Extended Study Name: "INNOVATIVE POLICIES FOR IMPROVING CITIZENS' HEALTH AND WELLBEING ADDRESSING INDOOR AND OUTDOOR LIGHTING"

Protocol Title: Population based lighting study on older adults

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Study Acronym: ENLIGHTENme (Coordinator: Simona Tondelli, Full Professor of Urban Planning at the Department of Architecture of the University of Bologna).

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The funding body had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data or decision to submit results.

Protocol Title: Population based lighting study on older adults

Coordinating Center: IRCCS Istituto delle Scienze Neurologiche di Bologna, AUSL di Bologna

Principal Investigator: Dr. Francesco Nonino

Protocol version: 3.0 (24-02-2023)

Roles

The **coordinating center,** with the role of supervision and auditing of trial conduct (check in the recruiting phase and in the follow-up phase), data monitoring and final statistical analysis, will be the IRCCS Istituto delle Scienze Neurologiche di Bologna, AUSL Bologna (three epidemiologists and three statisticians).
Data will be collected by study staff at one recruitment center in each city:

- IRCCS Istituto delle Scienze Neurologiche di Bologna, AUSL Bologna
- Vrije Universiteit Amsterdam
- Tartu Ulikool

One MD for each centre will be in charge of decision on eligibility, recruitment and follow-up of participants. One study coordinator for each centre will manage any organisational activity of the trial (eg, participant reception, scheduling of follow-up visits, data entry supervision, etc).

1. Background and Rationale

A major, albeit substantially underestimated, byproduct of urbanization has been an exponential increase of human exposure to electric light at night. In fact, in addition to public outdoor illumination and the artificial sky glow created by highly urbanized areas [1], people are also increasingly exposed to light at the individual level (domestic lighting, light-emitting screens including computers, smartphones, etc.).
It is now firmly established that inappropriate and disruptive light exposure at night or too little light during the day, may profoundly affect people's circadian rhythms and sleep, health and wellbeing, impacting on epigenetics and metabolism [2,3], predisposing to diseases including cancer [4,5], neurodegeneration [6,7] and psychiatric morbidity [8], particularly affecting fragile subgroups like older adults [9, 10].

Moreover, light shapes urban spaces and social life, whether at home or in public spaces, thus affecting people's behavior, mood, sense of security as well as social relationships. Lighting can increase citizens' sense of trust towards the city and the people who inhabit it, thus encouraging people to remain and to interact with each other, as well as promoting socialization within the urban community. Furthermore, lighting impacts older adults' lives in terms of access to public space and participation in civic life, and through the quality of indoor spaces in which they spend more time than younger adults.
Although there is an increasing public awareness of light-related health and wellbeing issues, lighting policies and investment strategies still focus on cost, energy efficiency, safety, or city-branding with poor understanding of how the impact of urban lighting on health is mediated by social inequalities that may determine the kind and amount of light that citizens are exposed to.
The ENLIGHTENme project aims at collecting evidence about the impact of outdoor and indoor lighting on human health and wellbeing through the development and testing of innovative solutions and policies that will also counteract health inequalities in European cities. In particular, through an open-online Urban Lighting and Health Atlas, ENLIGHTENme will collect and systematize existing data and good practices on urban lighting and will perform an accurate analysis on the correlations among health, wellbeing, lighting and socio-economic factors in three pilot cities: Bologna (Italy), Amsterdam (The Netherlands), and Tartu (Estonia).

In this context, the ENLIGHTENme project will also include an interventional, multicenter, prospective, randomized, controlled, unblinded trial involving one target district, selected based on its artificial light characteristics, in the urban areas of each of one of the three pilot cities. Within each target district, a random sample of individuals aged 65 years or older (intervention group) will be exposed to modifications in domestic indoor lighting and compared with a control group, living in the same target district, unexposed to domestic electric light modifications. At the same time, in a specific area of the target district, outdoor lighting will be modified by the local municipal authority. The hypothesis to be tested in this study is that light interventions may improve individual physical and mental health by affecting circadian entrainment, sleep pattern, and mood. Thus, the study is aimed at providing evidence whether the planned change in electric light exposure at both urban public outdoor and domestic indoor lighting levels may impact on physical and mental health by improving photo-entrainment of circadian rhythms to the light-dark cycle.

2. Study Objective(s)

2.1 Primary Objective

The primary aim of this study is:

- To assess the impact of lighting strategies on health, sleep, and wellbeing of adults of age 65 years or older. In particular, experimental interventions on indoor and outdoor lighting will be tested on population samples living in selected urban areas.

2.2 Secondary Objective(s)

The secondary aims of the project are:

- To assess the impact of lighting strategies on people's circadian rhythms photoentrainment and sleep as measured by actigraphic-derived metrics such as inter-daily stability (IS), intra-daily variability (IV), and relative amplitude (RA) of rest-activity rhythms, sleep efficiency (SE) and fragmentation index (FI), and the phase angle between dim light melatonin onset time (DLMO) and sleep onset time.

- To assess the impact of outdoor and indoor lighting innovations on mental and physical health as measured by validated and self-reported questionnaires for mental and physical health assessments.

3. Study Endpoint(s)

3.1 Primary Endpoint

The primary endpoint of the study is to evaluate the impact of electric light on sleep quality as measured with the Pittsburgh Sleep Quality Index (PSQI), a 19-item self-reported questionnaire that measures sleep quality over the previous month. A higher score indicates poorer sleep quality, therefore the hypothesis to be tested is that a participant's sleep quality will improve (i.e. PSQI score will decrease) at the end of the light intervention period compared to baseline.

3.2 Secondary Endpoints

The secondary endpoints will be:

- Change in photoentrainment as measured by the phase angle between DLMO and sleep onset between the baseline and the end of the light intervention period
- Change in circadian rest-activity measures (IS, IV and RA) as well as sleep measures (SE and FI) as derived from actigraphic measurements between the baseline and the end of the light intervention period
- Change in mental and physical health as measured by validated and self-reported questionnaires between the baseline and the end of the light intervention period
- Correlation between clinical parameters derived from actigraphic and melatonin assays and the polygenic risk score derived from publicly available summary statistics of Genome-Wide Association Studies (GWAS), as well as the mitochondrial DNA (mtDNA) haplotypes

4. Study Plan

4.1 Study Design

This is a European interventional, multicenter, unblinded, randomized, controlled trial on citizens 65 years of age or older living in urban target districts of the cities of Bologna (Italy), Amsterdam (The Netherlands) and Tartu (Estonia). The study is aimed at evaluating the impact on health and wellbeing of an artificial indoor and outdoor light intervention, compared to no intervention. The effect of tailored interventions on outdoor public illumination will be evaluated in specific areas nested within the target urban districts of the three cities mentioned above.

The indoor light intervention will be conducted over a period of 15 months. During this period, 5 groups of participants will be exposed each to a 12-week indoor lighting intervention/control condition, preceded by two weeks of baseline assessment and followed by two weeks endpoint assessment, bringing the total to 16 weeks.

Such assessments will include multiple instrumental, clinical and self-reported parameters, as well as sampling of biological material (saliva).

4.2 Study Setting

Within the 3 participating cities, the recruitment of the cohorts will be operated in a target urban district selected through a step-by-step process, which had combined a number of criteria including socio-economic considerations, population stratification, health epidemiological profile, urban and lighting conditions. In particular, the selected urban districts have to be highly exposed to artificial light, to cover a urban area populated by about 20,000-25,000 individuals, to be fairly homogeneous in terms of characterized social

inequalities and have a well-represented density of older individuals as a fragile subgroup of interest, thus with a determined profile in terms of health determinants distribution.

The selected districts, according to the above mentioned criteria are: Quartiere Savena (Bologna), Wildeman, (Amsterdam) and Anneline (Tartu).

5. Study Population

5.1 Number of subjects

To compensate for participants' drop-out , we will consider for the population sampling an area within the target urban districts including at least 600 individuals aged 65 or older. This number has been determined based on sample size and study power calculation (see section "**7. Statistics**").

5.2 Inclusion Criteria

- Living in the three selected cities within the target district chosen for the study
- Women and men
- Age 65 years or older
- Signing informed consent

5.3 Exclusion Criteria

- Lack of or inability to provide informed consent
- Lack of or inability to allow data collection over the course of the study

5.4 Representativeness of sample

Since the recruitment will be voluntary, encouraged through an informative campaign curated by the municipalities of the participating cities, the sample may not be fully representative of the target population to which the results of the study will be applied (the whole European population aged 65 or older). Therefore, the inevitable sample selection in a population-based study may limit the generalizability of its results. However, the criteria adopted to select the target district (distribution of health determinants, urban lighting conditions with high exposure to artificial light, urban area fairly homogeneous in terms of socio-economic inequalities, well represented population of older individuals as a fragile subgroup of interest) is among the strengths of the study. To avoid systematic errors in the sample selection among the three cities, we will adopt consistent strategies of promotion and communication of the project. Internal validity will be warranted by the randomized design and by adjustment procedures during the analysis.

6. Description of Study Procedures (study procedure description by visit)

6.1 Pre-Recruitment phase (Informed Consent to be contacted by telephone - January 2023)

Participation in the study will be encouraged by planning public events and conferences aimed at informing the population of each urban district and at fostering recruitment. A campaign including thematic meetings in the framework of the so-called "Urban Lighting Labs (ULLs)" held in locations administered by the Municipalities will be scheduled. Multiple media (TV, local newspapers, social media) will be exploited to maximize participation. Local researchers and health opinion leaders will participate in the promotion campaign. Mobile stands will be placed outside specific locations of social aggregation (churches, malls,etc.) in the selected urban districts to offer information and the possibility of joining the study. A written informed consent to be contacted will be collected during public events and conferences by study staff from persons

willing to participate in the study. People expressing their signed consent will be then contacted by telephone to set an appointment at the recruitment center of each city, during which they will receive detailed information about the study (purpose, schedule, mode of participation, sample collection, etc.).

6.2 Recruitment phase (Informed Consent to participate to the study)

After the pre-recruitment phase, those who expressed interest in the participation, will be asked to sign a written consent to be contacted, and will be therefore contacted to set an appointment with the researchers, during which they will:

- receive detailed information about the study (purpose, schedule, mode of participation, sample collection, etc.) and the information sheet,
- sign the GDPR compliant informed consent to participate to the study,
- sign the GDPR compliant informed consent of data collection, management, storage and analysis.

After signing the informed consent, each participant will be randomly assigned to the intervention or control arm with an allocation ratio of 1:1. Assignment will be performed according to a randomization list generated by an automated web-based system.

Periods of recruitment will be scheduled depending on the city latitude in order to warrant homogenous exposure to daylight cycle among the three cities during the intervention.

6.3 Intervention

Persons allocated to the indoor light intervention arm will be given a lamp with specific instructions. They will be asked to place the lamp in the room of their home where they spend most of their time (lounge or dining room/living room) in order to supplement the existing indoor lighting.

Thus, all people of the intervention arm will be equipped with the LUMIE Halo lamp.

https://www.lumie.com/products/halo

The Halo lamp is marketed by Lumie, a UK-based company producing light equipment, and can be purchased on line.

Utilizing both warm-white and cool-white LEDs, Lumie Halo delivers 10,000 lux at 20 cm at maximum brightness in "Day" Mode. The touch slider allows the user to adjust the brightness while mixing the color temperature of the light.
As for safety standards Halo was independently tested to EN 62471: 2008 (Photobiological safety of lamps and lamp systems) and was found to comply with the requirements of Exempt Group. As part of this test, ultraviolet hazards for eyes and skin were assessed (Hazards assessed: Actinic UV skin & eye and Eye UV-A).
Lumie Halo has been tested to meet:
• Directive 2014/35/EU relating to the making available on the market electrical equipment designed for use within certain voltage limits (Low Voltage Directive – LVD)
• Directive 2014/30/EU relating to electromagnetic compatibility (EMC Directive)

The instructions for the indoor light intervention in all three cities will be:

- to put the Halo lamp in the room where the participant spends most of his/her time with no need of setting a minimum distance;
- to turn the Halo lamp on in the "Day" Mode (full bright dim) for at least 2 hours, even if not consecutive, during the day, for 12 weeks when the participant is indoors
- to switch on the Halo lamp at least 1 hour after waking up but not before sunrise
- to turn off the Halo lamp 4 hours prior to bedtime.

The total exposure time to the light of the HALO lamp will be assumed basing on the self-administered questionnaires that participants will fill during the 2-week baseline assessment, the assessment after six months and the final 2-week assessment at the end of the study. Such self-administered questionnaires will collect information on the time spent at home and out of home. Persons allocated to the control group will receive no indoor light supplementation and will undergo assessment procedures only. Exposure to the outdoor intervention will be taken into account in the analysis in three ways, (1) by locating the distance between people's home and the modified outdoor light, (2) by a question about the awareness of the change in outdoor light, timing, and duration of exposure and what they think of it, and (3) by recording the amount of light exposure over the 24 hours during two weeks at baseline and after the 12 weeks of interventions.

In the statistical analysis, the effect of indoor lighting will be evaluated both individually and adjusted for the effect of outdoor lighting, if available. Therefore, any possible interaction effect between indoor and outdoor light exposure will be considered.

6.4 Baseline and Follow-up assessment procedures

Biomedical assessment protocol description

We will carry out a two-week period assessment immediately before the light-intervention (baseline assessment) in the pilot area, and after 12 weeks from intervention (follow-up assessment). All subjects of the intervention and control groups will undergo the same study procedures at both time-points (baseline and follow-up assessment). For study procedures and timelines please see table on **section 11**.

At the baseline time-point, a first meeting (**meeting 1**) will be conducted during which subjects will be informed on the overall design and aims of the study and asked to sign the informed consent form (ICF procedure). Individual medical history will also be retrieved by means of standardized (self-administered) **questionnaires** on the following domains:

- summary dietary lifestyle
- overall lifestyle
- socio-economic status
- overall sleep features and mood;
- visual function and home lighting conditions
- sleep diary (bed/rise time, time to sleep onset, and daytime naps)

Furthermore, each enrolled subject during meeting 1 will also receive:

- wrist **actigraph** for recording their rest/activity rhythm. Instructions for use will be provided verbally and in written form.
- **light sensor** placed as a brooch. Instructions for use will be provided verbally and in written form.
- saliva sampling **tubes** for melatonin quantification, with instructions (i.e. all participants will collect saliva during the evening of the 13th day, after meeting 1, before the light supplementation stage starts).
- saliva collector **tubes** for DNA extraction, with instructions.

In the same context, a 2nd meeting (**meeting 2**) will be scheduled, after 14 days, at which all subjects will return the questionnaires delivered at visit 1, and completeness of the forms will be checked. The actigraph and sensors delivered at meeting 1 and saliva samples will be also returned. At the end of this meeting the indoor light (**LUMIE Halo lamp**) will be given to participants of the intervention group to be placed at home, with instructions for use given verbally and in written form.

At the follow-up time-point, after 12 weeks of light-intervention, a 3rd meeting (**meeting 3**) will be conducted during which individual medical history will be updated and registered. During the same 3rd meeting, standardized (self-administered) questionnaires will be administered on the following items:

- summary dietary lifestyle
- Overall lifestyle
- socio-economic status
- Overall sleep features and mood
- visual function and home lighting conditions
- sleep diary (bed/rise time, time to sleep onset, and daytime naps)

Furthermore, each enrolled subject will also receive, as for meeting 1:

- wrist **actigraph** for recording their rest/activity rhythm. Instructions for use will be provided verbally and in written form.
- **light sensor** placed as a brooch. Instructions for use will be provided verbally and in written form.
- saliva sampling **tubes** for melatonin quantification, with instructions

In the same context of the follow-up assessment, a 4th meeting (**meeting 4**) will be scheduled, after 14 days, at which all subjects will return the questionnaires delivered at meeting 3, and completeness of the forms will be checked. The actigraph and light sensors delivered at meeting 3 and saliva samples will be also returned.

Overall, the study procedures will imply the collection of four different sets of data:

1. Instrumental (wrist actigraphy recordings to evaluate rest-activity circadian rhythms and wearable light sensoring device recording)
2. Biological material (saliva) for genotyping and circadian biomarker (melatonin) assessments
3. Clinical history of life-long morbidities

4. Self-reported questionnaires for mental and physical health assessments with particular reference to quality of sleep and chronotype, and socio-economic questionnaires.

1. Instrumental recordings

The instrumental recordings described in this section aim to evaluate the actigraphic sleep-wake cycles as a proxy of circadian photoentrainment [6], tightly correlated to the light exposure, with particular reference to the individual light exposure at night.
Spontaneous motor activity, indicative of rest-activity cycles, will be assessed for each enrolled individual by a **wrist actigraph** and worn on the non-dominant wrist for 14 days.
Simultaneously, personal light exposure will be recorded over the same 14 days by a **light sensor** placed as a brooch, for monitoring the light exposure of each participant.

Both the actigraph and the light sensor, are produced by Condor Instruments (www.condorinst.com.br).
This wearable system includes three hardware parts: 1) The Mini-AT Actigraphy device that is capable of measuring acceleration (used for rest/activity and sleep estimation) to be placed on the wrist. 2) The Mini-AT Light Sensor device that measures RGB Light exposure to be placed on clothes as a brooch. 3) The Mini-AT Gateway, responsible for creating a seamless interface with both sensors and the Cloud System through wifi connection.
Both sensor devices have a battery capable of withstanding a 1 month with a single charge and uses Bluetooth communication to transmit the data acquired. The actigraph will upload the collected data to the cloud (see section "8. Data Management Plan") using the gateway automatically when connected on the charging dock. Sleep algorithm will be automatically processed, and all raw data will be available.
Data will be assessed through a dedicated web-based Cloud Platform (see section "8. Data Management Plan").
To complement the actigraphic recording, each participant will also complete a **sleep diary** over the 14 days of actigraphic monitoring indicating bed/rise time, time to sleep onset, and daytime naps to allow assessment of the actigraphic sleep-wake patterns. Additional information about sleep, chronotype, mood disorders and wellbeing will be obtained by self-administered questionnaires (see point 4. below on self-reported questionnaires).

2. Biological sample (saliva)

Biological samples (saliva) will be collected for two separate investigations.

The first is aimed at providing further information on the status of circadian rhythms of the enrolled individuals by measuring in a simplified way the rhythm of melatonin secretion at night, one of the key proxies of circadian rhythm.

The second is aimed at correlating nuclear and mitochondrial DNA genotypes with the circadian phenotypes we anticipate to observe at baseline in our cohort of elderly individuals. Over 80 genes are associated with circadian rhythm regulation [11] and others impinge on chronotype, whereas population-related mtDNA variation has been shaped by environmental selective pressure including climate, infections, diet, and ultimately exposure to light. Thus, the genetic profile underlying the circadian phenotypes, with particular focus to pathological misalignment eventually related to artificial light over-exposure, and responses to the diurnal light intervention after 12 weeks, are the objectives of the genetic investigations.

Saliva samples for melatonin measurement (at least 1 ml) will be collected using Salivettes® (code 51.1534, manufactured by Sarstedt AG &Co., Germany, www.sarstedt.com), in the evening of the last day prior to meeting 2.

Measurement of dim light melatonin onset (DLMO): 7 sequential saliva samples will be collected at baseline in all 500 recruited subjects. The collection of saliva samples after the intervention will be performed in 100 randomly selected subjects in the control group and 100 in the experimental group in each city. Such random sampling after the intervention is due to the need of adjustment to the budget.

The saliva sampling will be performed hourly starting 5 hours prior to each individual's usual sleep onset until 1 hour after usual sleep onset. During sampling, the participant will remain in dim light conditions (< 10 lux) so that melatonin levels are not suppressed. Samples will be stored at 4ºC at people's homes for a maximum of 7 days. Samples will be sent to the laboratory or handed over during meeting 2, centrifuged and aliquots will be stored at -80 ºC.

Frozen samples will be transported to the laboratory for melatonin measurement by radio-immuno-assay (RIA), carried out by Chrono@Work, reagent materials from Novolytix, Switzerland. The melatonin profile will allow calculation of the DLMO, a reliable proxy of circadian timing (phase). The time interval between DLMO and the participant's habitual bedtime will allow calculation of the phase of entrainment.

One sample of saliva will be separately collected in a different collection device (GeneFixTM Saliva Collector GFX-01, Isohelix) for DNA genotyping, . DNA will be extracted from 1 ml stabilized saliva sample using GeneFiX Saliva-Prep 2 DNA Isolation Kit (GSPN-50, Isohelix). The collection of this sample is aimed at carrying out global genotyping of all participants included in the study, including both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA).
mtDNA haplotype analysis will be aimed at studying markers fixed during evolution as adaptation to environmental factors (climate, diet and infections), while nDNA SNP array will be aimed at deriving the polygenic risk scores.

SNP-Array (nDNA genotyping): SNP array will be carried out by using Infinium Global Screening Array-24 on the Illumina GSA platform at the Core Genotyping Laboratory of the Institute of Genomics, University of Tartu, Estonia. PLINK format files will be created using Illumina GenomeStudio. Prephasing will be done using Eagle software and imputation will be done using Beagle.

Mitochondrial genome sequencing (mtDNA haplotype): sequence analysis of mitochondrial genome will be carried out on total DNA extracted from saliva, by Next Generation Sequencing (NGS) method.

The library will be constructed by xGen DNA Lib Prep EZ UNI (IDT) protocol with xGen™ UDI 10nt Primer Plates 1-16 (IDT), followed by capture protocol with xGen Human mtDNA Hybridization Panel (IDT). Library will be sequenced on Novaseq 6000 System (Illumina), using flow cell S4 (300 Cycles), pooling 1536 samples and obtaining a mean coverage of about 6000x. Reads generated will be demutiplexed and quality checked using bcl2fastq2 and FastQC tool, then aligned to rCRS (NC_012920.1) reference mtDNA genome and variants called using MitoScape. The mtDNA haplogroup, private and heteroplasmic variants will be defined by Haplogrep. All mt DNA analyses will be performed at the laboratory of neurogenetics of the IRCCS Istituto delle Scienze Neurologiche di Bologna, Italy.

3. Clinical history

A summary medical history of the main past and current conditions of the participant will be recorded in a structured questionnaire and any specific health documentation that the subject may consider as relevant will be also recorded. Concurrent morbidities that may potentially impact the variables of interest will be considered for sensitivity analyses.

4. Self-reported questionnaires

In order to complement the objective measures and clinical information gathered in the previous points, we will administer a series of self-reported questionnaires (validated in Italian language) in the following areas:

I. Quality of sleep and sleepiness: Epworth Sleepiness Scale (ESS), Pittsburgh Sleep Quality Index (PSQI).
II. Chronotype: limited Horne-Östberg Morningness/Eveningness, and ultrashort Munich Chronotype questionnaire
III. Quality of life and wellbeing: Satisfaction with life scale, Subjective happiness scale, Flourishing scale, EuroQol 5D (EQ5D)
IV. Mental health: Hospital anxiety and depression scale (HADS), Three-item Loneliness Scale

7. Statistics

7.1 Sample Size

To determine the sample size, we assumed for the PSQI score in an elderly population an average score at baseline of 4.75 and a standard deviation of 3 [12].
Based on such assumptions, to detect a difference in mean PSQI score (primary outcome measure) of 0.75 points between the intervention and control groups at the end of follow-up, with 80% power and 5% significant level (two sided), 500 participants (250 in the intervention and 250 in the control group) will be needed (**Table 1**).

	Intervention group	**Control group**	**Total recruitment participant**	**Detectable mean difference**
In each one of the three cities	250	250	500	0.75

Table 1 – Study power (significant level 5%, power 80%)

We expect a dropout rate of about 20% (baseline assessment and follow-up assessment); therefore, in order to avoid attrition bias, about 100 more individuals will be recruited, with a total recruitment of about 600 individuals (300 for group) per city.

7.2 Statistical Methods

As descriptive analysis, continuous variables will be presented as mean ± standard deviation (SD) or median and Interquartile Range (IQR), while categorical variables as absolute frequency and relative frequency (%). Student T-test, Kruskal Wallis test, Chi-square test, and Pearson or Spearman correlation will be used to evaluate the univariable associations between the variables depending on the type and nature of variables. We will compare the variables in the same participants before and after the "intervention" and in a concurrent control group without intervention. For the comparison before-after intra groups we will use McNemar's test for binary and nominal outcomes, paired T-test for quantitative outcomes with normal distribution and Wilcoxon test for discrete or quantitative outcomes with non-normal distribution.

For the primary end-point, the PSQI-score, the comparison between intervention and control group will be performed by a linear regression model, which can be summarized by the following equation:

Follow-up PSQI-score = constant + (a x baseline PSQI-score) + (b x group)

where a and b are estimated coefficients and group is a binary variable coded 1 for intervention and 0 for control. The coefficient b is the effect of interest: the estimated PSQI mean difference at the end of follow-up between the two groups, adjusted from the PSQI-score at baseline [13].

Nonparametric methods will be used for the analysis of **circadian rhythm rest-activity measurements** [14]. Calculations will include: interdaily stability (IS; an index ranging from 0 to 1 reflecting the strength of the rhythm to supposedly stable environmental zeitgebers), intradaily variability (IV; an index ranging from 0 to 2 measuring the frequency and extent of transitions between rest and activity), and relative amplitude (RA; the normalized difference between the most active 10-hour period [M10] of the rest–activity rhythm and the least active 5-hour period [L5] in the average 24-hour pattern). Sleep efficiency and fragmentation index will also be derived as will the time of L5 and M10. Similarly, light information (in lux) will be processed from the sensor recordings.

Outdoor light exposure will be added to the model as an additional factor. If in the descriptive analyses some other variables are found to be unbalanced between the two groups with/without indoor light modification, we will add them as covariates in the regression model as sensitivity analysis. The results will be presented as beta coefficients and 95% Confidence Interval (95% CI).

For the secondary end-points we will use multivariable models (with the end-point as dependent variable and the groups as independent variable) to adjust the estimates with the potential confounders (e. g. unbalanced variables after randomization). Different types of multivariable models will be used depending on the type and distribution of the specific outcome.

Primary analysis will be undertaken on an intention to treat basis. A per-protocol analysis of the primary and secondary end-points will also be carried out, excluding participants with protocol deviation.

7.3 Subgroup Analyses

Subgroup analysis will be performed for clinical, biological and instrumental variables. The presence of interaction between the intervention and the other variables will be computed from the log-likelihood ratio test comparing models with and without interaction. In a possible sub-group analysis, outdoor light exposure will be added to the model as a covariate, for example we will compare the following models:

Follow-up PSQI-score = constant + (a x baseline PSQI-score) + (b x group) + (c x outdoor light)

vs. Follow-up PSQI-score = constant + (a x baseline PSQI-score) + (d x group * outdoor light)

where outdoor light is a binary variable coded 1 for presence and 0 for absence of outdoor light exposure, c is the direct effect of the outdoor light exposure and d is the interaction effect between indoor and outdoor light exposure. The interaction term will then allow to assess four levels of exposure: indoor plus outdoor light exposure, only indoor light exposure, only outdoor light exposure and neither indoor nor outdoor light exposure. Stratified estimates will be presented if the log-likelihood ratio test (comparing these two models) will be significant.

Polygenic prediction and mtDNA analysis

In order to investigate the sources of individual differences in the phenotypes under study we will calculate polygenic risk score using the collected DNA samples and publicly available summary statistics of GWAs.

Polygenic scores will be estimated using LDpred, a method that takes into account the level of linkage disequilibrium between measured single-nucleotide variants (SNVs; often called single-nucleotide polymorphisms) to avoid inflation of effect sizes. The method LDpred requires the inclusion of prior probabilities corresponding to the fraction of SNVs thought to be causal, which allows for testing varying proportions of SNVs associated with the outcome of interest. We thus will test a range of priors (0.75, 0.50, 0.30, 0.10, and 0.03) to assess the prior at which assessment is optimal. We will restrict analyses to common variants, using SNV inclusion criteria of minor allele frequency greater than 5% and imputation quality of R2 greater than 0.90.
Similarly, mtDNA haplotypes will be correlated with individual phenotypes under study at baseline, and responder analysis will be carried-out at the follow-up assessment.

8. Data management plan

The project will involve personal/sensitive data collection, namely:
- Name, surname, telephone number, address including postcode, status, age, ethnicity and gender
- Individual health and well-being data coming from the biomedical and clinical research activities, such as:
- Instrumental recordings (actigraphic recordings assessing rest-activity circadian rhythms and wearable light sensors)

- Genetic and biological data (saliva samples for genotyping and melatonin measurements
- Medical history data
- Self-reported parameters for mental and physical health assessments, with particular reference to quality of sleep, mood and chronotype, socioeconomic questionnaires
- Sleep/lifestyle diary
- visual function and home lighting conditions

Data will be collected for the following purposes and collected/stored in the following ways:

Types of data	Purposes	Collection/storage
Contact data	To favor participation in the study (see the “6.1 Pre-Recruitment phase”).	Stored separately from other data and accessible only to each PI of each participating city.
Demographic data (age, ethnicity and gender)	To interpret the experimental results and reach the correct conclusion about the impact of lighting strategies on health, sleep, and wellbeing (see section 2.1 and 2.2 of the protocol)	Pseudonymized data will be collected and stored directly into the electronic data-capture (EDC) system. All participating sites will have access to the data entered for the subjects enrolled at that specific site. Sites will be responsible for

		entering extracted patient data into a secure internet-based EDC database via the eCRF.
Individual health, socioeconomic and well-being data	Biomedical and clinical research activities of this clinical study (see section 6.4 of the protocol).	Pseudonymized data will be stored in 2 clouds belonging to provider companies based in an European economic area (EEA) country. . Cloud to store pseudonymized data (including genetic data) protected through end-to-end encryption. . Cloud system to which data collected by actigraph sensors will be sent. Only PBs (Project Beneficiaries) involved in biomedical and clinical research activities of each pilot cities will have access to the cloud (as joint controllers).

In particular, the aforementioned repositories will be:

- A cloud based in an EEA country to store and share personal data collected during the study protected through end-to-end encryption.
- Cloud System belonging to a provider company based in an EEA country to which data collected by actigraph sensors will be sent: Amazon AWS Datacenter located in Europe.

All data will be collected and entered directly into the EDC system. All participating sites will have access to the data entered for the subjects enrolled at that specific site. All sites will be fully trained on using the online data capture system, including eCRF completion guidelines and help files. Sites will be responsible for entering extracted patient data into a secure internet-based EDC database via the eCRF. The HCP (Health Care Practitioner) and site personnel will be able to access their account with a username and password. All eCRFs should be completed by designated, trained personnel or the study coordinator, as appropriate.

Considering that special categories of data (including health-related and genetic data) are involved, the highest level of security will be ensured to comply with the relevant national and European regulations, and especially with the GDPR's strict requirements on implementing data security measures and privacy by design. In particular:

- Personal data will be protected through end-to-end encryption during the whole research project.

- Only PBs involved in biomedical and clinical research will have access to the cloud (as data joint controllers). Permission settings will be used to guarantee that personal data are shared with only those who need to work with them.
- To minimize the risk of data exposure, encryption keys will be controlled by the end-users (i.e., the PBs involved in the biomedical and clinical research), and they will not be accessible to other PBs nor to the service provider.
- No personal data will be transferred/shared outside the cloud (e.g., via email) between PBs or between them and the service providers.
- The cloud will be based in an EEA country.

Data collected by actigraph sensors will be sent to a cloud system belonging to a provider company based in an EEA country. In particular, a Mini-AT Gateway is responsible for creating a seamless interface with both sensors and the Cloud System through wi-fi connection. Both sensor devices have a battery capable of withstanding 1 month with a single charge and uses Bluetooth communication to transmit the data acquired. The cloud platform is a web-based system that gives access after authentication to control the hardware devices.

With reference to biomedical and clinical research specifically, before uploading personal data to the shared cloud, personal data must be stored locally on encrypted parts of a hard disk and backups shall be made in local and password-protected networks.

As a rule, if not strictly necessary to achieve a specific purpose of the project, the data will be collected in anonymous form from the origin and contact data will be kept separate and not shared with other PBs.

The PBs carrying out qualitative analysis do not need identifiable data. Therefore, data will be collected with anonymous forms by the PBs that will meet the participant for the first time, while the other PBs will have access only to aggregated data.

The PBs will introduce pseudonymization procedures to implement the GDPR principles for all other data, especially for individual health and well-being data.

As a rule, samples collected in biomedical and clinical research will be identified by a unique code. The code – name list will not be available to PBs not directly involved in the sample collection. PBs involved in biomedical and clinical research will define a common pseudonymization method in advance of the beginning of the biomedical and clinical research.

Principal Investigators will have unlimited access to the final trial data set.

9. Diffusion of the findings

Researchers are committed to publishing study findings in aggregate form, to warrant the participants' privacy. Findings will be published as abstracts, oral communications at conferences and papers on peer-reviewed biomedical journals. Authorship of scientific papers reporting the study will be granted to those who will give a substantial contribution to the design and conduct of the study, and to the concept, analysis, discussion or writing of the report. No professional writers will be involved.

Actions will be taken to grant public access to the full protocol, data set and statistical code.

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11. Study procedures and timelines

Activity	**Performed by (partner)**	**Phase 1A (Basal)**	**Phase 1B (Basal+14 days)**	**Midterm A**	**Midterm B**	**Phase 2A (follow up)**	**Phase 2B (follow up+ 14 days)**	**Phase 3 (post-clinical study ending**
Timing		**T 0 (2/2023)**	**2 weeks**	**24 weeks**	**26 weeks**	**T1 (2/2024) 52 weeks**	**54 weeks**	**December, 2024**
Informed Consent release	AUSL	X						
Randomization	AUSL	X						
Delivery of equipment for biological sample collection and equipment for motor activity and light exposure monitoring	AUSL	X				X		
Delivery of self-administered questionnaires on health, circadian entrainment data and socio-economic status.	AUSL	X		X		X		
Delivery of LUMIE lamp (intervention group only)	AUSL		X					
Biologic sample collection (saliva)	AUSL		X				X	
Actigraphic and light exposure (badge) data collection	AUSL		X				X	
Biologic sample (saliva) analysis (melatonin) completion	Chrono@work					X		X
Biologic sample (saliva) analysis (nDNA genotyping) completion	UTartu, VUA, UNIBO					X		X
Biologic sample (saliva) analysis (mtDNA haplotype), completion	UTartu, VUA, UNIBO					X		X
Actigraphic data analysis completion	USurrey					X		X
Light exposure data analysis (badge) completion	USurrey					X		X
Self-administered questionnaires on **health, circadian entrainment** analysis completion	AUSL					X		X
Self-administered questionnaires on **socio-economic status** analysis completion	LSE							X
Returning equipment to obtain biological samples and equipment for motor activity and light exposure monitoring	AUSL		X				X	
Returning self-administered (filled) questionnaires on health, circadian entrainment data and socio-economic status.	AUSL		X		X		X	