijn complexe, multifactoriële processen met aanzienlijke persoonlijke en maatschappelijke impact. Multidomein leefstijlinterventies hebben de potentie om als preventieve methode toegepast te worden om het ontstaan en het verloop van cognitieve achteruitgang te vertragen door verschillende leefstijl-modificeerbare cardiovasculaire risicofactoren te beïnvloeden.

Het uitgebreide multimodale onderzoeksprotocol van dit proefschrift maakte het mogelijk om de stapsgewijze associatie tussen leefstijl en hersengezondheid via cardiovasculaire gezondheid aan te tonen in één geïntegreerd, omvattend model. Tegelijkertijd onderstrepen de bevindingen uit dit proefschrift de uitdagingen bij het isoleren van uitkomstmaten van hersenstructuur- en functie die samenhangen met cognitieve verbetering door multidomein leefstijlinterventies.

Hoewel dit proefschrift zich voornamelijk gericht heeft op mechanismen die gerelateerd zijn aan cardiovasculair risico en functionele neuroimaging uitkomstmaten, zijn vasculaire factoren waarschijnlijk niet de enige route die de relatie tussen perifere gezondheid en de integriteit van de hersenen verklaart. Toekomstig onderzoek, waaronder lopend werk binnen de HELI-studie en het bredere MOCIA-consortium, zal verder kunnen verkennen in hoeverre modificeerbaarheid van het verouderende brein mogelijk is, en welke onderliggende mechanismen in de periferie en de hersenen hierbij betrokken zijn. Het vinden van een evenwicht tussen praktische toepasbaarheid van multidomein leefstijlinterventies en het verbeteren van ons begrip van de betrokken hersenmechanismen is van essentieel belang om te komen tot een betekenisvolle preventieve impact op de wereldwijde toename van ouderdomsgerelateerde cognitieve achteruitgang en dementie.



APPENDICES

Research Data Management
Curriculum Vitae
Portfolio
Acknowledgements
Donders Graduate School (DGS)

Research Data Management

The work described in **Chapters 2, 3 and 4** are based on two human intervention studies conducted at the Donders Centre for Cognitive Neuroimaging. Both studies have been provided with formal, written approval by the medical ethics research committee (MERC) Oost-Nederland, and were carried out in accordance with the principles of the Declaration of Helsinki.

Handling of personal data complied with EU privacy law, GDPR and the Dutch Act on Implementation of the General Data Protection Regulation. (in Dutch: Uitvoeringswet AVG, UAVG).

The Donders Institute has established a data management infrastructure (<https://data.donders.ru.nl>) to which all relevant data will be stored online in a central storage space during data collection. Restricted access to study data on the central storage system is implemented by means of specific Unix user groups. Participant metadata was stored in password-protected databases. At study completion, a second copy of the data is stored on the Donders Institute Research data repository (RDM), located on the Radboud university campus. Raw data will be archived according to institutional standards. For data processing, raw data will be uploaded to network attached storage that, in compliance with institutional guidelines, only researchers directly involved in the processing of data from the specific project will be able to access.

At all times, subject's anonymity was and will be maintained. In all documents, subjects were identified by an identification code only (i.e., not by their names or any other feature by which subjects can be identified). A separate Subject Identification Code List (Key-file) was kept, which matches identifying codes with the subjects' names. Key-files were maintained in a strict confidential way and are preserved for a certain period of time meeting with demonstrable legitimate interest (according to GDPR). Subject anonymity will be maintained throughout all archiving steps. The identification code is shared with the WUR, so the data gathered at the Donders Institute and at Wageningen University is placed under the same identification code of an individual.

The data will be stored and archived through the data management infrastructure of the institute, which will be kept for at least 10 years after last publication at the institute (following guidelines for minimal risk research Kwaliteitsborging mensgebonden onderzoek 2.0 + institutional DR policy).

After completion of the study, data are backed-up on password protected or encrypted hard drives. Personal identifiers are stored separately from other metadata in a document safeguarded by the principal investigator. Other essential documents are archived in a study Master file. Following publication, raw and processed data will be archived for scientific integrity.

The data use agreement gives access to the data, provided that certain conditions set by the local institutional guidelines as well as (inter)national (EU) law are met.

Data sharing will be done using the Donders Institute research data repository (<http://data.donders.ru.nl>) in accordance to institutional and EU guidelines.

Digitized data archive (ICT) is in place for this project to store collected data in a firewall-protected network-attached storage at the institute. Access to data is managed by the researcher(s) responsible for the data and he/she/they decide(s) to give access rights to other individuals when necessary or requested. Data can be used for other research questions outside this particular study, however this is exclusive to research regarding cognitive aging.

Data will be published in such a way that data cannot be linked to related to personal identification. The handling of personal data will comply with the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation (in Dutch: Uitvoeringswet AVG, UAVG).

Curriculum Vitae

Mark van Loenen was born on the 17th of June, 1997, in Apeldoorn. After completing his secondary education in 2014, he pursued a teaching degree in Biology at Windesheim University of Applied Sciences in Zwolle. Alongside this program, he completed a pre-master's in (Medical) Biology at Radboud University Nijmegen, graduating in 2017, and subsequently enrolled in the MSc Medical Biology at Radboud University, with the specialization track Human Biology.



As part of his master's program, he completed a research internship examining the effects of food-borne psychotropic bacteria on behaviour, cognition, and mitochondrial function in a mouse model, under the supervision of dr. Anouk Tengeler and prof. dr. Amanda Kiliaan. This experience introduced him to brain research and, in particular, neuroimaging — an area that captured his interest and shaped the direction of his subsequent work. To deepen his expertise in neuroimaging within the context of Human Biology, he secured an internship position at the Department of Neurology of the Radboudumc, where he investigated predictors and the cognitive impact of incident lacunar infarctions in cerebral small vessel disease, under the supervision of dr. Mina Jacob, dr. Mengfei Cai, and prof. dr. Frank-Erik de Leeuw.

In January 2021, he began his PhD at the Donders Centre for Cognitive Neuroimaging, focusing on the brain mechanisms underlying lifestyle-induced change in a population at risk of age-related cognitive decline, under the supervision of prof. dr. Esther Aarts and prof. dr. Joukje Oosterman. Together with Lianne Remie, he was involved with conducting two human intervention studies (HELI and COMBI): from finalising the design and methods, through recruitment, data collection, to analysis. Central to his thesis was the HELI study, in which he also served as an active coach, guiding participants through an intensive six-month multidomain lifestyle intervention. His thesis primarily sought to identify brain mechanisms that may explain reported cognitive improvements following lifestyle changes, by employing an extensive MRI protocol.

Mark is currently a postdoctoral researcher at Amsterdam University Medical Center, where he combines research on mapping adverse events and patient safety in Dutch hospitals with teaching activities in patient safety courses within the bachelor's and master's programs in Medicine.

Portfolio

Courses and training activities		Organizer
2023	Donders Toolkit: Advanced (f)MRI	Donders Institute
	‘Scientific Integrity’ course	Donders Graduate School
2022	BROK course for clinical investigators	Radboudumc
	Graduate School Day 2	Donders Graduate School
2021	‘Achieving your Goals’ course	Radboud University
	Graduate School Day 1	Donders Graduate School
	Graduate School Introduction Day	Donders Graduate School
	‘Statistics with JASP’ course	Radboud University
Teaching and supervision activities		
2023 – 2025	Teacher Food and Brain Health, MSc Medical Biology, Radboud University	
2021 – 2025	BSc and MSc internship daily supervision	
2021 – 2025	BSc and MSc internship thesis supervision	
2022	Literature Thesis supervision, MSc Biomedical Sciences, Radboud University	
Presentations at (international) scientific conferences		
Oct 2024	Society for Neuroscience (SfN), Chicago IL, USA [poster presentation] “The relation between physical activity, blood pressure, cerebral perfusion and episodic memory function in Dutch elderly at-risk of cognitive decline”	
Jun 2023	Dutch Neuroscience Meeting (DNM), Tiel, the Netherlands [poster presentation] “The effects of a multidomain lifestyle intervention on brain functioning in cognitive aging: the HELI study”	

Outreach activities	
2022 – 2023	Donders Institute representative at the Start-of-Academic-Year Introduction Markets of Radboud University and HAN University of Applied Sciences
2022 – 2024	Speaker for visiting secondary school classes and university students during tours and introductory events at the Donders Centre for Cognitive Neuroimaging
2021 – 2023	Academic contact person for secondary school student graduation research projects (Dutch: profielwerkstukken)
2022	MOCIA consortium representative at the 50PlusBeurs

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Tobias, mijn zoon, mijn kleine man. Jouw uitgerekende geboortedatum was een uitstekende (harde) deadline voor het afschrijven van de volledige draft van dit proefschrift, en dat is me welgeteld één dag voor die datum gelukt. Jouw geboorte heeft me met beide benen op de grond gezet en laten zien en ervaren wat echt belangrijk is in het leven. Ik geniet van iedere dag met jou, en van de blijdschap en het geluk dat je niet alleen ons brengt, maar ook jouw overgrootouders, opa's en oma's, ooms en tantes, en neefje. Ik kan niet wachten om je te zien opgroeien.

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