Trials

STUDY PROTOCOL

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Older adults exercising ON TIME: protocol for a randomized controlled cross-over study to assess the effect of physical activity timing on insomnia severity

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Abstract

Background There are increased indications that physical activity timing, irrespective of intensity, impacts insomnia and circadian clock function. Here, we describe the rationale and design of a randomized cross-over study, called ON TIME, to examine the effects of (changing) physical activity timing on insomnia severity and on multiple exploratory outcomes that are linked to circadian clock function.

Methods We will conduct a randomized cross-over trial in 40 healthy older adults (aged 65 to 75 years) with subclinical or clinical insomnia (Insomnia Severity Index (ISI) scores of ≥ 10) from the Dutch municipality of Leiden and surroundings. Participants will undergo 3 intervention periods (14 days each) consecutively: one sedentary period and two periods of increased physical activity (one period with morning activity and one period with evening activity). The intervention periods are separated by a wash-out period of 1 week. In both active intervention arms, participants will follow coached or uncoached outdoor physical exercise sessions comprising endurance, strength, and flexibility exercises for 14 days. The primary outcome is change in insomnia severity as measured by the ISI. Additional exploratory outcomes include multiple components of objective sleep quality measured with tri-axial accelerometry and subjective sleep quality assessed by questionnaires as well as dim light melatonin onset and 24-h rhythms in heart rate, heart rate variability, breathing rate, oxygen saturation, mood, and objective emotional arousal and stress. Additionally, we will collect diary data on eating patterns (timing and composition). Finally, fasting blood samples will be collected at baseline and after each intervention period for measurements of biomarkers of metabolic and physiological functioning and expression of genes involved in regulation of the biological clock.

Discussion We anticipate that this study will make a significant contribution to the limited knowledge on the effect of physical activity timing. Optimizing physical activity timing has the potential to augment the health benefits of increased physical exercise in the aging population.

Trial registration Trial was approved by the Medical Ethics Committee Leiden, The Hague, Delft, The Netherlands (June, 2023). The trial was registered in the CCMO-register https://www.toetsingonline.nl/to/ccmo_search.nsf/Searchform?OpenForm under study ID NL82335.058.22 and named ("Ouderen op tijd in beweging" or in English "Older

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adults exercising on time"). At time of manuscript submission, the trial was additionally registered at ClinicalTrials.gov under study ID: NL82335.058.22 and is awaiting approval.

Keywords Exercise, Timing, Zeitgeber, Sleep, Biological clock, Physical activity, Crossover randomized controlled trial

Background

A good running circadian clock is associated with healthy aging [1]. Yet, proper circadian organization (also referred to as "circadian health") [2] is threatened by our modern 24-h society. Shift work, social jetlag, increased exposure to blue light-emitting devices, and globalization of a 24/7 society facilitated by advances in technology and social media jeopardize circadian health [3]. When behaviors such as physical activity, sleep, food intake, and exposure to light are not timed appropriately relative to the circadian timing system, a state of misalignment can occur in which internal circadian rhythms are not synchronized with the environment or with each other [4–6]. Studies consistently show that experimental disruption or desynchronization of circadian rhythms has negative effects on health and lifespan [7–10]. Decreases in our circadian health may be partly responsible for the rising prevalence of sleep problems in (older) adults [11, 12]. In the Netherlands, self-reported sleep problems occur in 20% of all people of 12 years and older and in 28% of older adults aged 75 years and older [13]. 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 (ischemic) stroke, and neuropsychiatric diseases such as depression, Parkinson disease, and dementia [3, 4, 12, 14–16].

Zeitgebers, which are defined as regular cyclic environmental signals and include food intake, light exposure, and physical activity, can calibrate our circadian clock [17–19]. Regular physical activity is known to positively affect the circadian clock and is associated with healthy aging [18, 20]. Guidelines provide indications about the intensity, frequency, and duration of physical activity required to maintain or achieve health. However, less is known about the optimal timing of physical activity. A growing number of research studies in the literature on the daily timing of lifestyle behaviors already marked the importance of timing of food intake and light exposure [3, 21–27]. Yet, intervention studies on the timing of physical activity (which we refer as "chronoactivity") 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 interventions and faster improvement of health outcomes, such as recovery from a period of poor sleep quality and/or mental health problems. Several studies in mice with central circadian disruption reported that exercise restores circadian rhythms in heart rate and core body temperature [28, 29]. Additionally, studies in humans have revealed that physical exercise in the morning and afternoon increases the expression of several core clock genes such as Cry and Bmal [30, 31]. Observational studies that specifically focused on chronoactivity found associations between morning physical activity and increased metabolic health [32], increased cardiometabolic fitness [33], and an approximately 16% lower risk of incident atherosclerotic cardiovascular and cerebrovascular disease [34]. However, the results from intervention studies on chronoactivity are inconclusive [35–37]. Multiple intervention studies observed a seemingly positive effect of morning physical activity on body composition and metabolic health [37, 38]. Contrarily, in several other intervention studies, evening exercise had positive effects on heart rate recovery, blood pressure, and blood glucose levels [35, 36, 39]. One of these studies observed that morning physical activity had a deleterious effect on blood glucose levels in diabetic men [35]. Nevertheless, these intervention studies do suggest that timing of physical activity can change the phase of the molecular clock, specifically in peripheral tissues, and might therefore be considered as an important Zeitgeber [28]. Yet, despite the increasing interest and mounting evidence for the importance of chronoactivity, the optimal circadian timing of physical activity for circadian health remains unclear and little is known about the influence of chronoactivity on sleep in older adults which frequently suffer from sleep problems [13, 40].

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

Methods and design

Aims and hypothesis

The primary aim of the ON TIME study is to examine the effect of physical activity timing on insomnia severity in older adults with self-reported sleep problems. The

secondary aims are to explore the effect of physical activity timing on rhythmic parameters of (1) biological clock function, (2) physiological parameters, (3) mental health, (4) behavioral factors, and (5) biochemistry. Our primary hypothesis, based on previous observational studies [32, 34, 41–43], is that morning physical activity will have a greater beneficial impact on insomnia severity symptoms and related circadian health parameters than evening exercise. SPIRIT guidelines were used to create this article [44].

Study design and setting

The ON TIME study will be conducted in the Leiden University Medical Center (LUMC, the Netherlands) by researchers and nurses of the department of internal medicine and the subdepartment of Geriatrics and Gerontology. We will perform a randomized two-armed cross-over study (Fig. 1) with a superiority framework with an allocation ratio of 1:1. The study consists of an 8-week protocol including a baseline visit, three follow-up visits, and three interventions that participants will follow consecutively. Both groups will start with the sedentary period. Depending on the intervention arm, participants will subsequently 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 a previous intervention effect. The sedentary period and the two interventions 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 relevant endpoints will be measured. The study will largely take place at the participants' home except for the training sessions that will take place at an outdoor sports facility in the municipality of Leiden and the study site visits that will take place in the LUMC. Figure 1 shows a schematic overview of the study outline, and Fig. 2 shows an example of the study schedule during the active morning intervention period. This study protocol was approved by the Medical Research Ethical Committee of Leiden, Den Haag, Delft (MREC-LDD), The Netherlands in June 2023.

Participants and recruitment

We will recruit Dutch-speaking, retired older adults aged 65 to 75 with long lasting (≥ 3 months) sleep problems (scoring ≥ 10 on the Insomnia Severity Index ISI) [45]. In Table 1, an overview of all inclusion and exclusion criteria of this study is given. Potential participants will be recruited through advertisements in flyers, newspapers, websites, and local radio. For practical reasons, participants will be recruited from the municipality of Leiden and surroundings.

Screening, baseline, and randomization

In Fig. 3, an overview of the screening and baseline flow is presented. 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, (iii) physical activity level, and (iv) availability during the study period via a screening questionnaire. If the person seems eligible based on their answers

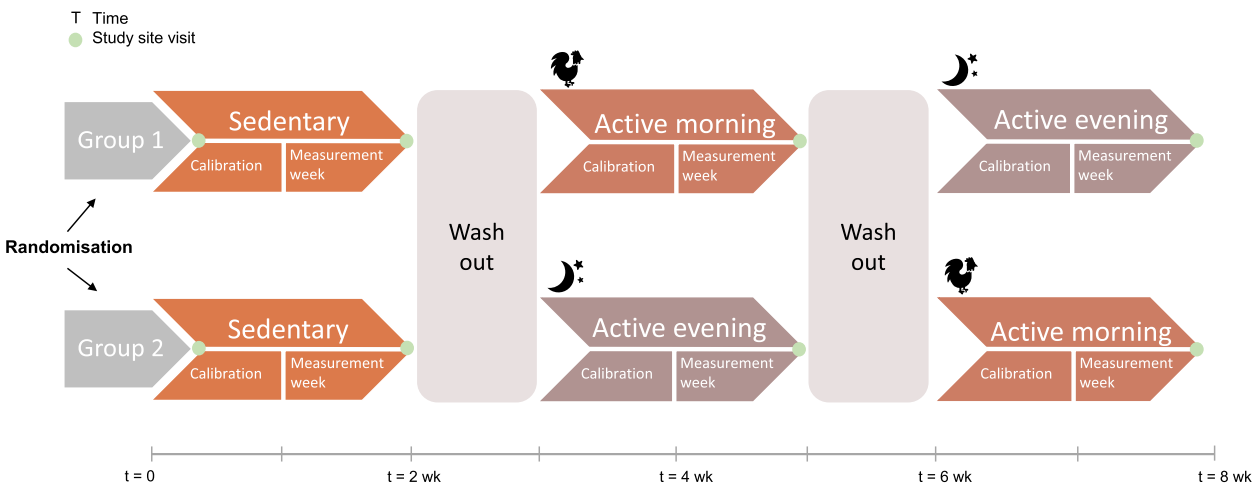

Fig. 1 Schematic overview of the study

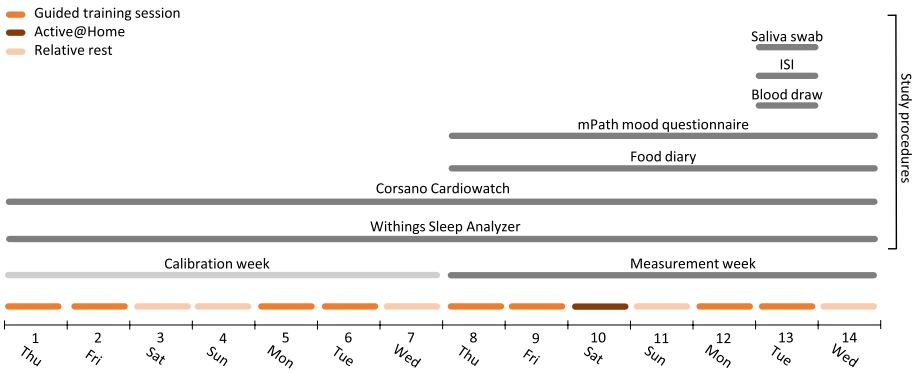

Fig. 2 Example of a study schedule and procedures. ISI, Insomnia Severity Index. This overview represents the schedule during the active morning and active evening interventions. It shows all study procedures and measurement tools participants will use as well as when these will be used

Table 1 Inclusion and exclusion criteria

Inclusion criteria
Aged between 65 and 75 years old
Retired
Long lasting (≥ 3 months) sleep problems (≥ 10 on the ISI)
Access to and ability to use a smartphone (Android or Apple)
Exclusion criteria
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
Clinically diagnosed neurodegenerative diseases (dementia or Parkinson's disease)
Diagnosed sleep apnea
Diagnosed restless legs syndrome
Use of beta-adrenergic blocking agents
Sporadic use of sleep medication
Injuries or other severe physical conditions (such as active arthrosis) that inhibits physical activity
Traveled across time zones one week prior to start of study

to these questions, a participant information form (PIF) and informed consent (IC) will be sent to them by mail. A second phone call will be scheduled at least 1 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 1 h, participants can give written informed consent. Thereafter, two non-fasted blood samples will be drawn (3.5 ml + 2 ml = 5.5 ml), and the ISI will be filled out. We will include participants scoring ≥ 10 on the ISI. Data collected on this day will be used for the final screening measurements. Only when participants meet our criteria measured by the ISI and if no abnormalities in biochemical measures are found in the blood samples, participants are officially enrolled in the study. These participants will receive an invitation for the baseline visit. During this visit, the participants will undergo physical tests, a blood draw and they will fill out the ISI once again. Participants will also receive the remainder of the questionnaires digitally and will be visited at home by one of the researchers, research nurses, or students. During this home visit, questionnaires will be checked for completeness, and all measurement devices will be installed and their proper use will be explained. Shortly after this last visit, the participant will start with the first intervention period. Randomization will be performed at the moment of enrollment by a research staff

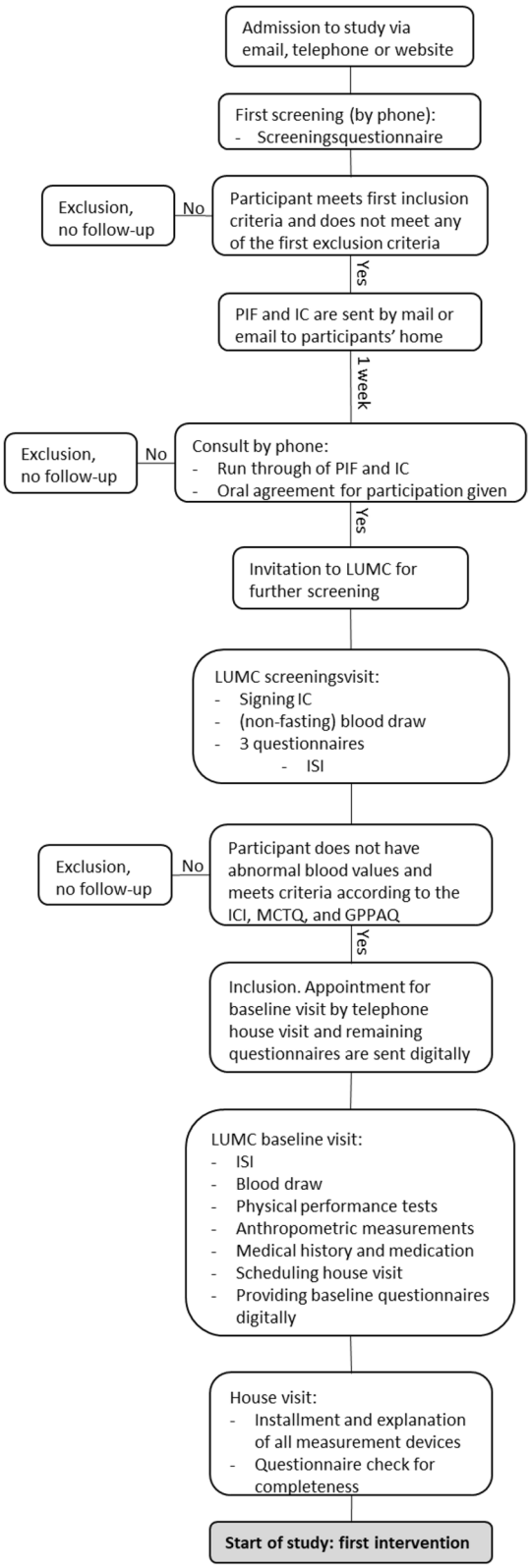

Fig. 3 Screening and baseline flow. PIF, patient information form; IC, informed consent; ISI, Insomnia Severity Index. This figure shows all steps in the screening and baseline procedure of this study

member who was designated in advance with help from a computer-based randomization program. The design and nature of this study makes blinding impossible for all participant-reported outcomes and for observer reported outcomes assessed by research nurses as they are in close contact with the participants. Blinding is also not feasible for observer reported outcomes assessed by researchers based on data collected by the Withings sleep analyzer and Corsano Cardiowatch as these data are likely to reflect the type of intervention. Blinding is only possible for external parties who will process outcome values from the blood draw, saliva samples, Withings sleep analyzers, and Corsano Cardiowatch. The data analysts can also not be blinded since the outcomes collected have a clear pattern which may be indicative of the intervention arm the participants are in.

Interventions

Participants will take part in three interventions, each consisting of a 14-day physical (in)activity regimen.

Sedentary period

All participants will start off with a sedentary period. Literature shows that physical activity (irrespective of the timing) improves insomnia severity [46]. Yet, in previous studies, the follow-up period was at least 3 months [46]. As this study will have a shorter follow-up period, we have included a sedentary period to assess the effect of physical activity versus no physical activity. This period will also serve as a baseline period as participants of this study have a sedentary lifestyle. 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. During the 14-day sedentary period, participants will be instructed to refrain from any moderate to vigorous exercise and are asked to adhere to a maximum step count of 4000. Next, the intervention will examine the effect of physical activity timing.

Active morning and active evening intervention

The active morning and active evening intervention are similar in content except that in the active morning group, participants will be instructed to be active between 10:00 and 11:00 in the morning while in the active evening group they 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 to ensure their physical activity peak will only be in the morning or evening (depending on the intervention). Per intervention, a total of eight training sessions will be organized given by a trained physical therapist specialized in geriatric physical therapy. Although the training sessions will

	STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			
TIMEPOINT**	$-t_1$ screening	0	t_1 baseline	t_2 sedentary	t_3 exercise 1	t_4 exercise 2
ENROLMENT:						
Eligibility screen (phone)	X					
Participant information send	X					
Informed consent (phone-oral)	X					
Eligibility screen (LUMC)	X					
Informed consent (written)	X					
Randomization		X				
INTERVENTIONS:						
Group 1-Active morning first			←	—	—	→
Group 2- Active evening first			←	—	—	→
ASSESSMENTS:						
Pittsburgh Sleep Quality Index (PSQI)	X					
Munich Chronotype Questionnaire (MCTQ)	X					
General Practice Physical Activity Questionnaire (GPPAQ)	X					
Blood Sodium and Potassium	X					
Insomnia severity index (ISI) Questionnaire	X		X	X	X	X
Blood Hematology, HbA1C, TSH, fT4, eGRF/creatinin, CRP, Gamma GT, ASAT, ALAT)	X		X	X	X	X
Katz Activities of Daily Living (ADL) Questionnaire			X			
Lawton Instrumental Activities of Daily Living (IADL) Questionnaire			X			
Quality of Life Questionnaires (EQ-5D-3L) and EQ-VAS)			X			
Charlson Comorbidity Index (CCI)			X			
Frailty (CSF and G8)			X			
Gait speed (4 MWT)			X			
Hand grip strength			X			
Body composition			X	X	X	X
Resting blood pressure			X	X	X	X
Anthropometric measurements (height, weight, hip and waist circumference)			X	X	X	X
Sleep quality			X	X	X	X
Mood (eEMA) (objective)			X	X	X	X
Electrodermal activity (emotional arousal and stress)			X	X	X	X
Continuous heart rate monitoring and heart rate variability			X	X	X	X
Core body temperature			X	X	X	X
Breathing rate and oxygen saturation			X	X	X	X
Objective sleep quality			X	X	X	X
Objective physical activity			X	X	X	X
Food diary			X	X	X	X
Blood insulin and glucose			X	X	X	X
Blood clock gene expression			X	X	X	X
Blood metabolomics			X	X	X	X
Blood proteomics			X	X	X	X
Blood Biobanking			X	X	X	X
Saliva melatonin				X	X	X

Fig. 4 SPIRIT figure. The schedule of enrolment, interventions, and assessments of the ON TIME study

be different in character (e.g., a game of volleyball and a circuit training), these will all comprise a combination of strength and endurance training at moderate to vigorous intensity tailored for older adults [47]. During the weekends, there will be no training 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 training session. Yet, since we do not want to lose the effect of the intervention, we ask the participants to perform any type of light activity during the specified time window of the intervention arm. For one weekend day (Saturday) in the measurement week, or when participants are unable to participate in the coached training session, they will be asked to perform physical activity/exercise at home (the "Active@Home" program) in the above-mentioned time windows (depending on the intervention). Participants will be handed multiple training schedules/options for the Active@Home option corresponding to a metabolic equivalent of task (MET) of 3–8 (moderate to vigorous physical activity) for 1 h. To ensure that participants stay well-informed and motivated, they will be handed a booklet with all necessary information and a day-by-day description of all measurements and exercise programs.

Discontinuation of interventions

When an adverse event is reported that is clearly linked to one of the interventions (either caused by of affecting it), the intervention will be discontinued for this individual and reported to toetsingonline.nl. A possible example would be a bodily injury that prevents safe physical activity. Participants are free to stop their participation to the study at any given time.

Measurements, outcomes, and materials

The SPIRIT figure (Fig. 4) gives an overview of the enrolment schedule, interventions and the measurements from this trial. All outcomes and measurements can be divided into three categories: (1) baseline measurements, (2) repeated measures and pattern assessments, and (3) follow-up measurements.

Baseline measurements

At the start of the study, we will collect information to characterize the participants. The following will be collected:

- Questionnaires:
    - Sleep chronotype measured by the MCTQ [48]. 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
- Physical activity level measured by the GPPAQ. The GPPAQ is a commonly used and validated questionnaire which consists of 7 questions. It provides a 4-level physical activity index (PAI) including: "Inactive," "Moderately inactive," "Moderately active," and "Active."
- Daily functioning measured by the Katz Activities of Daily Living (ADL) and Lawton Instrumental Activities of Daily Living (IADL) questionnaires [49, 50]
- Quality of life measured by the EQ-5D-3L and EQ-VAS [51]
- Comorbidities measured by the Charlson Comorbidity Index (CCI) [52]
- Frailty measured by the clinical frailty scale and the short, multidimensional Geriatric 8 assessment [53, 54]
- Medication use will be collected from the participants' pharmacy, and medical history will be requested from the participants' general practitioner (GP)

- Anthropometric measurements including height, weight, waist, and hip circumference will be measured by a trained research nurse
- Body composition will be assessed with Bioelectrical Impedance Analysis (BIA, InBody 720, InBody Europe, Amsterdam, the Netherlands)
- Handgrip strength measurement will be performed three times with the left hand and three times with the right hand using a hand dynamometer
- Gait speed will be assessed with the 4-m walking test (4 MWT)
- Resting blood pressure is measured twice after a 10-min rest with a 2-min interval on the dominant arm by a trained research nurse using a validated blood pressure device (ProBP3400, Welch Allyn, Wisconsin, United States)

Repeated measures and pattern assessments

Repeated measures and pattern assessments will be performed to collect the exploratory outcomes of this study. We hypothesize that these outcomes will either reflect biological clock function or a response to a changing circadian rhythm. These measurements will take place throughout the intervention periods and include:

- Sleep quality parameters measured by the Withings Sleep Analyzer (Withings, Issy-les-Moulineaux, France). Prior to the study, the Withings Sleep Analyzer will be installed in the participants bed and these measurements will continue throughout the whole study period. This device will provide information on the following sleep traits:
  - Sleep latency
  - Sleep duration
  - Efficiency (% of sleep duration relative to total bed time)
  - Sleep phases (deep, light, rapid eye moment (REM)
  - Number of awakenings at night
- Mood measured by electronical Ecological Momentary Assessment (eEMA) [55, 56]. For 7 days during the measurement week, participants will be asked to fill out the eEMA questionnaire in the m-Path app (m-Path, Leuven, Belgium) 4 times a day on set times. Participants will receive a pop-up on their smartphones and will have a window of 2 h to fill out the questionnaire. The questionnaire consists of 18 questions that gather information on current mood/feelings divided into 4 dimensions: positive affectivity, negative affectivity, energetic arousal, and cognition. A 7-point rating scale will be used to answer the questions. This repeated questionnaire will approximately take 4 min each time to minimize time burden
- Food intake will be measured using a food diary for 7 days during the measurement week. Participants are asked to record the timing and content of their meals throughout the day. This will take approximately 2 min per meal.
- Dim light melatonin onset (DMLO) is known to be the single most accurate marker for assessing the circadian pacemaker [57]. Therefore, on day 14 of each intervention (last day of the measurement week), participants will be asked to collect 7 buccal swabs in a time range of 5 h prior to bedtime to 1 h after bedtime to assess the melatonin pattern DLMO [58]
- Participants will wear a wristband wearable (Corsano Cardiowatch 287–2, Corsano Health, The Hague, the Netherlands) [59] day and night during the 2 weeks of each intervention period. This wristband will monitor the following:
  - Continuous heart rate and heart rate variability (HRV) using a PPG (photoplethysmogram, i.e., optically obtained volumetric measurement) and ECG
  - Continuous breathing rate and oxygen saturation. Breaths per minute count and oxygen saturation (%SpO2) will be derived
  - Core body temperature (CBT). This parameter will be derived through an algorithm of combined data

Table 2 Overview of blood sampling

Sampling periods (number)	Tubes (type)	Tubes (number, volume)	Blood volume (total)	Type biomaterial	Measured parameters
Screening	Serum	1 × 3.5 ml	3.5 ml	Serum	TSH, fT4, sodium, potassium, eGFR/creatinine, Hs-CRP, Gamma GT, ASAT, ALAT
Screening	EDTA	1 × 2 ml	2 ml	Plasma	HbA1c (direct)
Baseline, and after each of the three interventions (4)	EDTA	1 × 2 ml	8 ml	Plasma	HbA1c. hematology (direct)
After each of the three interventions (4)	Serum	1 × 3.5 ml	14 ml	Serum	Insulin, glucose, eGFR/creatinine, Hs-CRP, Gamma GT, ASAT, ALAT, TSH, ft4, ft3, bilirubin, CTX, P1NP (in batch)
After each of the three interventions (3)	Paxgene	1 × 2.5 ml	7.5 ml	RNA	Gene expression clock genes (in batch)
After each of the three interventions (3)	EDTA	1 × 2 ml	6 ml	Plasma	Metabolomics and proteomics (in batch)
After each of the three interventions (3)	Serum	2 × 3.5 ml	21 ml	Serum	Biobanking
Total amount of blood			**62 ml**		

from heat flux 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

- Objective emotional arousal and stress will be continuously measured by the wristband through skin conductivity (electrodermal activity)
- To monitor objective physical activity, raw accelerometry will be derived from the wristband. Raw data will be collected at 32 Hz and will be derived from the wristband similar to the heart rate monitoring and HRV

Follow-up measurements

After the baseline measurements, follow-up measurements will be performed (at the study site) on the 13th day of each intervention period. Each visit at the study site will take 30–45 min and will take place in the morning. The following measurements will be performed (once at baseline and) during follow-up (3 times):

- Insomnia severity measured by the Insomnia Severity Index (ISI), a 5-item index on insomnia and sleep quality. Based on the ISI questionnaire which comprises 7 questions, participants can be categorized as having: "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 an appropriate threshold when detecting insomnia cases in a community sample [30].
- Fasting blood samples will be collected and stored at − 80 °C. A total of 62 ml blood will be drawn during the study period. Table 2 gives an overview of the blood parameters that will be determined after storage, and those that will be determined during screening in freshly sampled blood. Participants will be asked not to eat or drink (except for water) during the 12 h before the blood draw

Study administration

Study data will either be recorded on Case Record Forms and transferred to or directly collected and managed in Castor Electronic Data Capture (EDC), in accordance to General Data Protection Regulations (GDPR). Data that is collected by external partners (Corsano Cardiowatch 287-2, Withing Sleep analyzers, and DLMO analysis by Chrono@Work, Groningen, The Netherlands) is stored in General Data Protection Regulation (GDPR) proof cloud services and transferred encrypted to the researchers. External partners processing of storing data derived from the Corsano Cardiowatch 287-2 or Withings Sleep analyzer will sign a data processor agreement and are obliged to remove all collected data after transfer to the researcher and at the researchers request. All data except traceable personal data will be collected on an encrypted disk in the LUMC and an SPSS file will be composed. More information on our data management plan can be found in the CCMO register (https://www.toetsingonline.nl/to/ccmo_search.nsf/fABRpop?readform&unids=E322CB2A5196384BC1258A950005D247).

Safety and monitoring

According the risk classification, this study has a negligible risk. This was determined in consultation with the monitoring coordinator of LUMC after approval of this research protocol. Due to the negligible risk, a data safety and monitoring board will not be appointed, and there will be no interim analysis. Given the short duration of the study, an independent monitor will monitor this study once during the recruitment period. Additionally, the monitor will guide the start and closing of the study period to ensure quality. The monitor process is documented in a monitoring plan. This trial may also be subject to internal or external and independent auditing or inspections procedure to ensure adherence to good clinical practice. Access to all trial-related documents including direct access to source data will be given at that time.

Serious adverse event

A serious adverse event (SAE) is any untoward medical occurrence or effect that

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

Power calculation and statistical analysis

The required 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 intervention as measured by the ISI, a validated and sensitive questionnaire for mapping insomnia severity [45]. 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 the treatment effect size of guideline recommended physical activity on insomnia severity (measured by ISI) found in a previous randomized controlled trial [46]. 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.

All data that will be collected in this study will result in (or be transformed into) quantitative data that will either be categorical or continuous. Normal distributions will be checked by plotting histograms and QQ plots of the residuals. 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 repeated data for analysis of trajectories or 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 standard algorithms statistical software packages.

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

Changes in the explorative secondary endpoints mentioned above will be measured between the three interventions. Twenty four-hour patterns will be calculated through the hourly mean level of for example heart rate and will be displayed for each condition. Additionally, we will perform sensitivity analyses for both the primary endpoint as well as all secondary endpoints by analyzing the changes between the sedentary period and morning activity and between the sedentary period and evening activity. Assuming the data is normally distributed, paired *t*-tests will be used for all analyses, and data will be presented as mean difference, SD, and *p*-value.

Discussion

Most biochemical, physiological, and behavioral processes are orchestrated by the circadian timing system to synchronize these with the 24-h day/night cycle [60]. Processes related to energy metabolism, hormone secretion, sleep wake cycles, and regulation of core body temperature are strongly governed by circadian rhythms [61]. Physical activity is known to act as a Zeitgeber to synchronize our circadian rhythms with each other and with the environment. However, little is known about the optimal timing for physical activity or about its effect on insomnia and sleep problems in the elderly population. The ON TIME intervention study aims to assess the effect of physical activity timing on sleep and parameters related to biological clock function.

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. Largely removing inter-participant variation, it allows for a more efficient and precise effect estimation for which less participants are needed [62, 63]. As we study the effect of timing on change in insomnia severity and changes in the rhythms of multiple processes hypothesized to be altered rapidly through environmental cues, such as daylight changes over the season, we considered a randomized cross-over study which is usually bound to short-term outcomes the most suitable design for this study.

There are several challenges to consider when designing a study on behavioral aspects within the field of circadian medicine. For example, influences of seasonal and even daily timing effects are important to consider [64]. This study is planned to take place from April until and June 2024. Yet, since seasonal changes in daylight hours may interact with the primary and secondary outcomes of this study, it is essential that all participants participate in the study simultaneously. Moreover, since all participants undergo the study procedures at the same time and this data all needs to be collected at approximately the same time of day, this study requires a relatively large research crew. For feasibility purposes, we have divided the study population in two groups which will start one day apart. We chose a randomized cross-over design to increase the feasibility of this study. Such design allows for fewer participants and is especially suitable to assess short-term effects [63].

Another point of attention in planning such a study is the daylight-saving time. When this event occurs during the study period, it is likely to affect results and to complicate the interpretation of the results. For this reason, we will maintain at least 7 days between daylight saving time and the baseline measurements. Moreover, it is known that the 3 major Zeitgebers (light exposure, food intake, physical activity) interact with each other [23]. When studying one of these Zeitgebers, it is important to either standardize, control, or register the others. Since this study will only examine the effect of physical activity timing, we have chosen to register food intake (through a food diary) and to standardize light exposure as much as possible in a "free" daily living situation. This is done by carefully choosing the seasonal timing of the study period and to ascertain that light exposure will be as similar as possible between the active morning and active evening exercise.

To the best of our knowledge, this is the first randomized cross-over study on the effect of physical activity timing on insomnia and biological clock function in older adults with sleep problems. We anticipate that this study will make a significant contribution to the currently limited knowledge on the effect of physical activity timing by its assessment of a comprehensive variety of continuous outcomes.

Abbreviations

ADL	Activities of daily living
BIA	Bioelectrical Impedance Analysis
CBT	Core body temperature
CCI	Charlson comorbidity index
DLMO	Dim light melatonin onset
ECG	Electrocardiogram
EDC	Electronic Data Capture
eEMA	Electronic Ecological Momentary Assessment
EQ-VAS	EuroQol visual analogue scale
EQ-5D-3L	EuroQol 5 dimension 3 levels
GP	General practitioner
GDPR	General Data Protection Regulations
GPPAQ	General Practice Physical Activity Questionnaire
HRV	Heart rate variability
IADL	Instrumental activities of daily living
IC	Informed consent
ISI	Insomnia Severity Index
IQR	Interquartile range
LUMC	Leiden University Medical Center
MCTQ	Munich Chronotype Questionnaire
MET	Metabolic Equivalent of a Task
MREC-LDD	Medical Research Ethics Committee Leiden Den haag Delft
N	Number
PAI	Physical Activity Index
PIF	Participant information form
PPG	Photoplethysmography
REM	Rapid eye movement
SAE	Serious adverse event
SD	Standard deviation
4 MWT	4-Meter walk test

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13063-024-08310-7.

Supplementary Material 1.

Acknowledgements

Not applicable.

Trial status
Version 8 is, to this date, the latest approved version of the study protocol. It was approved in February 2024. The recruitment period for this study was from January 8 until March 28, 2024. In April 2024, the baseline measurements were carried out. The study interventions (as well as data collection) ran from May 2, 2024, until June 28, 2024.

Authors' contributions
Leiden University Medical Centre (Albinusdreef 2, 2333 ZA Leiden, The Netherlands) is the sponsor of this trial. This study was conceptualized by GA, RN, DvH, DvB, and MvdE. VE and LK provided their expert opinions during conceptualization. GA and MvdE will manage the day-to-day administration and progress of the study. DvH, MvdE, and GA will undertake the intervention and oversee research assistant. DvH, RN, GA, and MvdE will conduct participant home and clinic visits. GA and RN are responsible for the accelerometer aspects of the study. RN and GA designed the statistical plan and will undertake all statistical analyses. MvdE, GA, and DvH are responsible for clinical review of the screening- and study outcomes. GA drafted this manuscript. All authors read and approved the final protocol manuscript.

Authors' information
GA is a PhD student at the department of internal medicine, subdepartment of Geriatrics and Gerontology. She will be the coordinating researcher for the ON TIME study. Raymond Noordam is an assistant professor, and MvdE is a research nurse at the abovementioned department. LK is an assistant professor in cell and chemical biology and consortium coordinator of the BioClock consortium. VE is a researcher and product manager at the Centre for Human Drug Research (CHDR). DvB is a professor in vitality and aging. DvH is the principal investigator for the ON TIME study and an associate professor at the department of internal medicine, subdepartment of Geriatrics and Gerontology.

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Availability of data and materials
In the future, the datasets used and/or analyzed during the current study available from the corresponding author on reasonable request. Trial results will be shared or published on multiple websites within the target group and in open access journal as scientific reporting.

Declarations

Ethics approval and consent to participate
This project received ethical approval from the Medical Research Ethics Committee Leiden Den Haag Delft (reference # NL82335.058.22). Version 8 of the protocol is the latest approved version. It was approved by the abovementioned ethics committee in February 2024. The ethics committee as well as the research team will be informed by writing of all modification to the research protocol. Written consent will be obtained from the participant included in this study by a qualified member of the research team.

Consent for publication
Not applicable. No identifying images or other personal or clinical details of participants are presented here or will be presented in reports of the trial results. The participant information materials and informed consent form are available from the corresponding authors on request in coded form.

Competing interests
The authors declare that they have no competing interests.

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