Ouderen op tijd in beweging studie

Older adults exercising on time

(February 2024)

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PROTOCOL SIGNATURE SHEET

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[redacted]	[redacted]	[redacted]

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
ADL	**Activities of daily living**
AE	**Adverse Event**
AET	**Aerobic Endurance Training**
AR	**Adverse Reaction**
BIA	**Bioelectrical Impedance Analysis**
CA	**Competent Authority**
CBT	**Core Body Temperature**
CCI	**Charlson comorbidity index**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CFS	**Clinical frailty scale**
Chronoactivity	**Timing of physical activity**
CV	**Curriculum Vitae**
CVD	**Cardiovascular Disease**
DLMO	**Dim Light Melatonin Onset**
DSMB	**Data Safety Monitoring Board**
ECG	**Electrocardiogram**
EMA	**Ecological Momentary Assessment**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
EQ-VAS	**EuroQol visual analogue scale**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
G8	**Geriatric-8**
HR	**Heart Rate**
HRV	**Heart rate variability**
IADL	**Instrumental activities of daily living**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**

IMPD	**Investigational Medicinal Product Dossier**
IMD	**Investigation with Medical Device**
IMDD	**Investigational Medical Device Dossier**
ISI	**Insomnia Severity Index**
IQR	**Interquartile Range**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
LUMC	**Leiden University Medical Center**
MCTQ	**Munich Chronotype Questionnaire**
MET	**Metabolic Equivalent of a Task**
Mg	**Miligravity**
MRI	**Magnetic Resonance Imaging**
PAI	**Physical Activity Index**
PBMC	**Peripheral Blood Mononuclear Cell**
PPG	**Photoplethysmography**
QOL	**Quality Of Life**
RET	**Resistance Exercise Training**
(S)AE	**(Serious) Adverse Event**
SCN	**Suprachiasmatic Nucleus**
SD	**Standard Deviation**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
SQUASH	**Short Questionnaire to Assess Health enhancing Physical Activity**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: There are indications based on epidemiological cohort studies and animal experiments that timing of physical activity (also referred as “chronoactivity”), irrespective of intensity, impacts health status and disease risk. Here, we hypothesize that timing of physical activity has a beneficial impact on insomnia symptoms and related circadian health parameters (e.g., metabolic, psychosocial) in older people.

Objective: Main: To examine the effect of physical activity timing on insomnia severity in older adults with self-reported sleep problems.

Additional: To examine the effect of physical activity timing on exploratory rhythmic parameters of biological clock function, physiology and metabolism, mental health, behavioural factors, and immune and cell signalling functions.

Study design: Randomised cross-over study.

Study population: 40 community dwelling older adults aged between 60 and 80 years old with long-lasting (≥3 months) self-reported sleep problems from Leiden and surrounding areas in close proximity to the study center.

Intervention: The intervention will comprise one sedentary period and two periods of increased physical activity with different daily patterns: 1) active morning; 2) active evening; with a duration of 14 days each. In both active intervention arms, participants will follow 8 coached outdoor physical exercise sessions (Vitality Club) comprising endurance and strength exercises. The training sessions will be held first either in the morning or evening (depending on the intervention arm) and will be one hour long. On days participants are not able to attend the Vitality Club, they will be asked to follow the ‘Active@Home’ program; a 1-hour training session of various moderate to vigorous activities. Since this will be a randomized cross-over study, all participants will follow the sedentary period as well as both exercise timing interventions consecutively with a 7-day wash-out period between all interventions.

Main study parameters/endpoints: The primary outcome is change in insomnia severity as measured by the Insomnia Severity Index (ISI). Secondary outcomes parameters include multiple components of objective sleep quality measured with the Withings Sleep Analyzer, tri-axial accelerometry and subjective sleep quality questions as well as circadian and diurnal patterns of melatonin, heart rate, heart rate variability, breathing rate, oxygen saturation, core body temperature, EMA, and objective emotional arousal and stress. Additionally, we will observe eating patterns (timing and overall intake). Finally, blood will be sampled at baseline and after each intervention and stored for measurements of metabolic and physiological functioning and expression of genes involved in the regulation of the biological clock.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Blood sampling, explanation of use of the measurement devices and the

assessment of participants well-being will be done by a trained nurse at the LUMC and at home to minimize burden for participants. During the complete study duration, participants will wear one wrist-worn wearable that is capable of measuring multiple physiological aspects in the participant. Moreover, participants are asked to perform three series of seven consecutive saliva swabs on set times on the last day of each intervention and they will be asked to rank their mood as well as log their meals multiple times a day. The risks of this study are considered minimal since this is a lifestyle intervention and only minimally invasive procedures are used (venous blood sample which may cause a small hematoma) that can be considered routine medical practice and are known to carry only minimal risks. The study may cause some burden since participants are required to change and monitor their daily routine for a longer period (42 non-consecutive days) of time.

1. INTRODUCTION AND RATIONALE

Essentially all biochemical, physiological and behavioural processes are orchestrated by circadian rhythms to respond to the 24-hour day/night cycle. Processes such as energy metabolism, hormone levels, sleep wake cycles, core body temperature are strongly governed by circadian rhythms.(1) Proper functioning according to the light/dark cycle of all bodily functions relies on the suprachiasmatic nucleus (SCN) also known as the central clock which is located directly behind the eyes in the anterior hypothalamus and primarily responds to light.(1) The SCN determines the phase of peripheral clocks through a variety of signalling relays.(2)

A good running circadian clock is associated with (healthy) longevity.(3) Yet, proper circadian organization (also referred as “circadian health”)(4) is threatened by our modern day 24-hour society. Shift work, social jetlag, increased exposure to blue-light emitting devices and globalization of a 24/7 society facilitated by advancing technology and social media, jeopardize a healthy biological rhythm.(4)

When internal circadian rhythms, which are normally approximately 24-hour cycles, are not synchronised with the environment or each other, disruption of circadian and diurnal rhythms can occur.(5) This ‘desynchrony’ occurs when behaviours such as physical activity, sleep, food intake, and exposure to light are not at an appropriate time relative to the central circadian clock.(5) Another cause of circadian misalignment or desynchrony is ageing. A substantial amount of studies shows the deterioration of the biological clock with increasing age (**Figure 1**).(1, 3, 6) Studies consistently show that experimental disruption or desynchronisation of circadian rhythms has detrimental effects on health and lifespan.(7, 8) Our decreasing circadian health may be partly responsible for the rising prevalence of sleep problems in (older) adults. In the Netherlands, 20% of all people of 12 years and older have self-reported sleep problems and even 28% of older adults aged 75 years and older.(9) Additionally, and perhaps through the pathway of poor sleep, clear associations of circadian misalignment are observed with cancer onset, cardiometabolic diseases such as obesity, diabetes, heart disease, and stroke, and neurologic disease such as depression, Parkinson disease, dementia, and sleep disorders.(4, 5, 10, 11)

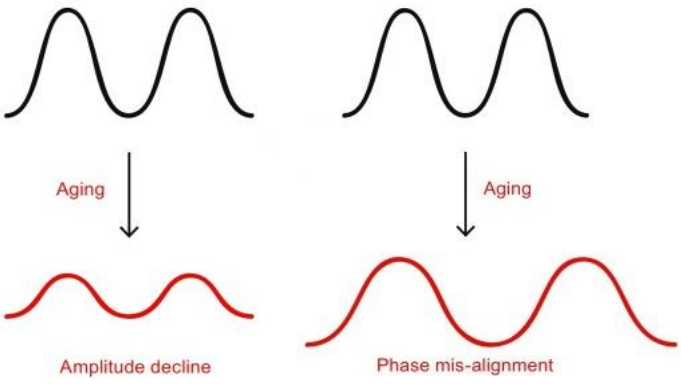

Figure 1. *Effect of ageing on circadian rhythms (*Liu *et al.*)(1)

‘Zeitgebers’, which are defined as regular cyclic environmental signals and which include food intake, light

exposure, and physical activity, can calibrate our circadian clock.(12, 13) Regular physical activity is known to positively affect the circadian clock and is associated with healthy ageing.(13, 14) Guidelines indicate the required intensity, frequency and duration of physical activity to maintain or achieve health. However, less is known about the best timing of physical activity. A growing field of research on 'chronobehaviour', (timing of lifestyle behaviours) already marks the importance of timing of food intake and light exposure.(4, 15-21) Yet, intervention studies on 'chronoactivity' (timing of physical activity) are largely lacking. Ideally, enriching current guidelines by adding the timing component of physical activity might improve these guidelines and facilitate for more effective physical activity and faster improvement of health outcomes such as recovering from a period of worse sleep.

Several studies in mice reported that exercise restores circadian heart rate and body temperature in mice with central circadian disruption.(22, 23) Additionally, studies in humans have revealed exercise stimulates the expression of core clock genes. Observational studies that specifically focussed on chronoactivity, demonstrate associations between morning physical activity and increased metabolic health,(24) increased cardiometabolic fitness,(25) and even an approximately 15% lower risk of incident atherosclerotic cardiovascular and cerebrovascular disease(26). Moreover, there are some intervention studies in this field.(27-29) However, their results are inconclusive. Multiple studies show the seemingly positive effect of morning physical activity on body composition and metabolic health.(29, 30) Contrarily, several studies show opposite findings indicating that evening exercise had positive effects on heart rate recovery, blood pressure, and blood glucose levels.(27, 28, 31). One of these even stated that morning physical activity had a deleterious effect on blood glucose levels in diabetic men.(27) This contrariety in existing literature could be partly explained by 1) different study outcomes, 2) different measurement methods and definitions of physical activity, and 3) small sample sizes and specific groups (e.g., diabetic men or menopausal women) in which the studies have been conducted. Nevertheless, these studies suggest that timing of physical activity serves as an important Zeitgeber, and changes the phase of the molecular clock, specifically in peripheral tissues.(22) Yet, despite the increasing interest and mounting evidence for chronoactivity, the optimal circadian timing of physical activity for circadian health remains unclear and little is known about the influence of chronoactivity in older adults which most frequently suffer from sleep problems.(9)

In view of the detrimental effects of circadian misalignment, the large group of older people suffering from sleep problems, and the seeming importance of chronoactivity, we will perform a randomised cross-over study that aims to uncover the effect of timing of physical activity on insomnia severity and related (circadian) health parameters in older adults with self-reported sleep problems.

2. OBJECTIVES

Primary Objective

To examine the effect of physical activity timing on insomnia severity in older adults with self-reported sleep problems.

Secondary Objective

To explore the effect of physical activity timing on rhythmic parameters of biological clock function, physiology and metabolism, mental health, behavioural factors, and immune and cell signalling functions.

3. STUDY DESIGN

We will perform a randomized two-armed cross-over study. Both groups will start with the sedentary period. Randomization will be performed at the moment of enrollment using a computer-based statistical program (depending on the intervention arm, participants will then undergo the active morning intervention or active evening intervention first, followed by the remaining intervention. There will be a total of two washout periods both lasting 7 days, one between the sedentary period and first intervention and one between the first and second intervention to prevent or minimize 'carry over' of previous intervention effect. The two interventions and the sedentary period will last 14 days each and contain a 'calibration period' (7 days) and a 'measurement week' (7 days). The former will serve as an adjustment period in which we hypothesize that our endpoints of interest will adjust to the new exercise regimen. After the calibration period, the measurement week starts in which all endpoints will be measured. Apart from the measurements, there are no differences in intervention between calibration and measurement week. The total study duration will be 8 weeks and the study will largely take place at the participants' home except for the training sessions (Vitality Clubs) that will take place at an outdoor sports facility in the municipality of Leiden and the study site visits which will take place in the LUMC. **Figure 3.1** shows a schematic overview of the study outline and **Appendix A** shows an example of the study schedule during the active morning intervention.

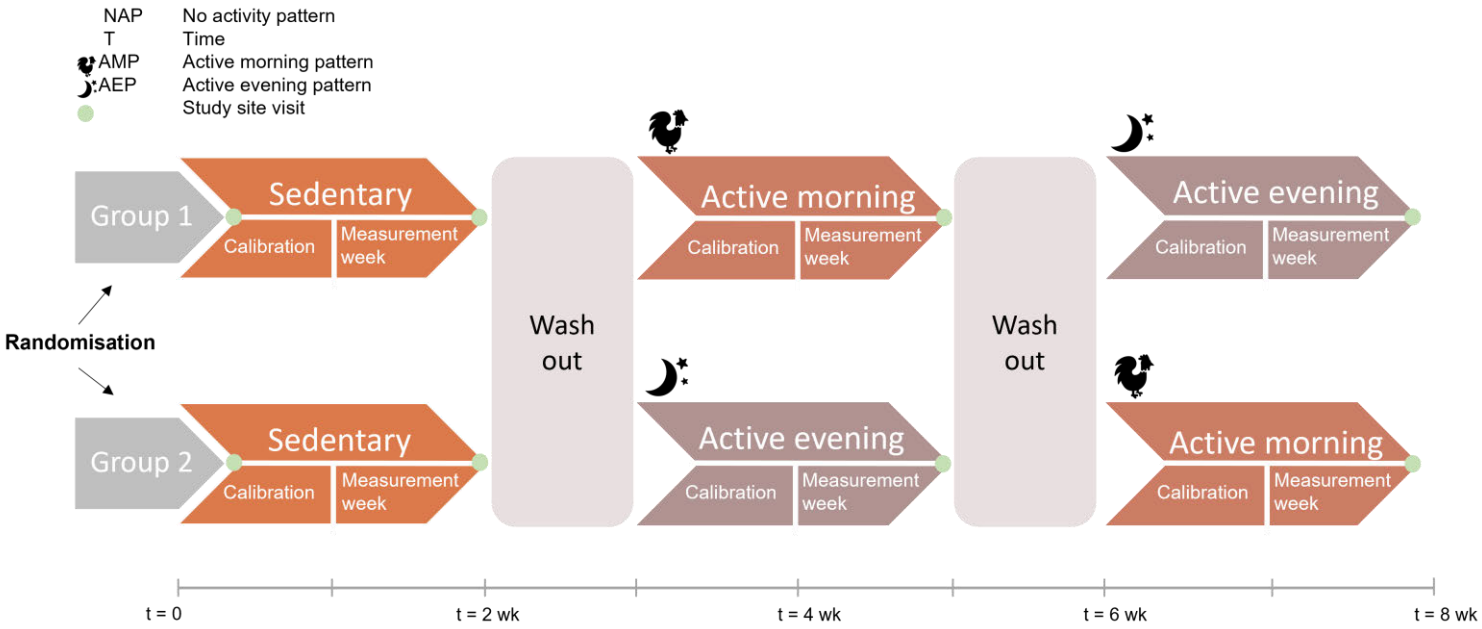

Figure 3.1. *Schematic overview of the study*

3.1 Design justification

A cross-over study allows the response of a participant to morning exercise to be contrasted with the same participant's response to evening physical activity and sedentary period. Removing inter-participant variation allows for more efficient and precise estimation than similar sized studies with parallel intervention groups.

As we study the effect of timing on change in insomnia severity and changes in the rhythm of multiple processes hypothesized to be altered rapidly through environmental cues, such as daylight changes over the season, a randomized cross-over study which is usually bound to short-term outcomes is the most suitable design for this study.

4. STUDY POPULATION

4.1 Population (base)

Dutch speaking older adults between 60 and 80 years old (male and female) from the general population in the Netherlands will be selected as participants for this study. Individuals who are interested in participating in the study based on the advertisements, will be screened by phone. During the first phone call questions will be asked about (i) personal data, (ii) presence of sleep problems, and (iii) availability during the study period via a screenings questionnaire. If the person seems eligible based on their answers to these questions, a PIF and IC will be sent to them by mail. A second phone call will be scheduled at least one week after the first phone call to address any questions related to the PIF. After receipt of verbal consent, during the second phone call, a screening at the study center will be planned. During the screening visit, which will take approximately 30 minutes, participants can give written informed consent. Thereafter, two non-fasted blood samples will be drawn (3.5 ml + 2 ml =5.5 ml), and two questionnaires will be filled out: (i) Insomnia Severity Index (ISI, to assess insomnia symptoms) and (ii) Munich chronotype Questionnaire (MCTQ, to assess chronotype). The latter will not be used as a screeningstool but is collected as a baseline characteristic. We will include participants scoring ≥10 on the ISI. Additionally, we believe that the possible outcomes of this study will be relevant for older adults with sleep problems since both ageing as well as sleep problems are closely linked to the circadian clock. Potential participants will be recruited through advertisements in newspapers and local radio. For practical reasons and since the 'Vitality Clubs' are located here, participants will be sought in Leiden and surroundings.

4.2 Inclusion criteria

In order to be eligible to participate in this study, all participants must meet all of the following criteria:

- Aged between 60 and 80 years old
- Retired
- Long lasting sleep problems (as assessed by a the screening questionnaire administered telephonically and by the ISI which will be filled out during the screening visit)
- Access to and ability to use a smart phone (Android or Apple)

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Currently employed or working
- Participation in any sort of fasting regimen (e.g. intermitted fasting or Ramadan)
- Experienced recent (<6 months) adverse life events (e.g., death of partner)
- Abnormal values in glucose metabolism, thyroid, liver or kidney function, or inflammation markers that after examination of the study doctor need immediate attention of a general practitioner or specialist.
- Diagnosed clinical depression
- Diagnosed neurodegenerative diseases (e.g. dementia or Parkinson's disease)
- Diagnosed sleep apnoea
- Diagnosed restless legs syndrome
- Use of beta-adrenergic blocking agents
- Use of sleep medication*
- Injuries or other severe physical conditions (such as active arthrosis) that inhibits physical activity
- Travelled across time zones one week prior to start of study

*If participants are willing to cease usage of sleep medication 2 weeks before baseline measurement until the end of the study, they can be included. An exception must be made for individuals with amitriptyline prescribes as sleep medication due to its side effects of discontinuing use.

For this study we want to use CTcue to facilitate the inclusion of patients.

4.4 Sample size calculation

The sample size was calculated for the primary outcome of mean change of insomnia symptoms severity from baseline and after completing the active morning vs. active evening measured by the ISI which is proven to be a well validated and sensitive questionnaire for mapping insomnia severity. All sample size calculations were based on statistical power of 0.80 and a 2-sided α of 0.05. The calculations were performed using GPower v.3.1 (Kiel University, Germany). To the best of our knowledge, there is no literature available on the effect size of physical activity timing on insomnia severity. Therefore, estimations were based on treatment effect size of guideline recommended physical activity on insomnia severity (measured by ISI) found in a previous randomized controlled trial(32). Assuming a standard deviation (SD) of 5.4, a total of 36 participants are required to detect a minimal difference of 2.6 points on the ISI. Considering a possible dropout rate of 10% during the study, the target sample size is 40 participants.

5. TREATMENT OF SUBJECTS

5.1 Investigational treatment

5.1.1 Active morning and active evening intervention

Participants will participate in three interventions consisting each of a 14-day physical activity regimen. The active morning and active evening intervention are equal in content but in the active morning group, participants will be instructed to be active between 10:00 and 11:00 hour in the morning and the active evening will be instructed to be active between 19:30 and 20:30 in the evening. Both groups will be instructed to refrain from any moderate to vigorous exercise for the rest of the day. Per intervention, a total of eight Vitality Club training session will be organised given by a trained sports coach or physical therapist specialized in geriatric physical therapy. These training sessions will be different in character (e.g., a game of volleyball and a circuit training) but will all contain a combination of strength and endurance training tailored for older adults. Literature is inconclusive whether it is more beneficial to participate in aerobic endurance training (AET) or resistance exercise training (RET). AET is more beneficial for body composition and endurance and RET is more beneficial for muscle mass and strength. Both types of exercises contribute to important components of QOL and healthy ageing and therefore we believe that it is most valuable/effective to compose a training regimen containing both AET and RET exercises.(34) During the weekends (Saturday and Sunday), there will not be a Vitality Club sessions. Instead, participants will be asked to perform a ‘relative rest’ session. On a ‘relative rest’ day, participants will be given the opportunity to recover from the vitality club. Yet, since we do not want to lose the effect of the intervention, we ask the participants to perform any light activity during the given window of time of the intervention arm. For one weekend day (Saturday) in the measurement week, or when participants are unable to participate in the Vitality Club, they will be asked to perform some sort of physical activity/exercise at home (the ‘Active@Home’ program) between the above-mentioned times (depending on the intervention). Participants will be handed multiple training schedules/options for the Active@Home option corresponding to a metabolic equivalent of 3-8 MET’s (moderate to vigorous physical activity) for one hour. Participants will also be asked to refrain from activities with equal MET’s for the rest of the day to ensure that their physical activity peak will only be in the morning or evening (depending on the intervention). To ensure participants will stay well informed, they will be handed a booklet with all necessary information and a day by day description of all measurements and exercise program (‘E4. Studieboekje’).

5.1.1 Sedentary period

Literature shows that physical activity improves insomnia severity.(32) In previous studies, the follow-up period was at least 3 months. As we will use a shorter follow-up period in this study, we have included a sedentary period, to assess the effect of physical activity versus no physical activity. This intervention additionally examines the effect of physical activity timing. In order to verify that the exercise intervention provided in this study has a similar positive effect, we will compare our interventions to this sedentary period. All participants will start with a 14-day sedentary period. They will be instructed to refrain from any moderate to vigorous exercise. Compliance to these instructions will be observed through the wrist worn accelerometer. Where necessary, participants will be reminded of the instructions.

5.2 Use of co-intervention

Not applicable

6. INVESTIGATIONAL PRODUCT

Not applicable

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

All measurement devices used in this study were reviewed by the 'medische technologie' department of the LUMC and were used before in other studies in the LUMC. A statement from the 'medische technologie' can be found in the researchdossier (D4. '**Verklaring meetinstrumenten medische technologie**').

Table for measurement devices of outcome variables

Name of device	**Manufacturer**	**Model**	**CE conform 93/42/EEG of EU/2017/745 (Medical devices) OR 98/79/EEG of EU/2047/746 (in-vitro diagnostics)**	**Conform intended use as described in the instruction manual?**
Multifunctional wearable	Corsano	Cardiowatch 287-2	Yes	Yes
Electronic Ecological momentary assessment tool	M-path Leuven	M-path app (*or similar platform)	-	Yes
Sleep Analyzer	Withings	Sleep Analyzer	No, conform 2014/53/EU	Yes

Table of measurement devices for baseline measurements

Name of device	**Manufacturer**	**Model**	**CE conform 93/42/EEG of EU/2017/745 (Medical devices) OR 98/79/EEG of EU/2047/746 (in-vitro diagnostics)**	**Conform intended use as described in the instruction manual?**
Bioelectrical impedance analyser	Akern	BIA ASE 101	Yes	Yes
Blood pressure device	Welch Allyn	ProBP3400 (or similar device)	Yes, conform 93-42/EEG	Yes
Dynamometer	JAMAR	Handdynamometer Hydraulic	Yes	Yes

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The main outcome of this study will be insomnia severity which will be measured by the Dutch version of the ISI ranging from 0-28 (0-7: no clinically significant insomnia; 8-14: subthreshold insomnia; 15-21: moderate severity clinical insomnia; 22-28: severe clinical insomnia).(35)

8.1.2 Secondary study parameters/endpoints

Secondary and exploratory endpoints include:

Biological clock function

- Clockgene expression
- Core body temperature (°C)
  - Diurnal pattern
  - Time of lowest temperature
- Melatonin
  - Dim light melatonin onset (DLMO)

Physiological parameters

- Heart rate
  - Diurnal pattern
  - Average
  - Resting heart rate
  - Minimum/peak heart rate (5 and 95 percentile)
- Heart rate variability (HRV)
- Breathing rate
- Oxygen saturation (SpO2)

Mental health

- Subjective mood
  - Diurnal pattern of:
    - Positive affectivity
    - Negative affectivity
    - Energy
    - Fatigue
    - Cognition
- (objective) Electrodermal activity (emotional arousal and stress)

Behavioural factors

- Food intake
  - Frequency
  - Timing
- Habitual sleep (**Table 8.1**)
  - Sleep latency (min)
  - Duration (min)
  - Efficiency (%)
  - Sleep phases (%, min)
  - Awakenings at night (x, min)

- Rested feeling (subjective, 0-7)
- General satisfaction with sleep (subjective, 0-7)

Biochemistry

- Glucose metabolism*
- Inflammatory markers
- Liver function
- Kidney function
- Thyroid function
- Bone metabolism markers**
- White blood cell count

* TSH and ft4 will be examined. If we find differences in these markers, ft3 will be examined as well.
** These markers will only be investigated when we find differences one of the primary outcomes (e.g. change in sleep/insomnia or food intake).

Table 8.1. components of habitual sleep and their measurement method

	Withings sleepmat	Accelerometer	Questionnaire
Sleep latency (min)	X	X	
Duration (min)	X	X	
Efficiency (%)	X	X	
Sleep phases (%)	X		
Awakenings at night (X, min)	X	X	
Rested feeling (0-10) *Subjective*			X
General satisfaction (0-10) *Subjective*			X

8.1.3 Other study parameters

The following phenotypic characteristics will be collected at baseline and/or during the study duration:

- General questionnaire on age, sex, marital status, education, diet, alcohol use, smoking, self-rated health, current and previous disease, emotional health, and family members
- Anthropometric measurements
  - Height
  - Weight
  - Hip and waist circumference
  - Body fat composition through Bioelectrical Impedance Analysis (BIA)
- Physical performance
  - Physical activity level through Short Questionnaire to Assess Health enhancing physical activity (SQUASH)
  - Hand grip strength using a dynamometer

    - Objective physical activity through accelerometry (*mg*)
    - 4-meter walk test
- Cardiometabolic measurements
    - Resting systolic and diastolic blood pressure
    - Standard biochemistry profile (e.g., glucose, HD, triglycerides)
- Daily functioning
    - Katz activities for daily living (Katz-ADL)
    - Lawton Instrumental activities of daily living (IADL)
- Quality of life
    - EuroQol five dimension questionnaire (EQ-5D-3L)
    - EuroQol visual analogue scale (EQ-VAS)
- Comorbidities
    - Charlson comorbidity index (CCI)
- Frailty
    - Clinical frailty scale (CFS)
    - Geriatric 8 (G-8)
- Subjective sleep quality
    - Pittsburgh Sleep Quality Index (PSQI)
    - Sleep chronotypeMunich ChronoType Questionnaire (MCTQ)
- Medication use
- Medical history

8.2 Randomisation, blinding and treatment allocation

As this is a cross-over study, participants will undergo all three conditions. Participants will be randomised into two groups at enrolment using a computer-based statistical program. Both groups will start with sedentary period where after the first group will start with the active morning first followed by the active evening. For the second group, the order of the two activity interventions will be reversed (sedentary period- active evening- active morning). Blinding will not be possible in this study. However, we will minimise the interference of the researchers that will perform the analyses during the study by hiring a research nurse to lead the study. If necessary, the research nurse will be assisted by medical students.

8.3 Study procedures

Appendix A shows a snapshot of the study and a schematic overview of its procedures. **Appendix B** shows the frequency and estimated duration of all study procedures. The table distinguishes between active and passive procedures and in this way also indicates the burden of all procedures (passive procedure=less burdensome). For example, filling out the ecological momentary assessments is marked as active study procedures (more burdensome) while heart rate measurement is marked as a passive procedure (participants wearing the wrist-worn heart rate monitor).Intervention procedures

Sedentary behaviour

During the sedentary period, participants are asked to attain their inactive/sedentary lifestyle. This means that they have to refrain from all moderate to vigorous (social) physical activities.

Physical activity interventions
During the active morning and active evening interventions, participants are expected to exercise on the set hour of the corresponding intervention. Chapter 5.1.1 contains a full description of the interventions

8.3.1 Questionnaires

Screening questionnaire
Questionnaire containing all screening questions to assess whether an individual might be eligible for participation, will be distributed prior to the study as part of the screening.

General Questionnaire
General questionnaire on age, sex, marital status, education, diet, alcohol use, smoking, self-rated health, current and previous disease, emotional health, and family members will be distributed and filled out at baseline.

Daily functioning
Daily (physical) functioning will be assessed by two questionnaires on performing daily activities. Katz Activities of Daily Living (ADL)(36) includes activities such as bathing and feeding, while the Lawson Instrumental Activities of Daily Living (IADL)(37) questionnaire includes mainly household activities such as cooking and doing laundry.

Quality of life
The EQ-5D-3L(38), self-reported measure of functional health and well-being will be distributed and filled out. It consists of 5 questions on different domains. Additionally, participants will fill out the EQ-VAS.(38)

Comorbidities
From the medical records, we will derive the number of medications used and the co-morbidities, from which we can constitute the Charlson Comorbidity Index.(39)

Frailty
Frailty will be measured by the clinical frailty scale.(40) Participants will be categorized on a scale from 1 (very fit) to 9 (terminally ill). Additionally, participants will fill in the G8(41), a short multidimensional assessment of 8 dimensions. The score ranges from 0-17 at which a score of 14 or lower suggests a full geriatric screening is necessary. Filling out the G8 takes approximately 3-5 minutes.

Medication use

At baseline, we will ask the participants to hand in a list of medications that they are currently using retrieved from their pharmacy or GP which will be checked by the research nurse together with the participant on the baseline measurement day in the research center.

Medical history
At baseline, we will ask participants to hand in a list of medical history and current medical diagnoses requested and retrieved from their GP which will be checked by the research nurse together with the participant on the baseline measurement day in the research center.

Subjective sleep quality
Sleep quality will be assessed by the Pittsburgh Sleep Quality Index (PSQI) which is a questionnaire with different questions on sleep quality in the past month. The questionnaire consist of 19 questions on 7 different domains of sleep problems; Subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction. Each of the sleep components yields a score ranging from 0 tot 3, with 3 indicating the greatest dysfunction.

Sleep chronotype
Participants will fill out the MCTQ about their sleep behaviour at baseline to assess their chronotype.(42) Individuals are also asked to subjectively rate themselves as one of seven possible chronotypes ranging from extreme early (preferring to rise much earlier than others) to extreme late. This information is combined to determine the time of day at which the respondent is likely to feel most alert, placing them objectively in a chronotype category. The scale is scored electronically by the Web site at which it is available. Total scores can range from 16 to 86, with the lowest values representing extreme-late chronotypes.

Insomnia Severity Index
Participants will be asked to fill out the ISI questionnaire (Dutch version) a total of 5 times; first time before the start of the study to assess if participants are eligible for the study (sleep problem yes/no), the second time on the baseline measurement day which we will use as baseline, and finally, they will fill out the ISI questionnaire on the last day of each intervention. The ISI questionnaire consists of 7 questions and outcomes include: ‘No clinically significant insomnia (0-7 points)’, ‘Subthreshold insomnia (8-14 points)’, ‘Moderate severity clinical insomnia (15-21 point), and ‘Severe clinical insomnia (22-28 points)’. According to the literature, a score of 10 points is and appropriate threshold when detecting insomnia cases in a community sample.(35) Therefore, we will include participants with a score of ≥10 in our study.

8.3.2 Mental health investigations

Ecological momentary assessment

During the measurement week, participants will be asked to fill out the eEMA questionnaire in the Leuven mPath app (or a similar platform meeting European privacy standards) 5 times a day on set times. Participants will receive a pop-up on their smartphones and will have a window of 1 hour to fill in the questionnaire. During this 1-hour window, participants will get multiple reminders to fill in the questionnaire. The questionnaire consists of multiple questions that gather information on current mood/feelings divided into multiple dimensions: positive affectivity, negative affectivity, energetic arousal and cognition. A 7-point rating scale will be used to answer the questions. To minimize the burden of this repeated questionnaire, the time needed to fill out the questionnaire is minimised/limited to approximately 4 minutes.

Subjective sleep quality
As an addition to the eEMA, two questions will be added to the morning questionnaire, one about general sleep satisfaction and one about the participants rested feeling.

Emography
Objective emotional arousal and stress will continuously be measured by the Cardiowatch 287-2 (Corsano Health, The Hague, the Netherlands) through electrodermal activity (measured by EDA and GSR).

8.3.3 Clinical test/procedures

Anthropometric measurements
Weight, height, and waist and hip circumference will be measured with a scale and measure in the research center which will be the same for all participants. Weight will be measured with the person dressed in light clothing and without shoes. Height will be measured with the person in standing position and wearing no shoes. Waist circumference will be measured at the midpoint between the lowest rib and the top of the iliac crest with a non-elastic tape and in standing position. Hip circumference will be measured at the widest part of the hip with a non-elastic tape and in standing position.

Body composition
Body composition will be assessed with bioelectrical impedance analysis (BIA, Omron BF508, Omron Healthcare Inc., Kyoto, Japan, or a similar device). This device can measure total body water, extracellular water, and intracellular water. The subject should be in a supine position for four minutes before the start of the measurement. Throughout the measurement the participants' arms and legs should be slightly apart and the subject should remain motionless. The measurement will last approximately one minute.

Hand grip strength
The handgrip strength measurement will be performed three times with the left hand and three times with the right hand using a hand dynamometer. This test will be performed with the participants sitting in a chair with armrests in an upright position with arms by their sides and the elbows at a 90-degree angle.

4 meter walking test
This test assesses gait speed. Participants are instructed to walk a distance of four meters at their normal pace. The time the individual needs to cover 4 meters is measured. Gait speed of longer than 5 seconds to walk 4 meters (0.8 m/s) suggests an increased risk of frailty and the need of further clinical review.

Resting blood pressure
After a 10-minute rest, systolic and diastolic blood pressure will be measured two times with a 2-minute interval on the dominant arm by trained staff members using a validated blood pressure device.

Continuous heart rate monitoring and heart rate variability
Participants will wear the Corsano Cardiowatch 287-2 (Corsano Health, The Hague, the Netherlands) wrist band on their right wrist continuously during the study period. The wristband is multifunctional and is waterproof. Participants will wear the wrist band 24 hours a day. The HR is measured using a PPG (photoplethysmogram, i.e., optically obtained volumetric measurement) and ECG. The manufacturer supplies the algorithm for converting the PPG/ECG signals into a set of HR values. All data from the Cardiowatch is available real time and is transferred from the watch to the smartphone using a clinical trial app, developed by the provider, which allows the researcher to retrieve live vital sign data while conducting a blinded study. From the app, it will be uploaded to the output server through a HIPAA/AVG compliant cloud service.

Core body temperature
Core body temperature will be continuously measured by the Cardiowatch 287-2. This parameter will be derived through an algorhythm of combined data from Heatflux sensor and PPG signal. Diurnal patterns of core body temperature as well as the time of lowest daily temperature will be derived from this data.

Breathing rate and oxygen saturation
Breaths per minute count and oxygen saturation (%SpO2) will also be derived from the Cardiowatch 287-2. Data will continuously be measured throughout the study period.

Objective sleep quality
Objective sleep quality will be monitored with the Withings Sleep Analyzer (Withings, Issy-les-Moulineaux, France) and an accelerometer (Corsano Cardiowatch 287-2 (Corsano Health, The Hague, the Netherlands)., see text below). Prior to the study, the Withings Sleep Analyzer will be installed in the participants bed. A smart phone is required to set-up the device and the devices must be connected to a Wi-Fi to be able to send data to the cloud. After set-up no link to the smartphone is required. The device must be connected to power.

Objective physical activity

To monitor objective physical activity, Raw accelerometery will be derived from the Corsano Cardiowatch 287-2 (Corsano Health, The Hague, the Netherlands) on their right wrist during the entire study period. Raw data will be collected at 32Hz and will be derived from the wristband similar to the variable heart rate monitoring and HRV.

Food capturing
Participants will log their daily food intake during each measurement week by using a food diary that will be distributed prior to the start of the study. Frequency and timing of food intake will be assessed.

8.3.4 Invasive procedures

Melatonin assessment
On day 13 of all interventions (second-last day of the measurement week), participants will be asked to perform/collect 7 buccal swabs in a time range of 5 hours prior to bedtime to 1 hour after bedtime to measure the melatonin pattern DLMO. This measurement will be done according to the standard protocol from Chron@Work (Groningen, The Netherlands) in dim light conditions. (43) Participants will be carefully instructed beforehand on the procedure

Blood samples
A total of 62 ml blood will be sampled during the total study period (see **Table 8.2**). For screening purpose, we will sample 3.5 ml (nonfasting) blood for direct measurements of thyroid function (TSH, fT4), electrolytes (sodium, potassium), kidney function (eGRF/creatinin), liver function (AST, ALT, gammaGT by the Central Clinical Haematology Laboratory (CKHL) of the Leiden University Medical Center. At screening and baseline (and subsequently also after each intervention) will collect one 2 ml sample of venous blood in a vacutainer on EDTA. This sample will be used for direct measurement of haemoglobin, hematocrite, erythrocytes, MCV, MCH, MCHV, thrombocytes, leukocytes (neutrofile granulocytes, lymfocytes, eosinofile granulocytes, basofile granulocytes, monocytes) by the Central Clinical Haematology Laboratory (CKHL) of the Leiden University Medical Center, as well as for direct measurement of HbA1c the Department of Clinical Chemistry and Laboratory Medicine (CKCL) of the Leiden University Medical Center. For each participant, at baseline, and after each of the three interventions, we sample 3.5 ml (fasted) blood for batch measurements after completion of the study of: Insulin, glucose, eGRF/creatinin, Hs-CRP, Gamma GT, ASAT, ALAT, TSH, and ft4. In addition, for each participant, we will also collect after each of the three interventions: 1 x 2.5 ml Paxgen tubes (for isolation of RNA and measurement of clock gene expression)and 1x 2 ml EDTA (for metabolomics and proteomics). In addition, for each participant, after each of the three interventions, we will collect 2x 3.5 ml serum for Biobanking purposes. Moreover, residual biomaterial from this study that has not been used in above stated analysis will be transferred to the LUMC Biobank if permission is given by the participant. The regulations of the LUMC Biobank will be applicable.

Table 8.2. Overview of blood sampling

Sampling periods (number)	Tubes (type)	Tubes (number, volume)	Blood volume (total)	Type Biomaterial	Measured parameters
Screening	Serum	1x 3.5 ml	3.5 ml	serum	TSH, fT4, sodium, potassium, eGRF/creatinin, CRP, Gamma GT, ASAT, ALAT
Screening	EDTA	1x 2 ml	2 ml	plasma	HbA1c, Hematology (direct)
Baseline, and after each of the three interventions (4)	EDTA	1x 2 ml	8 ml	plasma	HbA1c, Hematology (direct)
Baseline, and after each of the three interventions (4)	Serum	1x 3.5 ml	14 ml	serum	Insulin, glucose, eGRF/creatinin, Hs-CRP, Gamma GT, ASAT, ALAT, TSH, ft4(in batch)
After each of the three interventions (4)	Paxgene	1x 2.5 ml	7,5 ml	RNA	Gene expression clock genes (in batch)
After each of the three interventions (4)	EDTA	1x 2 ml	6 ml	plasma	Metabolomics and proteomics (in batch)
After each of the three interventions (4)	Serum	2x 3.5 ml	21 ml	serum	Biobanking
Total amount of blood			**62 ml**		

8.4 Withdrawal of individual subjects

Subjects 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 subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal (if applicable)

Not applicable

8.5 Replacement of individual subjects after withdrawal

If a subject withdraws from the study, the participant will not be replaced; the logistics of this study make it impossible to enter the study once it has started. We have taken this into account with the 10% drop-out rate.

8.6 Follow-up of subjects withdrawn from treatment

Not applicable

8.7 Premature termination of the study

Due to the COVID-19 pandemic and the consequential measures, this study could be postponed or temporally suspended. We aim to continue when possible and finish the study when all participants are included. To minimise the chance of premature termination of the study, the study duration will be relatively short.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / trial procedure/ the experimental intervention]. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded with an exception for minor bruising due to the blood draw.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that
- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

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

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not Applicable

9.3 Annual safety report

Not applicable

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.5 Data Safety Monitoring Board (DSMB)

According the risk classification (K6.), this study has a negligible risk. Therefore we will not be appointing a DSMB.

10. STATISTICAL ANALYSIS

All data that will be collected in this study will result in (or be transformed into) quantitative data and will either be categorical or continuous. Normal distributions will be checked by visual inspection of histograms and QQ plots. Normally distributed variables will be described as mean with standard deviation (SD) and non-normally distributed variables will be described as median with interquartile range (IQR). Categorical variables will be described as numbers (N) and percentages. As we collect data on patterns, we will descriptively present all patterns derived in line graphs to visually inspect pattern differences between interventions. Missing data will be interpolated/imputed with a standard algorithm in SPSS/ R Software.

10.1 Primary study parameter

The primary study endpoint will be change in insomnia severity measured by the ISI. We will perform a paired t-test (assuming normally distributed data) to examine the difference between active morning and active evening. Data will be shown as mean difference, SD (or 95% confidence interval), and p-value. A p-value lower than 0.05 will be considered as statistically significant.

10.2 Secondary study parameter(s)

Secondary endpoints include changes in:

Biological clock function

- Clockgene expression
- Core body temperature (°C)
  - Diurnal pattern
  - Time of lowest temperature
- Melatonin
  - Dim light melatonin onset (DLMO)

Physiological parameters

- Heart rate
  - Diurnal pattern
  - Average
  - Resting heart rate
  - Minimum/peak heart rate (5 and 95 percentile)
- Heart rate variability (HRV)
- Breathing rate
- Oxygen saturation (SpO2)

Mental health

- Subjective mood
  - Diurnal pattern of:
    - Positive affectivity
    - Negative affectivity
    - Energy
    - Fatigue

        - Cognition
    - (objective) Electrodermal activity (emotional arousal and stress)

Behavioural factors

- Food intake
    - Frequency
    - Timing
- Habitual sleep (**Table 8.1**)
    - Sleep latency (min)
    - Duration (min)
    - Efficiency (%)
    - Sleep phases (%, min)
    - Awakenings at night (x, min)
    - Rested feeling (subjective, 0-7)
    - General satisfaction with sleep (subjective, 0-7)

Biochemistry

- Glucose metabolism
- Inflammatory markers
- Liver function
- Kidney function
- Thyroid function
- Bone metabolism markers
- White blood cell count

Changes between active morning and active evening will be assessed. Diurnal patterns will be calculated through the hourly mean level of for example glucose and will be displayed for each condition. Additionally, we will perform sensitivity analysis for both the primary endpoint as all secondary endpoints by analyse the changes between the sedentary period & active morning, and the sedentary period & active evening. Assuming the data is normally distributed, a paired t-test will be used for all analyses and data will be shown in as mean difference, SD, and p-value.

10.3 Other study parameters

Not applicable

10.4 Interim analysis

Not applicable

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (version October 2013 as emended by the 64th WMA General Assembly, Fortaleza, Brazil) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and consent

This protocol will be submitted to the Medical Ethics Committee of Leiden Delft and The Hague and will not commence before formal approval has been granted.

Subjects will be recruited from the general population through advertisement in local media (e.g. newspapers and radio) and at community centres and local initiatives for older adults in Leiden and surroundings. After a subject has expressed interest and has met all inclusion criteria, subjects will be given oral and written explanation about the study after which they give written acknowledgement of informed consent to participate (E2.). Only hereafter, the study procedures will start.

11.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable

11.4 Benefits and risks assessment, group relatedness

All study components are minimally or non-invasive. **Appendix B** shows the frequency and duration of all study procedures. We include (repeated) questionnaires on well-being and mood and we ask the participants to repeatedly perform buccal swabs which can might be experienced as burdensome for some participants. Additionally, we ask participants to adhere to the different aspects regarding sedentary behaviour and exercise timing thus controlling and restricting their daily schedule to some extent. Further, blood withdrawal will be performed, which is a minimally invasive procedure which may cause a small hematoma.

Benefits of participation in the current study include free of costs training sessions guided by a professional that may lead to health benefits related to increased physical activity level, and in beneficial long-term behavioural changes.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

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

11.6 Incentives (if applicable)

Participants will get a financial compensation of €250,- including compensation of possible travel costs for completing the total study. During the centre visits participants will be provided with some refreshments.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Prerequisite is the voluntary approval of the participants by signing the informed consent prior to inclusion. For all participating subjects, a Case Record Form (CRF) will be completed in CASTOR. All subjects will be identified by a study ID-number, in a coded form (OPTID00). We will ensure that the privacy of the participants will be protected. The participant personal information (name, address) in CASTOR will be encrypted and only the principal investigator, dr. [redacted], and the main researcher, [redacted], will have access. During the study, the research nurses who are in contact with the participants will have access to this personal information in order to make the research schedule. The research data will be stored in a digital, protected, CASTOR database that is only accessible to the researchers of this study. After exporting the data from CASTOR, the databases will be stored on a secured local network drive of the LUMC with access by authorized persons. The collected data and biomaterials will be stored for 15 years. Residual biomaterial that has not been used for this study and data collected in this study will be transferred to the LUMC Biobank if permission is given. The regulations of the LUMC Biobank will be applicable. Data and biomaterials will be stored in the biobank indefinitely. Participants can at any moment withdraw from the study. Data that has been collected and measurements that has been performed in biomaterials can still be used, but residual biomaterials will be destroyed.
Data collected by the Withings Sleep is sent directly to the Withings server. Data will be stored on Withings servers and will subsequently be transferred to CHDR servers via

application programming interface (API). After data collection, CHDR creates an export of the data and shares it with LUMC. All data collected from the Corsano Cardiowatch will be exported through encryption (according to AVG regulation). No third party will have access to participant data. A data processor agreement (or joint controllership agreement) will be signed by all parties handling participant data. All data at the CHDR and Corsano servers will be deleted at the end of the study once all data is safely stored at a protected drive in the LUMC.

12.2 Monitoring and Quality Assurance

Intensity of monitoring depends on the risk classification of this study, which will be determined in consultation with the monitoring coordinator of LUMC after approval of this research protocol. Monitoring will be executed by (internal) monitors of the LUMC according to the monitor plan

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study

report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

journals and (inter)national conferences, according to the CCMO statement of publication policy. An overview of general results will also be communicated to the participants via newsletters. In accordance to the ICMJ guidelines, parties that have contributed significantly to the study, will be included as co-authors in publications when applicable.

13. STRUCTURED RISK ANALYSIS

Not applicable

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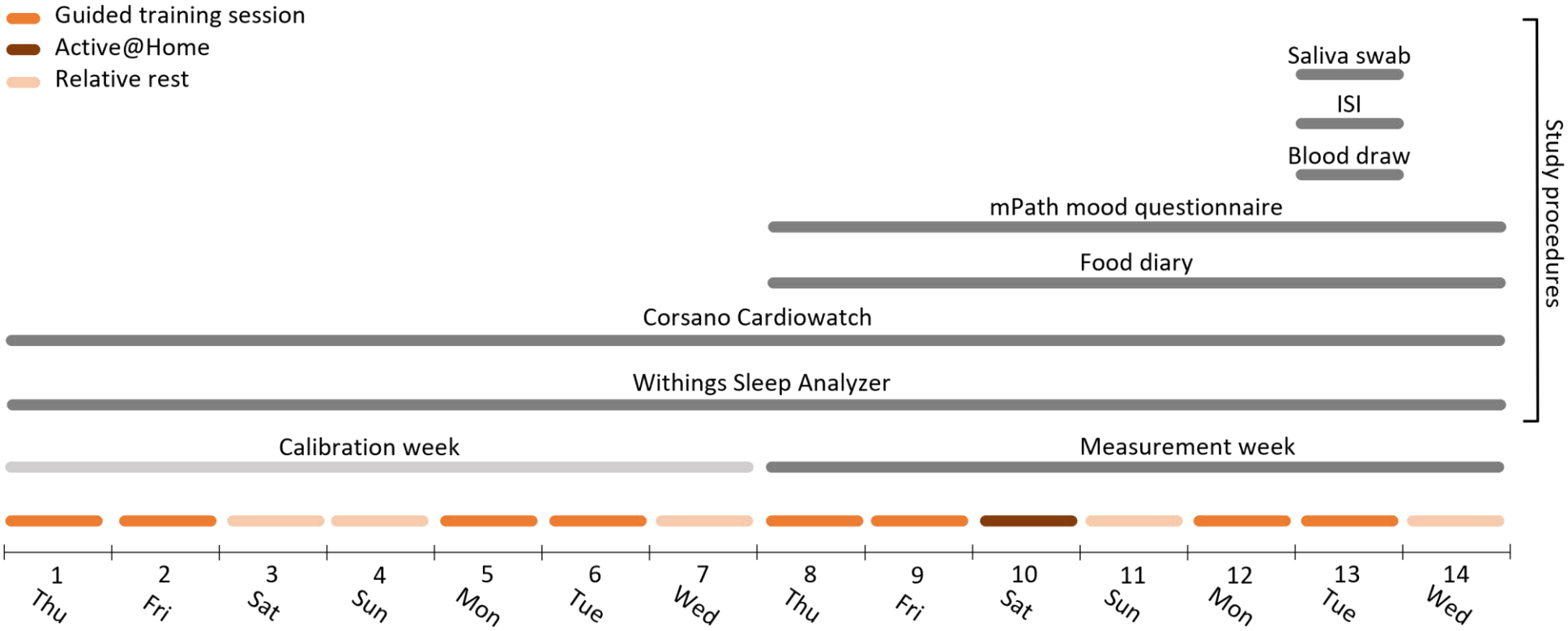
15. Appendix

15.1 Appendix A



Example of study schedule during an intervention

15.2 Appendix B

Overview of the frequency and duration of all study procedures

	Study procedure	How many times/days	Duration (+/- min, p/x)
Questionnaires	Screening questionnaire (by phone)	1x	10
	General questionnaire	1x	10
	Katz-ADL	1x	10
	Lawton IADL	1x	10
	EQ-5D-3L	1x	15
	EQ-VAS	1x	2
	CCI	1x	-
	CFS	1x	5
	G8	1x	3-5
	Medication use	1x	-
	Medical history	1x	-
	Pittsburgh Sleep Quality Index		
	Munich Chronotype Questionnaire	1x	10
	Insomnia severity index	5x	5-10
Mental health	Ecological momentary assessment	5x p/d, 21 days	3-5
	Emography	42 days	-
Clinical procedures	Anthropometric measurements	2x	15
	Body composition	2x	15
	Hand grip strength	1x	5
	4 meter walking test	1x	3
	Resting blood pressure	1x	5-10
	Heart rate variability	42 days	-
	Continuous heart rate monitoring	42 days	-
	Breathing rate	42 days	-
	Oxygen saturation	42	-
	Objective sleep quality	42 days	-
	Objective physical activity	42 days	-
	Food capturing	+/- 5x p/d, 21 days	3
	Melatonin assessment	7x p/d, 3 days	2

	Core body temperature	42 days	-
	Blood sampling	4x	10