Healthy ageing in older adults: an exploration of interactions between lifestyle, immune, metabolic, and gut microbial health

Morwani Mangnani, J.

CHAPTER 2

Design of the VOILA intervention study: A 12-week nutrition and resistance exercise intervention in metabolic or mobility compromised Dutch older adults and the response on immune-metabolic, gut and muscle health parameters

CS Kramer[1*A], A Monsegue[2*], J Morwani-Mangnani[3*], P Grootswagers[1], M. Beekman[3], PE Slagboom[3#], LB Verdijk[2#], L CPGM de Groot[1#]

[1] Wageningen University & Research, Wageningen Campus, Agrotechnology and Food Sciences Group, Division of Human Nutrition and Health, PO Box 17, 6700 AA Wageningen, The Netherlands
[2] Maastricht University Medical Center+, Department of Human Biology, NUTRIM Institute of nutrition and translational research in metabolism, PO Box 616, 6200 MD Maastricht, The Netherlands
[3] Leiden University Medical Centre, Department of Biomedical Data Sciences, Section of Molecular Epidemiology, Einthovenweg 20, 2333 ZC Leiden, The Netherlands
[*] Shared first authorship
[#] Shared last authorship
[A] Corresponding author

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ABSTRACT

Background: Exercise and nutrition interventions can slow ageing-induced decline in physiology. However, effects are heterogeneous and usually studied separately per outcome domain. In the VOILA study, we simultaneously study various health outcomes relevant for older adults and the inter-individual heterogeneity in response to a lifestyle intervention.

Methods: VOILA is a 12-week lifestyle intervention in 3 groups of older adults (≥60 years), with compromised mobility (n=50), compromised metabolic health (n=50), or recovering from total knee replacement (TKR, n=70, of which 20 randomized to standard care only). The intervention includes high-intensity resistance exercise training thrice weekly, nutritional counselling, and nutritional supplements every morning and evening (including 20-25g whey protein and (evening only) 5.5g Biotis™ GOS). We measure immune-metabolic, gut health, muscle mass and physical functioning at baseline and after completion of the intervention/standard care. An additional reference group of healthy older adults (n=50) will undergo baseline measurements only.

Discussion: Improvements in various physiological systems are expected, but with differences between groups/individuals. This study will provide insights into how the physiological state of older adults influences the extent of lifestyle-induced health improvements to create better tailored interventions to attenuate biological ageing and improve the health span of subgroups and individuals.

Keywords

Biological age, nutrition, gerontology, intervention, resistance exercise, metabolic health, gut health

LIST OF ABBREVIATIONS

1RM: 1-Repetition Maximum
1H NMR: Proton Nuclear Magnetic Resonance
25(OH)D: 25-hydroxyvitamin D
5CST: 5-times Chair Stand Test
6MWT: 6-Minute Walking Test
ALM: Appendicular Lean Mass
DEXA: Dual Energy X-ray Absorptiometry
DNA: Deoxyribo Nucleic Acid
FRS: Framingham Risk Score
GLC: glucose
GlycA: Glycoprotein Acetyls
GOS: GalactoOligoSaccharides
GSRS: Gastrointestinal Symptom Rating Scale
HbA1c: Haemoglobin A1C
LLS: Leiden Longevity Study
MMSE: Mini Mental-State Examination
MEC: Medical Ethics Committee
Qol: Quality of Life
SCFA: Short Chain Fatty Acids
SD: Standard Deviation
SF-36: Short Form (36) Health Survey
SF-SarQol: Short Form Sarcopenia and Quality of life
PI: Principal Investigator
SPPB: Short Physical Performance Battery
SQUASH: Short QUestionnaire to ASsess Health enhancing physical activity
TKR: Total knee replacement
TUG: Timed Up-and-Go test
VLDL-D: Very Low Density Lipoprotein Diameter
VOILA: Vitality Oriented Innovations for the Lifecourse of the Ageing society
WOMAC: Western Ontario and McMaster Universities Arthritis Index

INTRODUCTION

Life expectancy has increased twofold to threefold in the last 200 years because of better living conditions, nutrition, hygiene, and medical care (1-3). Globally, population ageing progresses as the number of people over 65 years is projected to double between 2019 and 2050, and people aged 80 years and older are projected to triple to 426 million (4). As age is the most prominent risk factor for common diseases and multi-morbidity in humans, general health declines as we get older (5), leading to loss of functional capacity in physical, cognitive, and social domains. Yet, since individuals of the same chronological age (age in units of time) display a large heterogeneity in health status, it is the biological age (i.e. age measured as a biological process or deviation from chronological age) of individuals that influences the risk of disease more than their chronological age does (3). As such, this heterogeneity in biological age is expressed as different susceptibilities to morbidity and mortality. In relation to this, adults of all ages favour a long lifespan only if accompanied by good health or vitality (6, 7). Therefore, in our ageing society, it is important to extend health span by delaying or partially reversing the ageing process. To do this we must first record the biological age for early recognition of those individuals that are at risk of faster functional decline, to then attempt to delay multiple aspects underlying the ageing process simultaneously.

During ageing, multiple physiological systems are dysregulated (8). Especially in animal studies and model systems, a range of molecular hallmark mechanisms have been identified that drive the ageing process across species (9-11). Targeting these hallmarks illustrated that health and lifespan can be extended in animal models (12). In humans, the same hallmarks are expected to underly ageing, leading to physiological deterioration and morbidity (3). Several mechanisms of ageing have already been identified to underlie multiple age-related diseases (13) and multimorbidity simultaneously (14). Consequently, prevention or delay of age-related functional decline and disease is likely possible either by lifestyle or pharmacological intervention especially when targeting these ageing processes. Experts agreed that interventions targeting hallmarks of ageing "should halt further detrimental aspects of ageing and preferably improve phenotypes associated with ageing" (11). In line with this, the "Vitality Oriented Innovations for the Lifecourse of the Ageing society" (VOILA) intervention study we present here, focuses on the improvement of phenotypical markers of three physiological systems in humans, hypothesized to be important during ageing – muscle mass and physical functioning, immune-metabolic health, and gut health - via a multimodal intervention.

The VOILA intervention we describe here focuses on resistance exercise (RE) and a healthy diet, including a high-protein component and galacto-oligosaccharides (GOS), for their combined impact on the ageing-related domains of muscle health, immune-metabolic health, and gut health. Regarding muscle health, muscle protein turnover decreases (15) and skeletal muscle strength, power, mass and quality decline with age (16) which are in turn associated with compromised mobility (17, 18) and increased chronic disease risk (19). Resistance

exercise is recommended as a countermeasure (19-23), showing increases in muscle mass and strength in healthy older adults (24-26) as well as strength and physical performance in pre-frail and frail older adults (27, 28). Remarkably, all older adults show improvements on at least one muscle modality in response to RE, although inter-individual variation is substantial (29).

RE and its aforementioned effects on muscle health in turn positively affect many physiological and metabolic ageing-related processes. For example, age-related decline of muscle mass, quality and physical activity are thought to be the main drivers of insulin resistance (30-32). Whether increasing activity levels can fully restore insulin sensitivity is not yet clear (33-35), but progressive RE has been shown to improve glycaemic control in overweight prediabetic older adults (36, 37), lower the risk of developing Diabetes Mellitus type 2 (38), and reduce mortality risk in diabetic patients (39). In addition, RE has been shown to reduce cardiovascular risk (40, 41), by improving the Framingham Risk Score (FRS) (42), decreasing blood pressure (42, 43) and improving lipid profiles (44).

Other ageing-related health benefits of RE are thought to be related to the release of myokines and other molecules by skeletal muscle upon contraction (45). Moreover, exercise reduces multiple inflammatory cytokines that are important in low-grade inflammation present with ageing-related impairments such as insulin resistance, cardiovascular disease, and atherosclerosis (46-48). Due to its extensive positive health effects throughout the body, RE is a cornerstone of interventions in older populations.

Next to exercise, adequate protein intake is essential for muscle mass maintenance as well as to support muscle hypertrophy. Protein intake increases plasma amino acid concentrations, providing the necessary building blocks for muscle and activating signalling pathways that stimulate muscle protein synthesis (49). Among older adults, relative anabolic resistance to dietary protein is prevalent (50, 51). The PROT-AGE study group proposed to increase the protein intake recommendations for adults above 65 years of age who are exercising to at least 1.2 g/kg bodyweight/d (52), and the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines advice a protein intake up to 1.5 g/kg bodyweight/d for older adults who are malnourished and/or those with acute or chronic illness (53). Currently, Dutch older adults have a lower protein intake, with means for community-dwelling, frail and institutionalized older adults ranging between 0.8-1.0 g/kg bodyweight per day (54). It remains unknown whether increasing dietary protein intake alone may counteract anabolic resistance (55-59). It might not be ageing itself, but accompanied chronic inflammation and especially reduced physical activity levels that cause anabolic resistance (60). Therefore, increasing dietary protein intake combined with RE is more likely to result in increased muscle mass and strength in older adults (61-65).

In addition to muscle and immune-metabolic health, gut health plays an important role in ageing, especially through the influence of gut microbiota on immunity. Gut microbial composition is influenced by genetics and non-genetic factors such as geographic location,

exercise, nutrition, disease, and medication (66, 67). Certain gut microbiota profiles are associated with healthy ageing (68) and diet can alter the gut microbiome in the long term (69). Increased fibre and prebiotics intake improve microbial composition and short-chain fatty acid (SCFA) production (69-71). One prebiotic of interest is GOS: carbohydrates produced from lactose, with various lengths and linkages (72). GOS enter the large intestine intact, where they serve as substrates for bacterial fermentation, producing SCFAs (73, 74). SCFAs are absorbed via the portal vein (75) and play a role in many physiological functions, ranging from energy balance and metabolism to the modulation of adipose tissue, immunity, liver tissue and skeletal muscle function (69, 76). Double-blind, placebo-controlled studies with various doses of GOS show increased gut *Bifidobacteria* count in healthy older adults (77-79), overweight adults (80) and patients with Diabetes Mellitus type 2 (81). Several specific strains of *Bifidobacteria* have been associated with positive immune and disease outcomes, potentially via the production of SCFAs, other metabolites or by improving gut barrier function (82, 83). In support, lower prevalence of *Bifidobacteria* has been associated with older age (84) and a number of diseases (82, 83). However, the health implications of increasing *Bifidobacterial* presence are less well-established, with some (77, 78, 80), but not all (77, 81, 85, 86) studies showing improved immune-metabolic profiles. Additionally, the potential immunomodulation via gut microbial alteration by GOS or fibre in general (87) might also work synergistically with protein and exercise, as specific gut bacteria, such as *Bifidobacteria*, may have anti-inflammatory effects (88) and inflammation could be an underlying factor for anabolic resistance (88, 89).

Beyond the health outcomes of specific physiological systems, overall markers of biological age have been developed in the past decade to be predictive of health decline during ageing. However, no gold standard exists yet for biological age (90). A novel biomarker indicating immune and metabolic health, the MetaboHealth score, was recently generated from blood-based metabolomics data in 44.000 people. The score includes lipids, lipoprotein particles, fatty acids, lactate, histidine, branched chain amino acids, phenylalanine, acetoacetate, albumin and glycoprotein acetyls and predicts age-related disease, frailty and mortality in prospective studies (91-93). A previous combined lifestyle intervention in older adults demonstrated the beneficial metabolomic response (94, 95), but it remains to be established to what extent the MetaboHealth score can be used as a response marker in interventions.

Considering the above, exercise and dietary components have been shown to improve various health parameters relevant in older age, but effects on domains of ageing in different groups of older adults needs to be studied. It is important to note that especially in older adults, inter-individual variability in health status and physiology is present between individuals (44, 96-98). Understanding this variability is essential to better predict intervention responses. The VOILA intervention is therefore applied to different groups of mobility and metabolically compromised older adults (≥60 years of age). We will select these groups primarily on the basis of walking ability, recent total knee replacement (TKR) and MetaboHealth score to

include groups of older individuals who are at risk of fast biological ageing for different reasons, who would likely benefit from lifestyle interventions.

The intervention consists of optimized combined resistance exercise and nutritional components to improve outcomes in three major ageing related domains: muscle, immune-metabolic and gut health. The goal is to study the effect sizes on these ageing-related health domains in mobility and metabolically at-risk groups as well as the response heterogeneity to this intervention and the potential underlying actors.

METHODS

Aim

The VOILA intervention study aims to improve different health domains underlying biological ageing by a combined nutrition and RE intervention in three groups of community-dwelling older adults with varying degrees of compromised mobility or metabolism (≥60 years of age) and to measure the extent to which these groups respond. Three major ageing related domains will be studied: immune-metabolic health, gut-microbiome composition, muscle mass and physical functioning. Additionally, we aim to explore the factors that are responsible for the heterogeneity in responsiveness in these groups, as well as on an individual level. An additional aim is to investigate the differences between baseline variables of the health-compromised groups compared to the reference group of active "healthy" older adults and assess the extent to which the VOILA intervention can diminish this difference. The objectives are as follows:

1. Primary Objective: To quantify the effect of a state-of-the-art multimodal intervention in individuals aged ≥60, segmented by metabolic health status or mobility impairment on 1) muscle mass (appendicular lean mass (ALM)) and muscle function (5-times chair stand test (5CST)); 2) the gut microbiome composition (*Bifodobacteria);* and 3) immune-metabolic health (fasting blood glucose (GLC), glycoprotein acetyls (GlycA) and very low-density lipoprotein diameter (VLDL-D), within each segment.

2. Secondary Objective: To determine to what extent each intervention segment compares to the active reference group, regarding muscle mass and muscle function, gut microbiome, and immune-metabolic health (using the same main parameters as the primary objective).

3. Exploratory Objectives: To obtain insight in the heterogeneity of responsiveness, including the factors that may explain such heterogeneity, by comparing the effect on all primary outcomes (muscle/functional, gut microbiome and immune-metabolic markers) both in individuals within the different segments and the complete sample. Additionally, in the future we want to include comparisons of genetic and epigenetic variation with existing datasets of other lifestyle intervention studies.

Additional to these objectives, a second set of objectives includes the same 3 objectives but with the secondary outcome parameters: short physical performance battery (SPPB), timed

up-and-go test (TUG), 6-minute walking test (6MWT), 1-repetition maximums (1RM) as measured on exercise machines (muscle function), Dual X-ray Absorptiometry (DEXA) whole body and regional lean mass and fat mass (body composition), Glycated Hemoglobin (HbA1C), insulin, blood pressure (immune-metabolic health); and muscle cross-sectional area for TKR group.

Design and setting

This exploratory study is a 12-week combined exercise and nutritional intervention study (Figure 1) in the Netherlands (ClinicalTrials.gov Identifier: NCT05354310. Registered on April 29th, 2022). The intervention groups are: a metabolically compromised group, a compromised mobility group, and a specific patient group who recently received TKR. Additionally, there is a reference group of active, healthiest older adults. A separate control group receiving usual care will be included for the TKR group.

All groups will be measured at both baseline and at 12 weeks, except the active reference group, who will only be measured at baseline. The reference group is only used as a reference representing the most active and healthy older adults in our society, i.e. those ageing relatively successfully. The TKR control group is necessary because several outcome improvements are expected regardless of the intervention, due to the nature of recovery after surgery.

The study will take place at three main study sites at universities and university hospitals of Leiden (LUMC), Maastricht (MUMC+) and Wageningen (WUR). RE training will take place at the study centers, local gyms, and health centres under study supervision. The active reference group and metabolically compromised group will be recruited mainly from the vicinity of Leiden, the mobility compromised group near Wageningen, and the TKR patients from around Maastricht (the province of Limburg). The measurements and intervention activities will be organised to suit the local facilities available, but will follow standardised protocols created through collaboration of the three centres. All study procedures have been approved by the medical ethics committee (MEC) of LUMC (NL76879.058.21).

We aim to recruit fifty individuals for each of the three intervention groups and active reference group, and twenty for the TKR control group. Men and women will be included in approximately equal numbers, aiming for no more than 20% difference between the numbers of men and women within each group.

Study Population

Participants will be selected for the groups on the basis of physical functioning-related aspects (mobility compromised and TKR groups), metabolic profiles (metabolically compromised group) and physical activity and metabolic health (active reference group). Other criteria were chosen for study safety and feasibility. Only the participants for the TKR

groups will be randomized into the intervention and control group with a ratio of 5:2. TABLE 1 shows all inclusion and exclusion criteria.

Sample size

The appropriate sample size was calculated to pick up within-group changes for each intervention group, for each of the primary parameters. GPower 3.1.9.4 was used to estimate the number of participants needed for a difference between two dependent means, with a power of 0.80 and a one-sized alpha of 0.05. For all calculations mean and standard deviation (SD) of change were used when possible. If these were not available, pre and post means and SDs and a correlation coefficient from other studies were used. A dropout rate of 15% is expected and taken into account in the final sample size (103).

Based on interventions with protein and vitamin D, RE, and physiotherapy treatments respectively, physical function as measured by 5CST would require n=22 in the mobility compromised group (104), n=12 in the metabolically compromised group (105), and n=35 in the total knee replacement (TKR) group (106, 107).

Muscle mass, as measured by ALM, is estimated to require n=25 in the mobility compromised group (108), n=24 in the metabolically compromised group (109, 110), and n=55 in the TKR group (111). This is based on interventions with protein, protein and RE, or elastic-band resistance training respectively.

TABLE 1. VOILA general inclusion and exclusion criteria, and additional criteria per group. *Not applicable to the active reference group. BMI: Body mass index; COPD: chronic obstructive pulmonary disorder; LLS: Leiden longevity study; SQUASH: Short Questionnaire to Assess Health-enhancing physical activity

Inclusion criteria	Exclusion criteria
General, all groups	**General, all groups**
Age ≥60 years	Already using prebiotic fibres*
BMI 18.5-35.0 kg/m^2	Use of laxatives that interfere with outcomes, as determined by study medical doctor
Able to give written informed consent	Not willing to stop using dietary supplements in high doses (high doses: 0.10*Upper Level or more)*
Community-dwelling	Following a structured, intense exercise programme (currently or in the last year)*
MMSE of ≥24 points	Allergic, intolerant or hypersensitive to milk/lactose (self-reported)
Able to follow the study protocol	Dietary restrictions on milk/lactose/prebiotics/vitamin D/calcium consumption
Active reference group	Abnormal hepatic or renal laboratory parameters (estimated glomerular filtration rate <30 ml/min/1.73 m^2 (screening) or contra-indication by treating medical practitioner (data from hospital) (52)
Meeting the Dutch physical activity guidelines (i.e. partaking in at least moderate exercise for at least 30 minutes 5 days per week) as determined by the SQUASH questionnaire **(100)**.	Diagnosis of disorders/diseases in which a high protein intake can be harmful, such as renal impairment or failure, liver disease, or diabetes

MetaboHealth (91) Score below the threshold for being considered metabolically-compromised (i.e. the bottom half scoring participants ≥60 years old from the LLS cohort).	associated with nephropathy (treating medical practitioner has the decisive voice). Diseases, conditions or disorders which may affect the ability to follow the study protocol and which cannot be overcome with help of a caregiver: Uncontrolled hypertension (blood pressure above 160/100 mmHg) Unstable angina Uncontrolled dysrhythmias Recent history of congestive heart failure that has not been evaluated and effectively treated Severe stenotic or regurgitant valvular disease Hypertrophic cardiomyopathy (all above: (99)) COPD (stage II/III/VI), Peripheral artery disease (or: Fontaine III or IV), Previously known cardiac abnormalities for which high-intensity exercise is contra-indicated (based on exercise ECG as determined by cardiologist) Surgical intervention in past 12 weeks for which high-intensity exercise is contra-indicated (except for TKR group) Unintentional weight loss of ≥3kg in the last 6 months Active cancer / chemotherapy or radiotherapy / end-stage malignancy in the last year Uncontrolled diabetes mellitus Cognitive impairments (MMSE <24) Recent (<3 months prior to intervention) immobilisation for >1 week (except for the TKR groups) Psychiatric or behavioural problems Use of unstable thyroid medication (i.e., changes in the last 3 months), immunosuppressive drugs (e.g. prednisone, methotrexate, biologicals (TNF-alpha antagonists)) Crohn's disease or ulcerative colitis, ileostomy or colonostomy Any other medical condition that may interfere with the safety of the participants during training or any condition that interferes with assessment of the outcome parameters, in the investigators' judgement Current participation in other scientific research that conflicts with this study (e.g. intervention studies or weight management studies) Not signed up to a general practitioner No permission to request information from the general practitioner/ treating specialist(s) about medical history, medication use.
Mobility compromised group People able to walk but needing walking aids to walk anywhere outside their house and/or attaining a five-times chair stand test time ≥15 seconds **(16, 101, 102)**.	
Metabolically compromised group MetaboHealth score in the top half scoring participants ≥60 years old either from the LLS cohort or the general population.	
Specific for TKR groups Patients who have recently undergone elective knee replacement surgery (baseline testing starts ~6 weeks after surgery)	**Specific for TKR groups** Osteoarthritis of the knee secondary to septic arthritis, osteonecrosis, fracture, osteochondritis dissecans, or malignant processes Collagen disorders, e.g. Marfan and Ehler-Danlos syndrome

Fecal bifidobacterial count is estimated to require n=5 participants (77) in the mobility compromised group, and n=15 in the metabolically comprised group (80), based on studies with GOS.

Immune-metabolic outcomes in the mobility compromised group are estimated to require n=23 for GlycA and n=38 for VLDL-D based on a study in which participants increased their physical activity level (112). The estimated sample size for the metabolically compromised group is n=56 based on GLC, n=31 based on GlycA and n=54 for VLDL-D readings respectively, based on a study in which energy intake was reduced and energy expenditure was increased through physical activity (94).

Taking the above into account we chose to aim for a sample size of n=50 for every intervention group and the reference group. All calculated sample sizes are well below n=50, except for the estimation of ALM in TKR, and some metabolic markers in the metabolically compromised group. However, the studies used for those calculations applied mild interventions. Hence, we expect the intervention effects in the VOILA study to be larger due to the application of a high intensity exercise program in combination with a nutritional intervention. The choice to aim for a larger sample size than needed for most of the primary outcome measures will facilitate the exploration of heterogeneity in the adaptive responses to the intervention between individuals, and the different biological factors that may explain such heterogeneity.

Additional calculations were performed taking into account expected changes in the TKR control group (i.e., standard care) (111); n=13 (with dropout) patients should be included in the control group to detect differential changes in physical functioning, whereas n=25 patients should be included in the control group to detect differential changes in ALM. However, we expect the increase in lean mass to be greater with the proposed combined RE and nutritional intervention than in the study by Liao et al (111). Therefore, we aim to include n=20 patients in the TKR control group, receiving standard care only.

Participant timeline

Once identified as eligible (see below), all study participants will undergo baseline measurements at the university/university medical centre, at the training location/gym and at home up to 2 weeks before starting the intervention period. These measurements will be divided over a total of 2-4 visits depending on the logistics of each centre.

Within one week of finishing baseline measurements, the 12-week nutrition and exercise intervention will begin for the three intervention groups. These groups will undergo supervised resistance exercise training, 3 times per week, and consume nutrition supplements twice per day. Additionally, participants will choose 2 nutrition goals to work on. During this 12-week period, the TKR control group will not receive any intervention, and only receive standard care from their own health-care providers. At the end of the 12-week period, the baseline measurements will be repeated, except for the baseline questionnaire and

familiarisation session. Materials for the at-home measurements (i.e. activity and dietary measurements, questionnaires, and fecal sample) will be provided at the training locations or delivered to the participant's home (TKR control group), to be measured during the final week of the 12-week period. The final measurements will take place in the week following the end of the 12-week period, and will be spread over 1-3 measurement days, depending on the logistics of the study centre.

Study procedures and data collection

Recruitment, consent and inclusion

Recruitment strategies of the study will differ per study group. The metabolically compromised and active reference groups will be recruited firstly from the Leiden Longevity Study (LLS) (113). If they consent, their previously collected immune-metabolic parameters will be used to determine if they are suitable for the metabolically compromised group, or active reference group. Additionally, participants for these groups will be recruited from the general public via advertisements. The mobility compromised intervention group will be recruited via the university research volunteer database, as well as through local advertisements. The patients undergoing elective total knee arthroplasty will be recruited via the outpatient orthopaedic clinic at Maastricht University Medical Center (MUMC+) or other hospitals in the surrounding areas, physiotherapy practices, and advertisements.

Potential participants will contact or be contacted by the researchers. The full written study information and an example informed consent (IC) form will be sent to those who are interested (group specific), and they will be given at least 1 week to consider participation. They will be invited for either an on-site information session or a telephone call during which the entire experimental trial will be explained and questions will be answered. Potential participants who want to join will then sign the IC form, before any measurements can be taken (Supplement 1).

Screening

After both the study participant and the local researcher sign the IC form, participants will be assigned a unique study number in the electronic participant database and continue to the screening. The screening questionnaire created for this study contains questions on living situation, age, mobility status, disease details and comorbidities, medication (type, frequency, duration, dose), recent vaccination status and types, Corona Virus Disease-19 history, involvement in other research, weight loss, allergies and diets, nutritional supplement and prebiotic use, following exercise programmes and having a general practitioner (Dutch questionnaire available upon request). FRAIL questions (114), and Mini Nutritional Assessment short version questions are also included(115). Additionally, participants will complete the Short QUestionnaire to ASsess Health-enhancing physical activity (SQUASH), a validated questionnaire including duration, intensity, and type of physical activities, to

assess adherence to the Dutch guidelines for physical activity (100). The MMSE will be applied to exclude participants with cognitive problems, using a cut-off of ≥24 (116).

For participant safety, kidney function will be assessed by calculating the estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration prediction equation. This calculation is based on age, sex, and serum creatinine levels (117). Therefore, a non-fasted blood sample will be drawn via venepuncture and levels determined locally within 24 hours. Participants with an eGFR below 30ml/min/m^2 will be excluded.

The 5CST (mobility compromised group only) will be carried out in the way described in the SPPB (118). Height, weight and blood pressure will be carried out as described in "Physical functioning and muscle strength".

Study measurement procedures

All study sites will follow the same standard operating procedures and biweekly meetings are held for alignment. For an overview of the measurements, see TABLE 2.

TABLE 2. **Overview of VOILA outcomes and collection methods per timepoint**. [*]Reference group does not have any measurements after baseline; [a]Mobility compromised group only; [b]Total knee replacement groups only; [c]Metabolically compromised and reference group only; [1]Measured in week 12. 1H NMR: Proton Nuclear Magnetic Resonance; 25(OH)D: 25-hydroxyvitamin D; 5CST: 5-times Chair Stand Test; 6MWT: 6-Minute Walking Test; ALM: Appendicular Lean Mass; CT: Computed Tomography; DEXA: Dual X-ray Absorptiometry; DNA: DeoxyriboNucleic Acid; GSRS: Gastrointestinal Symptom Rating Scale; MMSE: Mini Mental-State Examination; RNA: RiboNucleic Acid; SQUASH: Short QUestionnaire to ASsess Health enhancing physical activity; SF-36: Short Form (36) Health Survey; SF-SarQol: Short Form; Sarcopenia and Quality of life; SPPB: Short Physical Performance Battery; TUG: Timed Up-and-Go test; WOMAC: Western Ontario and McMaster Universities Arthritis Index

Outcome	Collection method	Screening	Baseline[*]	Intervention		Endline
				Week 4 & 8	Week 6	
Primary study parameters						
Muscle mass	ALM in kg, DEXA		✓			✓
Muscle function	5CST in seconds	✓[a]	✓			✓
Gut microbiota	*Bifidobacteria* (stool sample), in log10 cells per gram dried feces		✓			✓[1]
Immune-metabolic health	Fasting blood glucose in mmol/L, glycoprotein acetyls in mmol/L very low-density lipoprotein diameter in nm (from Nightingale 1H NMR platform)		✓			✓

Secondary parameters					
Muscle function	SPPB, TUG test, 6MWT		✓		✓
	1 repetition maximum test as measured on exercise machines		✓	✓	✓
Body composition	Whole body and regional lean and fat mass, DEXA		✓		✓
Immune-metabolic health	Fasting haemoglobin A1c, Fasting insulin,		✓		✓
	Blood pressure (systolic, diastolic)	✓	✓		✓
Muscle mass post-surgery	single-slice CT-scans midway the upper legs		✓[b]		✓[b]
Physical functioning post-surgery	WOMAC		✓[b]		✓[b]
Other study parameters					
Physical activity	7-day accelerometry (Actigraph)		✓		✓[1]
	SQUASH questionnaire	✓			
Hand grip strength	JAMAR Dynamometer		✓		✓
Anthropometrics	Height	✓	✓		✓
	Bodyweight	✓	✓		✓
	Waist circumference		✓		✓
Questionnaires					
Screening questionnaire	Demographics, medical history, supplement use, diet, medication, oral health	✓			
Baseline questionnaire	Education, alcohol intake, smoking, mini-nutritional assessment, FRAIL score		✓		
Gut health	Bristol Stool Chart		✓		✓[1]
	GSRS		✓		✓[1]
Sleep quality	Pittsburgh Sleep Quality Index questionnaire		✓		✓[1]
General quality of life	SF-36		✓		✓[1]

Muscle related quality of life	SF-SarQol		✓		✓[1]
Vaccination status	Sars-Cov-2 vaccination status, recent vaccinations (<1y)	✓			
Global cognition	MMSE	✓			
Nutrient and food intake	3-day pre-structured dietary records on paper		✓		✓[1]
Dutch Healthy Diet Index adherence	Digital EETSCORE		✓	✓	✓
	Biological measurements and scores				
Gut microbiota composition and function	shotgun metagenomics in fecal samples		✓		✓[1]
Vitamin D status	Fasted serum 25(OH)D levels		✓		✓
Kidney health	Estimated Glomerular Filtration rate from creatinine	✓			
Biological age	MetaboHealth Score (from Nightingale 1H NMR in serum)	✓[c]	✓		✓
	MetaboAge from Nightingale 1H NMR in serum		✓		✓
Cardiometabolic risk	Framingham Risk Score		✓		✓
Intervention response predictors and measures of response heterogeneity	Algorithms will be created with Nightingale platform metabolites, predicting relevant primary and secondary endpoints		✓		✓
Intervention responses in RNA expression and DNA methylation, and the influence of genetic variation on the response heterogeneity	In reference and metabolically compromised groups for comparison with other lifestyle interventions in the future.		✓[c]		✓[c]

Questionnaires

Health related quality of life will be assessed with the short-form health survey 36 (SF-36) (119), a measure of functional health and wellbeing with good reliability in community-dwelling older adults (120). Specific muscle-related quality of life will be measured by the short version Sarcopenia Quality of Life (SF-SarQoL) questionnaire (121), which was created to measure sarcopenia-related quality of life (122, 123).

Sleep quality over the past month will be measured with the Pittsburgh Sleep Quality Index (124). It measures several aspects of sleep quality (125, 126) and has been validated in older adults (124, 127).

Gastrointestinal health will be measured by the Gastrointestinal Symptom Rating Scale (GSRS) questionnaire, which evaluates a wide range of gastrointestinal symptoms by letting people rate the severity of symptoms over the past week and is sensitive to change (128). It consists of 15 questions covering 5 common symptom clusters (abdominal pain, reflux, indigestion, constipation and diarrhoea) on a 7-point Likert scale (129).

In the TKR groups, the Western Ontario and McMaster Universities Arthritis Index (WOMAC) will be applied. The WOMAC-index is specifically developed for patients with knee or hip osteoarthritis and represents the gold standard to assess functional outcome following TKR. It consists of three domains that assess pain, stiffness and physical functioning (130).

Physical activity

At baseline and during week 12, physical activity levels will be assessed with a three-axial accelerometer (Actigraph GT3x-BT) with a sampling rate of 30Hz, which will be worn on the waist for 7 days during waking hours (131, 132). In parallel to wearing the accelerometer, general physical activity will be recorded in an activity diary in order to account for potential extreme values recorded by the accelerometer.

Blood measurements

All baseline and endline blood samples will be collected in a fasted state via venepuncture. Whole blood will be collected in an EDTA tube for the measurement of HbA1c, locally at each centre. Serum samples will be collected in serum gel tubes, processed locally, and aliquoted and stored at -80C. These serum aliquots will be used to assess Vitamin 25(OH)D levels and Nightingale metabolomics at the end of the study. Plasma samples will be collected in EDTA tubes and processed locally. Plasma as well as buffy coat will be aliquoted and frozen at -80C. At the end of the study insulin levels will be determined in plasma and deoxyribonucleic acid (DNA) extracted from the buffy coat. Lastly, blood will be collected into PAXgene tubes, processed, and stored at -20 locally for later mRNA extraction.

Biological age and metabolic health

The primary immune-metabolic read-outs of fasting GLC, GlycA and VLDL-D have been chosen for their association with three aspects of metabolic health; blood glucose homeostasis, inflammation and lipid profiles, respectively (133-135). These will be measured as part of the high-throughput Proton NMR metabolomics platform Nightingale Health (136). Thirty-seven of the 250 measured biomarkers are clinically validated, which include GLC and GlycA. The MetaboHealth score will be calculated from 14 of the Nightingale biomarkers: total lipids in chylomicrons and extremely large VLDL, total lipids in small HDL, mean diameter for VLDL particles, ratio of polyunsaturated fatty acids to total fatty acids (%), GLC, lactate, histidine, isoleucine, leucine, valine, phenylalanine, acetoacetate, albumin and glycoprotein acetyls. The MetaboHelath score predicts 5- and 10-year mortality as well as several other health outcomes(91).

Other metabolomics-based scores will be investigated for exploratory studies into baseline and response heterogeneity, such as the MetaboAge score. MetaboAge is associated with chronological age and is calculated using 56 measurements of the Nightingale platform alongside total cholesterol, HbA1c, fasted insulin level, systolic and diastolic blood pressure (137). Additionally, the FRS score will be investigated, as it predicts cardiovascular disease by using metabolic data (total cholesterol, HDL cholesterol), blood pressure, current smoking, diabetes status, antihypertensive medication use, age, sex and systolic blood pressure (138).

Fecal measurements

Fecal samples are collected by the participants at home at a chosen time in the baseline/endline week with the materials provided. Participants will be provided with a cooling element (for cooled-transport of the fecal samples), which they will freeze >24 hours before sample collection in their home freezer (-20C). With a “feces catchers” (Tag Hemi) stool is caught, and participants are instructed to fill two-thirds of each of two 3ml tubes (Micronic MP52961, NBS Scientific Holding BV) using small spatulas (BIDFOOD 231-0416 or VWR International BV 848930). The tubes will then be immediately stored in the frozen cooling element (SARSTEDT 951123) in their freezer. Within one week, the tubes will be transported, via the cooling element placed inside a styrofoam container, to the university and stored at -80C. Participants will complete a short questionnaire at the time of stool collection, containing the Bristol stool chart on stool consistency (139), date and time of defecation, storage time, recent antibiotic use (<12 months), probiotic use (<3 days), current laxative use, milk/lactose tolerance and travel abroad (<1 month). Fecal *Bifidobacteria* numbers will be used as main gut outcome (78-80, 140). Additionally, when all data is collected, additional analyses (shotgun metagenomics (141)) will be carried out to determine the microbiota composition, gene diversity and functional pathways.

Anthropometrics

Height (to nearest cm; SECA stadiometer) and body weight (to nearest 0.1 kg; on SECA or Welch Allyn – HOM599) will be measured with light clothing on and used to determine body mass index (weight/m^2).

Waist circumference will be measured using non-stretching measuring tape, midway between the superior iliac crest and lowest rib in standing position with feet ~30cm apart, on exhalation and recorded twice. The mean of the two measurements will be rounded to 0.5 cm or repeated if the difference is >1cm (142).

Blood pressure will be recorded 3 times consecutively on the right arm once the participant has been seated with uncrossed legs for at least 10 minutes, with a minute rest in between measurements (OMRON HEM-907 or Welch Allyn – ProBP3400). If systolic measures differ >12 mmHg, or diastolic >10 mmHg, one (if only the first is much different) or three extra measurements are taken.

Physical functioning and muscle strength

Several aspects of physical performance will be measured. The 5CST, the Balance Test and the Gait Speed test together make up the SPPB, measuring lower extremity function (143). The total SPPB score has been shown to predict disability and mortality (118).

The TUG test will be applied to measure basic functional mobility (107, 144). During this test, the amount of time needed for the participant to rise from an arm chair, walk 3 meters and return to back to the chair in the seated position, is recorded (107, 144, 145).

In the 6MWT two cones are placed 30 metres apart (Wageningen: tape crosses 14 meters apart) and participants walk around these. Using walking aids or sitting down to rest is allowed. Total distance is measured to the nearest metre after 6 minutes (146).

Maximal handgrip strength will be measured with a handheld dynamometer (JAMAR hydraulic dynamometer)(147). Participants are seated on a chair without armrest, holding their elbow at 90, separate from their body. The dynamometer is set so that the middle knuckle of the middle finger is at a 90° angle around the dynamometer. Per hand, three maximal effort measurements will be taken with at least 30 seconds rest in between, alternating between left and right hands. Participants are encouraged verbally. The maximum of all 6 repetitions will be used. Handgrip strength is an indicator of overall health as it has predictive validity for mobility, functional status, cognitive decline and mortality (148).

Muscle strength will be measured by 1RM tests on weightlifting machines (leg press, leg extension, chest press, horizontal row, shoulder press and vertical pull) (149). The 1RM testing procedure has been shown to be valid, reliable, and safe in older adults (149). 1RM strength determines the maximum amount of weight that can be lifted in a single repetition. Before the baseline assessment of 1RM strength, participants will be familiarized with the

exercise equipment, ensuring proper lifting technique. An estimation of 1RM will be obtained using the multiple-repetitions testing procedure and Brzycki formula(150). After warming up, weight is increased until a weight is reached that participants can move ≤6 times. In a second session (at least 2 days after the estimation), the actual 1RM will be determined as previously described (149).

Muscle mass and body composition parameters

DEXA whole body scan (GE Lunar Prodigy advance, Hologic Horizon or Hologic Discovery A) will be used to measure ALM as a measure of muscle mass (151). Participants will be fasted and wearing light clothing. Concomitantly, whole body lean mass, whole body and regional fat mass and bone mineral density will be recorded in the same scan using the systems' software package.

In the TKR group, CT-scans will be additionally performed to assess thigh muscle cross-sectional area in both the operated and non-operated leg simultaneously, to compare legs. Participants will undergo a CT scan (IDT 8000; Philips Medical Systems, Best Netherlands) at mid-thigh level of both legs performed at the radiology department of MUMC+ to determine (anatomical) cross sectional area of all thigh muscles, as well as the quadriceps femoris and hamstring muscles separately. While the participants are supine with their legs extended and their feet secured, a 3-mm thick axial image will be taken 15 cm proximal to the top of the patella. The scanning characteristics will be as follows: 120kV, 300mA, rotation time of 0.75 sec, and a field of view of 500 mm. The exact scanning position will be measured and marked for replication 12 weeks later.

Nutrition measurements

Nutritional intake will be measured with a structured 3-day food diary. Days are randomized over a week to obtain a good distribution of the different days. Participants are instructed to fill out as much detail as possible on type, brand and portion size in household measures. Food diaries are checked for completeness upon handing in, and standardized glass models of cups, glasses, and bowls are used for estimating portion sizes of protein-rich food if description is insufficient. Data will be processed in Compl-eat^{TM} (152)(Human Nutrition WUR, Wageningen, NL), which uses the Dutch Food Composition Database (153) and Dutch portion sizes (154).

Additionally, participants will fill in the EETSCORE digitally for monitoring and guidance. This is a 34-item food frequency questionnaire that tests adherence to the Dutch Healthy Diet guidelines (155, 156). An advice module is attached that informs people how well their intake matches the guidelines and informs them how they could be improved.

Intervention

Nutrition intervention

The aim of the nutritional intervention is to reach sufficient intake of dietary protein while improving compliance to the general guidelines for healthy eating later in life (Dutch Healthy Diet guidelines (157)). The intervention is aimed at a total protein intake of 1.5 grams per kg bodyweight per day, with a sufficient amount (20-25 g) at the three main meals and pre-sleep (158), and additional GOS fibre intake of 5.5 g per day on top of the regular intake. The targets will be reached with dietary counselling and a protein-rich supplement (FrieslandCampina, the Netherlands) which will be consumed twice a day; at breakfast and pre-sleep. Breakfast is chosen because protein intake of older adults is especially low during breakfast (54, 159). The pre-sleep moment is chosen to introduce an additional moment of protein ingestion as regular protein peaks throughout the day are proposed to be especially important for supporting muscle protein synthesis in older adults (52). The morning powder contains 25g whey protein, 5.5g Biotis™ GOS, 800 IU vitamin D, and 250 mg calcium per portion. The evening powder contains 20g whey protein and 116mg calcium per portion (160). The powders have a mild vanilla flavour.

At baseline, participants will meet with trained researchers/dieticians to discuss their diet and how to adapt this towards the Dutch Healthy Diet guidelines by choosing 2 goals based on the EETSCORE filled out digitally (161) which will show the participant how well they adhere to the different components of the Dutch Healthy Diet Index (156).

During the 12 weeks intervention, regular contact will take place at the training location to increase compliance. Adherence to the intervention powders will be checked and stimulated. At week 6 of the intervention, each individual participant of the intervention groups fills in the EETSCORE again to monitor change, support compliance and choose a new goal if desired. Compliance to the intake of ‘breakfast’ and ‘before sleep’ products will be checked with intake logs on which participants fill in their daily supplement intake.

Resistance exercise intervention

Participants will go to the training location three days per week for 12 weeks of whole body RE training under experienced supervision, with always at least 1 rest day in between training days. Seven resistance type exercises will be performed using regular weightlifting machines and weight-bearing exercises: leg press, leg extension, calf raises, chest press, horizontal row, shoulder press and vertical pull. Focus in sessions 1 and 3 of each week are on 1-2 seconds of concentric and 1-2 seconds of eccentric contractions as to specifically train for muscle mass and strength. Session 2 of each week focusses on speed of contraction as to specifically train for muscular power, by making concentric contractions quick and explosive. Before and after each session, a 5-10 min warm up / cooling down at low intensity will be performed on a cycle ergometer/stepper/cross-trainer or equivalent.

Intensity and workload of the program will gradually increase from 3 sets of 10-15 repetitions at 50-70% of one-repetition maximum (1RM) in week 1, to 4 sets of 8-12 repetitions at 70-80% of 1RM in week 4 and beyond for the leg press and leg extension. For the remaining exercises, 2 sets will be performed throughout the entire training period. All sets and exercises will be separated by 1.5 - 2.5 minutes of rest, resulting in a total session time of approximately 45-60 min.

All final sets of each exercise (excluding calf raises) will be performed until voluntary failure. Training intensity (weight lifted) will be adapted based on 1RM assessment in week 4 and 8, as well as when more than 10 repetitions can be performed in the final set until failure. Apart from these general characteristics, several group specific aspects apply for each intervention group separately. The metabolically compromised group will train in groups of 3-5 participants with at least one trainer. The mobility compromised group will start training in groups of 2 participants with one trainer in week 1-3, which can be increased to 2-4 participants per trainer from week 4 and beyond. The intervention group recovering from total knee replacement will have supervised training on top of standard care and/or any physiotherapy sessions that patients follow. Training will commence with one-on-one supervision in week 1-2, increasing to groups of 2-4 participants when possible in week 3-4 and beyond, depending on inclusion rate. Intensity, workload and progressiveness of the program will be designed on an individual patient level (i.e., taking post-surgery trainability and pain of the individual patient into account) and in consultation with the treating physician and physiotherapist when appropriate.

Statistical analysis

Both intention-to-treat, as well as per protocol analyses will be carried out. For the primary analysis, linear mixed models will be used to analyse within-group changes of the primary study parameters (5CST, ALM, *Bifidobacteria*, GLC, GlycA, VLDL-D). Individuals will be added as random factors. To study the intervention in the TKR group, the TKR intervention group will be modelled with control group (both fixed factors). Secondary analyses will compare the primary study parameters of each group to the active reference group values with linear models, using age and sex as covariates.

Data-driven exploratory analyses will be carried out to study heterogeneity in response within each group and in the total sample post-hoc. The within-group heterogeneity will be analysed by studying the changes in the primary outcomes for the concerning group, through methods such as Principal Component Analysis (PCA) or a penalized linear regression model. Using this approach we investigate which secondary/exploratory measurements associate the strongest with the primary outcome measurements, at baseline as well as when comparing the deltas imposed by the intervention study, i.e. to explore which factors play a role in the response heterogeneity. The heterogeneous changes between the different groups will be explored by the construction of a composite score consisting of all the primary outcomes. We

will use this, along with a PCA and regression approach to investigate the differences between the individuals at baseline and heterogeneity of the different intervention groups.

Future analyses may include the influence of genetic variation on the response and changes in mRNA expression and DNA methylation in comparison to other existing lifestyle studies in older adults.

Compliance and withdrawal

Training participation will be deemed as 'compliant' when 80% or more of the training sessions have been followed. An intake of 90% or more of the powders will be taken as 'compliant'. Participants can leave the study at any time for any reason if they wish to do so, without any consequences. The investigator can decide to withdraw a participant from the study for urgent medical reasons. Participants dropping out of the study will be asked for permission to contact them for inclusion in the endline measurements as to enable intention-to-treat analyses, if the reason for dropout does not limit the possibility for such post-measurements. Drop-outs are not replaced because the sample size calculations include an expected 15% dropout.

Logging, data collection and management

A data management plan is available at DMP online and can be shared upon reasonable request (162). All measurements are carried out by trained researchers according to the standard operating procedures created for this study. The privacy of the potential participants that are screened and the participants of the study will be protected according to the European Union General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation.

All necessary personal information collected material and study results will be related to the unique participant number. Measurements are entered directly into Castor when possible and include range checks when possible (163). Personal information such as name and address are encrypted. Measurements recorded on paper or measuring device are stored in locked locations at each university or at university network drives. Biological material will be stored locally. Data collected and biological materials will be stored for 20 years. If participants revoke their consent, the information stored up to that point in time will be kept, but no new data will be collected or stored. Biological materials will be destroyed. We will archive and save all data that underlie publications, including data collection, cleaning and analysis methods. All research data will be pseudonymized and brought together in a data warehouse, so that researchers of each participating centre have access to all generated research data.

Adverse events

All adverse events related to the study protocol (measurements and intervention) reported spontaneously by the participant or observed by the investigators will be recorded. Severe adverse events are reported to the coordinating investigator and MEC digitally within a week.

Oversight and monitoring

The coordinating investigator at LUMC is the sponsor of this investigator-initiated research. The principal investigators (PI) at LUMC, MUMC+ and WUR are responsible for the trial at each of the respective locations. Yearly updates are provided to the MEC. A data management plan was agreed upon beforehand and approved by the MEC and data steward at LUMC. Data managers at LUMC built and maintain the Castor database used at all locations. PIs and other researchers involved have met monthly to create the protocol, discuss progress and decide on issues and changes in the protocol. Protocol amendments need to be approved by the original MEC before implementation. New IC forms will be provided if necessary.

The study has been approved by the MEC as having a negligible risk, therefore no data safety monitoring board needs to be installed. A monitor is appointed by LUMC who checks data integrity at every location.

Reporting and dissemination

Results of the study will be made public through peer-reviewed international scientific journals, according to the Dutch "Centrale Committee on Research Involving Human Subjects" statement of publication policy and disclosed via their register.

DISCUSSION

This paper describes the design of the VOILA human intervention study that explores the effects of a healthy lifestyle intervention with nutrition and RE in selected groups of older adults at risk for adverse ageing outcomes. This intervention study has been designed to better understand the response in several groups of older adults with different health statuses to a lifestyle intervention, as well as the heterogeneity of response, with the response measured by physical function, muscle, immune-metabolic and gut health parameters and novel biomarkers of ageing.

The intervention was designed using a combination of lifestyle interventions likely to improve health by targeting multiple physiological systems relevant to ageing. The novelty in the approach of this study is represented by i) preselection of three segments of older adults based on their health status to allow exploration of heterogenous responses and ii) simultaneously measuring outcomes in three major ageing related domains including immune-metabolic health, gut-microbiome composition, and muscle mass/physical functioning.

This study will give us the opportunity to study the effect of the intervention on a range of markers of biological ageing possibly enabling improved and individual response monitoring of interventions in older adults. Currently, no gold standard biomarkers are available in human ageing research, neither for defining the rate of biological ageing nor for recording the response to interventions to slow down that rate. Despite the lack of consensus on the most informative biomarkers (164), it is largely (>80%) agreed upon that ageing is

heterogeneous, cannot be measured by only a single metric, and proceeds differently in different tissues. Furthermore, the type of intervention that received the highest agreement (91%) in terms of its potential to attenuate biological ageing was the lifestyle intervention (164), in line with our approach. Very few interventions combining nutrtion and exercise have been carried out in which biological age is measured, but exploratory first results mainly using epigenetic clocks look promising (165, 166). In the VOILA intervention we will largely focus on metabolomics and proteomics biomarkers of biological age in addition to the major outcomes of muscle, immune-metabolic, and gut health. The components of the composite score reflecting biological age, such as those in the MetaboHealth and proteomics scores, are biologically well understood and will allow mechanistic explorations into the response heterogeneity in VOILA.

Our VOILA intervention study has the potential to improve our current understanding of the positive changes induced by a healthy ageing lifestyle intervention in different subgroups of older adults with varying degrees of compromised mobility or metabolism. This allows for exploration of the heterogeneity in individual responses within and across the groups. Better understanding of this heterogeneity should enable further tailoring of intervention efforts towards specific needs of (subgroups of) older individuals as to maximize the beneficial health effects. The VOILA intervention study we report on here is embedded within the larger Dutch Society for Research on Ageing (DuSRA)-VOILA project, in which different disciplines of nine institutes and eight private partners collaborate (167). The overarching project aim is to improve the recording of the current and forecast the future biological age of the population aged ≥50 years and reinforce physiological reserves that help maintain functional ability. Data and samples from the intervention study we report on here will allow for interdisciplinary research within the DusRA-VOILA project to advance the field in new ways. At the time of writing, no ageing biomarkers have been approved for clinical application (90), but there is a need for biological age markers responsive to intervention (90, 168). With VOILA we plan to create biomarkers to predict intervention response, contributing one step towards preclinical or clinical use of biological age biomarkers.

In the intervention study, the main research questions focus on the three age-related outcome domains of muscle/physical function, immune-metabolic and gut health. Several additional measurements will allow for better phenotypic characterisation of individuals in exploring the heterogeneity in ageing and intervention response, including objective measurement of activity levels via accelerometry, and detailed 3-day food diaries from which intake of most nutrients can be extracted. Moreover, the questionnaires on gut complaints, sleep quality, as well as general and sarcopenia-related quality of life add information on patient characteristics potentially contributing to differences in outcomes, as well as measuring well-being which is a very relevant but often overlooked paradigm in intervention studies focusing on a single specific domain. Comprehensive blood samples are collected so that future analyses may include the influence of genetic variation on the response, and changes in

mRNA expression and DNA methylation in comparison to other existing lifestyle studies in older adults.

The VOILA intervention study is not a randomized controlled trial, as our aim is not to study the effectiveness of specific subparts of the intervention. In the within-group changes, no distinction can be made between the separate components of the intervention (RE, nutritional powders and their components, and dietary advice). Instead, we use a positive reference group consisting of a sample of the healthiest older adults based on activity level and immune-metabolic profile. The health of the intervention participants can be compared to the reference group to assess in which ways these groups differ at baseline, and to assess to what extent the outcomes of the health-compromised groups can shift towards the profile of the healthiest counterparts following the intervention. Of main importance is that the intervention groups all receive the same intervention and are measured according to the standardized protocol so we can study response heterogeneity both between and within groups.

This human lifestyle intervention is innovative in that we target domains hypothesized to underly overall biological ageing, as opposed to a single domain or disease as outcome. Additionally, this study will provide valuable insight into the heterogeneity in effect of a healthy diet and exercise intervention aimed at improving healthy ageing in various different groups of health compromised community-dwelling older adults.

Results will provide information on the usefulness of markers of biological ageing in an intervention study and pave the way to develop improved response biomarkers for interventions. Insights from this intervention study can be used as the basis to further our understanding of factors underlying the heterogeneous ageing process, to better tailor future preclinical interventions and to fuel novel measurements such as biological ageing markers and intervention response markers. The results of this VOILA intervention study will be relevant for improving health in older adults and recording and forecasting which interventions effectively improve biological ageing and wellbeing.

DECLARATIONS

Ethics approval and consent to participate

All procedures are performed in accordance with the World Medical Association Declaration of Helsinki and in compliance with relevant laws and institutional guidelines, including national and European privacy rights. Ethical approval was obtained from the Medical Research Ethical Committee Leiden Den Haag Delft, the Netherlands, on 20th October 2021 (registration: “P21.049” locally and “NL76879.058.21” nationally at the Central Committee on Research Involving Human Subjects). Additional amendments were approved on 27th May 2022, 21st July 2022, 11th November 2022, 21st July 2023 and 27th of February 2024. Written consent is obtained from all participants before starting.

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AUTHORS' CONTRIBUTIONS

ES, MB, LdG and LV created the study and obtained funding. CK, LV, LdG, ES, MB, SM, AM and JM designed and wrote the protocol and acquired ethical approval. LV, ES, PG and LdG are responsible for the overall study coordination and JM, AM and CK are running the day-to-day trial, recruitment and sample collection. CK drafted the manuscript and all authors revised it. All authors read and approved the final manuscript.

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