The Exposome Project for Health and Occupational Research

Protocol of exposome case study on night shift work and health

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EPHOR. Protocol for WP7: Exposome case studies on night shift work and health

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1. Background

Experimental and epidemiological evidence shows that long-term disruption of endogenous circadian rhythms, in particular due to exposure to light during the biological night, may be associated with a wide range of common non-communicable diseases (NCDs), including cancers, cardiovascular diseases and major metabolic disorders (obesity and type 2 diabetes) [1-7]. The prevalence of circadian disruption in human populations is high and increasing due to expanding human activities over the 24-hour day in both the working and the general population. With approximately 19% of the European population engaged in night shift work, the continued study of potential health impacts of night shift work is of importance [8]. It is a priority to establish the long-term impact of circadian disruption on health, to further examine which biological pathways drive these associations and to create population and individual prevention policies across all age groups.

Evidence suggests that night shift work is associated with poor cardiovascular health outcomes [9, 10], although further epidemiologic evaluation is needed. In addition, night shift work may exacerbate pathways of chronic stress. The term allostatic load (AL) has been used to describe mechanisms of chronic stress including autonomic, endocrine, metabolic and immune changes that can lead to a physiological 'wear-and-tear' [11]. Measures of allostatic load have been associated with higher all-cause mortality and several negative health outcomes [12].

There is also evidence that night shift work may accelerate aging [13, 14]. Aging is characterized by a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death. This deterioration is the primary risk factor for major human pathologies, including cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases. Aging research has experienced an unprecedented advance over recent years, particularly with the discovery that the rate of aging is controlled, at least to some extent, by genetic pathways and biochemical processes conserved in evolution [15].

Individual chronotype is a human attribute with genetic basis that reflects the circadian phase of entrainment [16]. Chronotype correlates with diurnal preference, an attribute reflecting personal preference for activities in the morning or evening. Diurnal preference and chronotype may affect night work adaptation with evening types (subjects with a later circadian phase) possibly adapting faster to night shift work [17]. Genetic variation, related to chronotype, may also affect adaptation and effects of circadian disruption on health [18]. Circadian disruption may additionally affect behavior and lifestyle. To date, these topics are understudied, which limit the prediction of individual risk and planning of prevention policies.

There is a need for better insights in relevant exposure metrics and biological pathways, particularly relevant in the context of exposome research. This complex exposure situation involves both occupational and non-occupational risk factors and genetic make-up. We will be measuring several biomarkers in addition to cardiovascular and aging-related outcomes. Because night shift work can influence hormone levels [19, 20] and can also alter inflammatory

responses [21, 22], we will be measuring several hormones at various times throughout the day and will also be measuring cytokines/chemokines and growth factors to assess changes in the profile of these biomarkers in night shift workers compared to day shift workers. Furthermore, we will incorporate the use of sensors to measure light, noise, pollution and other exposures. By complementing the evidence on night shift work in the EPHOR mega cohort with state-of-the-art methods for assessing the external and internal exposome, we will uniquely advance knowledge on how occupational and non-occupational environment (including behavior and lifestyle) interact with genetics to impact disease risk. Finally, we will focus on identifying mechanistic pathways and potential differences by chronotype, genetics or gender.

Population-based mechanistic data is needed to address how night shift work impacts health. There may be few advances to be expected in the short term from animal model or small human-based studies. For instance, although more than a decade has been spent examining night shift work and cancer, many questions still remain. In 2007 the International Agency for Research on Cancer (IARC) classified night shift working as a probable carcinogen to humans (2A) [1]. This is primarily based on animal experimental evidence with only limited human evidence on shift work and breast cancer [4, 23]. A new evaluation of the evidence in 2019, 12 years later, resulted in a similar conclusion that there was also “limited evidence” that night shift work causes cancer in humans. One major problem was that numerous cohort studies had poor exposure assessment on night shift work and population studies examining key characteristics of carcinogens as endpoints were scarce and frequently small [24]. In this context mechanistic data become dramatically important. WP7 will focus on improving methods for measuring internal and external exposures and providing population based mechanistic data that will advance our knowledge on the cardiovascular and aging-related health effects of night shift work and eventually will help in designing and applying efficient prevention policies.

2. Aim and objectives

2.1 Aim.

To evaluate how the disruption of endogenous circadian rhythms, as a result of night shift work, impacts health.

2.2 Objectives.

1. To apply new targeted and agnostic exposome methods to collect individual level data on the external and internal exposome (developed in WP1&3) among night shift workers in both existing cohorts and newly established cohorts.
2. To identify key biological pathways for health effects associated with night shift, conducting biomarker analyses using biomonitoring, targeted assays and agnostic genetics, proteomics, metabolomics, epigenetics and transcriptomics.

3. To examine how the long- and short-term external working-life exposome among night shift workers affects key body functions and ageing in relation to the development of NCDs and investigate if this is influenced by chronotype, age, or gender.

3. Populations and eligibility criteria

3.1. Populations.

Three populations were included in the main application (Spanish, Swedish and Dutch cohorts). One additional cohort in Denmark had received external funding and we have been in frequent communication to add this cohort to the EPHOR project so as to expand the geographic coverage of the study and to improve statistical power. A basic description of the populations is shown in the table below (Table 1). While limiting the populations examined to the health sector would facilitate comparisons, it is a priority to also include non-health sectors since co-exposures may differ substantially and because the majority of prior research on this topic has focused on the health care sector.

Description of the cohorts in EPHOR:
There are three cohorts that are included in the EPHOR-funded project, which include participants from a new cohort that will be formed in Spain, participants from a pre-existing cohort in Sweden and participants from a pre-existing cohort in the Netherlands. In addition, researchers at the National Research Center for Work Environment in København, Denmark are interested in following the EPHOR protocol in a cohort of healthcare workers and they have received external funding in order to do so. We are in the process of formalizing a partnership with this new center. At the present time, this center is included in the protocol document, but the formal partnership is still to be finalized. The cohort in Denmark will be built using data from the Danish Working Hour database. Descriptions of participant characteristics from each contributing site are included in the table below.

Table 1. Description of the cohorts in EPHOR

Country	Spain	Sweden	The Netherlands	Denmark (in the process of adding this cohort)
Cohort	New cohort	Cohort based on existing study [25]	Cohort based on existing Nightingale study [26]	Cohort sampled from the Danish Working Hour database
Sex	Both	Both	Women	Women
Age at enrolment	25-65	25-65	25-65	25-65

Number subjects in EPHOR study	400	200	200	200
Population type	Occupational non-health sector (transportation workers) and healthcare workers	Occupational health sector county council healthcare workers	Occupational health sector nurses	Occupational health sector

3.2 Inclusion criteria.

This study will include adults (between ages of 20-65) who are employed in either health sectors (nurses, physicians) or non-health sectors (transportation, factory settings). Each study will contribute data on 200+ participants. We will have two groups of participants/samples:

a) Exposed (night shift workers). For the purposes of the present study, night-shift workers will be defined as those currently working a **minimum** of two consecutive night shifts per week. We will also require exposed participants to have worked night shifts for 3+ years prior to enrollment to be considered exposed. A **night shift in this study will be defined as a working schedule that involves working partly or 4+ hours of the shift between 00:00 hr and 06:00 hr).**

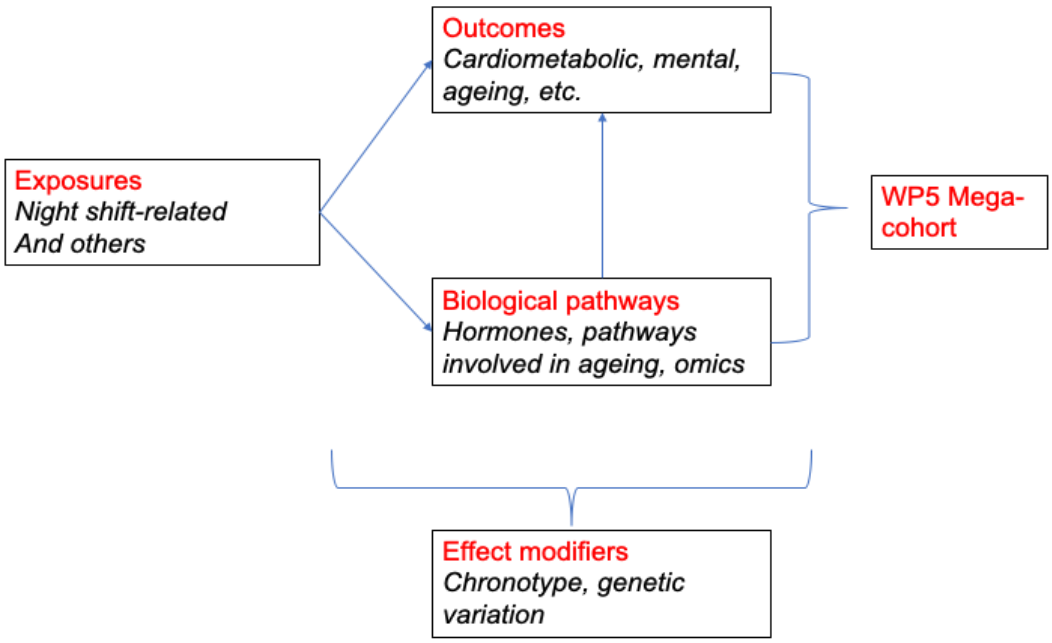
b) Non-exposed (day workers). Day shift workers must not currently work night shifts and must not have worked night shifts for the prior 3+ years at the time of enrollment. Incidental night shift work will be allowed, with incidental night shift work being defined as working less than one night shift per month. However, ≥ 1 week must have elapsed after an incidental night shift before a day shift worker enrolls in the study and begins data collection to allow any residual effects of night shift work to resolve and not alter normal data values for day shift workers. A **day shift in this study will be defined as a working schedule that involves working partly or 4+ hours of the shift between 07:00hr and 18:00hr (shift must start at 6:00am or afterward).**

4. Methods

4.1 Study design.

The basic design of the EPHOR WP7 study is shown in the figure (Figure 1) in connection with WP5.

Figure 1. Basic design EPHOR, WP7 Night shift work



Half of the enrolled sample will be night shift workers and half of the enrolled sample will be day shift workers. For example, the following sampling scheme will be utilized: If each site has 200 participants (for example), 100 would be night shift workers **and biologic samples will be on the ≥ 2nd consecutive night of work**. Then 100 would be non-exposed controls who are not night shift workers (and for comparability, biologic samples will again be taken on the ≥ 2nd consecutive day of work) —to help address how night shift work relates to chronic health outcomes.

4.2 Data collection overview.

To address feasibility constraints related to COVID-19 regulations that differ by country and impact the ability to collect some types of data (difficulty with in person data collection), and to address financial constraints imposed by the available budget to cover a finite number of biologic samples, we are developing a "core" protocol. This core protocol will be completed by all contributing partners. In addition, we have included elements of additional interest and that will be collected in a sub-set of the contributing cohorts. A table outlining which aspects are core versus non-core data collection activities is included below (Table 2). The sensor-collected variables will mainly be measured using an EPHOR-designed sensor system. These sensor-derived exposure variables are classified as core, but exceptions will be made when not feasible in workplace setting, as is the case in some of the hospital and health-care working settings where the use of sensors across the body or on the wrists is not permitted due to hygiene standards.

Table 2: Core data collection activities:

Variables/samples	**Core**	**Variables/samples**	**Core**
Baseline questionnaire info:		**In person height, weight, BP**	
Medication use	x	Height-in person	
Medical history	x	Weight-in person	
Alcohol use	x	Waist to hip ratio- in person	
Smoking	x	Blood pressure-in person[a]	x
Physical activity	x	**Cognitive**	
Sleep	x	Mental/cognitive	x
Chronotype	x	**Biologic samples**	
Diet and meal timing	x	Blood samples- self collected via finger prick for DBS	x
Socio-economic status, education and income	x	Blood samples- in clinic collected, venipuncture larger volume sample	
Working time, job title, duration of night work	x	Saliva	x
Psychosocial	x	**Variables generated from analyses of biologic samples**	
Height and weight (self-report)	x	Metabolic syndrome (measured using HbA1c, triglycerides, cholesterol, blood pressure, waist circumference/BMI)- will need blood samples, preferably whole blood (if not feasible, DBS may be used)	x
Hypertension	x	Diabetes (measured using HbA1c)- will need blood samples, preferably whole blood (if not feasible, DBS may be used)	x
Residence	x	Allostatic load (measured using hormone and inflammation biomarkers, lipids, blood pressure and heart rate)- will need saliva samples, blood samples -preferably whole blood (if not feasible, DBS may be used), and heart rate monitor	
Ecological momentary app (daily app to assess commuting to work, sleeping habits, diet, lifestyle and psychosocial factors)	x	Telomere length	
Sensor-collected[b]		Hormones (melatonin, cortisol, TSH, steroid) from saliva	x

Fine dust	x	Cytokines/chemokines	x
Temperature	x	C-reactive protein	
Relative humidity	x	DNA methylation	
Noise	x	Omics- genomics and proteomics	
Light	x	COVID-19	
UV	x	Leptin and Ghrelin	
Activity from accelerometer	x		
Heart rate	x		
Chemical exposures (passive sampler)			

[a] in-person blood pressure done by medical staff, or by enrolled nurse participants themselves. However, if nurses will be taking their own blood pressure, we will need to coordinate them using the same BP measuring device.

[b] Sensor collected variables including fine dust, relative humidity, sound, light, UV, activity, heart rate *are core, but exceptions will be made when not feasible in workplace setting*

Data will be collected on participants during the course of 5 consecutive days. The baseline questionnaire may be administered sometime during the 1-2 weeks before the 5-day period of intensive data collection activities commence. The following table (Table 3) provides an example of which data collection activities will occur on which day. Because the different sites include populations with varied working schedules, some participants will work on a given day and others will not. The most important aspects that are unchangeable are **that biologic samples need to be taken on a day following a shift (the second + consecutive night shift for night shift workers or the second + consecutive day shift for day shift workers).** This design will ensure the capture of acute changes in biomarkers that may relate to both acute and long-term impacts on aging and cardiovascular health. Ideally, the biologic samples will be taken on day 4 or day 5 so that we have detailed information from the ecological momentary app about how many night shifts a person worked and the sleep habits leading up to sample collection.

Table 3: daily data collection activities during the 5 days of the EPHOR data collection process

	Day 1[a]	Day 2	Day 3	Day 4 (work day)	Day 5 (2nd work day in a row)
Baseline participant info					
Baseline questionnaire					
Anthropometry	x				
Blood pressure	x				
WP1 Sensor/EMA					
EMA	x	x	x	x	x
Sensor 1, passive pen	x	x	x	x	x
Sensor 2, box	x	x	x	x	x
Sensor 3, heart rate	x	x	x	x	x
Sensor 4, activity	x	x	x	x	x
Collect sensors					x
Biologic sample collection					
Biologic sample 1, saliva					x
Biologic sample 2a, DBS from finger prick (non-fasting, fixed time and day)-used for methylation and telomere length					x
Biologic sample 2b, venous (non-fasting)					x
Cognitive testing					
Cold cognition tests with staff remotely[b]		x			
Hot cognition test online remotely	x				

[a]The baseline questionnaire can be administered and completed during the 1-2 weeks leading up to the first day of field work activities.

[b]Cold cognition tests can be completed on any day off, do not need to occur necessarily on the 2nd day of field work.

4.3 Exposures

4.3.1 Baseline questionnaires.

Subjects will complete a baseline questionnaire (Appendix 10.1) to provide information about: lifestyle, medication use, medical history (including menopausal status for women), alcohol use, smoking, physical activity, sleep, chronotype, diet, meal timing, education (for socioeconomic [SES] assessment), working time, chemical/physical exposures, workplace organizations, duration of night-shift work and psychosocial factors. We will also ask participants whether they had a confirmed COVID-19 test result or experienced symptoms consistent with COVID-19.

To assess chronotype, participants will respond to a question on the Morningness-Eveningness questionnaire (MEQ) [27]. The MEQ asks participants questions about what time they would wake up or go to bed if they were entirely free to plan their day, not including demands such as work or family.

4.3.2 Ecological momentary app (EMA).

Participants will be asked to download the "How am I" App (developed by project partners at TNO) to their smartphone. Participants will provide information on: commuting to work, sleeping habits, diet, lifestyle and psychosocial factors using the EMA. Through this app, participants will be asked to respond to a set of questions every day for 5 days in a row. Some questions related to dietary habits will be asked twice per day. To use the app: 1. Participants will receive login details, 2. Participants will be instructed to download the TNO HowAmI app. 3. Participants will log in with the specific study code and login details. 4. Usually, the app automatically chooses the language corresponding to the language that is set on the phone itself (otherwise, people can select the language in the app). 5. When participants have logged in, they can start with answering the daily questionnaires. The EMA will send participants daily notifications to respond to the daily questions. 6. Afterwards people can stop, do a final data upload [if necessary], log-out and delete the app. 7. All participants should give their consent before they receive credentials for the app. The app communicates via a representational state transfer API with the server where the data will be stored in a PostgreSQL database. The server is owned and managed by TNO. All data are constantly stored (whenever people are connected to the internet) on protected TNO servers.

4.3.3 Anthropometry.

Height, weight, waist/hip ratio will be measured for participants. Participants will have their height measured while standing from highest point of head to the floor. Participants will also have their weight measured. To measure waist to hip ratio, participants will be instructed to stand up straight and breathe out. Using a tape measure, the distance around the smallest part of the participant's waist, just above the belly button (waist circumference) and the distance around the participant's largest part of the hips- including around the widest part of the buttock (hip circumference) will be recorded. For sites that cannot perform in-person measurements, participants will also self-report height and weight and will be asked to measure their own waist and hip circumference.

4.3.4 Job records.

This is only feasible in some settings but may be used by some cohorts to assist in sampling of participants. For cohorts that do not have access to job records, eligibility screening should take place to identify eligible participants.

4.3.5 Residence.

Current residence will be recorded. This information may be used to generate geocoded environmental exposure assessment data. This information may include area-based air

pollution, green spaces near the home, exposure to road traffic noise and outdoor artificial light-at-night (and specifically blue-light spectrum that suppresses melatonin) will be analyzed using ISS images available for all major cities in Europe, downloaded from the Earth Science and Remote Sensing Unit, NASA Johnson Space Centre. In addition, residence will be used to identify area-based income level for SES assessment. Strict procedures for securing confidentiality will be applied based on local protocols.

4.3.6 Sensors (WP1).

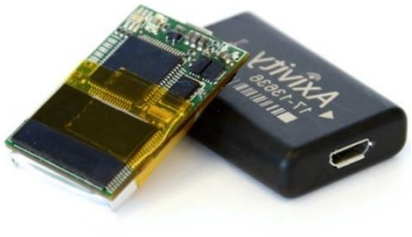
The sensor system being created by WP1 will include multiple devices. There will be a total of 25 sensors (25 sensor boxes, 25 activity sensors) made available to each case study, we will combine with the 25 in WP6 to have a total of 50 sensors for use in WP7. In addition, there will be 200 passive samplers for use in WP7 which will detect various chemical exposures. Finally, there will be 25 heart rate monitors purchased for the WP7 case study. The parts of the WP1-designed sensor system and brief instructions for use are described below (four parts: sensor box, activity sensor, passive sampler and heart rate sensor). Participants will be given a data sheet to log any instances where they forgot to put on the sensor, took it off for extended periods of time, or had technological difficulties with any parts of the sensor system.

a) Sensor box: There will be a sensor box which will measure:
1. ambient temperature, °C
3. relative humidity, %
4. sound, dB
5. light intensity, lux
6. UV intensity, W/cm^2
7. Particulate matter (1, 2.5, 10)

a) The sensor box (developed by VTEC) will be used in a subsample of participants depending on whether the sensor is feasible for use in a particular workplace and will be worn by the participants whenever possible or kept in their direct vicinity. The sensor box has an expected battery life of approximately 24 hours and will take 2.5 hours to charge. The sensor box will be worn using a belt-like strap that will fit the sensor around the participant's waist. The sensor must be worn on top of clothing and should not be blocked by any external clothing such as a jacket. The sensor box will be worn for 5 days during waking hours. While the participant sleeps, the box will be removed from the waist-worn strap and placed in close proximity to the gateway and plugged into charge so that data can be downloaded to the gateway while the sensor box recharges. In addition to the sensor box, a gateway unit will be provided (the sensor and gateway are shown in the figure below). The purpose of the gateway is to download the collected data from the sensor box via Bluetooth and store it. The gateway is a small box, that needs to be continuously plugged in (in order to maintain power) and connected to the internet via a LAN cable in the participants home. At the end of the study period, data will be downloaded from the gateway onto protected computers at the specific study site. The sensor box and gateway will be used in "offline" mode, and data will not be

transferred to any other non-study devices besides being downloaded by study staff to protected study-specific folders.

b) Activity sensor: There will be an activity sensor (Ax3, developed by Axitivity) which has an accelerometer and will measure activity as well as sleep. The battery life is 30 days. This activity sensor will be worn around the wrist. The activity sensor will be worn for 5 continuous days (including while a person sleeps). The activity sensor has sufficient battery and storage space to run for 5 continuous days. Following the data collection period, the data will be downloaded to protected study computers, wiped clean of old data and recharged before use by the next participant. These devices run off-line so that data is stored on the device and is not automatically transmitted to the company or elsewhere unless the researcher downloads data to a specific location.

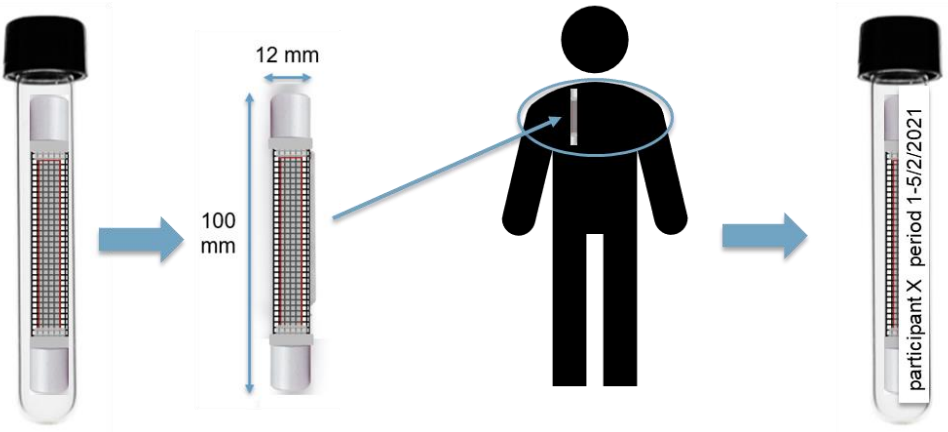


c) Heart rate monitor: There will be a heart rate monitor (specific type Zephyr, Polar or Wahoo TICKR TBD) will be worn around the sternum to measure heart rate. The heart rate monitor has an approximate battery life of 300 hours or more. These devices will be worn for 5 continuous days while a person is awake, and removed during sleep. At the end of the 5 days, the monitor will by synced with the gateway device to download data and then the old data will be wiped clean before the device is used by the next participant. These devices run off-line so that data is stored on the device and is not automatically transmitted to the company or elsewhere unless the researcher downloads data to a specific location.

d) Passive sampler: There will be 200 passive samplers (pen) for use by 200 participants. The majority of these (approximately 120) will be used by transportation workers in

Spain, since the possible chemical exposures in this setting are likely to be of greater interest than exposures in hospital settings. However, approximately 20 passive samplers will be used by the other contributing sites allowing for a comparison of exposures in different settings. Samplers are easy-to-use sampling devices for measurement of volatile organic compounds (VOCs) in the air. The uptake of chemicals is mainly based on molecular diffusion. The passive samplers for EPHOR are specially designed and consist of a small aluminum tube filled with an adsorbent (Tenax TA) to capture VOCs. The passive sampler with the size of a pen (12 x 100mm), is equipped with a clip and a buckle to attach the sampler to the clothes. Alternatively, the sampler can also be attached to a necklace. Preferably, the sampler should be placed in the breathing zone at chest- or shoulder height (see Figure). The passive samplers are sent to the participant in an airtight tube with screw cap with enclosed envelope for the return. The tube is provided with a label and unique code that is linked to the participant. Before application the sampler will remain in the airtight tube at room temperature. Only upon application the sampler is removed from the tube and attached to the clothes. The sampler is worn for five days continuously and must be attached to the outer layer of clothing (not covered by other clothes). Outside, the sampler must be attached to the jacket. While sleeping, the sampler must be placed in the bedroom (on the bedside table), while showering the sampler must be place in the bathroom outside the shower cubicle. While sporting, if it's not possible to attached the sampler on the clothes it can be placed in the vicinity of the participant. After 5 day use the sampler will be put immediately back in the same airtight tube with the screw cap attached. The tube is provided with a label where the participant's code and the period of sampling period must be entered. After that, the tube will be returned to TNO in the enclosed envelope.

Figure. Sending sampler in airtight tube to participant, remove sampler from tube before application and attach the sampler on clothes in breathing zone, after 5 days of use put the sampler back in the tube, fill in participant code and sampling period and return to TNO.



Alternative sensors will likely be incorporated into the study. Possible alternative sensors that may be used include:

e) Kronowise (developed by Kronohealth) wrist-worn device that measures UV, blue light and light intensity, activity and sleep and skin temperature. These wrist-worn sensors will be worn for 5 continuous days (including while the participant sleeps). They have sufficient data storage and battery life to run for 5 continuous days. At the end of the 5-day data collection period, data will be downloaded by study staff to protected study-specific folders and the device will be charged and old data wiped clean before the device is used by the next participant. These devices run off-line so that data is stored on the device and is not automatically transmitted to the company or elsewhere unless the researcher downloads data to a specific location.

f) HOBO light intensity data logger (HOBO-ware, Onset Computer Corporation) that will continuously (every 15 seconds) record their ambient light exposure. The HOBO light intensity data logger is a pendant that will be worn on the chest close to shoulder level. This position will be used to obtain measurements that would approximate the amount of light reaching the retina. During sleep, participants will be instructed to place the logger on a bedside table with the sensor facing upwards and while showering to place the logger nearby in the bathroom. The logger is relatively small in size (5.8 x 3.3 x 2.3 cm) and light in weight (18 g). The loggers are designed to record relative light intensity within the range of 0 to 320,000 lux and are designed for indoor and outdoor settings. The HOBO has sufficient data storage and battery life to run for 5 continuous days. These devices run off-line so that data is stored on the device and is not automatically transmitted to the company or elsewhere unless the researcher downloads data to a specific location.

4.3.3 Biological samples

Biologic specimens will be collected on a day where participants are working. *Note we are including protocol for self-sampling AND in clinic sampling. COVID-19 related precautions will likely not allow all sites to complete in-clinic sample collection. Therefore, we are making plans for the study to be done completely remotely if this is the case so sites can complete all of the core data collection activities.* A list of the planned biomarker analyses and the cell/tissue types are included in the table below (Table 4). Some additional related analyses may be conducted on stored samples pending further grant funding. Samples will be stored for up to 10 years following data collection, and after this time, materials will be destroyed.

Table 4: Biomarker analyses, matrix type and number of samples to be processed

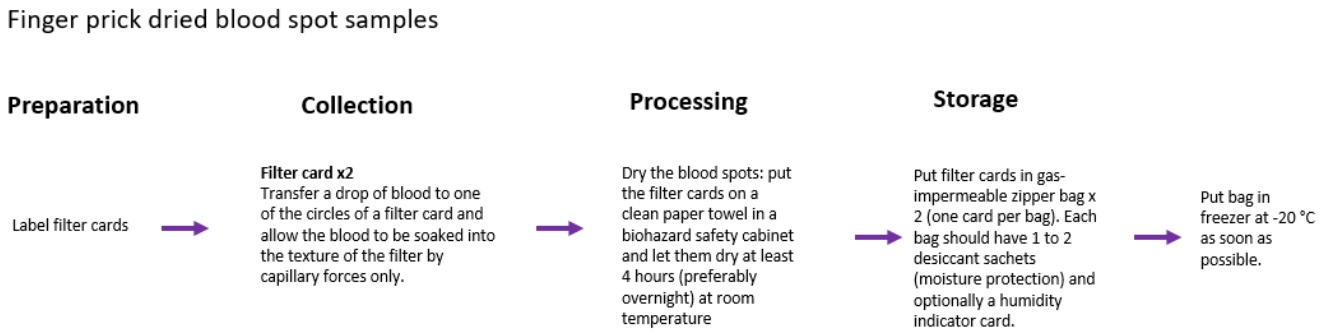
		Matrix			
Partner	**endpoints**	**saliva**	**plasma**	**DBS**	**# of participants**
At site	Differential cell count				600
CUT	Metals (ICP/MS-screening)				400
ISGLOBAL	Luminex/elisa (immune factors) YKL40 and CC16		x		600
ISGLOBAL	Melatonin	x			800
ISGLOBAL	Cortisol/other steroid hormones	x			800
Subcontracting (KUL)	Genome-wide association study (GWAS)		x		400
KI	Telomere length/mtDNA copy number			x	800
STAMI	Transcriptomic analysis (targeted RNA expression) ddPCR		x		400
Subcontracting (KUL)	Metabolomics (also HbA1c, lipids)		x		800
Subcontracting (KUL)	Arrays epigenomics in blood DNA(EWAS)		x		400
KUL	Pyrosequencing (SNPs)		x		200
KUL	cfDNA methyaltion		x		200
KUL	proteomics		x		200
ISGLOBAL?	CRP		x		600
KI?	leptin and ghrelin	x			400

4.3.1.1 Blood.

Blood concentrations will ideally be measured on two days (or one day only if two is not feasible, if one day, this will be a non-fasting sample at a fixed time of day) in all contributing cohorts. This sample will be taken during the early morning at approximately 7am (+/-1 hr), which will coincide with the end of a shift for night shift workers or the start of a shift for day shift workers. For this fixed-time point sample, there may also be other clinical examinations occurring at the same time (Spain, Sweden). The other time point (if it can be done) will be soon after waking on a day off/weekend off (fasting sample).

Self-sample. Finger prick dried blood spots (DBS) will be completed for all participants. This will be done at 8am and will be a non-fasting sample. DBS samples will be used to collect DNA for analysis of telomere length [28] and metal analyses. DBS offers several advantages over conventional whole blood, plasma or serum sample collection. For example, DBS sample collection is minimally invasive and easy to perform (e.g. finger or heel prick rather than venous puncture) and it can be done at home by volunteers themselves after minimal training. In addition, a low volume of blood (< 20 µL) is needed to spot onto filter paper. Furthermore, DBS is relatively stable at ambient temperature, thus facilitating easy shipping and storage of samples. As such, DBS samples have been used in many applications including therapeutic drug monitoring, pharmacokinetics, genomics, proteomics, and metabolomics [29]. However, capillary blood, when collected via finger prick, is often contaminated with skin tissue fluid,

applied cosmetics, and antiseptics, among others, and is prone to hemolysis. Therefore, the reliance on DBS from self-collected samples has limitations, and the use of plasma obtained from venous blood is more robust for comparing and interpreting physiological conditions [30]. A brief schematic for collection, pre-processing and storage of DBS samples is included below. A more detailed protocol for pre-processing, storage and analysis of DBS samples is included in Appendix 10.2.

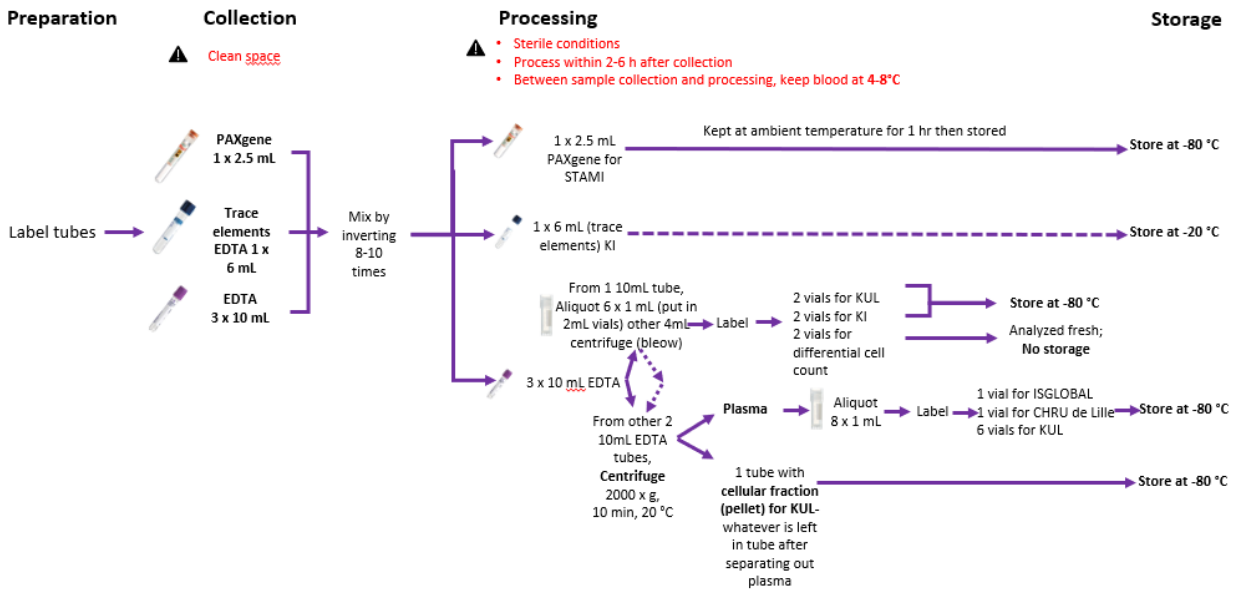


In clinic sample. In clinic non-fasting sample will be drawn at 7am (+/-1 hr allowed), so samples collected between 6:00am-8:00am are acceptable). A total volume of 38.5mL will be collected. The types of tubes and specific volumes are specified in the table below (Table 5).

Table 5: Volume of blood to be collected for in clinic sampling

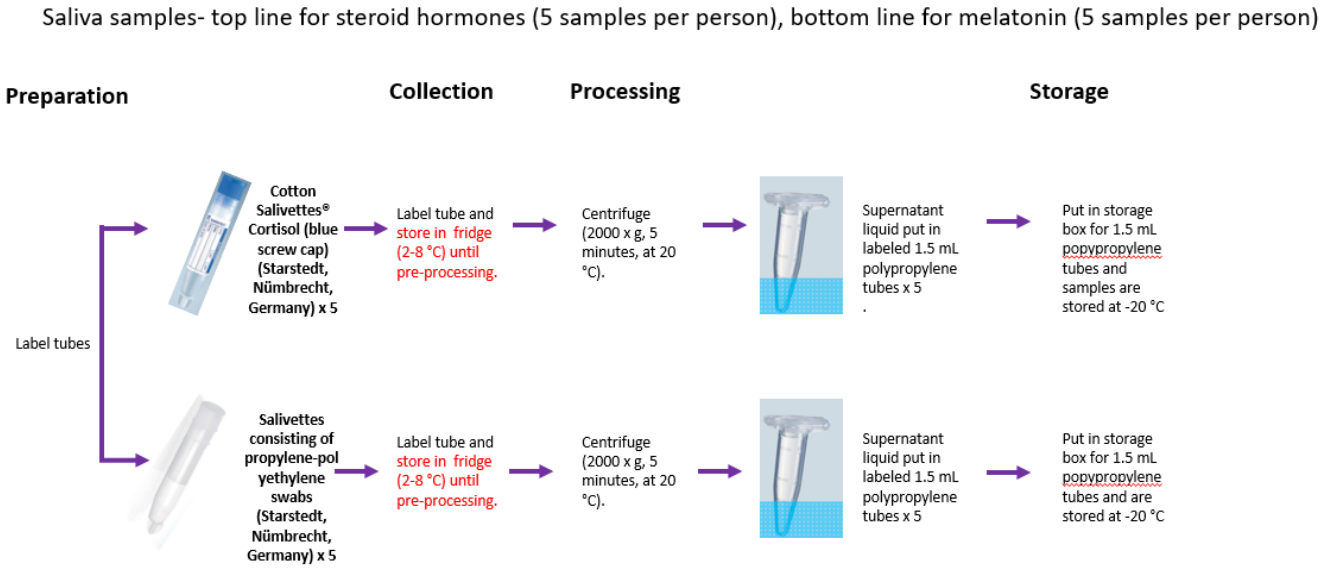
Type of tube	**Number of tubes**	**Volume of tubes (mL)**
Vacutainer® EDTA	3	10
PAXgene® blood RNA tube	1	2.5
Vacutainer® blood collection tube for trace element testing	1	6
Total volume		38.5

A brief schematic for collection, pre-processing, storage, and which EPHOR center will analyze specific analyses is included below. A more detailed protocol for pre-processing, storage and analysis of in-clinic collected blood samples is included in Appendix 10.3.



4.3.1.2 Saliva.

Participants will be asked to collect 5 saliva samples over the course of a single day in tubes numbered 1 to 5. Sample 1: right when waking up (while still in bed before brushing teeth), sample 2: 45 minutes after waking up, sample 3: approximately 4 hours after waking up (will likely occur during scheduled work), sample 4: approximately 10 hours after waking up (also potentially occurring during scheduled work, or right after a shift) and sample 5: right before going to bed BEFORE brushing teeth. Saliva samples will be used for hormone analyses (melatonin, cortisol, TSH and other steroid hormones) using protocol from a previous night shift study [31]. A brief schematic for collection, pre-processing and storage of saliva samples is included below. A more detailed protocol for pre-processing, storage and analysis of in-clinic collected blood samples is included in Appendix 10.4.



4.4 Outcomes:

Note that some outcomes are also considered as biological pathways (e.g. allostatic load, ageing) and therefore are included in the description of outcomes, and also in the following section describing pathways.

4.4.1 Mental/cognitive.

We will assess cognitive dysfunction using a combination of tests. The following cognitive tests indicated “a-d” assess “cold cognition.” These cold cognitive tests can be completed over the phone or in a remote setting with a study coordinator. Details on the content and how to administer these tests is included in Appendix 10.5. In an attempt to complete these cognitive tests while participants are in a relaxed state and when participants are not excessively tired, we will complete the cognitive testing sometime during a weekend/back to back double days off from work. We will make sure that the participant is able to isolate in a quiet room for the entirety of the testing (approximately 10 minutes), otherwise a different time to call back and re-try the tests will be scheduled.

In addition to assessing cold cognition, we will also assess “hot cognition,” as described below in “e.” Hot cognition refers to the stress- and emotional-induced impacts on cognition. The driving assumption of the attentional bias to threat tests is that people with anxiety or people experiencing stress will selectively attend to threating stimuli and recall threatening experiences [32]. For the purposes of this study, hot cognition will be assessed on a day when participants are working to see if night work versus day work is related to acute changes in attentional bias to threat. These tests will be completed on a computer or tablet and can be completed in person or remotely. Details on the content and how to administer the hot cognitive test is included in Appendix 10.6.

a) Digit Span (3 minutes). The Digit Span test is part of the Wechsler Adult Intelligence Scale [33]. In this test, the examiner reads a sequence of numbers and the test subject must repeat the same sequence back to the examiner in forward order. The Digit Span measures short term auditory memory, attention efficiency and capacity [34].

b) Digit Span reverse (3 minutes). This is again part of the Wechsler Adult Intelligence Scale [33]. In this test, the examiner reads a sequence of numbers and the test subject must repeat the same sequence back to the examiner in reverse order. The Digit Span reverse measures short-term memory and the ability to manipulate information while in temporary storage memory [34].

c) Semantic Fluency (1 minute, animals). In this test, subjects are asked to generate the name of as many species of animals as possible within 1min [35]. The semantic fluency test measures non-motor processing speed, language production, and executive functions [36].

d) Phonemic Fluency (FAS) Word Fluency (3 minutes). In this test, subjects were asked to list as many words as possible beginning with a specific letter. Three trials are conducted (each trial uses a different letter) with each trial lasting 1 minute [37]. The Phonemic Fluency test measures motor processing speed, language production, and executive function [36].

e) Dot probe test. The dot probe test will be used to assess attentional bias for threat [38]. In the dot probe test, participants are shown two images or words simultaneously, and then each new trial includes 2 different images or words. In some trials, one of the images or words is threatening (e.g. disease, violence) while the other is neutral. After the images or words disappear, a dot appears in the location vacated by the threatening image or word and the participant is told to push a button when they detect the dot's presence [39].

4.4.2 Cardiovascular.

We will examine blood pressure, heart rate, body mass index and waist to hip ratio as cardiovascular outcomes of interest in the present study.

a) Seated blood pressure (BP) will be assessed at room temperature using an automated blood pressure device and the time of day when BP is drawn will be indicated. The same standard procedures will be applied in all cohorts to reduce biological and observer variability in measured BP. BP is a major risk factor for coronary heart disease, congestive heart failure, and stroke. Three systolic and diastolic readings will be done. There should be 2-3 minutes of rest between each reading, and the average of the 2nd and 3rd readings will be recorded in order to reduce the impact of reactivity. Furthermore, smoking, caffeine and exercise should be avoided shortly before BP readings. In analyses focusing on hypertension, BP will be used to define hypertension

including any of the following (SBP≥140 mm Hg, DBP≥90 mm Hg, or use of an antihypertensive medication).

b) Resting heart rate reflects autonomic nervous system function and cardiovascular fitness. The heart rate will be measured using a wearable chest monitor. In addition to measuring heart rate, we will also examine heart rate variability (HRV) defined as the change in heart rate or in variability between the consecutive heart beats (generally measures as the time between R to R intervals in successive QRS complexes from an electrocardiogram [40]). Many wearable heart rate devices provide HRV information. Low HRV is generally considered to be a marker that the body is under stress from psychological events, internal or external stressors or from a bout of exercise. Meanwhile, a higher HRV indicates that the body is more resilient to stress and is able to recover from prior accumulated stress more robustly. HRV has been found associated with all-cause mortality [41], cardiovascular disease [42], and inflammation [43], among other things.

c) Body mass index, a proxy for obesity based on height and weight will be measured as kg/m^2. BMI continues to be used due to the ease and low cost of measurement.

d) Waist to hip ratio (WHR), a measure of central obesity and visceral fat will also be measured. WHR may be a better indicator of obesity than other anthropometric measures (such as BMI) because WHR can reflect a lack of muscle, as well as an increase in visceral fat, both of which have been found to be independently associated with cardiovascular disease risk [44-46]. Waist circumference should be measured at the end of a normal exhalation at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest. Hip circumference should be measured around the widest portion of the buttocks, with the tape parallel to the floor.

4.4.3 Metabolic.

We will examine metabolic syndrome and diabetes as measures metabolic outcomes of interest in the present study.

a) Metabolic syndrome (MetS) is a cluster of physiological and biochemical abnormalities that collectively increase the risk of CVD and T2 diabetes. MetS will be defined based on participants meeting three or more of the standard criteria including: 1) elevated fasting glucose (fasting blood glucose ≥100 mg/dl or antidiabetic medication use), 2) high triglycerides (triglycerides ≥150 mg/dl or triglyceride lowering medications), 3) low HDL cholesterol (HDL <40 mg/dl for males or <50 mg/dl for females), 4) elevated blood pressure (systolic ≥130/diastolic 85 mmHg or antihypertensive medication use), and/or 5) abdominal obesity generally indicated by increased waist circumference (≥102 cm for males or ≥88 cm for females) [47], but also can be defined using BMI to identify obesity (BMI ≥ 30 kg/m^2) [48]. If a fasting blood sample cannot be obtained, we will use

a varied definition of metabolic syndrome that includes three or more of the following criteria being met: 1) elevated glycated hemoglobin (HbA1c) ≥ 5.7% , which reflects the 3-month average glucose concentration, 2) high triglycerides (triglycerides ≥150 mg/dl or triglyceride lowering medications), 3) low HDL cholesterol (HDL <40 mg/dl for males or <50 mg/dl for females), 4) elevated blood pressure (systolic ≥130/diastolic 85 mmHg or antihypertensive medication use), and/or 5) abdominal obesity generally indicated by increased waist circumference (≥102 cm for males or ≥88 cm for females), or defined using BMI to identify obesity (BMI ≥ 30 kg/m^2). There is good agreement between metabolic syndrome defined using HbA1c as an alternative to plasma glucose level [49].

b) Diabetes will be defined using haemoglobin A1c (HbA1c) level. The threshold level of ≥48 mmol/mol (≥6.5%) will be used to define diabetes [50]. Although blood glucose levels measured in the fasting state are conventionally used as the basis for diagnosis of diabetes, HbA1c levels are an alternative option that will likely be easier to collect in all of the contributing cohorts.

4.4.4 Allostatic Load.

The allostatic load model evaluates chronic stress by combining many types of data together rather than examining only separate disease entities. We will evaluate 13 biomarkers (e.g. cortisol, inflammation) and functional tests (blood pressure and heart rate) that can lead to a physiological 'wear-and-tear' [11]. These measures include: from the neuroendocrine system: 1) salivary cortisol t1 and 2) salivary cortisol t1–t2, from the immune and inflammatory system 3) insulin-like growth factor-1 (IGF1), 4) C-reactive protein (CRP), 5) fibrinogen, 6) Immunoglobulin E (IgE), from the metabolic system: 7) high-density lipoprotein (HDL), 8) low-density lipoprotein (LDL), 9) triglycerides, 10) glycosylated haemoglobin (HbA1C), and from the cardiovascular and respiratory systems: 11) systolic blood pressure (SBP), 12) diastolic blood pressure (DBP) and 13) heart rate (we will not collect peak expiratory flow). There are some important primary mediators of allostatic load, such as adrenal steroids, catecholamines and DHEA-S, which we will not be collecting in the present study.

4.4.5 Ageing.

Telomere length will serve as a biomarker for aging. Telomeres shorten as a result of DNA replication, with length being inversely associated with chronological age [15]. Telomere length will be measured from blood samples. In addition to telomere length, we will examine DNA methylation as another marker of aging.

4.5 Biomarkers/Pathways

4.5.1 Hormones.

We will measure several hormones in this study including melatonin, cortisol, several steroid hormones as well as leptin and ghrelin.

a) Melatonin will serve as a biomarker for circadian disruption [51]. Melatonin will be measured from saliva samples. In order to create a melatonin profile, saliva samples will be collected multiple times throughout the day including during the expected rise in melatonin (occurring close in time to the onset of sleep).

b) Cortisol concentrations will serve as biomarker for the assessment of occupational exposure to stress [52]. Cortisol will be measured from saliva samples. Due to variability of cortisol throughout the day, multiple samples will be taken throughout the day (at same time intervals as melatonin saliva samples are taken).

c) Steroid hormones will be measured from saliva samples. Steroid hormones regulate a wide range of human activities, and the disruption of steroid hormones as a result of circadian rhythm disruption can have wide ranging impacts. The steroid profile (including endogenous steroid hormones and their main metabolites) is an important tool for the study of hormonal imbalances [53]. Using the analytical methods previously developed by the IMIM Bioanalysis group, the salivary concentrations of steroid hormones (androgens, estrogens, progestogens, glucocorticoids and mineralocorticoids) and their main metabolites will be determined.

Progestins	**Glucocorticoids**
Pregnanediol	Cortisol (F)
5-Pregnenediol	20a-dihydrocortisol
5-Pregnenetriol	20b-dihydrocortisol
16-androstenol	5α-tetrahydrocortisol
17-hydroxyprogesterone	Tetrahydrocortisol
17-hydroxypregnanolone	6b-hydroxycortisol
Pregnanetriol	a-Cortol
Pregnanetriolone	b-Cortol
21-deoxy-20a-cortol	11β-hydroxyetiocholanolone
21-deoxy-20b-cortol	Cortisone
Mineralocorticoids	20a-dihydrocortisone
Corticosterone	20b-dihydrocortisone
20a-dihydrocorticosterone	5a-tetrahydrocortisone
20b-dihydrocorticosterone	Tetrahydrocortisone
5a-tetrahydrocorticosterone	6b-hydroxycortisone
5b-tetrahydrocorticosterone	a-Cortolone
17-deoxy-b-cortol	b-Cortolone
11-dehydrocorticosterone	Deoxycorticosterone
20b-dihydro-11-dehydrocorticosterone	5a-tetrahydro-deoxycorticosterone
5a-tetrahydro-11-dehydrocorticosterone	5b-tetrahydro-deoxycorticosterone
5b-tetrahydro-11-dehydrocorticosterone	Cortexolone
17-deoxy-b-cortolone	20b-dihydrocortexolone
Androgens	5b-tetrahydrocortexolone
Testosterone	a-11-deoxycortolone
Epitestosterone	b-11-deoxycortolone
Androsterone	**Estrogens**
Etiocholanolone	Estradiol
5α-Androstan-3α,17β-diol	Estrone
5β-Androstan-3α,17β-diol	Estriol
5α-dihydrotestosterone	
Androstenedione	
Dehydroepiandrosterone	
11β-hydroxy-androsterone	
4-hydroxy-testosterone	

d) Leptin and ghrelin will also be measured. Methods are TBD. Both of these hormones have been recognized to have a major influence on energy balance. On the one hand, leptin acts as a suppressor of food intake, thereby acting as a mediator of long-term regulation of energy balance while on the other hand, ghrelin is a fast-acting hormone, which is involved in meal initiation [54]. Circadian disruption in shift work alters the

levels of both of these hormones, and this may explain to some degree the high rates of metabolic disorders among night shift workers.

4.5.2 Cellular immunity analytes.

We will measure cytokines, chemokines and growth factors and c-reactive protein.

a) Using a Luminex technique, we will measure 30 cytokines/chemokines and growth factors to assess changes in their profile in night shift workers compared to day shift workers. The interpretation of the findings is not straight forward and will be based on a comparison of levels and timing between workers in different shifts. Most studies have established differing "normal" cytokine profiles based on the characteristics of their study populations and the modes of cytokine measurement. The factors are as follows: (a) variation exists in what is considered to be a "normal" cytokine profile; (b) few conclusions have been drawn across studies to define normal cytokine levels; and (c) a variety of factors contribute to cytokine release and action. Hence, these studies often define cytokine levels only within the population of interest. Most studies do not use pre-established cutoffs for cytokine reference values but rather consider the median or mean cytokine levels of healthy subjects in a defined set of population to be "normal." This mean or median cytokine level for a particular study is then used as a reference cut-off to identify comparatively abnormal cytokine levels in some subjects. A preliminary list of cytokines, chemokines and growth factors that will be measured include: IL10, RANTES, FGF, IL13, EOTAXIN, IL1B, IL6, IL17, GCSF, IL12, MIP1A, GMCSF, MIP1B, MCP1, IL15, EGF, IL5, HGF, VEGF, IFNg, IFNa, IL1RA, TNFa, IL2R, IL2, MIG, IP10, IL7, IL4 and IL8.

b) C-reactive protein (CRP) is another commonly used biomarker for inflammation and will be sampled in a similar fashion to IL-6 or other interleukins.

4.5.3 Ageing.

Proposed hallmarks of ageing include telomere attrition [15] and epigenetic markings including DNA methylation.

4.5.4 Allostatic load.

We will measure 13 biomarkers and functional tests that relate to physiological 'wear and tear' (as described above in outcomes).

4.5.5 Omics.

We will quantify DNA methylation and will carry out other genomic and transcriptomic analyses in a limited number of participants (estimated 400 samples). We will include samples from each site in equal proportions. In determining which participants' samples will contribute to these analyses per site, we may base these decisions on exposure or biomarker values i.e. select participants of a particular range or who are non-smoking for instance. Cell-free DNA in blood

plasma is emerging as a powerful tool for disease diagnostics and biomarker development. This makes epigenetic marks, such as DNA methylation, a useful biomarker in the study of several disease outcomes [55]. In addition, some analyses (such as proteomics) will be conducted for 200 participants only. These 200 participants will be sampled form the 400 contributing to the other omics analyses. Evidence from animal models suggests that the circadian rhythm regulates thousands of protein-coding genes [56], and during circadian misalignment, as occurs with night shift work, the human plasma proteome is altered [57].

5. Data minimization and ethics

5.1 Informed consent.

In all centers, participants will be given information sheets and sign informed consent documents. Consent forms include specific clauses on personal data protection informing the study participants how their data is going to be treated and stored, the research purpose, information for relevant study contacts and a description of the participants' rights. Because each center has their own institutional review board requirements, these documents will vary slightly form center to center. Each site will have consent forms specific to the collection of new information from new participants for the WP7 project. In addition, sites that plan to leverage information from pre-existing cohorts additionally include the original consent forms from these earlier collected data projects. More information on the informed consent policies and documents is provided in ethics deliverable for WP7 (see D13.10).

5.2 Incidental findings policy.

Because of the minimally invasive and relatively standard approaches being applied in each site for the EPHOR study data collection, we do not anticipate many incidental findings. However, participants will be given information that incidental findings may occur and will be able to obtain this information from the study coordinator at their site. More information on the incidental finding policy is provided in ethics deliverable for WP7 (see D13.10).

5.3 Data minimization.

In following the data minimization principle, we are collecting only data that are relevant and limited to the purposes of this particular research project. The variable types we will collect and their purpose for this study are outlined in Appendix 10.8.

6. Confidentiality and data protection

6.1 Confidentiality and data protection.

There are multiple technical and organizational measures in place at the participating study centers to protect the rights of the research participants.

1. Data protection officers are involved.

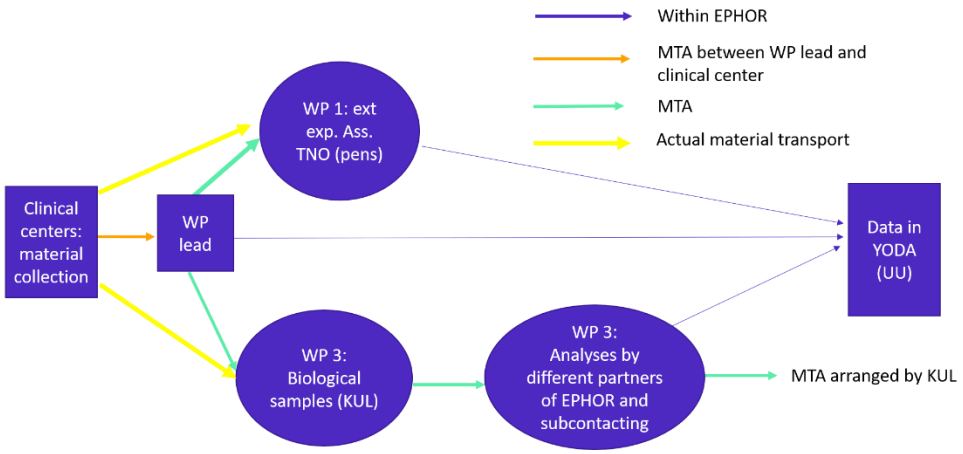
2. Pseudonymization will be implemented as a general standard, meaning that all material obtained in the framework of the project (questionnaires, biological samples, data from personal devices) will be identified through a code, the name and/or other personal data that could allow the identification of the participant will never be indicated. This unique identifier will link all basic data required for the study. The master key file linking the center's study numbers with personal identifiers will be maintained in a password protected file with limited access.
3. Whenever possible, anonymization will be applied. - All files containing personal data will be stored in encrypted and password-locked files. Access to these files will be limited to authorized project personnel; - In the case of tracking participants by geo-localization techniques, the geo-localization data will be store separately from the other participant's data (health, etc).
4. Only researchers linked to the project will have access to personal data.
5. Personal data will not be transferred to anyone outside of the study, except in the cases considered by law.
6. Reported study results will pertain to analyses of aggregate data. No individual's name will be associated with any published or unpublished report of this study.
7. All project personnel will be trained in the importance of confidentiality of individual records and required to sign a confidentiality agreement

6.2 Record retention.

Biological material collected for this study will be retained for a maximum of 10 years following data collection. Participant records will be maintained for at least 10 years following the end of data collection. The exact storage time will differ by study site and depend on institution-specific guidelines. After biologic samples are analyzed, additional biospecimens will be returned to the site whose participants they were collected form for storage.

6.3 Material transfer agreements and material transfer.

Material transfer agreements (MTAs) will be made between EPHOR partners and related centers responsible for collecting or analyzing data for night shift exposome case study. The following figure illustrates how data will flow between different EPHOR partners and related centers and the various MTAs.



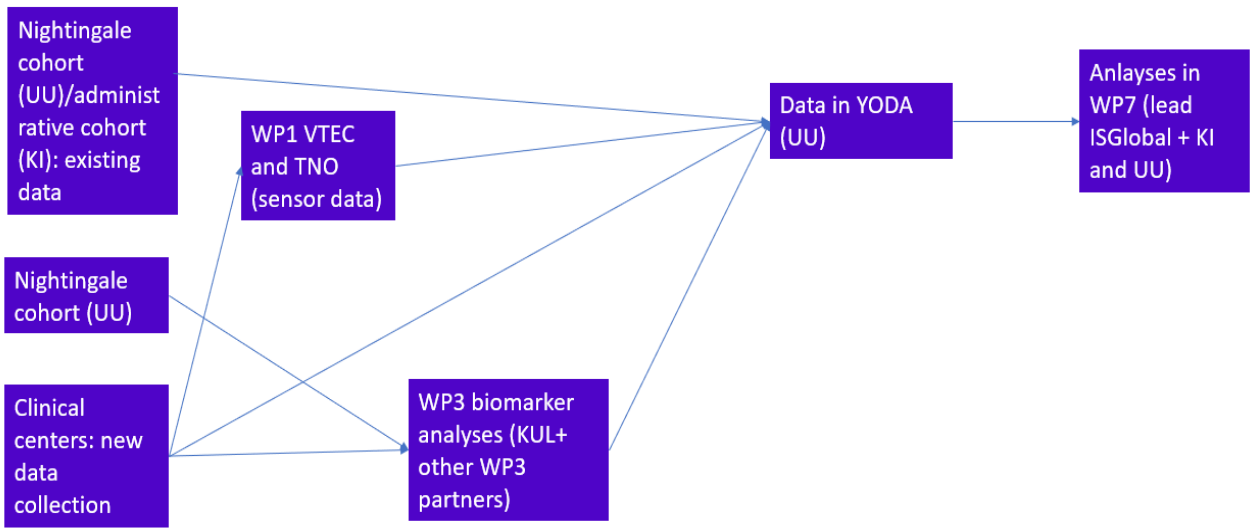
6.4 Digital data storage and transfer.

Data generated by the EPHOR project will be stored on the YODA platform, which is Utrecht University's institutional research data repository. Yoda complies with Utrecht University's Information Security policy for data classified as public, internal use, sensitive or critical. Data in the YODA platform can be shared within a closed user group, made accessible only to authorized users. All data in Yoda data is stored and transmitted in encrypted format, and is stored in two different geographical locations. The Yoda repository complies with the FAIR principles, following the guidelines for findability, accessibility, interoperability and reuse of data sets. More information on the Yoda platform is included in Appendix 10.9.

Analyses using data stored on Yoda will be analyzed on an EPHOR specified data analysis platform TBD allowing for the analysis of data without needing to download data from Yoda onto personal computers and therefore protecting study participant confidentiality and avoiding data being stored on many individual computers across different countries.

6.5 Data flow.

The WP7 project will include new data collection within WP7. In addition, data will be collected through WP1 (sensor-related data) and data will be collected through WP3 (data related to biological biomarker and outcome analyses). WP7 will also incorporate some previously collected information from pre-existing studies at some of our partner sites. All data will eventually be stored on the YODA system to allow for future WP7 led analyses. A figure outlining the flow of data through the study is provided below.



7. Statistical analysis and study power

7.1 Statistical analysis

We will utilize tools from WP4 (data management and analytics platform) for analyses. We plan to have a minimum of 1,000 participants included in this study. With the addition of participants from external, collaborating EPHOR sites (Denmark), we will likely have closer to 1,200 participants.

In addition to the data collected within WP7, job-exposure matrices from the EuroJEM (WP2) may be used to estimate various occupational exposures. In collaboration with WP1, we will work to integrate exposure information from the sensors and the EMA application and develop methods for calibration where needed. In collaboration with WP5, we will collectively work together to specify high priority research topics to be examined using data from the MEGA-cohort and being able to access these data for WP7-led analyses. Finally, with collaboration from the text mining group, we will work to integrate information uncovered about important additional pathways related to night shift work and cardiometabolic and aging related outcomes.

Because data collection will be staggered with one center beginning first and then the second and so on to accommodate the limited sensors we have available and the capabilities of each individual site, it will be important to adjust analyses for seasonality and/or hours of daylight which will differ based on the date of data collection and are known to impact normal circadian rhythms. In analyses, we will have different statistical power for different analyses based on which data elements are core and will be collected for everyone (approximately 1000) in the study versus data collection activities that are planned to only be conducted among 800, 400 (most OMICS analyses) or 200 (detailed exposure assessment e.g. VOCs, cell free DNA methylation, SNP pyrosequencing, targeted proteomics) participants. These different sample sizes will impact what analyses we can do.

For associations of shift work with biomarkers repeatedly collected during 24hrs (melatonin, steroid hormones), we will apply cosinor analysis [58]. The outcomes in these analyses will be the individual marker and grouped markers e.g. androgens and they key exposures investigated may vary depending on the specific research question. For associations of shift work and sleep with cardiometabolic and aging-related health outcomes, we will use linear or logistic regression models depending on whether the cardiometabolic or aging-related variable of key interest is examined as a continuous or binary outcome. For associations of shift work with biomarkers of interest that have been measured at one time point (e.g. cytokines, CRP), we will use linear regression models. We may also apply factor analysis methods [59] to group immune markers together and examine impacts of night shift work through these specific pathways. In OMICS analyses, we will apply standard GWAS approaches to examine shift work and genetic changes.

To identify how much biological pathways may explain changes in the occurrence of and extent of health outcomes among night shift workers, we will conduct mediation analyses [60]. Furthermore, we aim to apply exposome analysis methods to combine information on many exposures together to examine combined effects of the night shift exposome and gaining and cardiometabolic health. For these analyses, methods will be finalized in collaboration with WP4.

7.2 Study power

7.2.1 Continuous outcomes.

We will use linear regression to assess the associations between night shift work and various continuous outcomes. For a continuous outcome with 1000 participants, we will be powered to detect a difference of 10% between an exposure of interest and an outcome of interest, while 400 participants, we will be powered to detect a difference of 15% between an exposure of interest and an outcome of interest, assuming that the standard deviation of the covariate of interest is 1 and the standard deviation of the error term is also 1.

7.2.2 Binary outcomes.

We will use logistic regression to assess the associations between night shift work and various binary outcomes. With an enrollment ratio of 1:1 (exposed: unexposed), if the probability of an exposure of interest (e,g, levels of a biomarker) among day shift workers (unexposed) is 10%, we would be powered to detect an odds ratio of 1.5 or larger with a sample size of 1,000, while for a sample size of 1,200, we would be powered to detect an odds ratio of 1.4 or larger. Meanwhile, if the probability of an exposure of interest among day shift workers is 20% these corresponding numbers are 1.35 or larger for 1,000 and 1.32 or larger for 1,200, while if the probability of an exposure of interest among day shift workers is 30% the corresponding numbers are 1.32 or larger for 1,000 and 1.28 or larger for 1,200. Finally, if the probability of an exposure of interest among day shift workers is 50%, we will be powered to detect an odds ratio of 1.28 or larger with a sample size of 1,000 and an odds ratio of 1.25 or larger with a sample size of 1,200.

7.2.3 Omics analyses

For omics analyses such as targeted metabolomics, the same power calculations as above would apply. For the genetic analyses, power calculations were done using QUANTO, vs 1.2. for a GWAS with data from 400 workers (200 night, and 200 day). For main genetic effects assuming a 1-1 matching for night and day workers and a Minor Allele Frequency (MAF) of 10%, the study would have enough power (80%, p-value 0.05) to detect a relative risk of 1.6. For a MAF of 30%, the study could detect a RR of 1.4 to 1.5. These calculations are not considering FDA corrections. For Gene-Environment interactions, assuming a 10% MAF, a RR of 1.5 for the environmental component and 1.6 for the genetic effect, a study of 400 subjects would have power to detect a RR of 2.5 for the GxE interaction.

8. Discussion

The prevalence of circadian disruption in human populations is high, with approximately 20% of the workforce in Europe is doing some non-standard work schedule. Furthermore, circadian disruption is increasing due to the expansion of human activities over the 24-hour day. This study will contribute to our knowledge of the long-term impacts of circadian disruption on health, our understanding of key biological pathways underlying negative health outcomes among night shift workers and possible individual and population-level prevention policies. To date, the majority of the literature on health impacts of circadian disruption is based on animal experimental evidence, with limited studies conducted in humans. Furthermore, prior human studies have been limited by small sample sizes and a lack of information collected on important occupational exposures, environmental exposures and confounding lifestyle factors. We plan to collect questionnaire-based, sensor-based, biologic biomarker, omics and hormone level variables as we address how the working-life exposome of night-shift workers is related to cardiovascular, neurocognitive, metabolic, stress and other aging-related outcomes. With this rich data, we will be able to further examine important pathways that may explain how night-shift work relates to health.

The strengths of this study include the collection of many aspects of the working-life exposome. Further strengths include the incorporation of data from multiple work sectors and the collaboration between several experts in circadian disruption, occupational health and environmental health across multiple European countries. Furthermore, strengths include the application of novel sensor technologies to collect a multitude of exposures and the application of methods for virtual and self-collected data to adopt research methods that are safe and relevant during the COVID-19 pandemic.

There are also limitations of the present study to consider. First, because it is important to collect a wide range of data types to examine the working-life exposome as completely as possible, we are unable to enroll a large number of participants due to the cost of data collection. Although we anticipate between 1,000-1,200 participants, we may be limited in our ability to detect some important associations, especially between various exposures and pathways and binary outcomes. Second, some heterogeneity in populations will exist due to the work sector or due to some sites being limited to enrolling only women. We will attempt to sample with sufficient overlap to make comparisons of interest, but we may not be able to achieve perfect sampling. Third, as we embark on data collection amid the COVID-19 pandemic, there are many things to consider. Shifting data collection methodologies to go online will result in more reliance on self-collected biologic samples and will rely on participants using technology to complete questionnaires and various testing online. While the majority of our study sample (aged 25- 65) should be able to complete data collection online, this may be a barrier or create added difficulty for some of our participants. Furthermore, the impacts of COVID-19 are wide-ranging and include psychosocial changes. These changes may be particularly striking among health-care workers who have been intimately involved in addressing population health during the COVID-19 pandemic. Discerning the psychosocial impacts of COVID-19 from night shift work will be challenging. Finally, as there are multiple centers contributing to this study, strong communication within contributing partner sites will

be key to adapt and best support data collection in each contributing country, especially as COVID-19 related data collection regulations shift during the persisting COVID-19 crisis9.

9. References

1. *Painting, Firefighting, and Shiftwork IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 2010. 98: p. 818.*
2. Bass, J., *Circadian topology of metabolism.* Nature, 2012. **491**(7424): p. 348-56.
3. Scheer, F.A., et al., *Adverse metabolic and cardiovascular consequences of circadian misalignment.* Proc Natl Acad Sci U S A, 2009. **106**(11): p. 4453-8.
4. Schernhammer, E.S., et al., *Night work and risk of breast cancer.* Epidemiology, 2006. **17**(1): p. 108-11.
5. Parent, M.E., et al., *Night work and the risk of cancer among men.* Am J Epidemiol, 2012. **176**(9): p. 751-9.
6. Papantoniou, K., et al., *Rotating night shift work and colorectal cancer risk in the nurses' health studies.* Int J Cancer, 2018. **143**(11): p. 2709-2717.
7. Papantoniou, K., et al., *Shift work and colorectal cancer risk in the MCC-Spain case-control study.* Scand J Work Environ Health, 2017. **43**(3): p. 250-259.
8. Parent-Thirion, A.B., Isabella; Cabrita, Jorge; Vargas Llave, Oscar; Vermeylen, Greet; Wilczyńska, Aleksandra; Wilkens, Mathijn, *Sixth European Working Conditions Survey – Overview report.* November 17, 2016.
9. Vyas, M.V., et al., *Shift work and vascular events: systematic review and meta-analysis.* BMJ, 2012. **345**: p. e4800.
10. Karlsson, B., A. Knutsson, and B. Lindahl, *Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people.* Occup Environ Med, 2001. **58**(11): p. 747-52.
11. Castagne, R., et al., *Allostatic load and subsequent all-cause mortality: which biological markers drive the relationship? Findings from a UK birth cohort.* Eur J Epidemiol, 2018. **33**(5): p. 441-458.
12. Seeman, T.E., et al., *Allostatic load as a marker of cumulative biological risk: MacArthur studies of successful aging.* Proc Natl Acad Sci U S A, 2001. **98**(8): p. 4770-5.
13. Kondratov, R.V., *A role of the circadian system and circadian proteins in aging.* Ageing Res Rev, 2007. **6**(1): p. 12-27.
14. Belancio, V.P., et al., *The aging clock and circadian control of metabolism and genome stability.* Front Genet, 2014. **5**: p. 455.
15. Lopez-Otin, C., et al., *The hallmarks of aging.* Cell, 2013. **153**(6): p. 1194-217.
16. Roenneberg, T., et al., *Epidemiology of the human circadian clock.* Sleep Med Rev, 2007. **11**(6): p. 429-38.
17. Papantoniou, K., et al., *Circadian variation of melatonin, light exposure, and diurnal preference in day and night shift workers of both sexes.* Cancer Epidemiol Biomarkers Prev, 2014. **23**(7): p. 1176-86.
18. Jones, S.E., et al., *Genome-wide association analyses of chronotype in 697,828 individuals provides insights into circadian rhythms.* Nat Commun, 2019. **10**(1): p. 343.

19. Jensen, M.A., et al., *Changes in the diurnal rhythms of cortisol, melatonin, and testosterone after 2, 4, and 7 consecutive night shifts in male police officers.* Chronobiol Int, 2016. **33**(9): p. 1280-1292.
20. Schernhammer, E.S., et al., *Epidemiology of urinary melatonin in women and its relation to other hormones and night work.* Cancer Epidemiol Biomarkers Prev, 2004. **13**(6): p. 936-43.
21. Reinhardt, E.L., et al., *Night work effects on salivary cytokines TNF, IL-1beta and IL-6.* Chronobiol Int, 2019. **36**(1): p. 11-26.
22. Cuesta, M., et al., *Simulated Night Shift Disrupts Circadian Rhythms of Immune Functions in Humans.* J Immunol, 2016. **196**(6): p. 2466-75.
23. Hansen, J., *Increased breast cancer risk among women who work predominantly at night.* Epidemiology, 2001. **12**(1): p. 74-7.
24. *IARC (2020). Night shift work. IARC Monogr Identif Carcinog Hazards Hum, 124:1–371.*
25. Gustavsson, P., et al., *Occupational exposure and lung cancer risk: a population-based case-referent study in Sweden.* Am J Epidemiol, 2000. **152**(1): p. 32-40.
26. Pijpe, A., et al., *The Nightingale study: rationale, study design and baseline characteristics of a prospective cohort study on shift work and breast cancer risk among nurses.* BMC Cancer, 2014. **14**: p. 47.
27. Horne, J.A. and O. Ostberg, *A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms.* Int J Chronobiol, 1976. **4**(2): p. 97-110.
28. Stout, S.A., et al., *Validation of Minimally-Invasive Sample Collection Methods for Measurement of Telomere Length.* Front Aging Neurosci, 2017. **9**: p. 397.
29. Gao, F., et al., *Dynamic and temporal assessment of human dried blood spot MS/MS(ALL) shotgun lipidomics analysis.* Nutr Metab (Lond), 2017. **14**: p. 28.
30. Burla, B., et al., *MS-based lipidomics of human blood plasma: a community-initiated position paper to develop accepted guidelines.* J Lipid Res, 2018. **59**(10): p. 2001-2017.
31. Strohmaier, S., et al., *Maternal rotating night shift work before pregnancy and offspring stress markers.* Physiol Behav, 2019. **207**: p. 185-193.
32. Cisler, J.M. and E.H. Koster, *Mechanisms of attentional biases towards threat in anxiety disorders: An integrative review.* Clin Psychol Rev, 2010. **30**(2): p. 203-16.
33. D, W., *Wechsler Adult Intelligence Scale–Revised Manual.* 1981.
34. Fink, H.A., et al., in *Cognitive Outcomes After Cardiovascular Procedures in Older Adults: A Systematic Review*. 2014: Rockville (MD).
35. *Strauss E., Sherman E. M. S., Spreen O.. , A compendium of neuropsychological tests: Administration, norms, and commentary, 2006. 3rd ed. New YorkOxford University Press.*
36. Shao, Z., et al., *What do verbal fluency tasks measure? Predictors of verbal fluency performance in older adults.* Front Psychol, 2014. **5**: p. 772.
37. Greenaway, M.C., et al., *Mayo older americans normative studies: factor analysis of an expanded neuropsychological battery.* Clin Neuropsychol, 2009. **23**(1): p. 7-20.
38. MacLeod, C., et al., *Internet-delivered assessment and manipulation of anxiety-linked attentional bias: validation of a free-access attentional probe software package.* Behav Res Methods, 2007. **39**(3): p. 533-8.
39. McNally, R.J., *Attentional bias for threat: Crisis or opportunity?* Clin Psychol Rev, 2019. **69**: p. 4-13.

40. *Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.* Circulation, 1996. **93**(5): p. 1043-65.
41. Tsuji, H., et al., *Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham Heart Study.* Circulation, 1994. **90**(2): p. 878-83.
42. Tsuji, H., et al., *Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study.* Circulation, 1996. **94**(11): p. 2850-5.
43. Cooper, T.M., et al., *Heart rate variability predicts levels of inflammatory markers: Evidence for the vagal anti-inflammatory pathway.* Brain Behav Immun, 2015. **49**: p. 94-100.
44. de Koning, L., et al., *Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies.* Eur Heart J, 2007. **28**(7): p. 850-6.
45. Lear, S.A., et al., *Appropriateness of waist circumference and waist-to-hip ratio cutoffs for different ethnic groups.* Eur J Clin Nutr, 2010. **64**(1): p. 42-61.
46. Janssen, I., P.T. Katzmarzyk, and R. Ross, *Waist circumference and not body mass index explains obesity-related health risk.* Am J Clin Nutr, 2004. **79**(3): p. 379-84.
47. Alberti, K.G., et al., *Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity.* Circulation, 2009. **120**(16): p. 1640-5.
48. Grundy, S.M., et al., *Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition.* Arterioscler Thromb Vasc Biol, 2004. **24**(2): p. e13-8.
49. Annani-Akollor, M.E., et al., *Prevalence of metabolic syndrome and the comparison of fasting plasma glucose and HbA1c as the glycemic criterion for MetS definition in non-diabetic population in Ghana.* Diabetol Metab Syndr, 2019. **11**: p. 26.
50. Florkowski, C., *HbA1c as a Diagnostic Test for Diabetes Mellitus - Reviewing the Evidence.* Clin Biochem Rev, 2013. **34**(2): p. 75-83.
51. Bonmati-Carrion, M.A., et al., *Protecting the melatonin rhythm through circadian healthy light exposure.* Int J Mol Sci, 2014. **15**(12): p. 23448-500.
52. Bakusic, J., et al., *Study of temporal variability of salivary cortisol and cortisone by LC-MS/MS using a new atmospheric pressure ionization source.* Sci Rep, 2019. **9**(1): p. 19313.
53. Gomez-Gomez, A., et al., *Determination of the steroid profile in alternative matrices by liquid chromatography tandem mass spectrometry.* J Steroid Biochem Mol Biol, 2020. **197**: p. 105520.
54. Klok, M.D., S. Jakobsdottir, and M.L. Drent, *The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review.* Obes Rev, 2007. **8**(1): p. 21-34.
55. Mikeska T, C.J., *DNA methylation biomarkers: cancer and beyond.* Genes (Basel), 2014. **5**(3): p. 821-864.

56. Depner, C.M., et al., *Mistimed food intake and sleep alters 24-hour time-of-day patterns of the human plasma proteome.* Proceedings of the National Academy of Sciences, 2018. **115**(23): p. E5390-E5399.
57. *Kervezee L, Kosmadopoulos A, Boivin DB. Metabolic and cardiovascular consequences of shift work: The role of circadian disruption and sleep disturbances. Eur J Neurosci. 2020 Jan;51(1):396-412. doi: 10.1111/ejn.14216. Epub 2018 Dec 3. PMID: 30357975.*
58. Cornelissen, G., *Cosinor-based rhythmometry.* Theor Biol Med Model, 2014. **11**: p. 16.
59. Pripp, A.H. and M. Stanisic, *The correlation between pro- and anti-inflammatory cytokines in chronic subdural hematoma patients assessed with factor analysis.* PLoS One, 2014. **9**(2): p. e90149.
60. Richiardi, L., R. Bellocco, and D. Zugna, *Mediation analysis in epidemiology: methods, interpretation and bias.* Int J Epidemiol, 2013. **42**(5): p. 1511-9.

10. Appendix

10.1 Baseline questionnaire

WP7 BASELINE

Please complete the survey below.

Thank you!

WE WILL NOW ASK YOU GENERAL QUESTIONS ABOUT DEMOGRAPHICS AND RESIDENCE.

Please indicate your date of birth (DD-MM-YYYY).	_____
Please indicate your sex.	○ Male ○ Female
Please indicate your height (in centimeters). Note: if you do not know, please approximate.	_____
Please indicate your weight (in kilograms). Note: if you do not know, please approximate.	_____
Were you born in Spain? (updated to XX country based on location)	○ Yes ○ No
What country were you born in?	_____
Were your parents born in XX country?	○ Yes ○ No
Think of one of your parents first, which country were they born in?	_____
Think of your other parent, which country were they born in?	_____
Please indicate your address: street name and house or apartment number	_____
Which city or town do you live in?	_____
Please indicate your postal code.	_____
For how many years have you lived at your current address? (please round to the nearest whole number. If you have lived there less than 6 months, please indicate 0)	_____
Please indicate your marital status.	○ Married or living together with partner ○ Divorced/separated ○ Widow/widower ○ In a relationship but not living together ○ Single ○ Other

Please indicate the highest level of education you have achieved.	◯ < Secondary education completed ◯ Secondary education completed ◯ University degree completed ◯ Graduate degree completed
Please indicate the highest level of education that your partner has achieved?	◯ < Secondary education completed ◯ Secondary education completed ◯ University degree completed ◯ Graduate degree completed
WE WILL NOW ASK YOU QUESTIONS ABOUT YOUR CURRENT WORK AND YOUR WORK HISTORY.	
Do you always work the same shift or do you rotate?	◯ Permanent (always the same) day ◯ Permanent (always the same) night ◯ Rotate
Have you ever worked night shifts in the past?	◯ Yes ◯ No
What direction of rotation do you work?	◯ Forward (morning/afternoon/ evening/night) ◯ Backward (afternoon/evening/ morning/night) ◯ Other
What is the rate of rotation?	◯ Daily change ◯ Change every 2-4 days ◯ Change every week ◯ Change every 2-3 weeks
When does your day shift start?	_____
When does your day shift end?	_____
When does your night shift start?	_____
When does your night shift end?	_____
What is the main reason for working night shifts?	◯ Is part of my job ◯ Pleasant schedule, fits my rhythm ◯ Calmer work ◯ Financial compensation ◯ Other
How often do you work a long shift (defined as 12 or more hours at a time)?	◯ Never ◯ Once a month or fewer ◯ Every other week ◯ Once a week ◯ Multiple times a week ◯ Always

How many consecutive night shifts do you work on average?	○ Only work one night and then have a day off ○ 2 consecutive nights ○ 3 consecutive nights ○ 4 consecutive nights ○ 5 or more consecutive nights
On average, how many night shifts do you work per week?	○ Only work one night per week ○ 2 nights per week ○ 3 nights per week ○ 4 nights per week ○ 5 or more nights per week
Do you have the ability to self-roster?	○ Yes ○ No
How many days of rest (days off from work) do you have per week?	○ 0 ○ 1 ○ 2 ○ 3 ○ 4 or more
At what age did you start working night shifts?	_____
For how many years have you worked a night shift schedule for some or all of the year? (Add up all the periods you have worked night shift and indicate this number- if it is less than 1 year, count it as 1 whole year)	_____
How much travel time do you have on average between your home and work (one way travel time)?	○ Less than 15 minutes ○ 15-29 minutes ○ 30-44minutes ○ 45-59 minutes ○ 1-1.5 hours ○ More than 1.5 hours
How many hours per week do you work under your contract?	○ Less than 35 ○ 35-40 ○ 41-48 ○ 49-54 ○ 55 or more
WE WILL NOW ASK YOU QUESTIONS ABOUT YOUR SLEEP PREFERENCES AND HABITS.	
One hears about "morning" and "evening" types of people. Which ONE of these types do you consider yourself to be? (Morning types are early risers, perform mentally and physically at their best in the morning hours, and go to bed early in the evening. Evening types stay up late at night, rise at a later time in the morning, and perform best mentally and physically in the late afternoon or evening.)	○ Definitely a "morning" type ○ Rather more a "morning" type than an "evening" type ○ Rather more an "evening" type than a "morning" type ○ Definitely an "evening" type
During the past month, when have you usually gone to bed on a workday?	_____

During the past month, how long (in minutes) has it ususally taken you to fall asleep?	○ 15 minutes or fewer ○ 16-30 minutes ○ 31-60 minutes ○ 60 minutes or more
During the past month, when have you usually gotten up in on a workday?	_____
During the past month, how many hours of actual sleep did you get each day (this may be different than the number of hours you spend in bed).	○ More than 7 hours ○ 6-7 hours ○ 5-6 hours ○ Fewer than 5 hours

During the past month, how often have you had trouble sleeping because you:

	Not during past month	Less than once a week	Once or twice a week	Three or more times a week
Cannot get to sleep within 30 minutes	○	○	○	○
Wake up in the middle of the night or early morning	○	○	○	○
Have to get up to use the bathroom	○	○	○	○
Cannot breathe comfortably	○	○	○	○
Cough or snore loudly	○	○	○	○
Feel too cold	○	○	○	○
Feel too hot	○	○	○	○
Had bad dreams	○	○	○	○
Have pain	○	○	○	○
Other reasons	○	○	○	○

During the past month, how would you rate your sleep quality overall?	○ Very good ○ Fairly good ○ Fairly bad ○ Very bad
During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?	○ Not during the past month ○ Less than once a week ○ Once or twice a week ○ Three or more times a week
During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?	○ Not during the past month ○ Less than once a week ○ Once or twice a week ○ Three or more times a week
During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?	○ No problem at all ○ Only a very slight problem ○ Somewhat of a problem ○ A very big problem

During the week how often do you take naps?	○ Never ○ 1-2 times ○ 3-7 days a week
How long does the nap take on average per day?	○ Less than 30 minutes ○ 30 minutes-1 hour ○ More than 1 hour
Are you able to take naps DURING a night shift?	○ Yes ○ No
How dark is your bedroom when you sleep at night?	○ Not dark at all (daylight) ○ A little dark (dim) ○ Dark ○ Very dark (cannot see hand extended in front of face)
How dark is your bedroom when you sleep between two night shifts?	○ Not dark at all (daylight) ○ A little dark (dim) ○ Dark (can see shadows) ○ Very dark (cannot see hand extended in front of face)
WE WILL NOW ASK QUESTIONS ABOUT YOUR LIFESTYLE (SMOKING, ALCOHOL, DIET AND EXERCISE)	
Have you ever smoked regularly, that is, at least one cigarette per day, a couple of cigars a week, or ecigarette use each day for six months or more?	○ Yes ○ No ○ Do not know
Do you currently smoke or use smokeless tobacco?	○ Yes ○ No ○ Do not know
How many cigarettes do you currently smoke on average per week? (if none, indicate 0)	_____
How many ecigarettes do you currently smoke on average per week? (if none, indicate 0)	_____
How many cigars do you currently smoke on average per week? (if none, indicate 0)	_____
How many grams of pipe tobacco (with a pipe having on average 2 grams of tobacco) do you currently smoke on average per week? (if none, indicate 0)	_____
How many grams of smokeless tobacco do you currently use on average per week? (if none, indicate 0)	_____
Do you currently drink alcohol?	○ No, I quit ○ No, I do not currently drink and never drank ○ Yes
How many years ago did you quit drinking alcohol?	_____
The next set of questions will ask about alcohol use during the past year.	

How often do you have a drink containing alcohol?	○ Never ○ Monthly or less ○ 2-4 times a month ○ 2-4 times a week ○ 4 or more times a week
How many standard drinks containing alcohol do you have on a typical day when drinking? (one standard drink is equivalent to 12 fluid oz of beer or 5 fluid oz of wine or 1.5 fluid oz of hard liquor)	○ 1 or 2 ○ 3 to 4 ○ 5 to 6 ○ 7 to 9 ○ 10 or more
How often did you have 6 or more drinks on one occasion?	○ Daily or almost daily ○ Weekly ○ Monthly ○ Less than monthly ○ Never

THE FOLLOWING QUESTIONS ARE ABOUT THE TIME YOU SPENT BEING PHYSICALLY ACTIVE IN THE LAST 7 DAYS. THIS INCLUDE ACTIVITIES YOU DO AT WORK, AS PART OF YOUR HOUSE AND YARD WORK, TO GET FROM PLACE TO PLACE, AND IN YOUR SPARE TIME FOR RECREATION, EXERCISE OR SPORT.

During the last 7 days, on how many days did you do vigorous physical activitieis like heavy lifting, digging, aerobics, or fast bicycling? (Think about only those physical activities that you did for at least 10 minutes at a time.)	○ 1 day per week ○ 2 days per week ○ 3 days per week ○ 4 days per week ○ 5 days per week ○ 6 days per week ○ 7 days per week ○ none

How much time in total did you usually spend on ONE of those days doing vigorous physical activities?

How many minutes? _____

Again, think only about those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.	○ 1 day per week ○ 2 days per week ○ 3 days per week ○ 4 days per week ○ 5 days per week ○ 6 days per week ○ 7 days per week ○ none

How much time in total did you usually spend on ONE of those days doing moderate physical activities?

How many minutes? _____

During the last 7 days, on how many days did you walk for at least 10 minutes at a time?	○ 1 day per week ○ 2 days per week ○ 3 days per week ○ 4 days per week ○ 5 days per week ○ 6 days per week ○ 7 days per week ○ none

How much time did you usually spend walking on one of those days?

How many minutes?

_____

The last question is about the time you spent sitting on a workday, at home, while doing course work and during leisure time. This includes time spent sitting at a desk, visiting friends, reading, traveling on a bus or sitting or lying down to watch television.

How many minutes?

_____

THE FOLLOWING QUESTIONS ASK ABOUT YOUR DIET.

With what frequency do you eat the following types of foods?

	One or more times a day	4-6 times a week	3 x per week	Once or twice a week	Less than once a week	Never	Do not know
Fresh fruit (excluding juices)	○	○	○	○	○	○	○
Red meat (beef, pork, lamb)	○	○	○	○	○	○	○
Poultry	○	○	○	○	○	○	○
Eggs	○	○	○	○	○	○	○
Fish	○	○	○	○	○	○	○
Pasta, rice, bread, cereals	○	○	○	○	○	○	○
Vegetables	○	○	○	○	○	○	○
Beans and legumes	○	○	○	○	○	○	○
Nuts	○	○	○	○	○	○	○
Processed meats including sausages and cold cuts	○	○	○	○	○	○	○
Dairy products (milk, cheese, yogurt)	○	○	○	○	○	○	○
Sweets (cookies, pastries, jams, cereals with sugar, candies)	○	○	○	○	○	○	○
Sodas with sugar	○	○	○	○	○	○	○
Fast food (fried chicken, sandwiches, pizzas, burgers)	○	○	○	○	○	○	○
Snacks or savory finger foods (chips, crackers	○	○	○	○	○	○	○
Drink 100% fruit or vegetable juice	○	○	○	○	○	○	○

How many times per day do you eat fresh fruit?	○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10
How many times per day do you eat green leafy salad or other types of vegetables?	○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10
How many times per day do you drink 100% fruit or vegetable juice?	○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10
What type of dairy do you generally consume?	○ Skim/fat free ○ Low fat ○ Full fat ○ Do not know
What portion of your grains (rice, bread, crackers, pasta, oatmeal, etc.) are whole grains (brown rice, brown bread, whole wheat pasta, etc)?	○ Less than half ○ Half ○ More than half ○ All of my grains are whole grains ○ Do not know
What time to you generally eat breakfast?	_____
What time do you generally eat lunch?	_____
What time do you generally eat dinner?	_____
Which meal is your largest meal of the day?	○ Breakfast ○ Lunch ○ Dinner ○ Do not know

Have you ever received guidence about diet or meal timing at your workplace?	○ Yes ○ No ○ Do not know

WE WILL NOW ASK QUESITONS ABOUT YOUR MEDICAL HISTORY AND MEDICATION USE.

Have you ever had a pregnancy that lasted 24 weeks or more?	○ Yes ○ No
Have you had a period in the last 12 months?	○ Yes ○ No
If no, what statement best describes the reason you have not had a period in the last 12 months?	○ Menopause ○ Hysterectomy ○ Ovaries removed ○ Currently pregnant ○ Currently breast feeding ○ Taking birth control e.g. hormonal IUD, hormonal contraceptives, contraceptive implants ○ Chemotherapy ○ Other

Has your doctor ever told you you had any of the following conditions or diseases?

	Yes	No	Do not know
Myocardial infarction	○	○	○
Cardiac arrhythmia	○	○	○
Stroke	○	○	○
High cholesterol/triglycerides	○	○	○
Hypertension	○	○	○
COPD (chronic bronchitis, emphysema)	○	○	○
Asthma	○	○	○
Diabetes	○	○	○
Cancer	○	○	○
Depression	○	○	○
Anxiety	○	○	○
Neurological condition (head trauma, epilepsy, dementia, etc.)	○	○	○

What type of cancer(s) have you been diagnosed with?

_____

During the past year have you regularly taken any medications including prescription or over the counter?	○ Yes ○ No ○ Do not know

During the past 24 hours, have you taken any of the medications in the list below?	Yes	No	Do not know
Aspirin or some other non-steroidal anti-inflammatory NSAID (such as ibuprofen, diclofenac, piroxicam, etc), to treat any condition or to treat pain?	○	○	○
Any medications to treat heart disease or hypertension (such as type b-blockers, diuretics, MAO inhibitors, calcium blockers)?	○	○	○
Any medication to treat depression (such as Prozac, Zoloft, Paxil, Colexa, Elavil, Tofranil, Valium, Xanax, Librium, etc.)?	○	○	○
Melatonin supplements?	○	○	○
Any other medicines to treat insomnia besides melatonin (such as hypnotics)?	○	○	○
Lipid lowering medication (such as statins)?	○	○	○
Diabetes medications (such as metformin, sulfonylurea, insulin)?	○	○	○

Over the last 2 weeks, how often have you been bothered by the following problems?	Not at all	Several days	Over half the days	Nearly every day
1. Feeling nervous, anxious, or on edge	○	○	○	○
2. Not being able to stop or control worrying	○	○	○	○
3. Worrying too much about different things	○	○	○	○
4. Trouble relaxing	○	○	○	○
5. Being so restless that it's hard to sit still	○	○	○	○
6. Becoming easily annoyed or irritable	○	○	○	○
7. Feeling afraid as if something awful might happen	○	○	○	○

If you checked off any problems, how difficult have these made it for you to do your work, take care of things at home, or get along with other people?	○	○	○	○

Over the last 2 weeks how often have you been bothered by any of the following problems?

	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	○	○	○	○
2. Feeling down, depressed, or hopeless	○	○	○	○
3. Trouble falling or staying asleep, or sleeping too much	○	○	○	○
4. Feeling tired or having little energy	○	○	○	○
5. Poor appetite or overeating	○	○	○	○
6. Feeling bad about yourself or that you are a failure or have let yourself or your family down	○	○	○	○
7. Trouble concentrating on things, such as reading the newspaper or watching television	○	○	○	○
8. Moving or speaking so slowly that other people could have noticed? Or the opposite -- being so fidgety or restless that you have been moving around a lot more than usual	○	○	○	○
9. Thoughts that you would be better off dead or of hurting yourself in some way	○	○	○	○
If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?	○	○	○	○

Over the last month, how often have you felt the following things?

	Never	Almost Never	Sometimes	Fairly Often	Very Often
1. How often have you been upset because of something that happened unexpectedly?	○	○	○	○	○
2. How often have you felt that you were unable to control the important things in your life?	○	○	○	○	○
3. How often have you felt nervous and "stressed"?	○	○	○	○	○
4. How often have you felt confident about your ability to handle your personal problems?	○	○	○	○	○
5. How often have you felt that things were going your way?	○	○	○	○	○
6. How often have you found that you could not cope with all the things that you had to do?	○	○	○	○	○
7. How often have you been able to control irritations in your life?	○	○	○	○	○
8. How often have you felt that you were on top of things?	○	○	○	○	○
9. How often have you been angered because of things that were outside of your control?	○	○	○	○	○
10. How often have you felt difficulties were piling up so high that you coul dnot overocome then?	○	○	○	○	○

WE WILL NOW ASK YOU QUESTIONS RELATED TO THE COVID-19 PANDEMIC.

Between February 2020 until now did you have one or more of the following symptoms?
Cough
Sore throat
Headache
Muscle ache/pain
Fever
Dyspnoea (difficult breathing)
Reduced or loss of senses of taste and smell
Nausea or vomiting
Diarrhoea
Chest pains
Skin rashes

○ Yes
○ No

Have you been diagnosed with COVID-l9 on the basis of a test by a doctor/general practitioner /others?

○ Yes
○ No

Have you ever been hospitalised due to COVID-l9?

○ Yes
○ No

Do you use any of the following personal protective equipment during your work?

Face covering	○ No ○ Yes, it has been face fit tested ○ Yes, it is a home made or surgical mask
Gloves	○ Yes ○ No
Visor	○ Yes ○ No
In what way has your hours of work changed as a result of COVID-19?	○ Increased a lot ○ Increased a little ○ No change ○ Decreased a little ○ Decreased a lot

Which of the following control measures are in place where you work?

	Yes	No
Testing of staff/patientes/public	○	○
Social distancing	○	○
Ventilation	○	○
Barriers partial or complete	○	○
PPE	○	○
Intensified cleaning	○	○

Are you worried about catching the virus?	○ Not at all ○ Rarely ○ Sometimes ○ Often ○ Almost always
Are you worried that you can't keep your family safe from the virus?	○ Not at all ○ Rarely ○ Sometimes ○ Often ○ Almost always
How would you rate the changes in your working conditions before and after the beginning of the coronavirus crisis?	○ Significant worsening ○ Somewhat worsening ○ Somewhat improving ○ Significant improvement ○ No change
How would you rank the changes in your private life during the COVID-19 crisis?	○ Significant worsening ○ Somewhat worsening ○ Somewhat improving ○ Significant improvement ○ No change
To what extent have you perceived the coronavirus pandemic as a threat to yourself?	No threat at all to myself ——— Extreme threat to myself *(Place a mark on the scale above)*

10.2 Collection, pre-processing and storage of dried blood spot samples

1. The finger should be warmed to increase blood flow.

2. After finger prick, allow a large drop of free-flowing blood to collect, then quickly and gently touch the card to the blood, allowing blood to soak through. Repeat until at least 2 circles are filled in on the DBS card.

3. Specimens should be allowed to fully air dry horizontally for at least 3 hours at room temperature (keep away from direct sunlight) in a safe location avoiding possible contamination of samples or infection to field workers until the samples are dried and put in secure packaging.

4. Once dry, cards should be stacked (with filter paper between DBS cards) and inserted into a sealable envelope that has been labeled with the country and participant information and desiccant packets and a humidity card should also be inserted into the envelope.

5. DBS samples should be kept in sealed envelopes in a cool and dry place until transported to the lab (TBD) for further processing and analysis.

10.3 Collection, pre-processing and storage of blood

Introduction

Blood samples will be used for metals, cotinine, white blood cell count, cytokines, hydroxydeoxyguanosine, mitochondrial DNA copy number, telomere length, GWAS, epigenomics, targeted RNA expression, pyrosequencing, cell-free DNA methylation, proteomics and fluorescent oxidation products (FlOPs). Exposure to metals can result in oxidative damage. Therefore, Pb and As and possible other metals (e.g. Cd, Hg, Pt, Fe, Mn) will be measured in blood. Measuring cotinine gives an insight into the smoking behavior of the participants and will be done for samples from WP6 (asthma/COPD short-term study) and WP7 (shift workers). Furthermore, epigenome-wide association studies (EWAS) help elucidate associations between altered DNA methylation and health outcomes as well as environmental impacts on DNA methylation which is integral to the EPHOR study (WP6 and WP7). DNA methylation will additionally be studied using bisulfite Pyrosequencing. It is a technique based upon the "sequencing by synthesis" approach. It provides quantitative and highly reproducible methylation data at single-base resolution, and it requires relatively low quantities of DNA. This technique will be applied for samples from WP6 and 7. In addition, efficiently identifying circulatory proteins may provide a more mechanistic overview associated disease phenotypes, and at the same time help in development of predictive biomarkers (WP6). In this context another cell free marker, the cell-free DNA (cfDNA) in blood plasma is emerging as a powerful tool for disease diagnostics and biomarker development. And therefore, epigenetic marks found on cfDNA, such as DNA methylation, can be used as biomarkers for exposure and disease and will be validated in the present study (WP6). Moreover, 8-hydroxydeoxyguanosine (8-OHdG) will be used for measurement of oxidative damage. Blood samples from participants in WP6 and 7 cohorts will serve as matrix for 8-OHdG quantification. In addition, also FlOPs will be evaluated. FlOPs are a global marker of oxidation processes, including protein and DNA oxidation and lipid peroxidation are of growing interest in epidemiology. This biomarker of damage has been found to be associated with chronic diseases including asthma. Furthermore, relative mitochondrial DNA copy number (mtDNAcn) and relative telomere length (rTL) will both serve as biomarkers for senescence/DNA damage. In addition, mtDNAcn will be used for the assessment of oxidative stress, whereas rTL will be used for the assessment of biological ageing in relation to occupational exposure. mtDNAcn and rTL will be assessed in cases and controls from WP6 and shift workers from WP7. Moreover, digital droplet (dd)PCR provides high-precision, absolute quantification of nucleic acid target sequences in a wide-range of applications including analysis of gene expression, microRNA analysis and genomic alterations such as copy number variations (CNV). ddPCR will be used to assess biomarkers for occupational exposure to shift work and other exposures i.e. stress, etc. (WP6 and 7). Finally, investigations of the normal sleep–wake cycle showed that the production of pro-inflammatory cytokines exhibit peaks during early nocturnal sleep whereas the anti-inflammatory cytokine activity peaks during daytime wakefulness. Consequently, mistimed sleep and the associated alterations of circadian rhythms (like in a night shift work) are suggested to lead to disturbed immune responses which might contribute to the increased risk for infection, autoimmune diseases, cardiovascular and metabolic disorders, and cancer. A panel of 30 cytokines, chemokines and growth factors will be measured in plasma samples to assess changes in their profile in night shift workers compared to day shift workers. Concentrations will be measured at

multiple time-points for several days based on feasibility in all contributing cohorts. Certain other parameters (e.g. IgE, CC16, YKL40) that have an added benefit for the study could be measured in the samples that will be collected during the project.
Note: The exact time of sampling will be discussed with WP6 and 7 leaders.

Materials needed

Collection:	-10 mL Vacutainer® EDTA tubes -2.5 mL PAXgene® blood RNA tube -6 mL Vacutainer® blood collection tube for trace element testing -rack for tubes -BD Vacutainer® Safety-Lok blood collection set -Powder-free disposable gloves -70% alcohol swabs for skin disinfection -Garrottes/tourniquets -Adhesive bandages or tapes -Container for disposal of used needles after venipuncture -Labels: country ID – participant ID – sample ID (including date and time of collection) -Barcode scanner -Datasheet for information on time of sample collection and time since last meal
Pre-processing:	-Bench top Centrifuge (Eppendorf/Sigma) -Pipettes and tips -Nalgene™ General Long-Term Storage Cryogenic Tubes (Catalogue number: 5000-0020; Volume- 2 mL; ThermoFisher) -Labels: country ID – participant ID – sample ID (including date and time of collection)
Storage:	-Storage box -Freezer -80 °C

Collection of blood
Note: Clean space preferable; some experiments need sterile condition.
Participants are asked to refrain from drinking, eating and smoking before collection.

Type of tube	Number of tubes	Volume of tubes (mL)
Vacutainer® EDTA	3	10
PAXgene® blood RNA tube	1	2.5
Vacutainer® blood collection tube for trace element testing	1	6
Total volume		38.5

Note: Additional clinical paramters might be studied, thus an additional heparin tube might be collected. This will be added to the protocol after discussion with the clinical lab.

Instruction for collection

1. Label tubes
2. Select tube for sample collection (first BD Vacutainer® EDTA, then BD Vacutainer® blood collection tube for trace element testing, end with PAXgene tube). Place sample tubes on a rack in order of collection.
3. Blood samples are collected according to WHO's best practices in phlebotomy (annex 1).
4. Assemble a blood collection set with 12-inch tubing into a BD Vacutainer® One Use Holder. Be sure that blood collection set is firmly attached to holder and does not unthread during use.
5. Hands are washed (see annex 2) and a mask is worn. Volunteers are identified and prepared.
6. Select site for venipuncture; Apply tourniquet.
7. Prepare a venipuncture site with appropriate antiseptic. Do not palpate the venipuncture site after cleansing.
8. Perform venipuncture with limb downward and tube stopper up (for prevention of backflow).
9. Push tube onto non-patient-end (NP-end) of needle in one swift action. Hold tube on NP-end during drawing.
10. Remove tourniquet as soon as blood appears in the last tube.
11. Do not allow the contents of the tube to contact the stopper or end of the needle during the procedure.
12. Allow vacuum to be exhausted prior to removing the tube from the NP (non-patient) end of the needle.
13. Give the participant an adhesive bandage or tape to apply to the puncture site.
14. Discard the used equipment into a puncture-resistant container, discard sharps and broken glass into the sharps container and discard items containing blood or body fluids into the infectious waste.
15. Remove gloves and wash hands.
16. Mix specimen tubes with additives, by slowly inverting the tube 8 to 10 times immediately after blood collection.
17. Fill-in datasheet with information on time of collection and covariates (see annex 3).

Pre-processing and storage of blood at site of collection

Note: sterile condition required for most of the following steps.

Time allowed from collection and processing: 2-6 h.

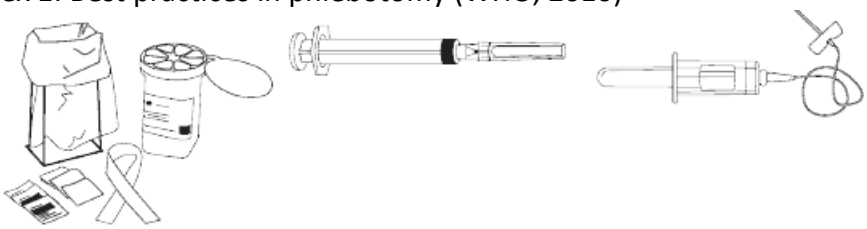
Note: It is very important to minimize the time between collection and separation of plasma; We want to study cell free DNA/ protein and prolonged storage of blood after collection without processing will result in haemolysis and cell death; Thereby, resulting in release of cellular DNA and proteins into the matrix and altering the profile.

- 1 PAXgene RNA tube of 2.5 mL is kept for 1 h at ambient temperature and then stored at -80 °C for ddPCR analysis.
- 1 Vacutainer® blood collection tube for trace element testing of 6 mL is X.

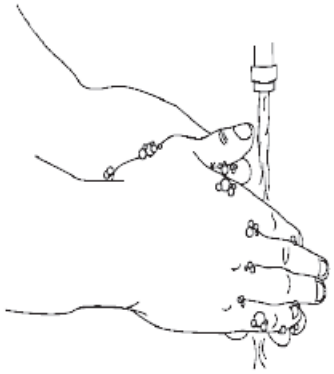
- Blood from EDTA tubes is aliquoted in 6 cryo-vials of 2 mL (1 mL per vial). 2 vials are sent for differential count of cells at the site of collection, 4 vials are immediately stored at -80 °C.
- The remaining blood samples from EDTA tubes are centrifuged (2000 x g, 10 minutes, at 20 °C). (Note: Shorter time between collection and centrifugation should be ideal)
- After centrifugation, plasma is aliquoted in 8 cryo-vials of 2 mL (1 mL per vial). All vials are carefully labeled.
- Store all cryo-vials at -80 °C.
- Leave the cellular fraction (pellet) in the EDTA tube and store at -80 °C.

Note: Please note that the final method for cotinine will be adapted based on method development, optimization and validation. Before the start of the experiments, the developed and validated protocol will be made available. The protocol for collection, pre-processing and storage of blood for cotinine analysis described here is therefore only a limited outline.

Annex 1: Best practices in phlebotomy (WHO, 2010)

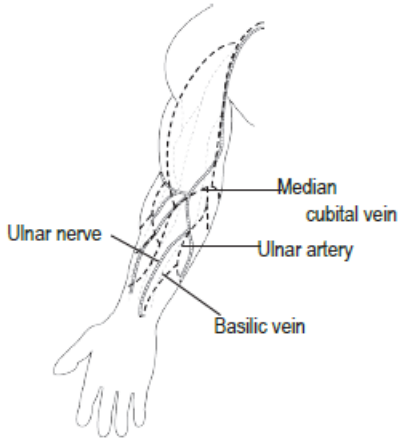


1. Assemble equipment and include needle and syringe or vacuum tube, depending on which is to be used.

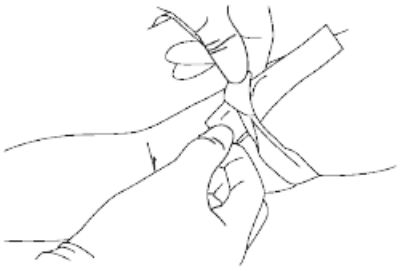


2. Perform hand hygiene (if using soap and water, dry hands with single-use towels).

3. Identify and prepare the patient.

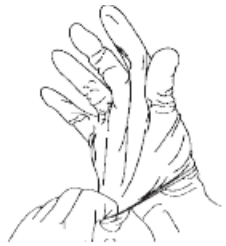


4. Select the site, preferably at the antecubital area (i.e. the bend of the elbow). Warming the arm with a hot pack, or hanging the hand down may make it easier to see the veins. Palpate the area to locate the anatomic landmarks. DO NOT touch the site once alcohol or other antiseptic has been applied.



5. Apply a tourniquet, about 4–5 finger widths above the selected venepuncture site.

6. Ask the patient to form a fist so that the veins are more prominent.

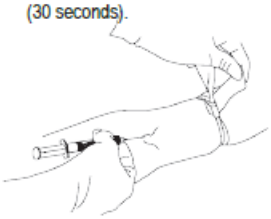


7. Put on well-fitting, non-sterile gloves.

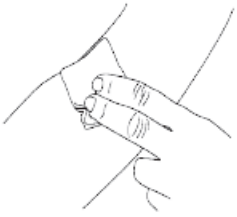
8. Disinfect the site using 70% isopropyl alcohol for 30 seconds and allow to dry completely (30 seconds).

9. Anchor the vein by holding the patient's arm and placing a thumb BELOW the venepuncture site.

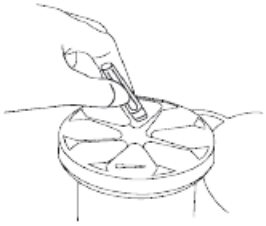
10. Enter the vein swiftly at a 30 degree angle.



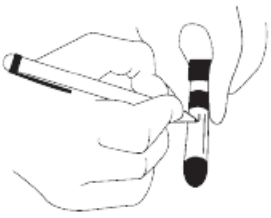
11. Once sufficient blood has been collected, release the tourniquet BEFORE withdrawing the needle.



12. Withdraw the needle gently and then give the patient a clean gauze or dry cotton-wool ball to apply to the site with gentle pressure.

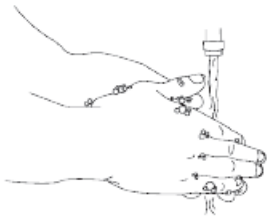


13. Discard the used needle and syringe or blood-sampling device into a puncture-resistant container.

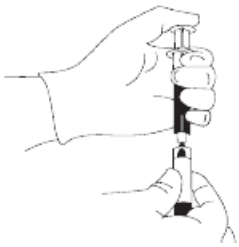


14. Check the label and forms for accuracy.

15. Discard sharps and broken glass into the sharps container. Place items that can drip blood or body fluids into the infectious waste.

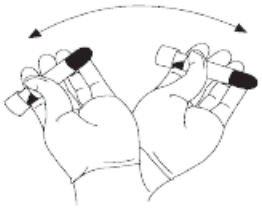


16. Remove gloves and place them in the general waste. Perform hand hygiene. If using soap and water, dry hands with single-use towels.



1. If the tube does not have a rubber stopper, press the plunger in slowly to reduce haemolysis (this is safer than removing the needle).

2. Place the stopper in the tube.



3. Following laboratory instructions, invert the sample gently to mix the additives with the blood before dispatch.

Annex 2: Instructions for hand washing

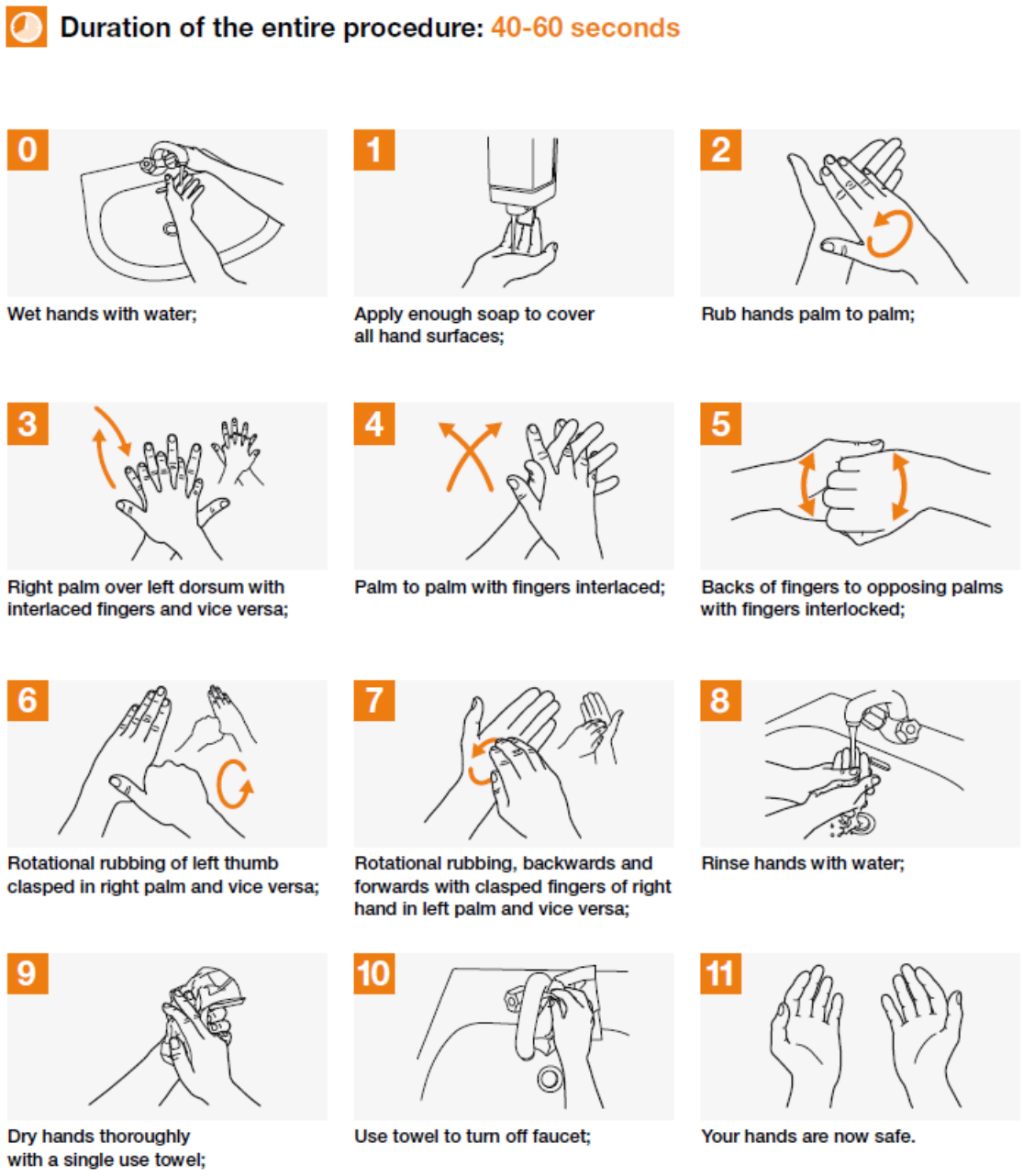


Figure copied from WHO guidelines on hand hygiene in health care (2009).

Annex 3: Datasheet

	First day of sampling	Second day of sampling
Day of the week and date		
Time-sample		
Duration of sleep		
Coffee/alcohol intake		
Physical activity		
Smoking		
Medication use		
Other remarks		

Sex: ☐ Male ☐ Female
Date of birth:

10.4 Collection, pre-processing and storage of saliva

Introduction

Steroid hormones (including glucocorticoids, androgens and progestogens) play important roles in key human functions. Their concentrations might be altered by night shift. Melatonin is a hormone mainly released by the pineal gland at nighttime. The main role of melatonin is the regulation of the sleep-wake cycle, although it plays additional roles such as antioxidant, body weight regulation or reproduction. Saliva is a well-established matrix for melatonin determination. Quantification of salivary melatonin is especially useful for capturing sharp changes in the pineal activity. Saliva will be used as a non-invasive matrix to measure steroid profile concentrations and melatonin concentrations in shift workers from WP7. Concentration will be measured at 5 timepoints during 1 day.

Materials needed

Collection:	-Cotton Salivettes® Cortisol (blue screw cap) (Starstedt, Nümbrecht, Germany) -Tap water -Labels: participant ID – sample ID (including date and time of collection) -Datasheet for information on time of sample collection and covariates (duration of sleep, time of awakening, alcohol/coffee consumption, smoking, medication use and working/non-working day, and sex) (see annex 1)
Pre-processing:	-Centrifuge -1.5 mL polypropylene tubes (Eppendorf, Hamburg, Germany) -Labels: country ID – participant ID – sample ID
Storage:	-Storage box for 1.5 mL polypropylene tubes -Fridge/Freezer (-80 °C)

Collection of saliva

Participants are asked to refrain from drinking, eating, brushing their teeth and smoking for at least 30 minutes before collection (1). Furthermore, participants should avoid vigorous physical activity for at least 60 minutes before each collection.

1) A total of ten samples will be collected over the course of a single day in tubes numbered from 1 to 10. Samples 1&2 will be collected at the first time point, 3&4 at the second, 5&6 at the 3rd , 7&8 at the 4th and finally 9&10 at the 5th time point. The time points are outlined below:

1. Right when you wake up (while still in bed BEFORE brushing teeth).
2. 45 minutes after you wake up.
3. About 4 hours after you wake up.

4. About 10 hours after you wake up.
5. Right before you are going to sleep, but BEFORE you brush your teeth.

*Note: each time a sample is taken, participants will take TWO samples. One will be put in one tube and used for cortisol analyses, the other will be put into a separate tube and used for melatonin analyses. In total each participant will collect TEN total samples (two at each time point) throughout one day.

2) Instructions for Taking Cortisol Saliva Samples

Step 1: Rinse mouth. Note: this step is only done for samples 2-5. Sample 1 should be taken while participant is in bed before brushing teeth or rinsing mouth. Before collecting samples 2 through 5; use cold water to rinse your mouth. Sample 1 will be collected while you are still in bed.

Step 2: Open vile. The participant removes the swab from the Salivette® and places it in the mouth (see annex 2).

Step 3: Coat the salivette with saliva. Keep the swab in the mouth for one minute and coat with saliva.

Step 4: put swab back in vile and close. After the swab is coated with saliva, the swab is placed back in the tube and the tube is closed immediately with the screw cap.

Step 5: The vial is labeled. Mark the tube (on the label) with the time and date of the sample collection.

Step 6: Log sample. Pull out the saliva sample log (included in this instruction packet). Complete the section that corresponds with the sample number, including the time it was taken. Be sure to indicate 'am' or 'pm' and to answer all the questions in the section. It is very important to complete the questions on sleep and the time you woke up for sample #1.

Step 7: Refrigerate! After collecting each sample, place it in the plastic specimen bag labeled 'Completed Cortisol Saliva Samples' that contains a folded paper towel and place into a refrigerator as soon as you can. The samples should be put in the refrigerator (2-8 °C) as soon as possible, but within 30 minutes following sampling

Step 8: The samples should be kept refrigerated (2-8 °C) until they are shipped to ISGlobal.

Step 9: Mail samples.

Pre-processing and storage of saliva at site of collection

Samples are centrifuged (2000 x g, 5 minutes, at 20 °C).

After centrifugation, saliva samples are transferred to 1.5 mL polypropylene tubes and labeled. The samples are stored at -20 °C.

References

1. Bakusic J, De Nys S, Creta M, Godderis L, Duca RC. Study of temporal variability of salivary cortisol and cortisone by LC-MS/MS using a new atmospheric pressure ionization source. Sci Rep. 2019 Dec 1;9(1).

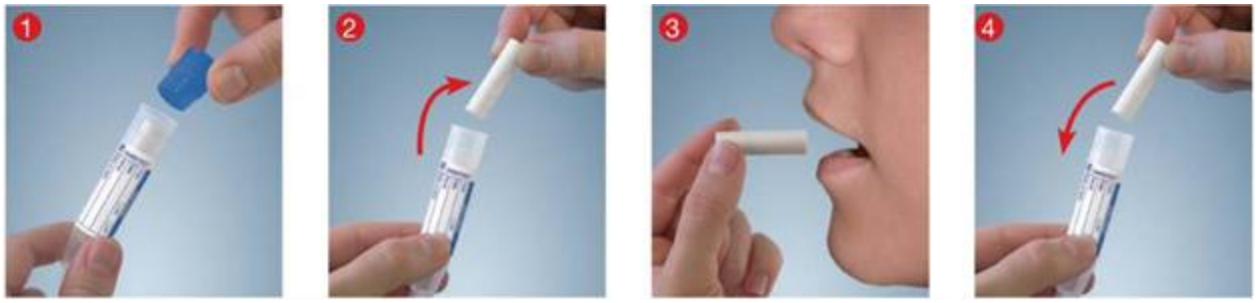
Annex 1: Datasheet for saliva collection

	First day of sampling
Day of the week and date	
Time-sample 1	
Time-sample 2	
Time-sample 3	
Time-sample 4	
Time-sample 5	
Duration of sleep	
Coffee/alcohol intake	
Physical activity	
Smoking	
Medication use	
Other remarks	

Sex: ☐ Male ☐ Female
Date of birth:
BMI:

Annex 2: Procedure for saliva collection

- ✔ Remove the swab from the Salivette® and place it in your mouth
- ✔ Chew on the swab for 1 minute until fully soaked with saliva
- ✔ Transfer the swab to the original tube and immediately close with blue screw cap
- ✔ Fill in the identification label on the tube: country ID – participant ID – sample ID (including date and time of collection)
- ✔ Place Salivette® immediately in the fridge (2-8 °C)



You will have to repeat the procedure 10 times by use of 10 different Salivettes®. Note: each time a sample is taken, participants will take TWO samples. One will be put in one tube and used for cortisol analyses, the other will be put into a separate tube and used for melatonin analyses. In total each participant will collect TEN total samples (two at each time point) throughout one day.

Before collection of all 5 samples, you have to refrain from brushing your teeth, eating, drinking (except for water), smoking. Rinse mouth extensively with water 15 minutes before collecting samples 2-5.

10.5 Instructions for administration and scoring responses from the "cold cognitive" exams

For each exam, the date and time should be recorded on the data sheet.
Test 1: DIGIT SPAN (forward)

- After saying the instructions administer the digit spans in order.
- Do not repeat a span once read.
- Administer both spans of the same length regardless of how the participant performs.
- Say the digits at a rate of 1 digit every 1 second
- Use a monotonic voice without inflections at the end
- Discontinue after failure on BOTH TRIALS OF ANY ITEM (e.g. 5a and 5b)

Examiner: "I am going to say some numbers. Listen carefully, and when I am through say them right after me. For example, if I say 7-1-9, what would you say?"

- If the participant responds correctly /7-1-9), say "That's right," and proceed to item 1.
- If the participant fails the example, say_ "No, you would say 7-1-9. I said 7-1-9, so to say it forwards you would say 7-1-9. Now try these numbers. Remember, you are to say them forwards. 3-4-8"
- Whether the participant succeeds or fails with the second example (3-4-8), proceed to Item 1. Give no help on this second example or any of the items that follow.

Scoring: Each span is scored "1" (pass) or "0" (fail). ONLY DISCONTINUE TEST WHEN PARTICIPANT HAS FAILED BOTH TRIALS OF THE SAME SPAN LENGTH (e.g. 5a and 5b).

Item	Digit Span	Pass	Fail
1 a.	1 - 7	○ 1	○ 0
b.	6 - 3	○ 1	○ 0
2 a.	5 - 8 - 2	○ 1	○ 0
b.	6 - 9 - 4	○ 1	○ 0
3 a.	6 - 4 - 3 - 9	○ 1	○ 0
b.	7 - 2 - 8 - 6	○ 1	○ 0
4 a.	4 - 2 - 7 - 3 - 1	○ 1	○ 0
b.	7 - 5 - 8 - 3 - 6	○ 1	○ 0
5 a.	6 - 1 - 9 - 4 - 7 - 3	○ 1	○ 0
b.	3 - 9 - 2 - 4 - 8 - 7	○ 1	○ 0
6 a.	5 - 9 - 1 - 7 - 4 - 2 - 8	○ 1	○ 0
b.	4 - 1 - 7 - 9 - 3 - 8 - 6	○ 1	○ 0
7 a.	5 - 8 - 1 - 9 - 2 - 6 - 4 - 7	○ 1	○ 0
b.	3 - 8 - 2 - 9 - 5 - 1 - 7 - 4	○ 1	○ 0
8 a.	2 - 7 - 5 - 8 - 6 - 2 - 5 - 8 - 4	○ 1	○ 0
b.	7 - 1 - 3 - 9 - 4 - 2 - 5 - 6 - 8	○ 1	○ 0

Test 2: DIGIT SPAN REVERSE

- After saying the instructions administer the digit spans in order.
- Do not repeat a span once read.
- Administer both spans of the same length regardless of how the participant performs.
- Say the digits at a rate of 1 digit every 1 second
- Use a monotonic voice without inflections at the end
- Discontinue after failure on BOTH TRIALS OF ANY ITEM (e.g. 5a and 5b)

Examiner: “I am going to say some numbers, but this time when I stop I want you to say them backwards. For example, if I say 7-1-9, what would you say?”

- If the participant responds correctly (9-1-7), say: "That's right," and proceed to Item 1.
- If the participant fails the example, say: "No, you would say 9-1-7. I said 7-1-9, so to say it backwards you would say 9-1-7. Now try these numbers. Remember, you are to say them backwards. 3-4-8."
- Whether the participant succeeds or fails with the second example (3-4-8), proceed to Item 1. Give no help on this second example or any of the items that follow.
- Discontinue after failure on both trials of any item (e.g., 5a and 5b)

Scoring: Each span is scored “1” (pass) or “0” (fail). ONLY DISCONTINUE TEST WHEN PARTICIPANT HAS FAILED BOTH TRIALS OF THE SAME SPAN LENGTH (e.g. 5a and 5b).

Item	Digit Span	Pass	Fail
1 a.	2 - 4	○ 1	○ 0
b.	5 - 7	○ 1	○ 0
2 a.	6 - 2 - 9	○ 1	○ 0
b.	4 - 1 - 5	○ 1	○ 0
3 a.	3 - 2 - 7 - 9	○ 1	○ 0
b.	4 - 9 - 6 - 8	○ 1	○ 0
4 a.	1 - 5 - 2 - 8 - 6	○ 1	○ 0
b.	6 - 1 - 8 - 4 - 3	○ 1	○ 0
5 a.	5 - 3 - 9 - 4 - 1 - 8	○ 1	○ 0
b.	7 - 2 - 4 - 8 - 5 - 6	○ 1	○ 0
6 a.	8 - 1 - 2 - 9 - 3 - 6 - 5	○ 1	○ 0
b.	4 - 7 - 3 - 9 - 1 - 2 - 8	○ 1	○ 0
7 a.	9 - 4 - 3 - 7 - 6 - 2 - 5 - 8	○ 1	○ 0
b.	7 - 2 - 8 - 1 - 9 - 6 - 5 - 3	○ 1	○ 0

Test 3: SEMANTIC FLUENCY

- In this exam participants will say the name of as many animals as they can think of.
- Participants will have 60-seconds to respond.
- Start the clock as soon as you have finished giving the directions.

Examiner: “Tell me the names of as many animals as you can think of, as quickly as possible.”
If the person says nothing for 15 seconds, say “A dog is an animal. Can you tell me more animals?”
If the person stops before 60 seconds, say “Any more animals?”

Scoring: Count all animals, including birds, fish, reptiles, insects, humans, extinct animals, etc. Credit can be given for general category terms (e.g., dog) and for specific instances (e.g., terriers) when both are given. Credit only one item when people name the same animal at different developmental stages (e.g., sheep, lamb).

Test 4: PHONEMIC FLUENCY

- This test will take approximately 3 minutes to complete.
- Participants will be given a letter of the alphabet and asked to respond with as many words as possible that start with this letter during one minute. Each letter is 1 "trial".
- They will be given a total of 3 letters and complete 3 trials.
- Start the clock for 60-seconds as soon as you finish saying the letter for that trial.

Examiner: "I will say a letter of the alphabet. Then, I want you to give me as many words you can that begin with this letter, as quickly as possible. For example, if I say B, you can say bed, big, but you can't say proper nouns like Brazil or Beatriz. Also you can't say the same word with a different ending".
Ask the participant if they have understood these instructions. If they have not, you can repeat them and give the same example.
Begin the test. Start with the letter "F" and write down every word that the person says. You will score it later. Keep track of time, measure 1 minute starting once you say the letter "F". Then after one minute, stop the clock.
Continue the test. Now, say the letter "A" and re-start the clock allowing the participant to generate as many words as possible within 60-seconds. Write down every word that the person says. You will score it later.
Continue the test. Finally, say the letter "S" and re-start the clock allowing the participant to generate as many words as possible within 60-seconds. Write down every word that the person says. You will score it later.
To score:

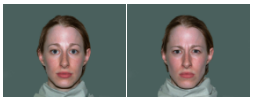
- Exclude proper nouns such as people's, city and country names and the same word with a different suffix.
- Words in other languages that are not included in the Spanish or Catalan dictionary should not be counted.
- If a participant originally says an incorrect word but corrects their response, this is not considered an error.
- The final score only includes correct answers, with one point awarded for each correct word given during the entire 180-second period.

The following items should be considered errors and will not receive a point:

- intrusions (i.e.: when appropriate answers for a letter were given, but inappropriate in terms of letter used at that time;
- perseverations (i.e. same words were repeated twice or more);
- derivations (i.e.: words that varied in number, size, gender and verb conjugations).

10.6 Instructions for the administration of the “hot cognitive” exam

Background: This will be completed by participants on a tablet at the job site at the end (or near the end) of their shift. This test will assess their attentional bias toward threat. This is done by presenting pairs of facial images followed by a probe “>” on one side of the page that participants need to respond to by clicking the corresponding key on the keyboard. Some of these image pairs will present a neutral face next to a threatening face and other of these image pairs will present two neutral faces side by side. A couple examples are shown below for reference.

 (L: neutral, R: threat)

 (L: neutral, R: neutral)

The sequence of presentation will be loops of the following:

1. a cross fixation point (+)
2. two images side by side
3. the probe (>)

The participants will be instructed before the test begins to place their left index finger on the “n” button on the keyboard and their right index finger on the “m” button on the keyboard. When the probe (>) is presented on the left side of the screen, they should press the “n”, while if the probe (>) is presented on the right side of the screen, they should press the “m”.
When the participant is given the tablet, a new trial should be started (approx. 30 seconds in length). They will input their study id, then they will receive instructions for the test. They will first complete a “trial” run to see if they understand how this test works. If they have any questions at any time, please respond to their questions so they are sure about how to complete the test correctly. Following the trial period, the participant will see a screen telling them that the main test is starting (approx. 2.5 minutes in length).
After the test is completed, they will see a thank you screen and they will be done with the test.

Instructions for administering the test:

The examiner will give a tablet to a participant and ask say: “You will now complete a cognitive test. This test will take approximately 5 minutes to complete.”
The test will start with a page of instructions. Please read these instructions and ask any questions you may have before pressing the space bar to begin the test.
As the instructions explain, you will first complete a short trial run to help you become familiar with how this test works. If you have any questions after the trial run, please ask them before you press the space bar to start the actual test.”

Important: before the participant begins the test, they should put their study id into the box so that their results can be recorded correctly. Please say: “Now, before we begin, please enter your study ID into the box. Then, press *run.*”
While the participant is reading instructions and completing the trial, be available to answer any questions they may have about the test. Once the test is complete, thank the participant and collect the tablet for the next study participant to use.

[participant completes the trial period]

Then instructions for starting the real test will be presented to the participant on the screen.

[participant completes the real test]

10.7 Information sheets and informed consent forms for each center

1) ISGlobal forms

ISGlobal Barcelona Institute for Global Health

INFORMACIÓ PARA EL ESTUDIO EXPONIT (EXPOSICIÓN AL TURNO DE NOCHE)

Introducción

Solicitamos su participación en un estudio para investigar los cambios en marcadores biológicos y los efectos sobre la salud debido al trabajo nocturno. El estudio está coordinado por el Instituto de Salud Global de Barcelona (ISGlobal) con la colaboración del Instituto Karolinska de Suecia y la Universidad de Utrecht en los Países Bajos. Su participación en este estudio es completamente voluntaria. Puede negarse a participar y puede retirar su participación en cualquier momento. Lea este documento detenidamente y haga todas las preguntas que pueda tener.

Explicación de procedimientos

Si acepta participar en este estudio, su contribución consistirá en contestar las preguntas de un cuestionario, donar muestras de sangre (tanto una pequeña muestra de sangre del dedo como una muestra más grande de extracción venosa) en un día, donar muestras de saliva durante un día, y llevar una serie de sensores (para recopilar información sobre la actividad, el sueño, el ruido, la luz, las exposiciones químicas y la frecuencia cardíaca) y contestar a una serie de breves pruebas cognitivas. Registraremos su altura, peso y signos vitales. El cuestionario recoge información sobre sobre características demográficas, hábitos de vida, historial médico, salud mental, sueño, factores relacionados con el trabajo y COVID-19; le llevará aproximadamente 15 minutos completarlo. Le proporcionaremos recipientes para recolectar 5 muestras de saliva, los materiales necesarios para tomar una muestra de sangre mediante una técnica de punción digital y también los sensores.

Las muestras de sangre y saliva recolectadas se utilizarán exclusivamente con fines científicos relacionados con los objetivos clave de este estudio. A todas las muestras se les dará un código numérico para identificarlas de modo que no contengan ninguna información personal después de ser recolectadas. Estas muestras se conservarán en congeladores de investigación adecuados hasta que se realicen los análisis. En caso de retirada del consentimiento del participante, las muestras serán destruidas. En este estudio, las muestras de saliva se analizarán para determinar, entre otros, los niveles de melatonina y hormonas esteroide; la sangre se analizará para determinar los niveles de citocinas y quimiocinas, colesterol, triglicéridos, metilación del ADN, longitud de los telómeros y genómica. Se guardarán las muestras sobrantes para análisis futuros.

Beneficios potenciales

No se beneficiará directamente de este estudio, pero su participación es muy importante, ya que permitirá realizar un estudio grande para identificar cómo el trabajo nocturno se relaciona con los cambios biológicos que pueden afectar a la salud y el bienestar. Al final de este estudio, se proporcionarán las conclusiones de nuestra investigación a todos los participantes. Además, si está interesado en conocer sus resultados individuales de niveles de melatonina, hormonas esteroides, citocinas / quimiocinas, colesterol o triglicéridos, podemos proporcionárselo.

Riesgos

No existe ningún riesgo para la salud de los participantes, excepto por una baja probabilidad de que se produzcan hematomas en el lugar de la extracción de sangre.

¿Cómo procesamos la información que recopilamos y cómo garantizamos la confidencialidad y protección de sus datos personales?

Sus datos serán tratados con absoluta confidencialidad y de acuerdo con el Reglamento (UE) 2016/679 del Parlamento Europeo y del Consejo de 27 de abril de 2016 sobre la protección de las personas físicas en lo que respecta al tratamiento de datos personales y sobre el libre movimiento de dichos datos y la Ley Orgánica 3/2018, de 5 de diciembre, de protección de datos personales y garantía de los derechos digitales.

Los datos de salud se separarán de los datos de carácter personal. La seudonimización (esto quiere decir que se le asignará un código y los datos que utilicemos no incluirán información que permita identificarle. También se conoce como disociación) de los datos significa que la información de salud no se puede rastrear hasta una persona identificable, ya que los datos personales se reemplazan por un código. La información seudonimizada será archivada y utilizada por los investigadores del proyecto y sus socios de investigación. Todos los resultados del estudio a nivel de grupo, nunca se presentarán individualmente.

A continuación, encontrará información sobre la protección de datos personales. Léalo detenidamente y contáctenos si tiene alguna pregunta:

¿Quién es el responsable del tratamiento de sus datos personales?

Responsable del Proceso de Datos: Fundació Privada Instituto de Salud Global Barcelona (ISGlobal), CIF: G65341695, Dirección: Carrer Rosselló, 132, 5º 2ª, 08036 Barcelona. Teléfono: (+34) 932271806. Email: lopd@isglobal.org
Delegado de Protección de Datos, email: lopd@isglobal.org

¿Por qué procesamos sus datos personales?

De acuerdo con su participación en el estudio EXPONIT (Exposoma del Turno de Noche), para examinar los impactos cardiovasculares y sobre el envejecimiento producido por el trabajo nocturno y la alteración del ritmo circadiano, el Responsable del Tratamiento de Datos le

informa que, en cumplimiento del Reglamento General de Protección de Datos (en adelante RGPD) y la Ley Orgánica 3/2018, sus datos personales serán utilizados para realizar el estudio de investigación en el que ha dado su consentimiento para participar.

Asimismo, el tratamiento de sus datos también incluirá el acceso a su información de geolocalización, rastreada por un GPS durante un número de días determinados, con el objetivo de realizar análisis ambientales en los lugares que está durante el día.

También le informamos que sus datos personales, en línea con el consentimiento que ha prestado, también podrían ser utilizados en otros estudios de investigación en el marco de este proyecto, o en estudios de investigación en salud global, como los relacionados con enfermedades infecciosas o no transmisibles, y en el área de salud ambiental.

¿Cuál es la base legal para procesar sus datos personales?

La base legal para el tratamiento de datos es el consentimiento del candidato que lo proporciona al aceptar la cláusula de tratamiento de datos. La obtención de sus datos personales es necesaria para la realización de este proyecto de investigación, que no podría realizarse sin sus datos. Sin perjuicio de esto, tiene derecho a retirar su consentimiento en cualquier momento, y sus datos serán eliminados.

¿Cuánto tiempo conservaremos su información personal?

Los datos facilitados se conservarán mientras esté activo el proyecto de investigación o cualquier proyecto dentro de la misma línea de investigación que necesiten sus datos personales, según los criterios establecidos por la legislación vigente.

¿Compartimos sus datos personales?

Esta información será utilizada por el grupo de Investigación responsable de este proyecto (en particular, el investigador principal y colaboradores del estudio, las autoridades sanitarias y el personal afiliado a la entidad financiadora y auditores), quien estará sujeto a la obligación de secreto profesional en cuanto a la validación de datos y procedimientos del estudio, y mantendrá siempre esta confidencialidad de acuerdo con la legislación pertinente. La información también será compartida con estamentos oficiales públicos o privados que requieran acceso a los datos, por responsabilidad o necesidad de cumplimiento, con el propósito de la buena marcha del proyecto de investigación, y de acuerdo con las buenas prácticas científicas.

En ningún caso sus datos personales serán compartidos con un tercer país u organización internacional, fuera de la Unión Europea.

¿Cuáles son sus derechos cuando nos facilita sus datos personales?

Usted será el único responsable de la autenticidad y veracidad de los datos que facilite, y tiene derecho de acceso, rectificación, supresión, limitación del tratamiento, cesión y oposición al tratamiento de sus datos, de acuerdo con la normativa de protección de datos. Para ejercer sus

derechos, debe escribir al Delegado de Protección de Datos (DPO) lopd@isglobal.org. En todo caso deberá adjuntar a su correo electrónico una fotocopia de su documento nacional de identidad o equivalente.

Por último, además de ejercitar sus derechos enviando un correo electrónico al DPO, si no está de acuerdo con el tratamiento de datos realizado por la Institución o considera que sus derechos han sido vulnerados, puede presentar una reclamación ante la Agencia Española de Protección de Datos en cualquier momento.

Gracias por leer esta hoja informativa.
Para obtener más información sobre el estudio, puede contactar con Barbara Harding:

ISGlobal
Instituto de Salud Global de Barcelona - Campus MAR
Parc de Recerca Biomèdica de Barcelona (PRBB)
Doctor Aiguader 88, 08003 Barcelona
ESPAÑA

Telefono: +34 664 31 38 83
Email: barbara.harding@isglobal.org

ISGlobal Barcelona Institute for Global Health

Consentimiento informado para el estudio EXPONIT (Exposición al Turno de Noche)
Investigador principal: Manolis Kogevinas

- He leído la hoja informativa del estudio EXPONIT.
- He tenido la oportunidad de hacer preguntas sobre el estudio EXPONIT.
- He recibido suficiente información sobre el estudio EXPONIT.
- He tenido la oportunidad de hablar con los coordinadores del estudio que me han aclarado todas mis dudas.
- Entiendo que mi participación es voluntaria.
- Entiendo que soy libre de retirarme del estudio en cualquier momento y sin necesidad de justificación y sin ningún impacto en mis derechos legales.
- Entiendo que el estudio EXPONIT está diseñado para aumentar el conocimiento médico.
- Entiendo que la información que proporcione será tratada con estricta confidencialidad y de acuerdo con la legislación vigente.
- Tengo conocimiento de que el estudio ha sido aprobado por el Comité de Ética del Parc de Salut Mar (CEIm).
- Entiendo que todos los resultados son confidenciales.
- Doy libremente mi consentimiento para participar en el estudio EXPONIT
- Tratamiento de datos personales: dada esta información que me ha facilitado el Responsable del tratamiento, y habiendo entendido esto, doy mi consentimiento para el tratamiento de:

	SI	NO
Mis datos personales, utilizados para la realización de este Proyecto de investigación.	☐	☐
Mis datos personales, utilizados para la realización de estudios de investigación que estén relacionados con este proyecto o proyectos dentro de la misma área de investigación.	☐	☐
Mi información de geolocalización, utilizada para realizar este proyecto de investigación.	☐	☐
Contacto en el futuro	☐	☐

Nombre del participante _____ Firma_____ Fecha_____

Nombre del investigador_____Firma _____ Fecha_____

Agradecemos sinceramente su cooperación en este proyecto de investigación. Si tiene alguna pregunta sobre el estudio, puede comunicarse con los investigadores del estudio Manolis Kogevinas (manolis.kogevinas@isglobal.org) o Barbara Harding (barbara.harding@isglobal.org).

COMPONENTE GENÉTICO: INFORMACIÓN PARA EL ESTUDIO EXPONIT (EXPOSICIÓN AL TURNO DE NOCHE)

Introducción

Esta hoja de información proporciona detalles del estudio genético que se llevará a cabo como parte del proyecto EXPONIT (Exposición al Turno de Noche). El estudio investiga los cambios en marcadores biológicos y los efectos sobre la salud debido al trabajo nocturno. Es de gran interés saber cómo el trabajo nocturno puede afectar al envejecimiento y si existen componentes genéticos identificables que puedan explicar el envejecimiento acelerado entre las personas que se dedican al trabajo nocturno. El estudio está coordinado por el Instituto de Salud Global de Barcelona (ISGlobal) con la colaboración del Instituto Karolinska de Suecia y de la Universidad de Utrecht en los Países Bajos. Su participación en este estudio es completamente voluntaria. Puede negarse a participar y puede retirar su participación en cualquier momento sin que ello afecte su atención médica posterior. Lea este documento detenidamente y haga todas las preguntas que pueda tener.

Explicación de procedimientos

Si acepta participar en este estudio, su contribución consistirá en donar una muestra de sangre y de saliva para analizar los factores genéticos y las interacciones entre éstos y los factores ambientales. Así, también es importante entender los mecanismos moleculares (epigenética y transcriptómica) a través de los cuales las exposiciones afectan la salud.

¿Qué se analizará y cómo?

En el estudio genético se analizarán las variantes genéticas del ADN (la molécula que contiene la información genética). También se analizará qué genes están activos en un momento determinado a través de estudios de epigenética (ADN) y de transcriptómica (ARN). Para ello utilizaremos parte de las muestras de sangre (u otros tejidos) que usted ha cedido al estudio en diferentes visitas.

Los ensayos realizados darán una visión global del genoma/epigenoma/transcriptoma, y, algunos de ellos, podrán basarse en la determinación de la secuencia del ADN/ARN.

¿Dónde se harán los análisis y qué se hará con las muestras restantes?

Las muestras biológicas y el ADN y ARN extraídos de ellas se almacenarán en los laboratorios del Instituto de Salud Global de Barcelona (ISGlobal).

Para analizar el genoma/epigenoma/transcriptoma, las muestras podrán ser enviadas a laboratorios colaboradores. Siempre que se envíen muestras a laboratorios colaboradores se seguirá la normativa vigente y se firmará un acuerdo para garantizar su anonimato y el uso de las muestras solamente para los objetivos del proyecto.

Las muestras biológicas y el ADN y ARN restante se almacenarán de forma segura para futuros estudios relacionados con el proyecto en los laboratorios del Instituto de Salud Global de Barcelona (ISGlobal).

¿Cómo procesamos la información que recopilamos y cómo garantizamos la confidencialidad y protección de sus datos personales?

Los datos se tratarán con absoluta confidencialidad y de acuerdo al Reglamento (UE) 2016/679 del Parlamento Europeo y del Consejo de 27 de abril de 2016 relativo a la protección de las personas físicas en lo que respecta al tratamiento de datos personales y a la libre circulación de estos datos.

Los datos del estudio (datos de salud, exposición o genómicos) se pseudonimizarán (se le asignará un código y los datos que utilicemos no incluirán información que permita identificarle), eso significa que se mantendrán disociados de los datos personales. Disociar los datos significa que su información del estudio no podrá asociarse a usted ya que sus datos personales se sustituyen por un código. La información disociada se archivará para ser usada por investigadores del proyecto e investigadores colaboradores. Siempre que se envíen datos a investigadores colaboradores se seguirá la normativa vigente y se firmará un acuerdo para garantizar su anonimato y el uso de los datos solamente para los objetivos del proyecto.

Los datos genómicos, de salud o ambientales obtenidos se podrán hacer públicos en repositorios científicos destinados a compartir información entre investigadores con el fin de acelerar la investigación. Siempre que se haga esto, se asegurará su anonimato evitando la publicación de datos sensibles (ej, no se publicarán mutaciones genéticas individuales que, junto con otra información, puedan identificarle).

A continuación le detallamos la información sobre la protección de datos personales, por favor léala detenidamente y consúltenos si tiene alguna duda:

Responsable del Tratamiento: Fundación Privada Instituto de Salud Global Barcelona (ISGlobal), CIF: G65341695, Dirección postal: Calle Rosselló, número 132, 6ª de Barcelona (08036). Correo electrónico: lopd@isglobal.org

Delegado de Protección de Datos, contacto: lopd@isglobal.org

De acuerdo a su participación en el proyecto de investigación EXPONIT, el Responsable del Tratamiento le informa que, en cumplimiento de lo establecido en el Reglamento General de Protección de datos (en adelante RGPD), sus datos de carácter personal serán utilizados, incluyendo los datos genéticos o los biométricos que consten o sean recabados, para llevar a cabo la investigación a la que usted ha consentido participar.

Asimismo, es importante informarle que los datos de carácter personal, con el consentimiento que ha otorgado, también podrán ser utilizados por otros proyectos / investigaciones dentro del área del presente proyecto, o bien en proyectos de investigación en salud global, tanto en enfermedades infecciosas como no-comunicables, y salud ambiental, para estudiar el efecto de los factores ambientales en la salud de las personas.

La comunicación de sus datos se convierte en un requisito necesario contractual para llevar a cabo el proyecto de investigación sin el cual no podría llevarse a cabo, sin perjuicio de que usted en cualquier momento tiene derecho a retirar los consentimientos prestados, sin que esto afecte la licitud del tratamiento realizado previamente a su retirada.

¿Por cuánto tiempo conservaremos sus datos personales?

Los datos proporcionados serán conservados mientras esté en activo el proyecto de investigación o bien los sucesivos proyectos de investigación dentro de la misma área o línea de investigación en los que se traten sus datos de carácter personal, de acuerdo a los criterios que establezca la legislación vigente.

¿A qué destinatarios se comunicarán sus datos personales?

Esta información será utilizada por el Grupo de Investigación encargado de la investigación / es (en particular, el investigador del estudio y sus colaboradores, autoridades sanitarias, y los monitores y auditores del promotor) los cuales estarán sometidos al deber de secreto inherente a su profesión, cuando lo necesiten, para comprobar los datos y procedimientos del estudio, pero siempre manteniendo la confidencialidad de los mismos de acuerdo con la legislación vigente. También será transmitida la información a los estamentos oficiales públicos o privados que, por obligación legal o necesidad material, deban acceder a los datos a efectos del correcto desarrollo del proyecto de investigación, de acuerdo a las buenas prácticas científicas. En ningún caso sus

datos de carácter personal, es decir, aquellos datos que permitan identificarle, serán transferidos a terceros países u organización internacional, fuera de la Unión Europea.

¿Cuáles son sus derechos cuando nos facilita sus datos personales?

Usted es el responsable de la veracidad y corrección de los datos que nos entrega y tiene la facultad de ejercer los derechos de acceso, rectificación, supresión, limitación del tratamiento, portabilidad y de oposición de sus datos de acuerdo lo dispuesto en la normativa en materia de protección de datos. Para ejercerlos, deberá dirigirse por escrito al Delegado de Protección de Datos a lopd@isglobal.org en cualquier caso se deberá adjuntar una fotocopia de su documento nacional de identificación o bien equivalente.
Por último, además de la posibilidad de ejercer sus derechos, si no está de acuerdo con el tratamiento realizado por la Entidad o considera infringidos sus derechos podrá presentar una reclamación en todo momento ante la Agencia Española de Protección de datos.

¿Qué posibles ventajas me puede proporcionar participar en el estudio?

El estudio no tiene como objetivo identificar variantes genéticas asociadas a una predisposición a sufrir algún tipo de enfermedad. Los análisis genéticos que van a realizarse no van a tener impacto sobre su salud individual, es decir, no se espera encontrar hallazgos inesperados que pudieran tener impacto sobre su salud. Usted puede indicar si desea o no ser informado de los resultados.
En caso de que indique que no desea ser informado, solamente le informaremos si detectamos un hallazgo genético que pueda tener implicaciones graves para su salud o la salud de un familiar biológico de acuerdo a la normativa legal vigente[1].

En caso de tener que informarle porque el resultado tenga implicaciones para su salud o su familia biológica, desde ISGlobal se le contactará para informarle de que se ha encontrado un hallazgo genético que le afecta y se le indicará que se ponga en contacto con su médico de

[1] La comunicación de los resultados genéticos se hará de acuerdo al Artículo 4.5 de la Ley 14/2007, de 3 de julio, de Investigación biomédica (https://www.boe.es/eli/es/l/2007/07/03/14) "Toda persona tiene derecho a ser informada de sus datos genéticos y otros de carácter personal que se obtengan en el curso de una investigación biomédica, según los términos en que manifestó su voluntad. El mismo derecho se reconoce a la persona que haya aportado, con la finalidad indicada, muestras biológicas, o cuando se hayan obtenido otros materiales biológicos a partir de aquéllos. Se respetará el derecho de la persona a decidir que no se le comuniquen los datos a los que se refiere el apartado anterior, incluidos los descubrimientos inesperados que se pudieran producir. No obstante, cuando esta información, según criterio del médico responsable, sea necesaria para evitar un grave perjuicio para su salud o la de sus familiares biológicos, se informará a un familiar próximo o a un representante, previa consulta del comité asistencial si lo hubiera. En todo caso, la comunicación se limitará exclusivamente a los datos necesarios para estas finalidades.

cabecera para que le remita al especialista en consejo genético que corresponda. Desde ISGlobal le prepararemos una carta con el resultado del análisis genético realizado.

¿Qué pasa si decido abandonar el estudio?

Usted tiene el derecho de revocar este consentimiento en cualquier momento del estudio sin que esto afecte la atención médica que esté recibiendo.

Si decide dejar de participar en el estudio, los datos y las muestras obtenidas hasta este momento se conservarán a no ser que usted indique lo contrario de forma explícita. Sólo en este caso, las muestras y los datos se destruirán.

Gracias por leer esta hoja informativa.

Para obtener más información sobre el estudio, comuníquese con Barbara Harding.

ISGlobal
Instituto de Salud Global de Barcelona - Campus MAR
Parc de Recerca Biomèdica de Barcelona (PRBB)
Doctor Aiguader 88, 08003 Barcelona
ESPAÑA
Telefono: +34 664 31 38 83
Email: barbara.harding@isglobal.org

ISGlobal Barcelona Institute for Global Health

Consentimiento informado para el estudio EXPONIT (Exposición al Turno de Noche)
Investigador principal: Manolis Kogevinas

- He leído la hoja informativa del estudio EXPONIT.
- He tenido la oportunidad de hacer preguntas sobre el estudio EXPONIT.
- He recibido suficiente información sobre el estudio EXPONIT.
- He tenido la oportunidad de hablar con los coordinadores del estudio que me han aclarado todas mis dudas.
- Entiendo que mi participación es voluntaria.
- Entiendo que soy libre de retirarme del estudio en cualquier momento, sin necesidad de justificación y sin ningún impacto en mi atención médica o mis derechos legales.
- Entiendo que el estudio EXPONIT está diseñado para aumentar el conocimiento médico.
- Entiendo que la información que proporcione será tratada con estricta confidencialidad y de acuerdo con la legislación vigente.
- Tengo conocimiento de que el estudio ha sido aprobado por el Comité de Ética del Parc de Salut Mar (CEIm).
- Entiendo que todos los resultados son confidenciales.
- Doy libremente mi consentimiento para participar en el estudio EXPONIT
- Tratamiento de datos personales: dada esta información que me ha facilitado el Responsable del tratamiento, y habiendo entendido esto, ofrezco mi consentimiento para el tratamiento de:

	SI	**NO**
Mis datos personales, utilizados para la realización de este Proyecto de investigación.	☐	☐
Mis datos personales, utilizados para la realización de estudios de investigación que sean relacionados con este proyecto o proyectos dentro de la misma área de investigación.	☐	☐
Quiero ser informado de los resultados del análisis genético	☐	☐
Contacto en el futuro	☐	☐

Nombre del participante_____ Firma_____ Fecha_____

Nombre del investigador _____ Firma_____ Fecha_____

Agradecemos sinceramente su cooperación en este proyecto de investigación. Si tiene alguna pregunta sobre el estudio, puede contactar con los investigadores del estudio Manolis Kogevinas (manolis.kogevinas@isglobal.org) o Barbara Harding (barbara.harding@isglobal.org)

2) Karolinska Institutet forms

Skiftarbete och determinanter för icke smittsamma sjukdomar – en longitudinell studie

Det är sedan en längre tid känt att arbete under natten kan ha negativ effekt på hälsan. Vi kommer att genomföra en studie bland hälso-och sjukvårdspersonal som jobbar skiftarbete inom Region Stockholm. Syftet är att bättre förstå de bakomliggande faktorer som ökar risken för ohälsa, för att bättre kunna anpassa arbetstider och arbetsmiljö.

Studien innebär att du som forskningsperson kommer att

1. Besvara ett frågeformulär.
2. Bära sensorer på kroppen och klädnad för registrering av hjärtaktivitet, omgivande exponering på arbetsplatsen (ex ljus, ljud och damm) under 5 dagar.
3. Dagligen under 5 dagar kommer svara på frågor (morgon och kväll) via en app som laddas ner till din smartphone.
4. Ta blodprov vid 2 tillfällen.
5. Lämna salivprov genom att hålla en svabb i munnen i några minuter och sedan stoppa ner denna i ett provrör. Dessa prov samlar du själv in på 5 olika tidpunkter under en dag.
6. Genomgå en läkarundersökning (blodtryck, lyssna på hjärta och lunga, vikt och längd).
7. Genomföra 5 kognitiva tester.
8. Omgivningsexponering vid din bostad (ljus, buller, luftkvalité och grönområden, samt socioekonomiska faktorer) kommer inhämtas från registerdata och modeller för luftföroreningar och trafikbuller.

Vi kommer även att göra en fördjupad studie bland ett begränsat antal studiedeltagare, som frivilligt anmäler sig till detta. Denna extra provtagning innebär:

1. Provtagning från fettvävnad med nålbiopsi.
2. Bära en kontinuerlig blodsockermätare som fästes på armen under 2 dygn (en dag och en natt).

Vi kommer använda oss av uppgifter i HEROMA för att kunna lägga upp ett bra schema för studien, se arbetstider och inhämta information om tidigare arbetade timmar (3 år tillbaka i tiden).

Provtagning kommer ske i anslutning till, samt under arbetstid på din arbetsplats.
Undersökningar och provtagning kommer genomföras av läkare, forskningssjuksköterska och psykolog på anvisad plats på arbetsplatsen.

Vi kommer efter två år göra en uppföljningsstudie för att kunna bekräfta våra eventuella fynd samt se på långtidseffekter. Genom att delta i denna studie binder man sig inte till att delta i uppföljningsstudien.

Syftet med denna studie är att bättre förstå olika mekanismer som ligger bakom riskökningen för vissa sjukdomar och tillstånd vid arbete under natten. Denna förståelse är nödvändigt för att sedan kunna optimera och anpassa arbetsscheman och därmed reducera riskerna.
De olika momenten i denna studie är väl beprövade och bedöms innebära en låg risk för komplikationer. Vid provtagning kan visst obehag förekomma. Under studien kommer du att vara försäkrad via Kammarkollegiet via Karolinska institutet.

All information är kodad så att endast ansvarig forskare kan härleda den till ett personnummer. All information från studien kommer att förvaras säkert på Karolinska Institutet i Stockholm och i enlighet med GDPR. De prover som tas kommer att förvaras på Karolinska Sjukhuset under 10 år och endast ansvarig forskare har tillgång till dessa.
Samtliga resultat från studien kommer att redovisas på gruppnivå och så att ingen enskild individ kan identifieras.

För att öka antalet studiedeltagare, antalet yrkeskategorier, samt få en ökad geografisk spridning, samarbetar vi med forskningscentra i Spanien, Danmark och Holland. Prover som skickas till laboratorier utanför Sverige (inom Europa) för analys hanteras i enlighet med strikta protokoll och kommer efter analys att förvaras på Karolinska Sjukhuset i Stockholm.

Studien är helt frivillig och du kan när du vill välja att avbryta utan att behöva uppge några skäl.
Du kan även begära att få dina lagrade prover förstörda.

Det kommer att utgå en ekonomisk ersättning **om 750 kr före skatt för de deltagare som ingår i studien. Beloppet utbetalas efter halva studietiden. För de som ingår i den fördjupade studien kommer extra ekonomisk ersättning om 1500 kr före skatt att utgå och betalas ut direkt efter genomförd provtagning.**

Vi som är genomför studien arbetar på Karolinska Institutet och Centrum för arbets-och miljömedicin i Region Stockholm.

Kontaktperson

Caisa Laurell
Leg läkare och doktorand
caisa.laurell@ki.se
Telefon: 0709783707

Huvudansvarig forskare
Karin Broberg, professor
karin.broberg@ki.se

Samtycket till att delta i studien "Skiftarbete och determinanter för icke smittsamma sjukdomar – en longitudinell fältstudie"

Jag har fått muntlig och skriftlig informationen om studien och har haft möjlighet att ställa frågor. Jag får behålla den skriftliga informationen.

Jag samtycker till att delta i studien
"Skiftarbete och determinanter för icke smittsamma sjukdomar – en longitudinell fältstudie" ____

Jag samtycker till att uppgifter om mig behandlas på det sätt
som beskrivs i informationsbrevet ____

Jag samtycker till att mina prover sparas i en biobank på det sätt som
beskrivs i informationsbrevet ____

Ja, jag är intresserad av en uppföljningsstudie och samtycker till att ni kontaktar mig om cirka
2 år för ett eventuellt deltagande i den studien ____

Plats och datum _____

Underskrift _____

10.8 Data to be collected, and purpose of each data element

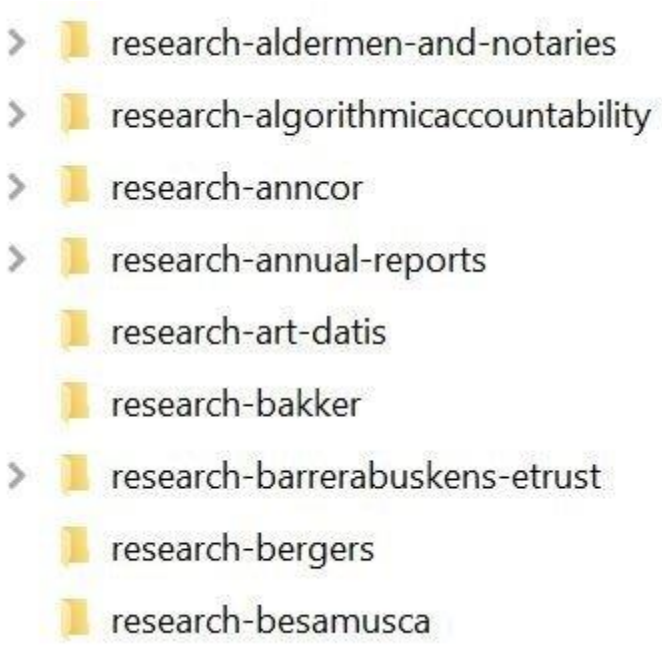
Variables/samples	**Purpose**
Baseline questionnaire info:	
Medication use	characterizing cohort, adjustment variable
Medical history	characterizing cohort, adjustment variable
Alcohol use	characterizing cohort, adjustment variable
Smoking	characterizing cohort, adjustment variable
Physical activity	characterizing cohort, adjustment variable
Sleep	exposure assessment
Chronotype	effect modifier
Diet and meal timing	exposure assessment
Socio-economic status, education and income	characterizing cohort, adjustment variable
Working time, job title, duration of night work	characterizing cohort, exposure assessment
Psychosocial	characterizing cohort, adjustment variable
Height and weight (self-report)	characterizing cohort, adjustment variable
Hypertension	exposure assessment
Residence	characterizing cohort, adjustment variable
COVID	characterizing cohort, adjustment variable
Ecological momentary-app	
commuting to work (time and type)	characterizing cohort, physical activity exposure assessment
sleeping habits	exposure assessment
diet	exposure assessment
physical activity	exposure assessment
psychosocial	characterizing cohort, adjustment variable
Sensor-collected	
Fine dust	exposure assessment
Temperature	exposure assessment
Relative humidity	exposure assessment
Noise	exposure assessment
Light	exposure assessment
UV	exposure assessment
Activity from accelerometer	exposure assessment
Heart rate	outcome assessment
Chemical exposures (passive sampler)	exposure assessment
Anthropomorphic measurements	
Height	characterizing cohort, adjustment variable
Weight	characterizing cohort, adjustment variable

Waist to hip ratio- in person	characterizing cohort, outcome assessment cardiometabolic
Cognitive	
Mental/cognitive	outcome assessment
Biomarkers	
HbA1c	outcome assessment
Metals (ICP/MS-screening)	exposure assessment
Luminex/elisa (immune factors)	outcome assessment
Melatonin	outcome assessment
Cortisol/other steroid hormones	outcome assessment
Genome-wide association study (GWAS)	outcome assessment
Telomere length/mtDNA copy number	outcome assessment
Transcriptomic analysis (targeted RNA expression) ddPCR	outcome assessment
Metabolomics	outcome assessment
Arrays epigenomics in blood DNA(EWAS)	outcome assessment
Pyrosequencing (SNPs)	outcome assessment
cfDNA methyaltion	outcome assessment
proteomics	outcome assessment
C-reactive protein	outcome assessment
leptin and ghrelin	outcome assessment

10.9 Yoda data management platform detailed instructions

Instructions for the Yoda data management platform are accessible here:
https://www.uu.nl/en/research/yoda/guide-to-yoda

Storing your data
Yoda allows you to store your data in a secure way. All data in Yoda is stored in research groups. These are the main folders. Their names always start with 'research-'.



Your data is stored in your own research group. If you want to download or upload bigger amounts of data or files (more than a couple of files / MB's), you need to use the Yoda network drive instead of the website portal.

To store data in your research group, first make sure that you have configured the Yoda network drive. This adds a network drive to your computer where you can store your data. Now that you have access to Yoda through the network drive, you can start working with your data in your research group. You can work with your data like you normally would via the file explorer. For example, you can copy, paste, drag, open, and edit your data. Use your existing applications and tools to work with the data.

You can only access files in another research group if you have been granted access to that group.

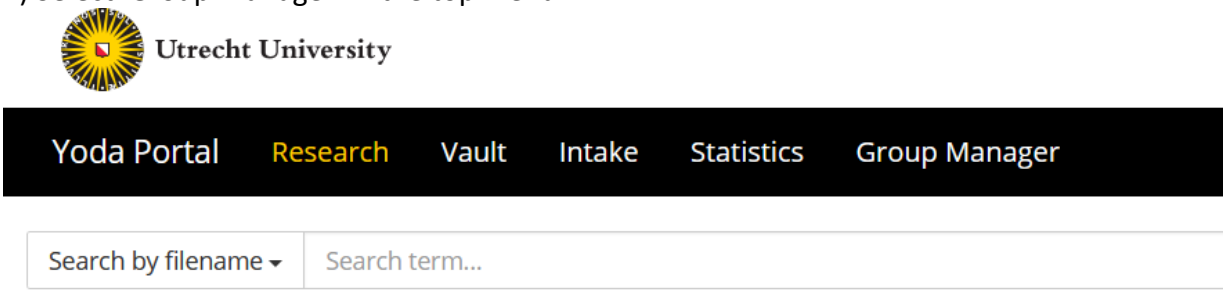
Creating a new research group
Sometimes you want to work with multiple research groups for one project. For example, you might want to have one research group with non-sensitive data that is shared with collaborators, as well as a second one with sensitive data that is only accessible to the principal investigator.

There are two prerequisites for creating research groups:
1. You need to have been granted a system-wide right to create groups.
2. You need to have a group manager role in an existing group in the same research environment.

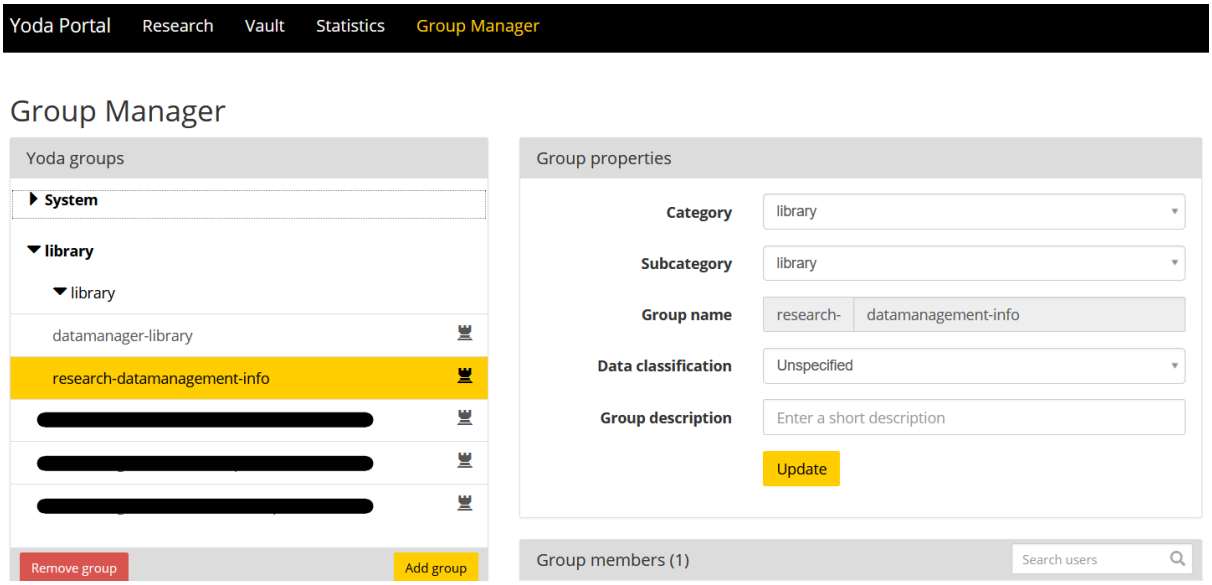
Please contact your data manager if you are not authorized to create a group yourself.

If you are authorized to create a new group, you can do so in the following way:

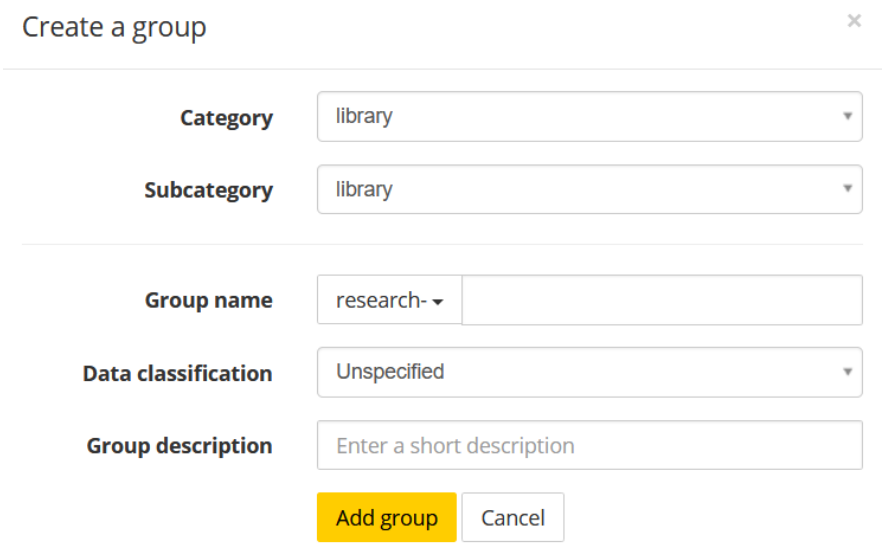
1) Go to the website portal and log in.
2) Select 'Group Manager' in the top menu.



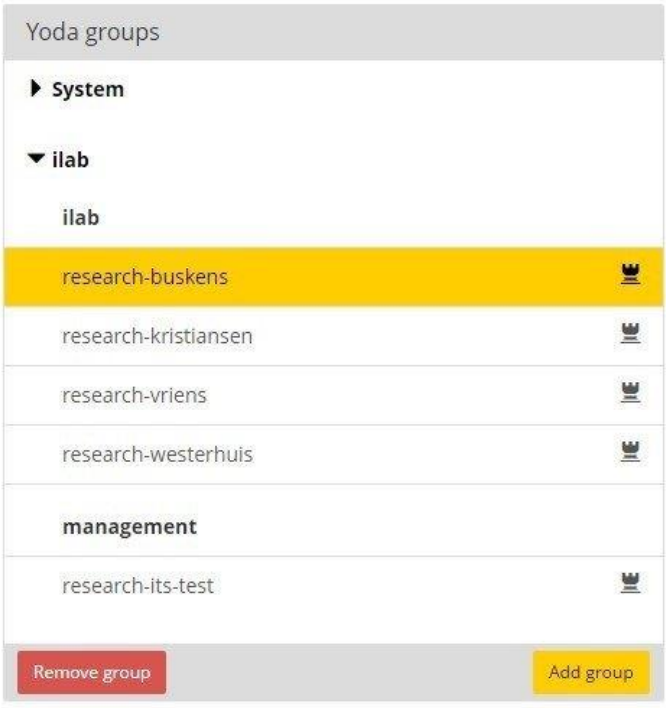
3) In the left pane of the Group Manager, select any existing group in the research environment.



Press the button 'Add group' at the bottom of the screen.
A dialogue will open. Enter the name of the group in the field 'Group name'. The 'research-' prefix is selected by default and should not be changed. Only lowercase letters and numbers can be used in group names.



Click on 'Add group' to finish the process. Be aware: once you have created the group, you cannot rename it.



Sharing your data

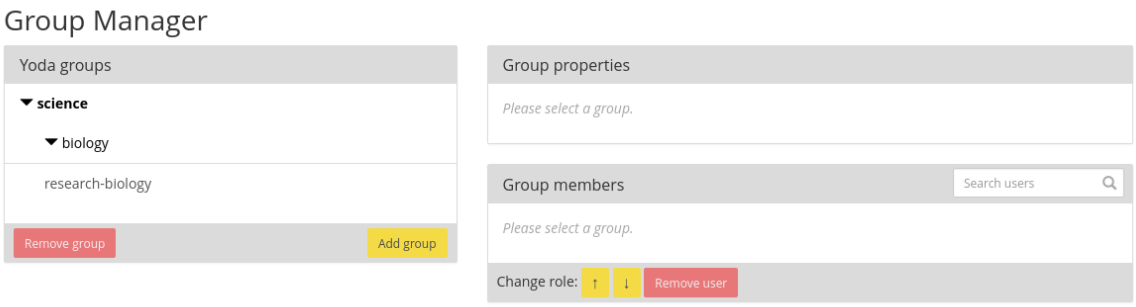
If you want to collaborate with others in your research project, it is possible to give others various degrees of access to your data.

Giving other researchers access to your data

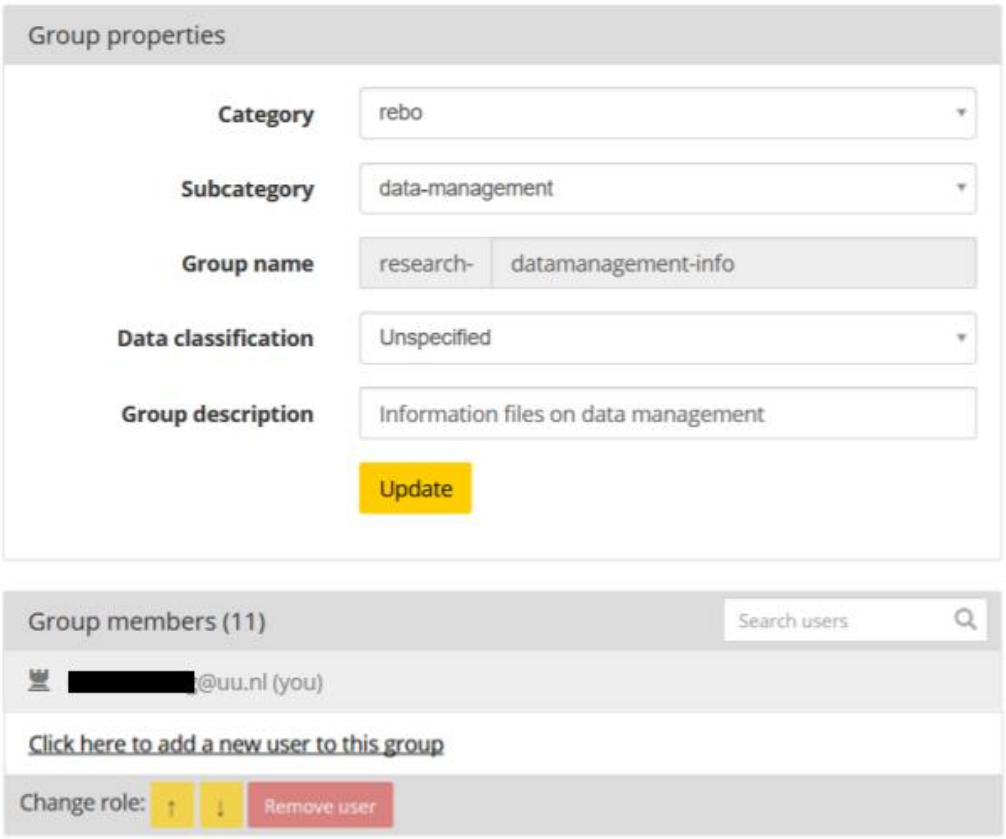
1) Go to the website portal and log in.

2) Click on 'Group Manager' in the menu (the black bar).

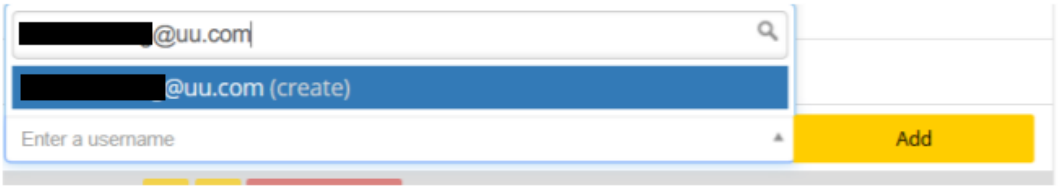
3) In the left pane of the Group Manager you now see the research groups that you have access to. Select your research group. Note: the image below is an example; you will see the name of your own research environment on your screen.



4) Now you will see information about your group. Under 'Group properties' you will find general information about your research group. Under 'Group members' you will see a list of the people that have access to your data.



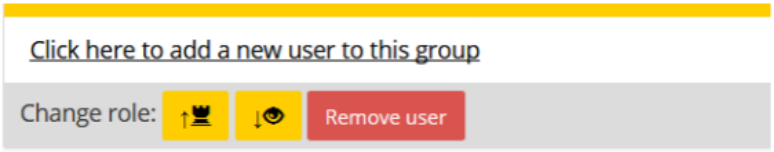
5) Select 'Click here to add a new user to this group'.
6) Enter the email address of the person you want to add. The address must be entered entirely in lower case. If the email address is not known by the system, you will see '(create)' behind the email address. Click on the email address that is shown to you, then click on add.



Changing the role of a user in your research group
Yoda-users can have three different roles in a group:
1) Members with read-only access.
They can view or download files and folders in the group.
2) Members with read-write access.
They can view, download, upload, modify or delete files and folders in the group.
3) Group managers.
They can grant and revoke access rights for the group. They can also view, download, upload, modify or delete files and folders in the group.

A group manager has read and write access and controls who has access to the data and which roles members have. Usually, it is best that only one person is the group manager. If required, assign a maximum of two group managers. Any more makes it difficult to follow who is giving access to whom.

You can see which role a person has. This is marked through an icon on the left of their email address. By default, a new user will be a member with read-write access to the group. If you want to change the role the group member has, then follow these steps:
1) Go to 'Group members'.
2) Click on the person of which you want to change the role.
3) Click on the role you want to assign to this person.



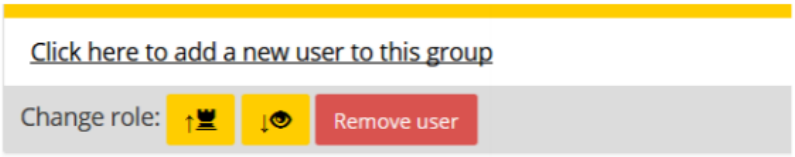
Explanation symbols:

The person has only-read access.

The person has read and write access.

The person is a group manager.
Revoking access to your data
As a group manager, you can revoke someone's access to your research group.

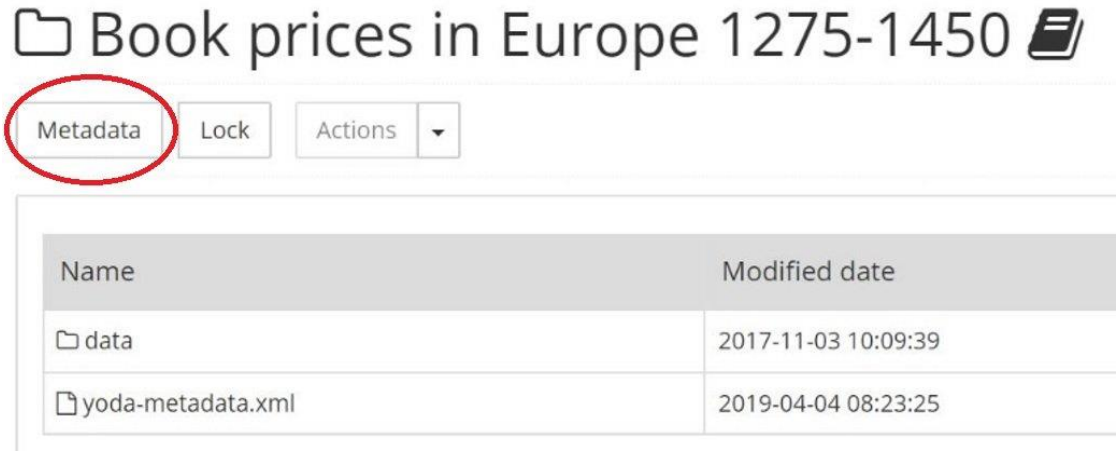
1) Follow the instructions for changing user roles.



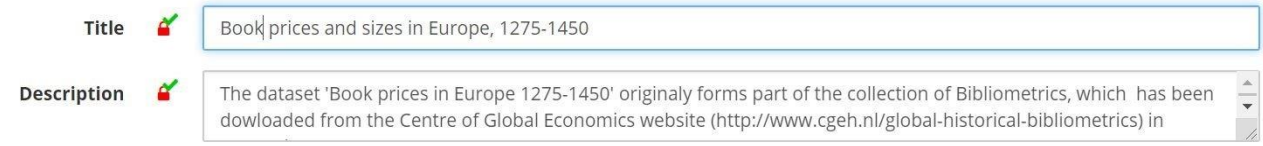
2) Click on the button 'Remove user'.
Now this person is removed from the research group. He or she has no longer access to the data.

Documenting your data
Metadata is 'data about data'. It is information about your data package necessary to:
make the dataset findable in data catalogues;
describe the contents of the dataset for a broad audience;
inform the audience whether the data can be reused and if so, under what conditions;
prescribe how the data should be cited and whom to acknowledge;
inform digital archivists and IT staff how long the data should be retained.

You have to add metadata before you can archive or publish your data. Follow these steps for the instructions:
1) Go to the website portal and log in.
2) Go to the folder you want to add metadata to. This can also be a subfolder.
3) Click on 'Metadata'.



4) Fill in the form with information about the contents of the folder, the creator, contributor, licenses, etc. For more information, see the Metadata Element List.



All mandatory fields are marked with a padlock icon. Some fields can have multiple values. Press the '+' sign to add a value. The data manager can help you filling in this form if needed.
5) After filling in the metadata form, click on 'Submit for archiving or publication''. If you are not ready to submit, click on 'Save'. That way you can continue adding metadata at a later phase.
6) Your submit request will go to the data manager who evaluates the request by checking the folder structure and metadata.

Adding extra metadata
You have the possibility to add more metadata besides the required **metadata form** This allows you to give additional information about the experimental design, data transformation, sampling method, etc. You add this extra metadata with, for example, a README.TXT or another file. This information is then included as part of the data package. Users can find it when opening and inspecting the data package.

Reusing metadata
You can reuse structured metadata. The metadata form includes a button 'Clone from parent folder'. Once you have filled in the metadata form in a parent folder, you can add the same metadata form in one of the sub-folders by going to the sub-folder, clicking on the metadata form, and then clicking on ‘clone from parent directory’. This will then copy the contents of the metadata form from the parent directory to the just opened metadata form in the sub-folder. The Yoda metadata form consists of approx. 33 fields. More information on the 33 fields can be found at: https://www.uu.nl/en/research/yoda/guide-to-yoda/i-am-using-yoda/documenting-your-data

Archiving your data
At specific milestones such as the completion of raw data collection, completion of your analysis, or a publication, you want to archive your data. This is a good way to secure your valuable data. You first need to add metadata to your data packages to be able to archive them. It is important that you add metadata conform certain criteria. This way you make your work better findable for interested researchers and to comply with privacy rules and regulations. The data manager will perform an assessment to help you make sure your metadata meets the requirements. However, you will always remain in charge of your own data.

When you choose to archive (a part of) your data, Yoda creates a 'snapshot' of the data you want to archive. This snapshot captures the data at a certain point in time. This package of data is copied to what is called the 'vault'. This is a main folder containing your archived data. After archiving your data, you will find the vault below the folder of your regular research group. In the vault, the data snapshot will be stored during its retention period. From the moment onwards your data is in the vault, you cannot change it anymore.

Take these steps to archive your data
Step 1. Archive your data through the website portal
Step 2. Organize your data
Step 3. Submit data to the vault
Step 4. Check for compliance, privacy rules and regulations
Copy data from the vault to the research group
Making your data future-proof
More information on each step of archiving your data can be found at:
https://www.uu.nl/en/research/yoda/guide-to-yoda/i-am-using-yoda/archiving-your-data