The effects of a multidomain lifestyle intervention on brain functioning and its relation with immunometabolic markers and intestinal health in older adults at-risk of cognitive ageing: study design and baseline characteristics of the HELI randomized controlled trial

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The effects of a multidomain lifestyle intervention on brain functioning and its relation with immunometabolic markers and intestinal health in older adults at-risk of cognitive ageing: 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 the mechanisms underlying these interventions could help explain these mixed findings.

Objective: The HELI 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 multicentre, 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 multidomain lifestyle intervention consisted of five domains (diet, physical activity, stress management/mindfulness, cognitive training, and sleep) and participants were randomized in one of two intervention groups: (1) a high-intensity coaching group consisting of weekly supervised online and on-site group meetings, exercises and learning materials from lifestyle-specific course modules, and (2) a low-intensity coaching group consisting of general lifestyle-related 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 an fMRI working memory task (2) ASL-quantified cerebral blood flow in dlPFC and hippocampus, (3) systemic inflammation from blood plasma (IL-6, TNF-?, 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 the relation between the effects found in the

aforementioned primary brain and gut outcome measures. Secondary study outcomes include (1) structural and neurochemical MRI, (2) anthropometric measurements, (3) neuropsychological test battery scores, (4) lifestyle-domain related measures from questionnaire scores and a smartwatch, and an array of peripheral measures from (5) faecal analysis, (6) blood analysis, and (7) breath analysis.

Results: Between April 2022 and October 2023 we have successfully included n = 102 older Dutch adults (mean age: 66.6 (SD: 4.3) years; 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 of cognitive ageing at baseline were overweight or obesity (74.5%), followed by hypertension (56.9%), hypercholesterolemia (55.9%), and physical inactivity (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. Clinical Trial: ClinicalTrials.gov ID NCT05777863
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The effects of a multidomain lifestyle intervention on brain functioning and its relation with immunometabolic markers and intestinal health in older adults at-risk of cognitive ageing: study design and baseline characteristics of the HELI randomized controlled trial

1. Abstract

Background

Studies of multidomain lifestyle interventions show mixed results on preventing or delaying cognitive decline in ageing. A better understanding of the mechanisms underlying these interventions could help explain these mixed findings. The HELI 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 multicentre, 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 multidomain lifestyle intervention consisted of five domains (diet, physical activity, stress management/mindfulness, cognitive training, and sleep) and participants were randomized in one of two intervention groups: (1) a high-intensity coaching group consisting of weekly supervised online and on-site group meetings, exercises and learning materials from lifestyle-specific course modules, and (2) a low-intensity coaching group consisting of general lifestyle-related 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 an fMRI working memory task (2) ASL-quantified cerebral blood flow in dlPFC and hippocampus, (3) systemic inflammation from blood plasma (IL-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 the relation between the effects found in the aforementioned primary brain and gut outcome measures. Secondary study outcomes include (1) structural and neurochemical MRI, (2) anthropometric measurements, (3) neuropsychological test battery scores, (4) lifestyle-

domain related measures from questionnaire scores and a smartwatch, and an array of peripheral measures from (5) faecal analysis, (6) blood analysis, and (7) breath analysis.

Results

Between April 2022 and October 2023 we have successfully included n = 102 older Dutch adults (mean age: 66.6 (SD: 4.3) years; 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 of cognitive ageing at baseline were overweight or obesity (74.5%), followed by hypertension (56.9%), hypercholesterolemia (55.9%), and physical inactivity (55.9%).

Discussion

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.

Keywords: lifestyle, cognitive ageing, risk factors, risk reduction, multidomain, intervention, randomized controlled trial, brain, gut-brain, magnetic resonance imaging

2. Introduction

The HELI study is a 6-month multidomain lifestyle intervention in older adults at risk of cognitive decline, focusing on involved central and peripheral mechanisms. Below, we explain the rationale of our objectives and hypotheses.

2.1 Lifestyle and cognitive ageing

In our ageing population, the incidence of cognitive decline and incurable neurodegenerative diseases like Alzheimer's dementia (AD) and other types of dementia are increasing drastically [1]. A substantial part of the risk factors for dementia, such as obesity, hypertension, type II diabetes and physical inactivity [2-5], are 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 [6, 7]. As the signs of neurodegenerative diseases - such as amyloid beta pathophysiology and tauopathy in AD - begin decades before the first symptoms become apparent [8, 9], 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 [10]. 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 [11].

Previous multidomain lifestyle intervention trials to prevent cognitive decline in ageing or dementia include the Prevention of Dementia by Intensive Vascular Care study (preDIVA-study) [3], Multidomain Alzheimer Preventive Trial (MAPT) [12], Japan-Multimodal Intervention Trial (JMINT) [13], AgeWell.de trial [14], and the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) [15-17]. The FINGER study was the first large, longitudinal randomized controlled trial showing very small positive outcomes of a 24-month multidomain lifestyle intervention (comprised of nutritional guidance, physical exercise, cognitive training, and cardiovascular risk management) in a large sample of older adults (n = 1260) with an elevated risk of cognitive decline [15, 18].

Uncertainties remain regarding the effectivity of multidomain lifestyle interventions in other randomized studies as in the preDIVA-study, MAPT, JMINT and AgeWell.de trial, no differences were found in cognition or dementia risk between the intervention groups. However, positive effects of a multidomain lifestyle intervention on cognitive measures were seen in specific at-risk subgroups. These subgroups consisted of participants with untreated hypertension in the preDIVA-study [3], and participants with a Cardiovascular Risk Factors, Aging and Incidence of Dementia (CAIDE) risk score ≥6 or a positive amyloid positron emission tomography (PET) scan at baseline in the MAPT [19]. Moreover, systematic reviews and meta-analyses have found that multidomain lifestyle interventions (i.e. interventions targeting at least 2 lifestyle domains) positively influence several cognitive functions (e.g., global cognition and subjective cognition [20, 21], memory-function and subjective memory performance [22]), especially compared to single-domain designs [23-25]. An important caveat is that, given the integration of a cognitive training component in some of these studies, a potential learning effect on the cognitive outcome measures has to be considered [26]. Currently, the amount of causal evidence provided by previous studies have been scarce, potentially caused by the heterogeneity between conducted intervention randomized controlled trials (RCT's) (e.g. intervention domains or components, intensity, duration, outcome measures, population characteristics). Another explanation may stem from the fact that we have a limited understanding via which brain mechanisms, and potential underlying peripheral pathways, these multidomain lifestyle interventions might exert their effects on cognition. Gaining a better understanding of the effect of multidomain lifestyle intervention on involved peripheral- and brain mechanisms could help in making future RCT's more effective by allowing them to be more targeted in their influence on these underlying mechanisms.

In order to elucidate these underlying brain mechanisms, neuroimaging techniques are required to analyse brain health and function *in vivo* in a longitudinal, intervention setting. To our knowledge, only few studies have investigated the effects of a multidomain lifestyle intervention on brain mechanisms using neuroimaging. An example is the MAPT, where PET- and structural magnetic resonance imaging (MRI) scans were acquired in a subsample (n = 68) of the study population [12]. Using PET-scans, it was found that participants in the intervention group showed elevated levels of cerebral glucose metabolism within, e.g., the hippocampus and the adjacent parahippocampal gyrus relative

to a placebo-control group without lifestyle coaching during the intervention period [19]. Additionally, structural MRI showed that the MAPT intervention was associated with morphological brain changes, namely decreased deformation of periventricular areas and temporal lobes, and decreased whole-brain atrophy, which were related to improved cognitive functioning [27]. However, in a subsample of the FINGER-study (n = 132), no intervention effects were found on white matter lesion volume, cortical thickness or regional brain volumes on structural MRI [28]. Therefore, it is important to better understand the effects of a multidomain lifestyle intervention (with the domains: diet, physical activity, stress management/mindfulness, cognitive training, and sleep) on the ageing brain, and explain individual differences in treatment effects. This could be achieved by using a broader neuroimaging protocol , which includes task-related fMRI and quantitative imaging of cerebral blood flow, along with peripheral measures related to the gut microbiome and the immune system. Below, we explain the rationale of our chosen outcome measures.

2.2 The ageing brain

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 grey and white matter volume, leading to loss of overall brain volume [29]. Grey matter changes are most often characterized by cortical thinning and decreased brain volume, caused by neuron body shrinkage and the decrease of dendritic spine and synapse count [29-32]. However, 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 [33-35]. These changes in grey matter volume of the hippocampus and PFC, in addition to age-related changes in perfusion and functional connectivity (further discussed below), have been consistently related to decreased executive functioning, processing speed and memory performance [36-41]. Likewise, alterations in the structural integrity of the white matter (e.g. demyelination and decreased fiber connections) significantly affect cognition in both normal and pathological ageing [29, 32, 42]. The structural integrity of the white matter is highly dependent on a process called myelination, as white matter is primarily comprised

of (myelin-sheathed) axons and myelin-producing glial cells. Variation in the quantity of myelin throughout the brain has been associated with increased age [43-46], and a broad array of age-related effects on white matter integrity has been previously reported (for review, see [47]). Especially in AD, strong associations between global and frontal white matter integrity disruptions and overall cognitive performance have been reported [48, 49].

These findings underline the link between age-related structural brain changes and specific effects on cognitive outcomes. Brain activation, as can be measured by functional MRI (fMRI), is significantly influenced by the structural properties of the brain [50]. The most notable cognitive changes associated with normal and pathological ageing can be found in the more complex cognitive domains such as executive functioning, working memory, episodic memory, as well as processing speed [51]. With ageing, changes in fMRI regional activation during performance of cognitive tasks occur, including increased activation in middle temporal and medial frontal gyrus during a (spatial) working memory task [52]. Additionally, one study in prodromal AD patients reported fluctuations in hippocampal brain activity (ranging from hyperactivity to hypoactivity) prior to AD symptom onset [53]. Multiple studies have identified increased activity in regions of the PFC in older adults who show comparable working memory performance to young adults [54, 55]. These studies indicate that differences in functional activation patterns, measured with fMRI, may already show a – perhaps compensatory – reorganization before detriments in cognitive performance are observed [51, 56]. It remains unclear how this potential functional reorganization is affected by a multidomain lifestyle intervention.

A decrease in blood flow is one of the potential causes underlying the age-related decline in cognitive functioning [57-60]. Multiple studies have reported the predictive value of quantified cerebral perfusion on cognitive performance in both normal and pathological cognitive ageing [57-60]. Moreover, increased local perfusion in specific brain regions such as the hippocampus and PFC, has been directly linked to better cognitive performance [57].

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 grey matter), although conventionally found in healthy ageing adults [61-63], has been directly linked with increased local oxidative stress leading to

neurodegeneration [64-67] and decreased cognitive functioning in subjects with amyloid-β pathology [68]. Similarly, and in addition to intracranial iron deposition, neuroinflammation contributes to neurodegenerative processes in the normally ageing brain [69], for example, by affecting the cerebrovascular endothelium leading to neurovascular dysfunction [70]. These impairments could eventually contribute to decreased brain functioning and cognitive decline [71]. 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 [72, 73], in mild cognitive impairment (MCI) and in AD [73, 74]. 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 [75-77] and functional connectivity [78]. 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.

2.3 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 [79, 80]. Furthermore, a substantial part of lifestyle effects on brain functioning is expected to arise via mechanisms in the periphery. 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 *e.g.* the modulation of immune signaling to the brain, affecting the hypothalamic–pituitary–adrenal axis, and transmission via the vagus nerve [81]. 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 [82]. They reflect microbiome function [83] and have demonstrated health effects on the host (*e.g.* (anti)inflammatory and metabolic processes) [84, 85], also in ageing [86].

We know that ageing is associated with changes in microbial composition- and diversity and gut health [87]. Older adults more frequently show gut microbiome dysbiosis (‘imbalance’) compared to younger individuals, potentially due to prolonged exposure to an unhealthy lifestyle [88, 89]. Particularly microbial diversity and uniqueness seem to correlate with ageing in Western societies [90]. A lower faecal microbial diversity predicted poorer cognitive function in healthy older adults [91]. Microbial metabolites are also affected by ageing, as older adults show reduced faecal total SCFA levels [92]. SCFAs, especially butyrate, are known for their beneficial anti-inflammatory effects [93, 94], and have been linked to improved cognition in mice [95, 96].

Importantly, these microbial changes become more pronounced in case of pathological cognitive ageing like AD. Compared to healthy aged-matched individuals, AD patients show a decreased microbial diversity and stability over time [97], differences in abundance of individual taxa [80, 98] and more often have small-intestinal bacterial overgrowth (SIBO) [99]. In addition, a further decrease in levels of faecal SCFAs and tryptophan metabolites was found, which correlated with cognitive decline [100].

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 integrity of the intestinal barrier via different mechanisms, such as disruption of the tight junctions by lipopolysaccharides or oxidative stress [101], thereby provoking a local inflammatory reaction which could eventually lead to systemic inflammation [102]. In addition, microbiota dysbiosis can also lead to the dysregulation of abdominal adipose tissue, affecting e.g. immune- and insulin pathways, promoting systemic inflammation even more [103, 104]. Low-grade, systemic inflammation is commonly seen in ageing [105], 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 [105, 106]. Importantly, low-grade, systemic inflammation due to impaired intestinal health can lead to elevated levels of neuroinflammation [107] and is linked with neurodegenerative processes in the normally ageing brain.

Faecal microbial transplantation (FMT) in mice has demonstrated a causal role for the gut microbiome in brain aging [108-111], highlighting that microbial modulation may be of therapeutic benefit in preventing age-related inflammation and cognitive decline [112]. 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 [113].

There are numerous studies showing that gut microbiome composition and immune-metabolic health effects on the host are affected by diet and nutritional components [114-116], including the Mediterranean-type diets [117, 118]. In addition, other lifestyle components such as physical activity [119, 120], stress [121] and sleep [122, 123] are known to affect the microbiota composition and intestinal health, as well as immune-, and metabolic health [124-131]. However, it is still largely unclear how gut microbiome, immune and metabolic factors link to brain health in ageing, and which mechanisms are involved. Importantly, 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.

2.4 Study relevance and objectives

To summarize, previous lifestyle intervention studies in ageing propose effects of lifestyle factors on cognitive performance, functional brain responses, cerebral blood flow, and peripheral markers. However, in the current literature, many lifestyle interventions focused on one domain, whilst multiple studies have indicated that multidomain (≥2 domains) interventions show a greater protective effect against cognitive decline in ageing. It is currently unclear what the underlying mechanisms are, as studies investigating the possible involved mechanisms behind multidomain lifestyle interventions are scarce. The potentially

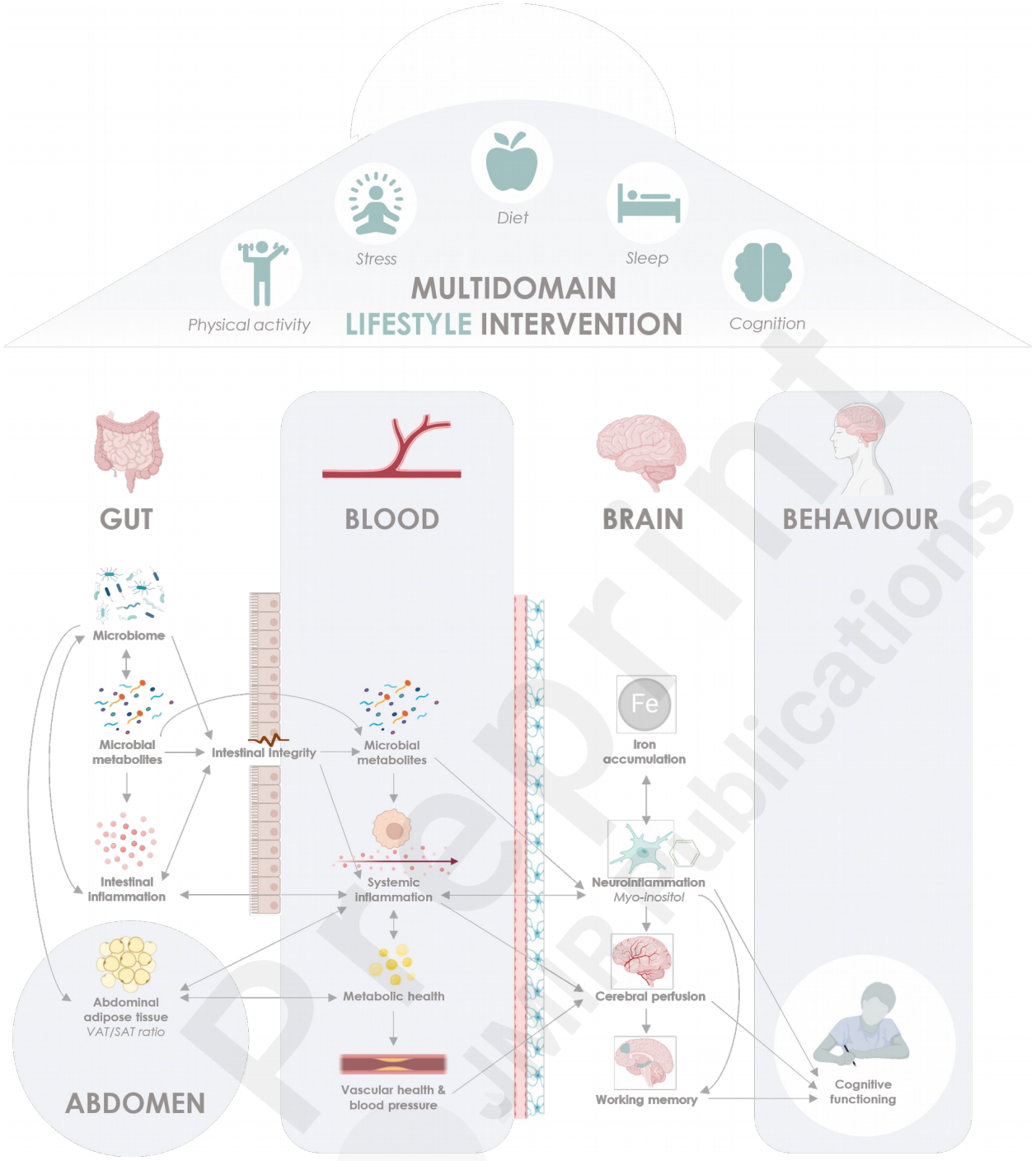
substantial role of peripheral factors, captured in the gut-brain axis, in explaining lifestyle intervention effects for cognitive ageing remain unresolved. Additionally, a large portion of ageing related research is based on cross-sectional studies (creating cohort effects), and are limited due to possibly including participants with silent or unknown disease (e.g. AD, vascular dementia). These limitations may overestimate the effect of ageing on brain changes and cognition in these studies, as some of these effects could be caused by disease instead of ageing.

To bridge these knowledge gaps, 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 potential immunometabolic-based individual differences in these neurocognitive effects.

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), combining five different lifestyle domains, namely diet, physical activity, stress management/mindfulness, cognitive training, and sleep. We will assess its effects on multiple structural and functional MRI-related brain measures, peripheral measures related to the gut-immunometabolic-brain axis, as well as their connections.

2.5 Hypothesis

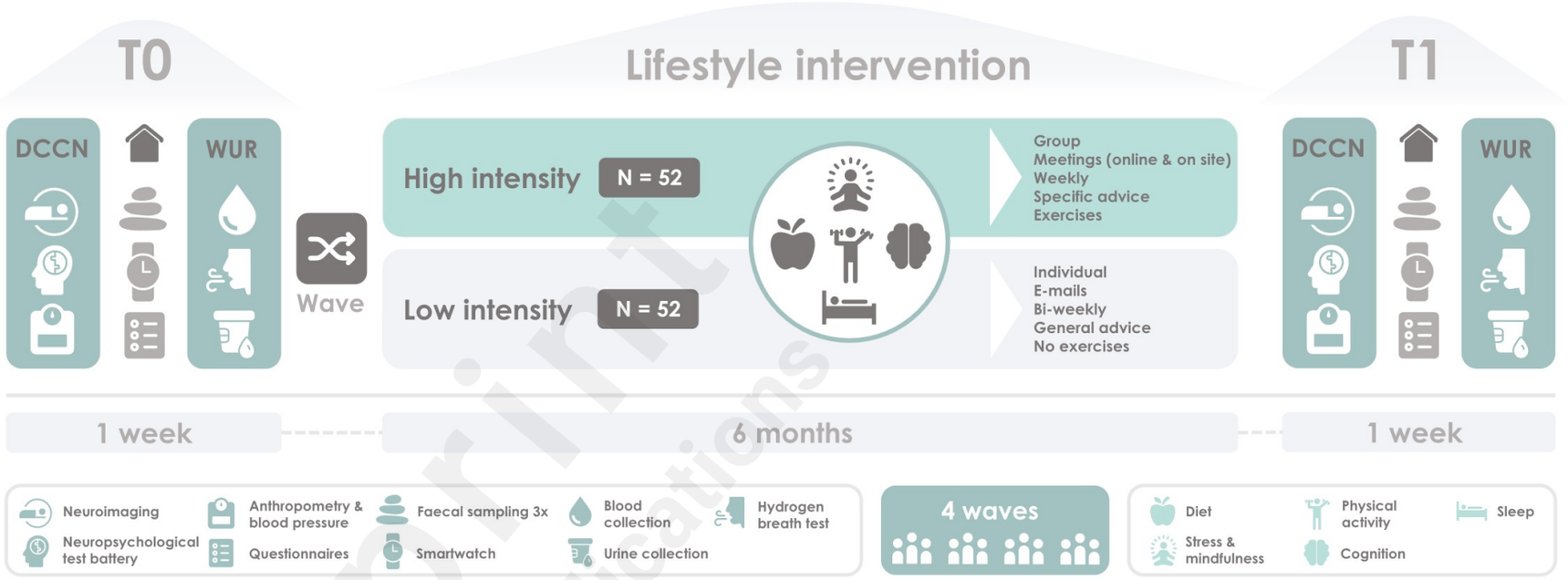
We hypothesize that a multidomain lifestyle intervention affects multiple peripheral and central mechanisms simultaneously, evident in microbiome, immune- and metabolic markers, which in turn relate to central mechanisms such as cerebral perfusion, brain activation, and neuroinflammation (particularly in prefrontal and hippocampal regions). In the figure below, a summary of our rationale and overall hypothesis is represented (Figure 1).



3. 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 high-intensity coaching intervention group, and (2) a low-intensity coaching intervention group. Since the high-intensity intervention participants were supervised in group context, we recruited and subsequently randomized participants in four 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 (WMO; '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).



4. Methods: Participants, interventions, and outcomes

4.1 Study setting

The HELI intervention is based on the FINGER-NL lifestyle intervention, a multicentre, randomized, controlled, multidomain lifestyle intervention effectivity trial in The Netherlands. The rationale and study design of FINGER-NL has been published previously [132]. Both HELI and FINGER-NL are part of the overarching Maintaining Optimal Cognitive function In Ageing (MOCIA) research program [133]. 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.

4.2 Recruitment

To achieve adequate participant enrolment and reach the target sample size of 104 included participants, we have utilized 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, 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.

4.3 Eligibility criteria

4.2.1 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 CAIDE [134]).

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 [135]: <300 minutes of moderate intensity aerobic physical activity, or <150 minutes of vigorous	1

intensity aerobic physical activity per week, spread out over several days)	
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 4.2.2 ‘Exclusion criteria’)	1

4.2.2 Exclusion criteria

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

Practicalities and participation

(1) Concurrent participation in other intervention trials;

(2) Technologically illiterate (i.e., complete incompetence in working with computers, smartphone, mobile applications, online questionnaires, etc.);

(3) No internet access from home;

(4) Cognitive impairment as determined by a score lower than 23 points on the Telephone Interview for Cognitive Status (TICS-M1) [136], 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:

(4) Cerebrovascular event (e.g. transient ischemic attack, cerebral hemorrhage, stroke);

(5) Neurological disease (e.g., mild cognitive impairment, multiple sclerosis, Parkinson's disease, epilepsy);

(6) Current malignant disease(s), with or without current treatment;

(7) Current psychiatric disorder(s) (e.g., depression, psychosis, bipolar episodes);

(8) Symptomatic, moderate to severe cardiovascular disease (e.g., stroke, angina pectoris, heart failure, myocardial infarction);

(9) Revascularization surgery in the last 12 months at moment of pre-screening;

(10) Inflammatory bowel disease (characterized by diarrhea);

(11) Visual impairment, which is uncorrectable with visual aids (e.g., total or partial blindness);

(12) Hearing or communicative impairment, which is uncorrectable with hearing aids;

MRI safety-specific criteria

(13) Metal objects located in the upper body (exceptions: tooth-fillings and/or dental crowns);

(14) Metal splinters in the body, in particular within the eyes (e.g. through labor work in the metal industry);

(15) Jewelry items or piercing that cannot be taken off;

(16) Undergone brain surgery in the past;

(17) Active implants within the body (e.g., pacemaker, neurostimulator, internal insulin pump, internal hearing aids that cannot be removed);

(18) Medical plasters or patches which cannot or may not be taken off (e.g., nicotine patch);

(19) Self-reported claustrophobia.

4.4 Interventions: description

In the HELI multidomain lifestyle intervention five distinct lifestyle domains were combined, namely (1) diet, (2) physical activity, (3) stress management & 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, provided through information leaflets sent by e-mail once every two weeks. 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.

4.3.1 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 on 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 of 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 straining 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/mindfulness) had one information session and three boost sessions. The lifestyle domains of cognitive training and sleep differed from the information/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.

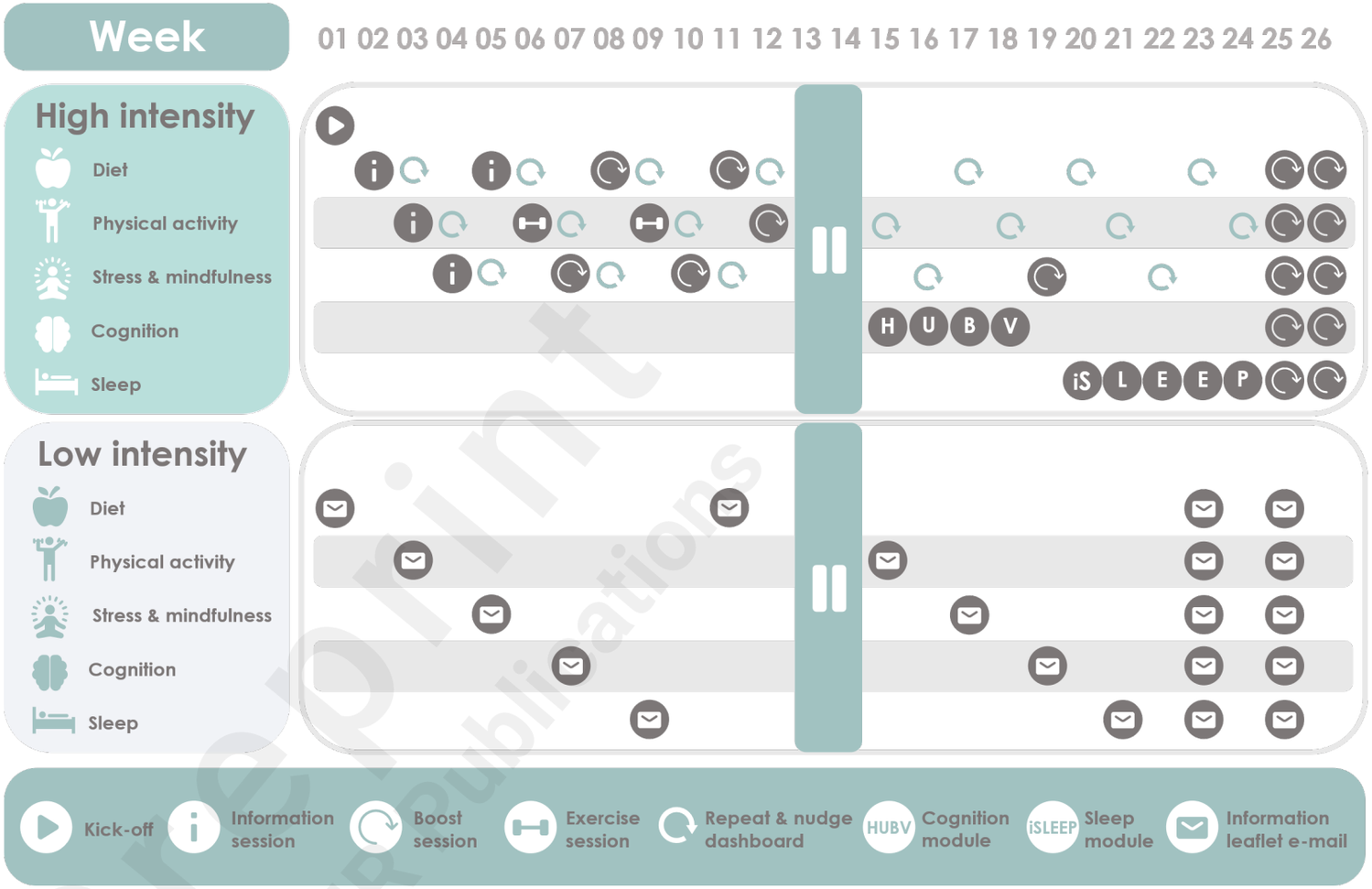


Figure 3 Intervention overview. High intensity: In the first week of the intervention, a kick-off session at location was used to give a general introduction and information about the study and the intervention set-up. The lifestyle domains of nutrition, physical activity, stress/mindfulness were structured 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 prepared 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 ageing-related cognitive decline [137], and the Dutch guidelines for a healthy diet [138] which is associated with protective effects on multiple major (chronic) diseases (e.g. coronary heart disease, stroke, diabetes, heart failure, dementia). Briefly, MIND-diet specific food recommendations comprise of: (1) green leafy vegetables, (2) other vegetables, (3) berries and strawberries, (4) whole-grain products, (5) unsalted nuts, (6) (unfried) fish, (7) poultry, (8) legumes/beans, and (9) olive oil. MIND-diet specific food restrictions comprise of: (1) cheese, (2) butter/margarine, (3) red or processed meat, (4) fried food or take-out, (5) pastries and candy, and (6) alcohol [137]. 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 [139, 140], 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 [135]. These guidelines recommend engaging in physical activity of moderate intensity (e.g. swimming, running, 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, 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, lying down) must be limited according to these guidelines.

3. Stress management & mindfulness

Ambition and goals

The goal of the stress management & 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 [141]), 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 [142]. 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) [143, 144]. 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.

4.3.2 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 by information leaflets (1-2 A4), through e-mail, 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.

4.5 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 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.

4.4.1 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 [145] and hippocampus (Harvard-Oxford atlas), using fMRI blood-oxygen-level-dependent (BOLD) activity and task accuracy during a numerical N-back task [146];

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.

4.4.2 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. grey/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) [147], (2) Verbal Fluency Test (VFT) [148], (3) Trail Making Test (TMT) part A&B [149], (4) Digit Symbol Substitution Test (DSST) [150], and (5) Digit Span Test (DST) [150];

d) Lifestyle domain-related questionnaire scores for (1) diet (MIND-adjusted Eetscore [151]), (2) physical activity (Short Questionnaire to Assess Health-enhancing Physical Activity (SQUASH) [152], LASA Sedentary Behavior Questionnaire (SBQ) [153] and SARC-F Sarcopenia Questionnaire [154]), (3) stress management & mindfulness (Perceived Stress Scale (PSS) [155], Hospital Anxiety and Depression Scale (HADS) [156], Five Facet Mindfulness Questionnaire (FFMQ) [157]), (4) cognitive training (Cognitive Failures Questionnaire (CFQ) [158] and Metamemory In Adulthood Questionnaire (MIA) [159]), (5) sleep (Pittsburgh Sleep Quality Index (PSQI) [160]). In addition, stress, physical activity and sleep were measured objectively using smartwatch measurements of skin temperature, galvanic skin response, and movement;

e) Faecal analysis, including intestinal transit time [161], stool water content, pH, microbiota composition (both relative- and absolute abundances), metabolite profiles, and inflammatory biomarkers;

f) 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;

g) Breath analysis to measure SIBO [162, 163].

4.4.3 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) [164];
d) Changes between baseline (T_0) and follow-up after 6 months (T_1) of the Montreal Cognitive Assessment (MoCA) [165];
e) Urine analysis of microbiota-derived bioactive compounds (changes between baseline (T_0) and follow-up after 6 months (T_1));
f) 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 [166]), (2) quality of life (5-level EuroQol-5D (EQ-5D-5L [167]), (3) modifiable dementia risk score ('Lifestyle for BRAin health' (LIBRA) [168]), (4) social contact and perceived social support (Lubben Social Network Scale [169]), (5) cognitive coping strategies (Cognitive Emotions Regulation Questionnaire [170]), (6) apathetic symptoms (Starkstein Apathy Scale [171]), (7) gastrointestinal symptoms rating scale (GSRS) [172], and (8) stool consistency (Bristol Stool Scale [173]).

4.6 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 [76, 77, 119, 130, 131, 174] (See Supplementary Table 3). 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 participants × 15% potential data loss = 103.5 = 104) participants in this study.

5. 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 [175].

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, deviations would be made (e.g. an unblinded researcher conducting the MRI protocol). Neuropsychological tests and trainings 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 of the group allocation and the nature of the study setting.

6. 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). FDR correction will be used to account for multiple comparisons of secondary and exploratory outcomes. FWE correction will be used for secondary whole-brain 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 as a measure of improvement for each respective domain as predictors in a 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 T1 (after week 26) follow-up outcome assessments. This means that we will use all available collected data, including collected (follow-up) data of participants that dropped out of intervention. Missing data or data with insufficient quality, however, will not be used in analysis.

7. Results

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 = 51 participants each.

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 (± 4.3) years, of which 65.7% (n = 67) was female (see Table 2). The median risk, measured by points on the self-reported lifestyle-modifiable risk factor scale, was 3.0 (IQR: 2.0 – 3.0) 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.

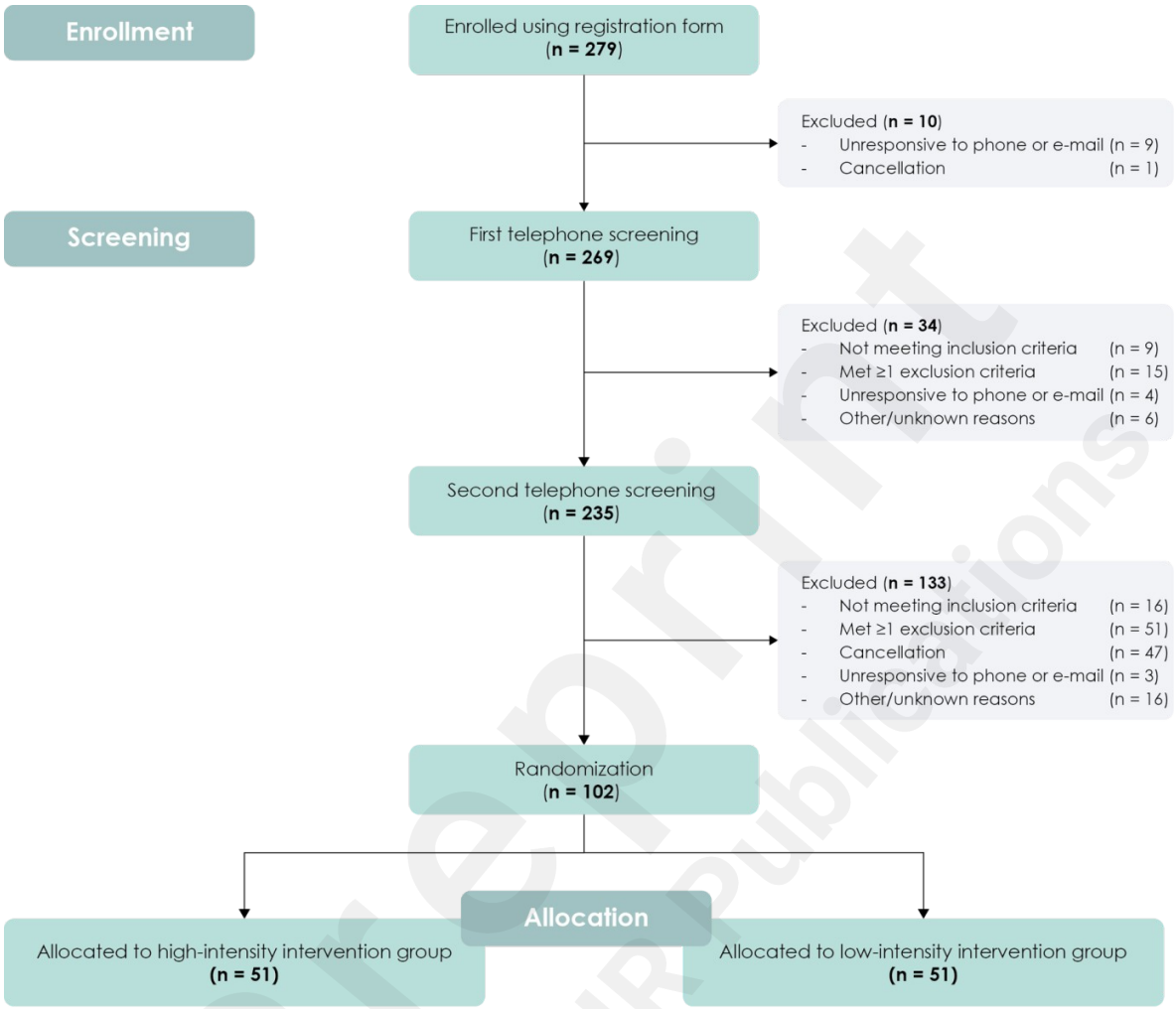


Figure 4. CONSORT study flowchart. Flowchart of HELI study participant enrollment, screening (inclusion and exclusion), randomization and allocation.

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	39	(38.2)
High	53	(52.0)
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 >5mmol/L or LDL-cholesterol >3mmol/L

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

8. Discussion

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 [132] 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 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 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, 12, 15, 16] and the FINGER-NL trial [132], 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 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 neuroimaging, the duration of HELI (26 weeks) is relatively long compared to other lifestyle intervention studies (6-9 weeks), which reported significant central mechanistic intervention effects [76, 78]. 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. To illustrate, previous lifestyle intervention studies found significant effects on brain functioning outcomes such as working memory after interventions with fewer integrated lifestyle domains [176, 177].

Altogether, the HELI study has the potential to provide novel mechanistic insights of lifestyle effects on cognitive ageing. These insights can be of great importance for targeting upcoming studies on lifestyle to prevent cognitive decline in ageing. Finally, potential predictors of individual effectivity of lifestyle interventions on gut-immuno-brain links can be used to develop personalized prevention strategies in the future.

9. Acknowledgments

Figure 1 was created with elements from BioRender [178], and adapted from BioRender, Remie L. (2015) [179]. Figures 1, 2 and 3 were created with elements from the Noun Project [180].

10. Authors' contributions

ML, LR, JO and EA have drafted the manuscript. All authors (ML, LR, MT, MJ, JM, JC, OR, YV, NS, SS, KD, MZ, WF, SK, WS, JO, EA) have contributed to the HELI study trial design and data acquisition, data interpretation, and have read and approved the final manuscript.

11. 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 [133]. There are no competing interests to be declared.

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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.

12. Abbreviations

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
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
FFMQ: Five Facet Mindfulness Questionnaire
FINGER: Finnish Geriatic Intervention Study to Prevent Cognitive Impairment and Disability
fMRI: functional magnetic resonance imaging
FMT: faecal microbial transplantation
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

JMINT: 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

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

TICS-M1: Telephone Interview for Cognitive Status

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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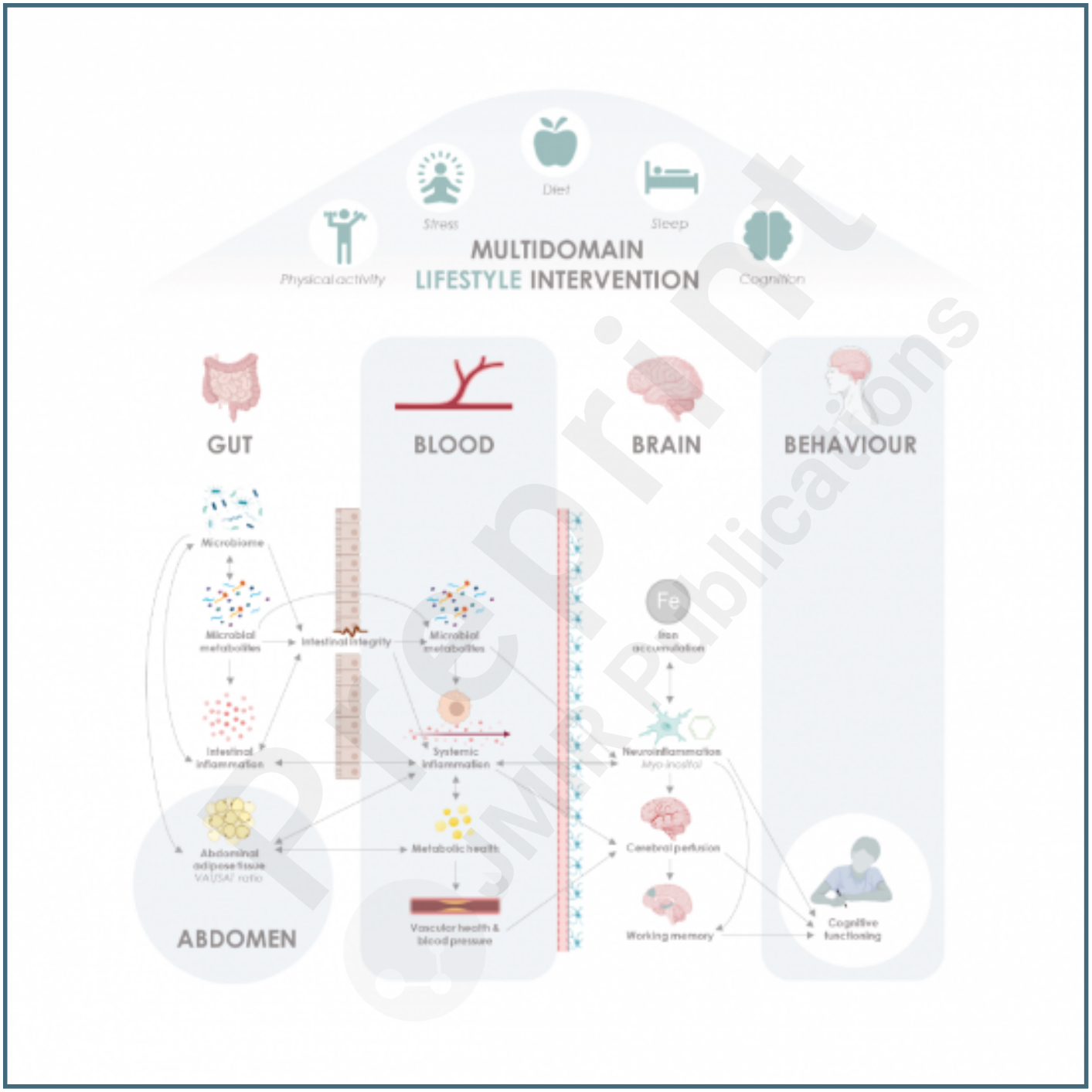
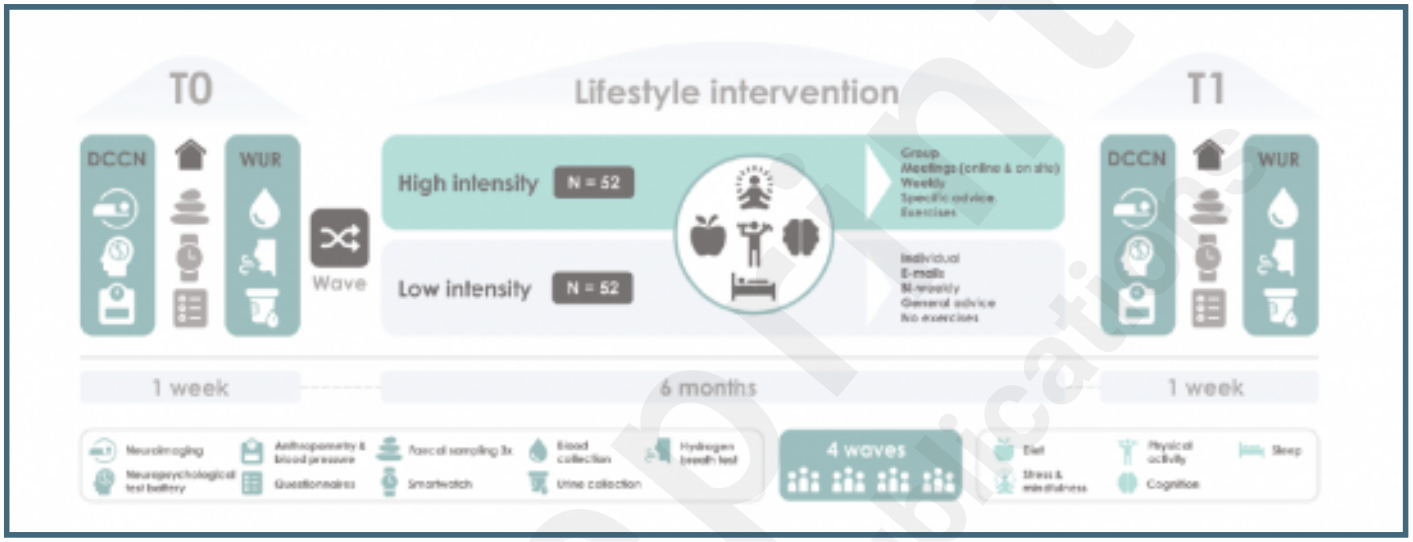
Supplementary Files

Figures

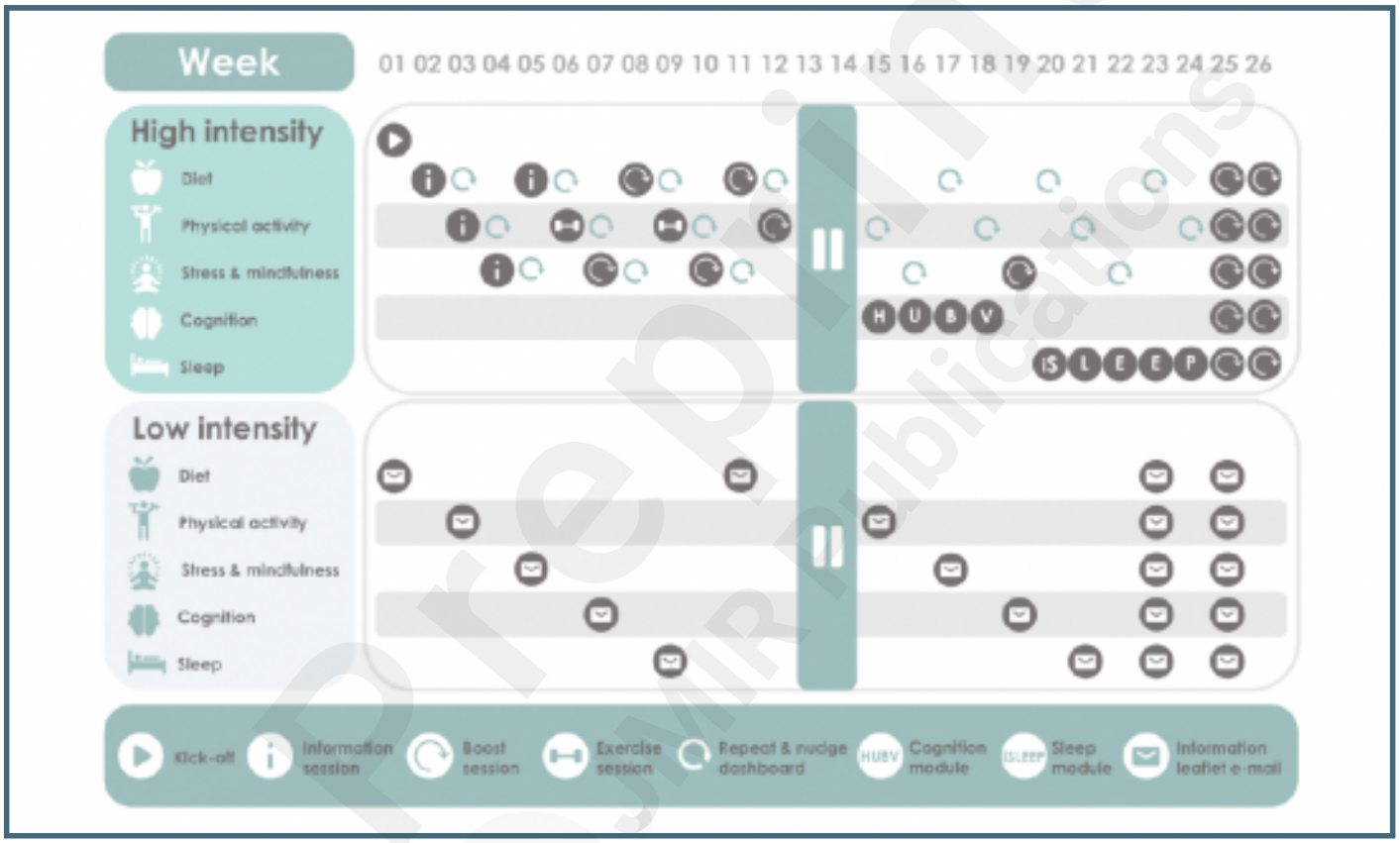
Hypothesized peripheral pathways from gut to brain affecting cognitive functioning, based on the existing evidence mentioned in the introduction. A multidomain lifestyle intervention is expected to affect these proposed pathways at different levels. Graphic is focused on HELI study outcomes. Not each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



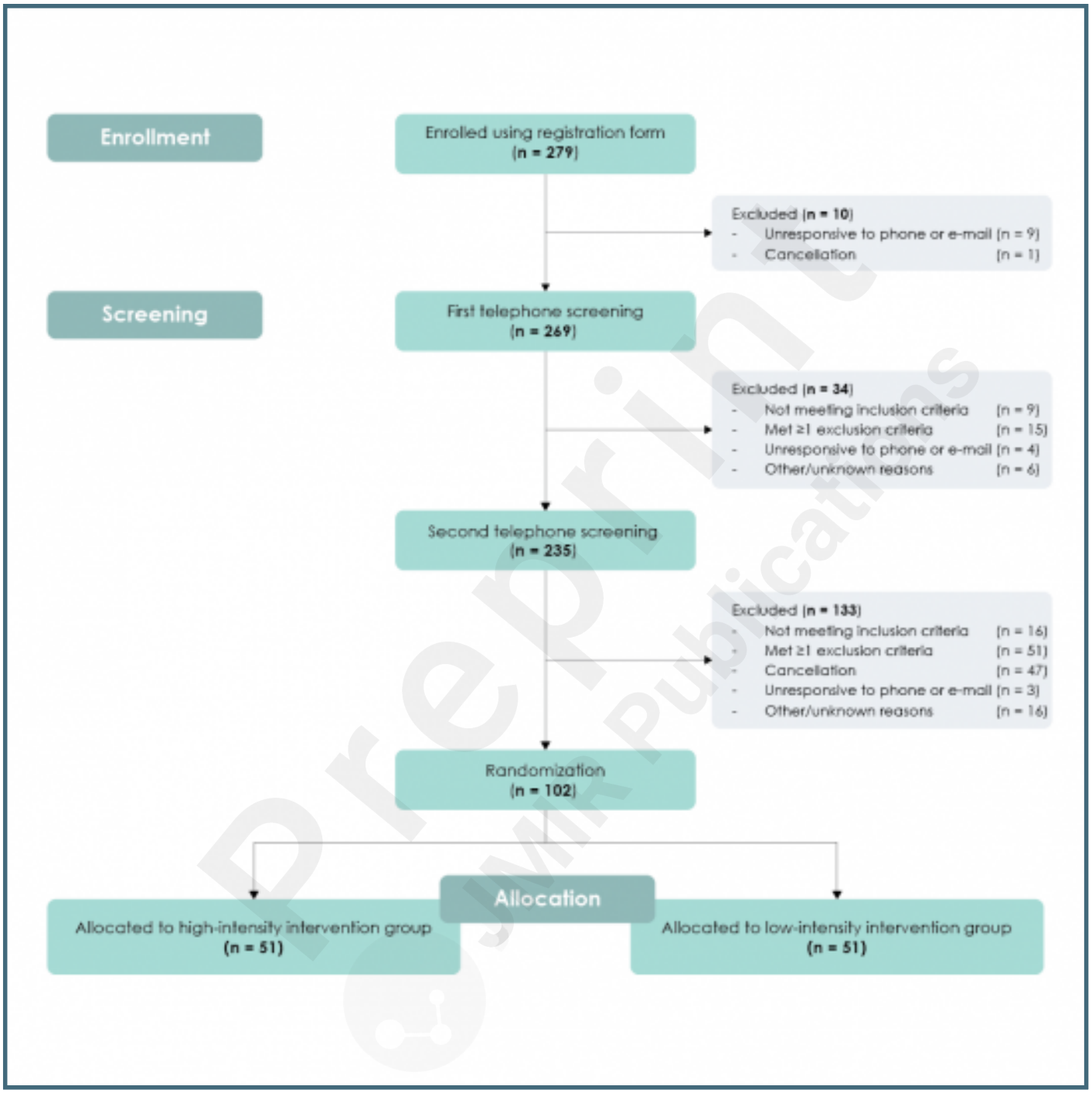
Schematic overview of study visits, assessments, and intervention groups. Before (T0) and after (T1) the intervention period of 6 months, participants visited the Donders Centre for Cognitive Neuroimaging (DCCN) and Wageningen University and Research (WUR) study centres with one week in between. Assessments 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.



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.



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



Multimedia Appendixes

Supplementary material.
URL: http://asset.jmir.pub/assets/fd426268f7a93faca6a563992e9c17b9.docx

CONSORT (or other) checklists

CONSORT checklist referencing to page numbers of main manuscript and supplementary material.
URL: http://asset.jmir.pub/assets/d27455f45569c6edc3082988f75ee266.pdf