Statement of Work
Amended 04-12-2020

TITLE :

Microgravity Research Activity **(MRA):** Understanding the Health impact of Inactivity- A bed rest study.

OBJECTIVE :

The purpose of this work is to provide the services and facilities necessary for the execution of a head-down tilt bed rest study. This is part of the Microgravity Research Activity project, implemented by the Canadian Space Agency (CSA).

BACKGROUND :

The CSA and the Canadian Institutes of Health Research (CIHR), with the Institute partners the Institute of Aging (IA), Institute of Circulatory and Respiratory Health (ICRH), and the CIHR Institute of Musculoskeletal Health and Arthritis (IMHA), in collaboration with the Canadian Frailty Network (CFN) are supporting an inactivity study using a bed rest paradigm that will provide new knowledge on the process of aging, the impact of inactivity on health and that will allow testing of a physical exercise countermeasure intervention. The bed rest paradigm is relevant to the CSA because it offers the best ground analogue of the physiological effects of weightlessness.

The CSA has the responsibility to contract the experimental facility for the study, including the recruitment of participants, the medical supervision of the participants, and the acquisition of the standard measures during the study. CIHR and the Frailty Network

are responsible for providing research support funds for eight scientific teams that will provide in-depth analysis of the effects of the bed-rest countermeasure prescribed to the participants.

Bed rest studies, in which healthy volunteers are confined to bed in a 6° head-down tilt position, are a well-established model for some of the adaptations experienced by astronauts during spaceflight. Further, the results obtained in these studies have obvious relevance and applications in terrestrial clinical contexts, which make them even more useful. Many space agencies (Institute for Space Medicine and Physiology-Medes, the German Institute of Aerospace Medicine-Envihab, Planica bed rest facility, Slovenia) and in some cases even individual investigator teams around the world are involved in organizing bed rest studies.

Few Head Down Tilt Bed Rest (HDTBR) studies have been conducted on subjects between 55 and 65 years old, and the present campaign will examine the effects of a 6 degrees head down bed rest on healthy volunteers in order to test an exercise countermeasure that aims to efficiently alleviate the symptoms of skeletal unloading in astronauts and in the sedentary population on Earth.

GENERAL DESCRIPTION:

The following information is closely based on the Guidelines for Standardization of Bed Rest Studies in the Spaceflight Context [PDF (5.3 MB) - external link] and has been adapted and modified to reflect the objective of the current study.

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Infrastructure facility and services:

The experimental facility will accommodate up to a minimum of 6 and a maximum of 8 volunteers at the same time, and offers all the biomedical equipment and medical personnel needed to guarantee the safety and welfare of the volunteers participating in the study including data analysis of standard measures and final reporting of the effectiveness of the countermeasure.

Campaign description:

The experimental campaign will consist of continuous exposure to a 6 degree head down bed rest for a total duration of 14 days of a minimum of 20 and a maximum of 24 healthy older volunteers between the age of 55-65 years old, male and female, non-smokers, dementia-free, drug-free, with no allergies, and no history of thrombosis or heart attack. It is possible for the infrastructure provider to complete the study by using successive cohorts in order to obtain the minimum number of 20 participants or the maximum of 24 participants. Participants who meet the inclusion/exclusion criteria will be continuously monitored for the whole duration of the study. The control group will receive minimal physiotherapy exercise while remaining in bed, while the experimental group will undergo periods of daily high-intensity exercise while remaining in bed (description of exercise below).

Each bed rest period will be preceded by an ambulatory control period to allow collection of baseline physiological data. Subjects will be housed in the facility for a 5 day ambulatory baseline testing and adaptation period, followed by the 14-day experimental head down tilt bed rest campaign, then by a 7-day ambulatory recovery period at the same facility (see example of timeline chart in

Appendix 2). The ambulatory recovery period is followed by follow-up visits (including data collection) scheduled at 4 and 16 weeks after the end of each bed-rest campaign.

Data collection will occur before, during, and after the bed-rest campaign. It is acceptable for the facility to conduct the study using successive cohorts (the timeline example displays a potential of three cohorts). Intervention and control groups must be in the same cohorts. Staggered starting time within cohorts for the participants may be needed within each cohort in order to accommodate the data collection performed by individual Principal Investigators (PI) from the 8 scientific teams collecting data to assess the effects of the exercise countermeasure.

The infrastructure provider is responsible for collection of 'Standard Measures'. These data are collected to facilitate comparison with previous bed-rest studies. The infrastructure provider will analyse the Standard Measures in order to provide an assessment of the efficacy of the exercise-based countermeasure (intervention).

INCLUSION AND EXCLUSIONS LIST

Inclusions: The infrastructure provider **is** responsible for collection of data required by the eight science teams selected by CIHR, for invasive procedures such as blood draws with or without a catheter (a) ,biopsies (b) or to provide access to bulky equipment such as Magnetic Resonance Imaging (MRI)(c) or High Resolution Peripheral Quantitative Computer Tomography (HRpQCT) scans (d) or Dual-energy X-ray absorptiometry (DEXA) scans (e) . Costs incurred by ,) infrastructure provider for the above inclusions (a, b, c, d, e will not be part of this proposal but will be charged to the science team requesting them.

Exclusions: The infrastructure provider is not responsible for non-invasive data collection (questionnaires, ultrasound, etc.) required by the researchers protocols as well as the analysis of the data collected by and for the CIHR-selected teams; these analyses will be done by the science teams themselves, who will provide independent assessments of countermeasure efficacy.

The infrastructure provider will integrate the information from the preliminary reports provided by the CIHR-selected teams into the infrastructure provider's report on countermeasure efficacy.

The exercise-based countermeasure is the only intervention that will be tested during the bed-rest campaigns.

Description of the exercise-based countermeasure:

During the experimental campaign, half of the subjects will participate in a 60 minute per day standardized countermeasures while the other half will serve as controls. The 60 minutes will be divided into 3 sessions that will include high-intensity interval training on a cycle ergometer. Upper body exercises will be conducted using resistance bands. The exercise must be completed, from start to finish, in the head-down tilt posture or horizontal position.

TECHNICAL SPECIFICATIONS

A complete application package should describe in detail the services available to support scientific research during the recruitment, baseline, head-down bed rest study, and recovery periods, and should include all associated costs. The resources and facilities

described below are required. When specific companies are referenced as sources of equipment, the bid may propose alternative suppliers if the equipment has equivalent function.

High-level requirements are listed in Table 1, and sub-requirements (e.g. 4.3: Inclusion and Exclusion criteria) are documented in Annex 1.

Table 1: High-level requirements for the bed rest study

Reference APPENDIX 1	Requirement
1	Infrastructure (e.g. accommodation for participants)
2	Study Management, Personnel and Communication
3	Development of the integrated study protocol and Informed Consent Form
4	Volunteer recruitment and selection
5	Volunteer rules and general conditions of bed rest
6	Subject Care
7	Nutritional Intake
8	Data Management
9	Standardized Measures and Schedule
10	Countermeasure Procedures
11	Mid-term Assessment Review
12	Final Report

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CLIENT SUPPORT

Meetings:

Prepare, support and provides minutes for the weekly teleconference meetings.

Milestones and deliverables

1) Kick off meeting an minutes
   1.1. Prepare and hold a face to face kick off meeting at facility location
   1.2. Provide a summary report of the meeting including the point discussed, the next steps and the actions items.
2) Investigator Working Group Workshop (section 2.4.2.3 of Appendix 1)
   2.1. Experiment data sheet (section 3.1.1 of Appendix 1)
   2.2. Information to participants before screening (refer to section 4.1.3 of the Guidelines)
   2.3. First investigator package (section 2.4.2.2 of Appendix 1)
   2.4. Second investigator package (Final version)
   2.5. Data sharing agreement (section 8.2 of Appendix 1)
3) Study protocol for the ethics submission (section 3 of Appendix 1)
   3.1. Integrated study protocol (initial version)
   3.2. Inform consent form (initial version)(section 3.2 of Appendix 1)

3.3. Participants rules and directives for pre, during and post-bed rest (initial version))(section 5 of Appendix 1)

4) Approval of final study protocol by the Research Ethics Board (REB) (section 3 of Appendix 1)
   4.1. Final submission of the Integrated study protocol and approval by the REB (final version)
   4.2. Integrated study protocol (final version)
   4.3. Inform consent form (final version)(section 3.2 of Appendix 1)
   4.4. Participants rules and directives for pre, during and post-bed rest (final version))(section 5 of Appendix 1)
5) Mid-term assessment review and report
   5.1. Volunteer recruitment and selection for the first and second group of participants (section 4 of Appendix 1
   5.2. Data delivery to science teams of standard measures collected for the 2 first cohorts following the signed agreements stipulations (section 8.2 of Appendix 1)
   5.3. Delivery of Mid-term assessment report of the evaluation of the results from the standardized measures at the mid-term with 12 to 16 subjects (section 11.2 of Appendix 1)
   5.4. Prepare and hold a meeting to present the mid-term summary report
6) Draft final report

6.1. Volunteer recruitment and selection for the third and fourth group of participants (section 4 of Appendix 1

6.2. Data delivery to science teams of standard measures collected for the 2 first cohorts following the signed agreements stipulations (section 8.2 of Appendix 1)

6.3. Delivery of draft of the Final Report on effectiveness of exercise using standard measure data and integrating the preliminary reports received from science teams (data processing, statistical analysis, discussions, and recommendations) (section 12 of Appendix 1)

6.4. Deliver the data set for standard measures to CSA in a non-proprietary, machine-readable, aligned to industry-based standards, and conventional format such as TXT or CSV (section 8.3 of Appendix 1).

7) Final Report

7.1. Final Report on effectiveness of exercise using standard measure data and integrating the preliminary reports received from science teams (data processing, statistical analysis, discussions, and recommendations) (section 12 of Appendix 1)

7.2. Prepare and hold a face to face closure meeting at the facilities to present the final report

LIST OF ACRONYMS

BDC: Coordinate Baseline Data Collection

BMC: Bone Mineral Content

BMD: Bone Mineral Density

CFN: Canadian Frailty Network

CIHR: Canadian Institutes of Health Research

CSA: Canadian Space Agency

CSV: Character Separated Values

CT-scan: Computerized tomography

DBP: Diastolic Blood Pressure

DEXA: Dual-energy X-ray Absorptiometry

ECG: Electrocardiography

EDS: Experimental Data sheet

GHQ: General Health Questionnaire

HDTBR: Head Down Tilt Bed Rest

HIIT: High-Intensity Interval Training

HR: Heart Rate

HRpQCT: High Resolution Peripheral Quantitative Computer Tomography

IA: Institute of Aging

ICRH: Institute of Circulatory and Respiratory Health

IMHA: Institute of Musculoskeletal Health and Arthritis,

MAP: mean arterial pressure

MRA: Microgravity Research Activity

MRI: Magnetic Resonance Imaging

PET: Positron Emission Tomography

PI: Principal Investigator

REB: Research Ethics Board

RER: Respiratory Exchange Ratio

SBP: Systolic Blood Pressure

SOT: Sensory Organisation Test

SPECT: Single-Photon Emission Computerized Tomography

TXT: Text Files

VO^2 max: Maximum Rate of Oxygen Consumption

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APPENDIX 1. Detailed Explanation of Requirements

1. Infrastructure requirements

1.1. Secured rooms and study area

Restricted access to facility. Access to facility needs to be limited to infrastructure working team in order not to disturb the data collection. CSA personnel and VIP (astronaut or other) will be provided access to visit the participants.

1.2. Participants' room and bed

The capacity to accommodate 6 to 8 participants is required. Some privacy for participants is needed, since they will be at the facility for a protracted period. Rooms need a secured storage space for participants' personal items.

It is mandatory to provide beds that can tilt the head down by 6 degrees with comfortable mattresses to avoid participants dropping out from the study. The use of one small size flat pillow is allowed as long as the shoulders touch the mattress.

1.3. Exercise equipment

The following equipment is required to perform high-intensity interval training (HIIT), aerobic exercise, and strength exercise:

- Two to three cycle ergometers to be used both supine during the bed rest period and sitting up right during the baseline and recovery periods as well as during the selection assessment periods.
- Resistance bands with or without a shuttle device will be required to perform the strength exercises. These

exercises need to be also performed in a supine head down tilt position.

1.4. Secure storage area

A secure room will be needed for storage of the science teams' equipment (portable computer, portable ultrasound machine, portable MRI, and small disposables laboratory equipment such as blood tubes, etc.) during the bed rest campaigns.

1.5. Meeting, working and social area

A meeting room will be needed as well as desk spaces for the science teams (2 or 3 people) to work and to allow periodic meetings of all science teams and study management team and personnel.

A social area (bedroom, room or area inside the building) is required to be accessible by the participants during the bed rest campaigns in order for them to talk to each other (at least 2 at the time) while maintaining the appropriate body position.

1.6. On-site access to medical instrumentation for science teams participating to the study:

Provide access and support to the science team for biomedical facilities (3-Tesla MRI, HRpQCT, and DEXA) in a head down tilt or recline position. These additional tests <u>are not part of the cost to be included in the proposal</u> but costs related to these tests need to be directly charged to the science team requesting them.

Provide access to a level 1 containment laboratory and adequate benchtop space to allow minimal sample

preparation for a maximum of eight science teams; with a refrigerated centrifuge, biological safety cabinet, and freezers (minimum capacity of 368L) at -20°C and at -80°C to store samples until shipment to the experimental team laboratory for analysis. Note that science teams will be responsible to bring their own disposables and small equipment needed when using these facilities.

1.7. Sample shipment

The Project Scientist (see Section 2.1) will describe the proper sample transport procedures that are possible from the facility, ensuring correct transport regarding temperature and other safety issues. It is the science teams' responsibility to cover the costs associated with transport, and to guarantee safe transport. The main objective is for the CIHR-selected teams to receive the samples in an optimal, frozen condition in their respective labs. To ensure that samples can be received, the Project Scientist will contact the science team before shipping.

2. Study Management, personnel and communication

Proof of specific training and qualifications (Curriculum vitae) need to be provided for all staff involved in the care of participants or support of participants-related activities to establish their eligibility to conduct a study including medical supervision and care of participants.

2.1. Management team

The facility will provide a study management team comprised of:

- Project Scientist: Person in charge of all scientific aspects of the study and the representative of all CIHR-selected teams in the study.
- Project Manager: Person in charge of the organization and management of the study and responsible in verifying qualifications of all personnel.
- Head Medical Doctor: Qualified medical doctor (not an associated Principal Investigator (PI) or Co-Investigator from the participating funded teams) representing the medical interest of the test subjects.

A backup person will be needed for each position.

2.2. Internal personnel

For a continuous level of monitoring of the participants and support of the scientific activities including logistical support for the CIHR-selected teams, qualified personnel will be required to:

- Provide well-trained caregivers (Registered nurse or nurse assistant) for 24-h round the clock supervision of participants during their entire presence in the facility, to monitor compliance of the subjects with the study requirements and provide support for the well-being of the participants and data collection .
- Provide Medical supervision by a qualified general practitioner either on-site or on call for the entire duration (24 hour around the clock) when the participants will be on site for data collection campaign. If on-call, the general practitioner need to

be on-site within 15-20 minutes or as instructed by the Research Ethics Board.

- Provide a medical monitoring routine comprised of the measurement of blood pressure, heart rate, body temperature, and body weight. Other measures should be performed if deemed necessary. It is the responsibility of the Head Medical Doctor to organize and provide the medical monitoring of the subjects' health status on a daily basis during the study. The protocols (including Standard Measures and protocols conducted by the CIHR-selected teams) for which this might be necessary are defined in advance by the Project Scientist and the Head Medical Doctor, who will also work with the CIHR-selected investigators to define termination criteria for the participants. The Medical Doctor can decide to stop any protocol if the well-being of a subject is jeopardized. Additionally, the medical doctors monitor the psychological well-being of the subject during the daily ward rounds and contact the psychologist if necessary.
- Provide physical therapy to help prevent contractures during the bed rest period and ensure quick recovery after the study. Physiotherapy can provide relief in some cases and should be considered by the medical doctors as treatment before using drugs. It is the decision of the Head Medical Doctor to schedule

physiotherapy sessions as a treatment, in addition to the mandatory physiotherapy (no countermeasure group) routine. It should be discussed with the PIs during the Investigator Working Group workshop before the beginning of the study whether physiotherapy could have a significant impact on any of the experiments.

- Provide psychological monitoring and support with the same psychologist(s) (specialised in older subjects) that participated to the screening of the participants. The psychologist should visit the subjects every 2 weeks during the bed-rest study unless requested by the medical doctor or unless the Research Ethics Board requires a different interval. The content of the interviews is considered confidential, unless it is the wish of the individual test subject to transfer anything to the Project Scientist.

2.3. External personnel

External personnel to support the study related activities may come from the PIs' team or may be recruited by the facility provider. Appropriate security clearance will need to be provided. Proper training regarding individual experiment-related procedures but also regarding general operations / rules of the facility and how to deal with the specific physical and psychological situations of the subjects shall also be provided. Training should include emergency, security rules, instructions for all devices, general goal of the study, and rules for subjects and staff.

2.4. Communications

Clear communications will need to be established both at the internal level of the facility and external level which includes the Principal Investigator (PI) teams, the Canadian Space Agency project team and facilities and laboratories required for data collection.

2.4.1. Internal communications

2.4.1.2. Proper communication tools need to be implemented to communicate between people working in different shifts or locations to ensure optimal communication between staff members and to report the progress of the study and to exchange problems or news with the next shift.

2.4.1.2. Documentation of unexpected events

To support the interpretation of data collected during each campaign and to protect subjects' rights and safety all unexpected events shall be documented.

2.4.2. External communications

2.4.2.1. Experiment data sheet (EDS)

Prepare and distribute EDS to collect the relevant information from the 8 Principal Investigators (PIs) from the CIHR-selected teams on their specific needs. These EDS will be used to prepare the Investigator Working Group Workshop.

2.4.2.2. Investigator Information Package (IIP)
Compile and distribute the First investigator information package (preliminary version, delivered before the IWGW (see below) and the Second investigator information package (final version, delivered after the IWGW). The investigator information package is the compilation of relevant information covering all aspects necessary for the joint creation of the integrated study protocol. The purpose of the investigator information package is to ensure all investigator team members' understanding of the rationale for and compliance with the key features of the specific bed rest campaign.
The IIP will include:

- Provide the general background of the study (inclusion/exclusion criteria, medical follow-up, physiotherapy, nutrition, etc.
- Provide organization of the study (key functions)
- List of equipment and hardware available on site for the science teams
- Define data rights, data access and publication priorities for the science teams (provided in the final IIP)
- Provide investigator rules :
  > Obligations (such as bed rest walkthroughs)
  > Respective responsibilities of the Facility provider and the PIs
  > Confidentiality

- The integrated study protocol (provided in the final IIP).
- Provide a list of capabilities available at the bed rest facility

All recipients shall treat the IIP as a confidential document.

2.4.2.3. Investigator Working Group Workshop (IWGW)

Prepare and participate to the IWGW (room rentals and meals of PI and their representatives will be covered by the CIHR and Partners) to optimize the final plan, and data sharing plan. The general plan will be prepared and distributed to all PIs in an effort to identify possible interferences between their experiment and others. The data sharing plan and a visit of the facility will need to be organized during the workshop. The topics to be discussed will be:

- Introduction and review of the investigator rules by the PI's. Each PI must sign consent to the rules at the start of the workshop.
- Introduction of the different investigator groups and their experiments
- Discussion about possible interferences between individual experiments
- Introduction of the general plan by the Project Scientist
- Discussion of the general plan

- Introduction and discussion of a data sharing plan
- Harmonization and cooperation between different experiments
- Discuss data rights, data access and publication priorities with the CIHR-selected teams
- Communication between each investigator and the management team during the planning of the study (Ex.: monthly email distributed about the progress of the study planning).

A final investigator package will be distributed following the workshop.

If necessary a second investigator meeting will be organized by videoconference or teleconference to discuss the final version of the integrated study protocol and agree on final consent on final data sharing agreement.

2.4.2.4. During the study campaigns

Conduct an integrated Test Readiness Review of the bed rest study, including a safety walk-through and check-out of final procedures and operations prior to the study. Organize a face-to-face meeting between the Project Scientist and each CIHR-selected team upon their arrival and before they actually perform their experiment in the running study. This meeting is meant to provide instructions on the processes related to the study, introduce them to the staff, and ensure that the investigator team will fulfill their obligations.

Additional meetings will be needed to inform the investigator teams of any unexpected event or modifications required during the study.

2.4.2.5. Press/Media

Media activities should be coordinated by CSA, CIHR and partners and the facility provider. Media communication with the test subjects will not be allowed until they have been informed and prior written consent has been obtained. Subjects and the facility provider is not allowed to contact media on their own authority. To avoid interference with data collection, all media contact should be limited to just before the pre-bedrest period or at the end of the rehabilitation period.

3. Development of the Integrated study protocol and informed consent form

3.1. The facility provider Project Scientist will be responsible to develop the integrated study protocol, in close collaboration with all eight individuals science teams, based on their experimental data sheet (EDS) describing their individual protocols. The development of the integrated protocol will also include a general plan and a daily schedule plan. 3.1.1. Experiment Data sheet (EDS)

The facility provider will be responsible to prepare and collect the EDS. The EDS should include a description of

the specific aims and hypotheses, and methodology details such as:

- Test descriptions and requirements
- Sampling schedule (time of day for each testing session, and time during each phase (adaptation, bed rest, and recovery)
- Experimental constraints (for example, fasting, requirements before an exercise session, body position during the experiment)
- Resources needed such as support for data/specimen collection (Biopsies, blood samples, MRI, DEXA) , access to biochemical lab level 1 (refrigerated centrifuge, biological safety cabinet, freezer at -20C and -80 C to store sample before shipment, benchtop space for minimal sample processing)

3.1.2. General Plan

A general plan will incorporate the study requirements of all experiments and be distributed to all PIs to identify possible interferences between their experiment and others. In addition to the general plan, a blood volume document including the amount of blood drawn from subjects for each test that requires blood samples will be prepared. The general plan and blood volumes document are usually submitted with the proposal to the Research Ethics Board (REB). The facility will coordinate any recommendations springing from the REB with the eight science teams.

3.1.3. Daily schedule

The facility provider creates a detailed daily schedule for each test subject for every day that the subject participates

in the study. The daily schedule documents all testing and activities (including showering and meals) that are planned for each individual subject. All study personnel should have access to copies of the general plan and the daily schedules. Changes to the documents can only be made by the Project Scientist and distributed to all PIs and study personnel. To be included in schedule:

- Time necessary for transportation to test sessions.
- Time allotted for tests.
- Time allotted for appointments for physiotherapy
- Time allotted for appointments for medical monitoring
- Time allotted for psychology assessment
- Time for other routine activities (meals, snacks, breaks)
- Time needed for personal hygiene (showering) of the test subjects
- Time necessary for setting up and calibrating specific experiment hardware

3.2. The facility provider is responsible for preparation of an integrated protocol that includes all infrastructure-provider protocols (for recruitment and standard measures collection, for example) and CIHR-selected team protocols and submission of this integrated protocol and associated informed consent form to their Research Ethics Board (REB). The REB must conform to the policies of the Tri-Council Guidelines for Ethical Research on Humans. The integrated protocol and informed consent form must be approved by the REB before starting recruitment of research participants (i.e. the research subjects). During the consent process,

subjects will be provided with a detailed description of the study, risks and benefits of participation, study schedule, participating researchers and all other items required by the site-specific REB.

4. Volunteer recruitment and selection

The final selection of the subjects should be a joint decision of the Psychologist, the Project Scientist, and the Head Medical Doctor.

4.1. Recruitment

The infrastructure provider is responsible for the recruitment of participants. Recruitment methods can include using test subject lists, announcements in print and electronic media in local and nationwide newspapers, radio and television, and the internet, to solicit as many interested volunteers as possible. The basic study information to include in the recruitment advertisements is: title and purpose of the study, eligibility criteria, location, study duration, and contacts for further information. Candidates will be contacted only after they have shown interest in participating in the study. All potential candidates will be provided with information relevant to the candidate's decision to proceed with the screening procedures including description of potential hazards, medical examination, medical issues etc. All material used in the recruitment campaign and to inform the candidates will have been approved by the ethics committee of the facility.

Provider shall include appropriate funds for a stipend, and will distribute these to participants according to standard institutional procedures, as approved by the REB.

4.2. Screening processes

Screening processes will begin with an initial screening via telephone, website questionnaires or other similar means of communication. The questionnaire should be prepared to select from the candidates the ones that fit the basic participants requirements. Candidates passing this part will be eligible to proceed with the medical and psychological screening. Psychological interview should be done after candidates have passed the medical screening. Based on information gained from these screening, a first decision can be made whether the interested person would be a potential candidate to bring for additional health assessment. Once qualified the candidate will sign a written and dated consent form including the signatures of the study personnel in order to document the process.

4.3. Inclusion and Exclusion criteria

4.3.1. Inclusion criteria:

- A minimum of 20 and a maximum of 24 non smoking participants in the age group of 55 to 65 years old, half male and half female.
- Female participants must be menopausal (See section 4.4.1)
- Height between 158 to 190 cm with a body mass index between 20 to 30 kg/m^2
- Physically and mentally healthy subjects that will have successfully passed the psychological and medical screening appropriate for the age group.
- Sedentary people and people that are addicted to exercise are excluded (see section 4..4.9).

Participants will include those that participate in at least 2.5 hours of exercise at a moderate to vigorous-intensity aerobic activity per week. Participants will be stratified according to physical capacities and distributed between exercise countermeasure and control groups (to match in each group the level of fitness of the participants).

- Willing to be assigned randomly either to the exercise or the control group
- Willing to sign informed consent

4.3.2. Exclusion criteria

The Project scientist, Head medical doctor, and Psychologist of the study can exclude a test subject from the study for any other reason than the ones below.

- Participants must be dementia-free, drug-free, with no history of heart attacks, no thrombosis risk, no severe allergies, no hypocalcaemia, no uric acidaemia, no orthostatic intolerance, no vestibular disorders, no considerable musculoskeletal issues, no chronic back pain, no head trauma, no seizures, no ulcers, no renal stones, no gastro-esophageal reflux disease or renal function disorder, no hiatus hernia, no migraines, no claustrophobia, and no mental illness.
- Applicants who cannot conform to the prescribed diet, or who object to frequent blood donation will be excluded.
- Participants must not have:
    - Electrocardiogram abnormalities,

- HIV
- Ferritin range outside 10 to 154 ng/ml (F); 20 to 245 ng/ml (M)
- A family history of thrombosis
- Bone mineral density (measured by DEXA) greater than 2.0 standard deviation ≤ t-score
- Medication requirements that may interfere with the interpretation of the results
- Recent substandard nutritional status
- Special dietary requests (e.g. vegetarian, vegan or some other diet)
- Metallic implants, osteosynthesis material
- Given blood in the past 3 months before the onset of the experiment
- Smoked within 6 months prior to the start of the study
- Abused drugs, medicine or alcohol within up to 30 days prior to the start of the study
- Participated in another study within 2 months before study onset

4.4. Screening test

4.4.1. Physical examination

The medical examination should include the following:

- Contact data of candidate

- Questionnaire including questions (additional questions can be added):
    - Headaches at regular intervals
    - Dizziness or fainting
    - Head accident or other
    - Allergies / hay fever
    - Asthma or bronchitis
    - Heart problems
    - High / low blood pressure
    - Stomach, intestine or liver problems
    - Blood or saccharides in urine
    - Epilepsy
    - Neurological problems
    - Suicide attempt
    - Alcohol or drug abuse (pharmaceutical or illegal)
    - Joint / spine disorders
    - Frequent skin rash
- History of medical treatments
- History of pharmaceutical usage
- For women: gynecological history and menopausal diagnostic (Menopause is defined as no menses for at least 1 year (or documented ovariectomy) and a serum FSH above 30 IU/L)
- Full physical exam including resting heart rate and blood pressure.

4.4.2. Blood Analysis

During the selection process the following parameter will be analysed:

Blood chemistry	Hematology
Fasting glucose	Red blood cells
Urea (BUN)	White blood cells (Eosino, Neutro, Lympho, Mono, Baso)
Uric acid	Hemoglobin
Creatinine	Hematocrit
Total bilirubin	Ferritin
Aspartate Aminotransferase (AST = GOT)	Mean corpuscular volume (MCV)
Alanine Aminotransferase (ALT = GPT)	Mean corpuscular hemoglobin concentration (MCHC)
Alkaline Phosphatase (AP)	Platelet count
Glutamyl transferase (GGT)	Red cell distribution width
Sodium	PT
Potassium	PTT
Chloride	Fibrinogen
Calcium	Antithrombin III
Phosphorous	Protein S
25-OHD (Vitamin D)	Protein C
TSH	Factor V Leiden
T4 (free)	Factor II
Total Protein	Lupus-like anticoagulant
Cholesterol	HIV screening
Triglyceride	Hepatitis B and C screening
High density Lipoprotein	FSH
Low Density Lipoprotein	
C reactive protein (CRP)	

The following vitamin-mineral status will be assessed:

- Retinol
- Retinyl-palmitate
- Beta-carotene / alpha-carotene
- Serum phylloquinone (Vitamin K)
- Alpha-tocopherol / gamma-totocopherol (Vitamin E)
- Erythrocyte glutathione reductase (Vitamin B1)
- Vitamin B6
- Folate
- Vitamin C

4.4.3. Urine analysis

The following parameters are analysed for the selection process:

- pH Blood
- Specific Gravity Bilirubin
- Appearance Urobilinogen
- Ketones Nitrite
- Protein Leukocyte
- Glucose
- Nicotine
- Other drugs (TCH, cocaine, opiates, amphetamines/methamphetamines, benzodiazepines, and barbiturates)

Candidates shall be screened for the use of drugs and nicotine during the selection procedure by using commercially available kits. Alcohol use shall be monitored by a breath analysis test.

In case they are selected for participation in the study, subjects shall be screened again for drugs, nicotine and alcohol use just before starting the baseline data collection period.

4.4.4. Stand test

This test will check the absence of orthostatic hypotension. The protocol for performing the test shall include: measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) in both supine position (after the subject has rested comfortably for at least 10 minutes) and standing position after 3 and 10 minutes.

4.4.5. Visual Test

- Visual acuity tests
- Fundoscopic examination
- Tonometry

4.4.6. Hearing test

- Physical examination
- Audiometry

4.4.7. DEXA scan

DEXA scans should be done once the candidates have successfully passed the screening tests listed under 4.4.1 to 4.4.6. The lumbar spine and hip should be included in the medical screening process.

The bone mineral density for each subject should not exceed the mean value of the specific t-score ≤ 2.0 standard deviation.

4.4.8. Psychological Screening

A psychologist (specialized in the age range of subjects) will assess if the candidates have the mental fitness necessary to complete all aspects of the respective bed rest study. The psychological screening should comprise a questionnaire on the biography and personal history of the subjects and a standardized test on personality and if

passed successfully be followed up by a face to face interview. The selection of specific tests used in the screening process will be done by the psychologist who will perform the psychological screening.

4.4.9. Fitness Level Assessment

It is important to evaluate the fitness level of the potential candidates since for this study sedentary people (less than 150 min/week of exercise) must be avoided, and superathletes (person that participate into sports competitions or training more than 1 hours per day 7 days a week or more than 420 min per week) must also be excluded.

To assess the level of fitness measurement of the VO^2peak needs to be performed using a bicycle step test with spirometry. For accurate collection of gases, subjects wear a nose clip and breathe through a respiratory valve. The graded exercise protocol provides an individualized approach to achieve subjects' maximum aerobic capacity using small increments in workload.

VO^2 max protocol: Subjects warm up cycling at a light workload (0 to 75 Watts) for 1 to 3 minutes. During testing, subjects maintain a pedalling cadence of 70 to 75 revolutions per minute (rpm). Workload begins at 50 Watts for 3 minutes and is then increased by 25 W every minute. Subjects who are small (women of 156 cm or less and men of 169 cm or less) or have low initial aerobic fitness (Women: 22 ml/min/kg < VO^2 max < 25 ml/min/kg, Men: 30 ml/min/kg < VO^2max < 34 ml/min/kg) may use an alternative light protocol starting at 45 W for 3 minutes followed by 15 W increases each

minute. Increasing workload in small increments with each minute of exercise allows for optimal evaluation of ventilatory threshold and VO^2 max. Using this protocol, maximal exercise is achieved in approximately 8 to 15 minutes.

Testing is completed when subjects:

1) are no longer able to maintain a pedalling rate of 70 rpm,
2) reach a plateau in oxygen uptake (VO^2) despite further increases in workload,
3) achieve a heart rate greater than 90% of age predicted maximum accompanied by a respiratory exchange ratio (RER) (VCO^2/ VO^2) greater than 1.10, or
4) indicate a desire to stop the test.

The range of VO^2 Max values according to the age of volunteers to be selected in this study (55 to 65 years) will be:

- Women: 22 ml/min/kg < VO^2 max < 29 ml/min/kg
- Men: 30 ml/min/kg < VO^2max < 41 ml/min/kg

Additionally, it is required to assess the real fitness level of the candidates by measuring the activity level using the total number of steps a candidate performs per day (for 2 normal weeks).

5. Volunteer rules and general conditions of bed rest

The area rules and activities listed below will need to be validated by the ethics committee and agreed upon with the potential candidates before they are selected to participate in the study. These rules are mandatory to guarantee identical conditions

between the different study campaigns required to complete the total number of subjects. Subjects should not leave the premises once the study has started unless they are no longer wanting to participate, or accompanied by infrastructure personnel to complete a test required by the study. (e.g. go for an MRI scan).

5.1. General conditions

5.2. Activities during the baseline and recovery phases of the study

5.2.1. General conditions

Subjects should not be allowed to lie on the couch or in their bed during the day in the ambulatory phases. All daily activities such as reading, eating, and watching TV are performed in a seated position with the feet on the ground rather than outstretched on the couch or chair. Additionally, subjects should be encouraged to spend most of their 'free time' in the group, rather than by themselves in their bedrooms. If subjects prefer to spend time for studying in their own rooms, the personnel will ensure that the subjects keep the specified body position. Subjects are allowed to have a little afternoon nap of 15 to 20 minutes in seated position in the baseline/recovery phase of the study.

5.2.2. . Exercise/activity intervention program

To standardize the activity between the different study campaigns, an individually tailored exercise/activity program will be developed and applied during the baseline and recovery phases. An exercise specialist or physiotherapist shall be available to discuss and tailor

exercise/activity interventions during the ambulatory phases.

The objective of the exercises/activities intervention is to keep the activity level as close as possible to the activity level before the study. The activity level should consider all experiments and other studies related activities and should be compared with the activity levels assessed during the fitness level assessment. This should be done for each day individually, as they usually are very different, especially in the recovery period after bed rest. This will result in an exercise/activity program that is tailored for each subject according to their usual activity habits.

Daily activity to be used in the program can be walking on a treadmill or using a step ergometer but these activities should consist of 'activity' rather than 'exercise'. Actual exercise period will also be planned to match the levels measured prior to the beginning of the study . Loss of body fluid and energy due to exercise/activity interventions has to be adjusted with dietary intake.

5.3. . Rules and conditions during the bed rest phase

The provider needs to apply the following rules required in order to guarantee identical study conditions for each cohort of subjects (subgroups of subjects).

Subjects 'live' in bed during that phase, meaning that all activities such as hygienic procedures, eating, reading, and "going to the bathroom" (use of bedpans and urine bottles)

take place in bed continuously for the duration of the study. Thus, the subjects privacy will need to be respected by permitting the participants to isolate themselves during hygienic procedures, dressing and undressing, and "going to the bathroom".

Subjects are allowed to move slowly without pushing from supine to ventral or lateral positions but are not allowed to get up, sit, or stand at any time. They need to maintain the head down tilt position at all times.

All activities that need to be performed outside of the subjects' room such as weighing procedures, showering, or transport to other experiment facilities, should be done with a gurney tilted 6° head down.

The subjects can choose appropriate clothing (to avoid sweating or chilling) in order to contribute to their well-being as long as it is respectful to others and complies with experiment requirements.

5.3.1. Start and end of the bed rest phase

The first Day of the 6°-HDT bed rest phase starts at approximately 9:00 am, after subjects get up at the predefined time, perform the normal morning procedures such as urine sampling, blood draw, weighing, showering, and breakfast in an upright position. The bed rest ends in the morning after subjects performed their daily morning procedures. The difference in "getting up" between subjects should not exceed 2 hours. This has to be considered when designing the study schedule.

5.3.2. Activities

Food will be served on a lower desk at the bedside. The subjects consume beverages (with a straw) and eat all meals in an HDT position leaning out of the bed to maintain the correct body position at all times.

Hygienic procedures (teeth brushing, shaving, washing with a washbowl in bed or showering) shall be performed in the HDT position.

When not performing experiments, subjects are allowed to read or write, watch TV, DVD, use the computer or listen to music, use a telephone (maximum of 2 hours per day) or talk to each other as long as the correct body position is maintained at all times.

Internet access for the test subjects shall be provided as long as this does not interfere with experiments. However, the subjects will have to sign a form wherein they confirm that they will agree to the regulations of the site in using the internet.

Internet access and phone calls are not allowed during the sleeping times from 11:00 pm (+/-1 hour) until 7:00 am (+/-1 hour) and during experimental sessions.

Other mood-enhancing activities should be permitted as long as they do not interfere with the experimental activities.

5.3.3. Day and night cycle

Subjects will maintain a strict day-night cycle. They will be awake for 16 hours, and asleep for 7 to 8 hours. They should awake around 7:00 am (+/- 1 hour) and go to

sleep around 11:00 pm (+/- 1 hour). Volunteers are allowed to have a little afternoon nap (15 to 20 minutes - in HDT position.

5.3.4. Visitors

The facility will have as a team member a psychologist whose responsibility is to coordinate development of a policy for visitors, in consultation with the rest of the facility team. For costing, the initial assumption is that visitors will only be allowed twice per week for a maximum of one hour each time during the study.

Visitors are not allowed to bring food or other items that could compromise the subject's participation in the study.

5.3.5. Media Events, Interviews, Tours

Media activities should be coordinated by CSA, CIHR and partners and the facility and service provider. The subjects shall be informed prior to the communication with media and their written consent shall be obtained. Subjects are not allowed to contact media on their own authority. To avoid interference with any experiment session, all media contact should be limited to just before the pre-bedrest period or at the end of the rehabilitation period.

6. Subject Care

6.1. Care givers

Provide a sufficient number of care givers (registered nurse or nurse assistant) at the bed rest facility on a 24-hour basis to support the subjects and supervise the adherence to the study

rules and schedule. The care givers shall be trained on the operating procedures to conduct measurements for medical monitoring (blood pressure, heart rate, temperature, body weight) as well as any other measurements according to the relevant standard operating procedures for the bed rest protocol data collection (urine, feces collection, etc.).

Care givers need to make sure that these measurements as well as any other experiment-related activities are on time with the help of a spread sheet from each experiment day for each test subject. The spreadsheet needs to be located in the subjects' rooms for information of the subjects and as well in the supervision area.

6.2. Daily Ward Round, Medical Monitoring and Psychological Support

Activities related to these care have been described under section 2.2 while describing specialized personnel provisions.

6.3. Hygienic procedures

Provide laundry services once a week for the participants clothing during the bed rest campaign.

6.3.1. Daily washing

Subjects will be provided with the opportunity to clean themselves daily. During the bed rest phases it is essential that subjects maintain the head down tilt position and are safe throughout the shower.

Participants can be moved to the shower area with the equivalent of a folding stretcher as long as they do not sit or stand during the head down tilt bed rest periods.

6.3.2. Collection of urine

Collection of urine samples will be done 8 times starting in the morning at 7:00 am for 24-hour urine according to the experiment requirements. The participants are asked to void into pre-weighed single-void containers at particular times. A protocol shall be developed in which subject code, date, and exact time of all voids, including tare weight of the urine bottles shall be noted. The urine bottle has to be stored in the dark, refrigerated immediately after each voiding, until it is transferred to the laboratory for analysis.

6.4. Environmental Conditions

The room temperature and humidity should be kept at comfortable levels (19°C to 22°C and 50% to 70% relative humidity) so that sweating or chilling is avoided as both would affect study results. Room temperature and humidity shall be documented throughout the whole study.

The exposure to daylight should be controlled to be similar for each study campaigns if not done during the same season, as variation in daylight exposure triggers physiological reactions that may influence the results of bed rest studies. Proper supplementation of vitamin D should be provided if exposure to sunlight is judged insufficient.

7. Nutrition Intake

7.1. Level recommended

Recommended values of nutrient intake levels are defined in Table 7-1, unless other nutritional constraints from specific

proposals are required. For the vitamins and elements, the recommended intakes should be achieved on an average per week.

The facility personnel will measure Resting Metabolic Rate of each participant prior to the study (WHO equation for Resting Metabolic Rate (RMR)). Ideally RMR should be measured via indirect calorimetry to get individual data × 1.1 (bed rest: HDT) or × 1.4 (ambulatory: BDC, Recovery) + 10% (of TEE) for thermogenesis. This RMR will be used to assure adequate intake of nutrients by each participant.

Table 7.1: Recommended levels of nutrient intake.

Nutrient	Adequate intake
Energy and Macronutrients	
Total fat (%TEE)	30% to 35 %
Saturated fatty acids (%TEE)	≤ 10
Monounsaturated fatty acids (%TEE)	≥ 10
Polyunsaturated fatty acids (%TEE)	≥ 7
Protein g/kgBW/d	1.2
Carbohydrates (%TEE)"	50 to 60
Total Fibre (g/d)	≥ 30

Electrolytes and Water	
Sodium (g/d)	3.5 to 4.5
Chloride (g/d)	6.0 to 7.5
Potassium (g/d)	3.5 to 5.0
Calcium (mg/d)	1000 to 1200
Water (ml/kgBW/d)	35 to 50
Vitamins	
Biotin (µg/d)	100
Pantothenic Acid (mg/d)	5
Folate (µg/d)	400
Niacin (mg/d)	20
Riboflavin (mg/d)	1.5
Thiamin (mg/d)	1.5
Vitamin B6 (mg/d)	2
Vitamin B12 (µg/d)	2
Vitamin K (µg/d)	80
Vitamin D (ug/d)	5
Vitamin A (µg/d)	1000

Vitamin C (mg/d)	100
Vitamin E (mg/d)	20
Elements	
Copper (µg/d)	1500-3000
Fluoride (mg/d)	1.5 – 4
Iodine (µg/d)	200
Iron (mg/d)	10
Magnesium (mg/d)	300
Phosphorus (mg/d)	700-1500
Zinc (mg/d)	Dec-15

Additional amino acids to be added automatically to control and exercise groups for better health maintenance. Leucine supplementation is necessary. The facility team will include a nutrition expert to define the level of supplementation.

To avoid seasonal differences all subjects have to be supplemented with 1000 IU vitamin D3 from final selection to the end of the study.

Besides matching the nutrient intake levels, some other dietary restrictions are mandatory. These are:

- Levels of methylxanthine derivatives (coffee, decaffeinated coffee, black and green tea, energy

drinks, chocolate, cola) will be set after the Investigators Workshop.

- No alcohol intake
- No flavor enhancer
- No sweat inducing spices (such as chili, hot curry)

7.2. Teaching Test Subjects on Constant Nutrient Intake

It is important to include in the informed consent forms a very detailed section on the importance of high compliance with appropriate dietary intake to prepare adequately the participants

7.3. Menu preparation

A balanced diet should be provided by the facility (3 times a day +snack) to the participants during the study. Meals are prepared in accordance with specific nutrients intake as specified in section 7.1. There is no requirement for the meal to be prepared at the research facility, a delivery service is also imaginable.

The weight of each food item/beverage to be offered to the test subject should be almost exactly the weight foreseen on the menu or the actual provided weight of the respective ingredients will be calculated in the nutrition software to be sure that at the end of the day that the nutrient requirements are met. In case of any leftovers on the test subjects' plate, the food items will be separated and weighed separately, it is understood that a very accurate measurement is impossible. However, based on the weighed food items, an estimation of the nutrient content in the leftovers is done by the nutrition

software and the missing part of the nutrients is provided to the participants with the next meal. This procedure results in standardized nutrient intake level for each day.

7.4. Documentation and evaluation of individual nutrient intake

The amount and time of meals will be documented on each individual hard copy of the daily menus and then entered and evaluated with a nutrition software regarding nutrient content.

8. Data management

8.1. Secure data storage

Provide a secure storage of the participants personal data and the standard measure data that follows appropriate national regulations for these type of data. Once the analysis are completed, the provider will transfer in a secure manner the standard measure data and the associated metadata to the CSA.

8.2. Data sharing of standard measures

Data sharing agreements are required to share the standard measures collected during the study. Before the study / data collection begins (primarily during the IWGW), the facility will establish data sharing agreements according to the required data of each individual PI team stated in their experimental protocol and approved by the ethics committee.

8.3. Delivery of standard measures dataset to CSA

The delivery of the dataset for standard measures to CSA in a non-proprietary, machine-readable, aligned to industry-based standards, and conventional format such as Text Files (TXT) or Character Separated Values (CSV) is required in order for CSA to make the data available on a public data repository for potential future use by the scientific community.

8.4. Data encoding

Every applicant participating in the selection process will be provided with a unique personal code allowing for anonymous data storage during selection and during the study. Random numbering scheme should be developed prior to the start of the study.

Use of the personal code is mandatory for all data transmissions between participating facilities / agencies,

8.5. Databases

Three strictly separated databases are required for data generated during the preparation and during the bed rest study.

8.5.1. Personal participants' data

This database will contain all personal information such as name, address date of birth and the relationship with the applicant's participants unique code. Data of rejected participants will also be kept in this database.

Access to this database needs to be restricted to personnel directly in charge of the organization of applicant selection and this database should not be connected to any other database containing the study data.

8.5.2. Medical Participants data

This database holds the subject code and all medical / psychological data acquired during the selection process and during the daily medical and psychological monitoring. It should include all medical / psychological interventions and off-nominal events occurring during the study. Data of applicants rejected during the selection process due to any reason will be kept separate from this database.

These data shall be accessible for all involved scientists as long as it is pertinent to their respective study.

8.5.3. Descriptive and study data

This database should include the main characteristics of the study including:

- Study name
- Scientific rationale and methods
- Study campaigns, date, time, and duration
- Facility name
- Participating scientists
- Participants codes

- Activities and events
- Study campaign
- Date and time
- Personnel involved
- Location
- Standardized data measurements
- Experiment measurements (if taken by the facility provider for a scientist)

9. Standardized measures and schedule

The following measures (and schedule) are the standard measures required for bed rest studies. They will be presented by discipline along with the methodology and when applicable suggested equipment manufacturers. Comparable equipment can be acquired from other manufacturers as long as they satisfy the testing needs. A summary of the required standard measures can be found in Table 9.1.

Table 9.1 schedule and required standard measures

Standard Measure	**Baseline Data Collection (BDC)**	**Head-Down Tilt (HDT)**	**Recovery period**
SENSORIMOTOR			
Postural Equilibrium Control (30 min)	BDC-1		R+1,
CARDIOVASCULAR			
Tilt test (50 min)	BDC-5		R+1, R+6, R+ 4 weeks, R+4 months
Maximal Aerobic Capacity (60 min)	BDC-2		R+2 R+ 4 weeks R+4 months

MUSCLE			
Vertical Jump (30 min)	BDC-5		R+2, R+ 4 weeks
Muscle strength (50 min)	BDC-5		R+2, R+4 R+ 4 weeks
BONE			
Bone mineral density (DEXA)	@ Screening		R+3, R+4 months
Bone markers in blood/serum (table 9.4-1)	BDC-4	HDT 10	R+1, R+4 weeks R+ 4 months
Bone markers in urine (table 9.4-1)	BDC-4	HDT3 HDT6 HDT10 HDT14	R+1 R+3 R+7, R+ 4 weeks R+4 months
NUTRITION/HEMATOLOGY			
Required blood measures (table 9.5-1)	BDC-4	HDT-10,	R+1 R+ 4 weeks R+4 months
Required urine measures (table 9.5-1)	BDC-4	HDT-10	R+1 R+ 4 weeks R+4 months
IMMUNOLOGY			
Immunology	BDC-4	HDT 10	R+1 R+ 4 weeks R+4 months
PSYCHOLOGY			
Positive and Negative Affect Scale (5min)	@ Screening, BDC-1	HDT6, HDT13,	R+1, R+ 4 weeks, R+4 months
General Health Questionnaire (10 min)	@ Screening, BDC-1	HDT7, HDT13,	R+1, R+6 R+ 4 weeks R+4 months

9.1. Sensorimotor

9.1.1. Postural equilibrium control

Postural stability will be evaluated using a computerized dynamic posturography system (Ex.: Equitest,

NeuroCom International, Clackamas, OR). For each Sensory Organisation Test (SOT) trial, data are recorded for 20 seconds or until there is a "fall". The standard SOT protocol with head erect is comprised of five conditions involving two support surface conditions (fixed and sway-referenced) and three visual conditions (eyes open, eyes closed and sway-referenced surround). Two modified SOT conditions are used to increase sensitivity and specificity by including dynamic head tilts with eyes closed on either fixed (2M) or sway-referenced support surface (5M). The dynamic tilts involve pitching the head at 0.33 Hz (± 20°) paced by an audible tone. See table below.

During SOT the subjects are instructed to maintain stable upright posture with arms folded across the chest. External auditory orientation cues are masked by white noise supplied through headphones. The more challenging SOT conditions involve disrupting proprioceptive and visual feedback by rotating the support surface and visual surround in proportion to body sway, referred to as sway-referencing. Sway is achieved by large forward and backward platform translations (400 ms, amplitude scaled to the subject height).

Table 9.1-1. Sensory Organisation Tests

Condition	**Support Surface**	**Vision**	**Head**
1	Fixed support	Eyes open, fixed surround	Erect
2	Fixed support	Eyes closed	Erect

3	Fixed support	Sway-referenced surround	Erect
4	Sway-referenced	Eyes open, fixed surround	Erect
5*	Sway-referenced	Eyes closed	Erect
2M	Fixed support	Eyes closed	Dynamic (0.33 Hz, ± 20°)
5M*	Sway-referenced	Eyes closed	Dynamic (0.33 Hz, ± 20°)

No exercise is permitted prior to testing on the day of the scheduled test. A normal or light meal may be consumed 3 hours before testing. However, no food should be eaten within 30 minutes of testing. Medications that effect sensorimotor performance should not be taken 24 hours prior to testing. No other medications should be administered within 12 hours of testing.

9.2. Cardiovascular Standard measures

9.2.1. Tilt test

The tilt test is used to assess orthostatic tolerance before and after bed rest. The tilt test is usually the first test on that day that brings the subject upright.

Subjects are instrumented while supine and this position is maintained while baseline data are collected for 5 minutes. The table is then tilted to 80° placing subjects in a head-up tilt position at a rate of approximately 7°/s. Subjects remain in this position for 15 minutes or until they exhibit symptoms of presyncope. Total time standing at 80° head-up tilt is recorded.

During the test, continuous measures are obtained for blood pressure, electrocardiography (ECG), and Doppler ultrasound of blood flow velocity at the suprasternal notch. Finger arterial blood pressure is sampled at 200Hz using a photoplethysmography device (Ex.: Finometer Pro, Finapres Medical Systems, Netherlands). This device uses a hydrostatic adjustment routine to provide an accurate estimation of blood pressure independent of sensor location with respect to the heart. Oscillometric brachial artery pressure is also measured using a cuff placed around the upper arm every minute.

Systolic and diastolic blood pressure are recorded and mean arterial pressure (MAP) is calculated as MAP = [(2 × diastolic)+systolic] / 3. ECG data are collected at 100Hz using a 5-lead system (Ex.: Escort II, Medical Data Electronics, Arleta, CA). Heart rate measures are derived from the ECG and ECG collection is synchronized with the suprasternal notch Doppler ultrasound signals for stroke volume and cardiac output calculations.

Two-dimensional echocardiography is used to obtain the aortic annulus diameter from the para-sternal long axis during supine rest prior to data collection. The aortic blood velocity time integral is measured for each beat during supine rest and during the period of 80° head-up tilt. These Doppler measurements are made at the suprasternal notch using a 1.9 MHz pulsed wave Doppler probe (Ex.: Biosound MyLab Gold, Indianapolis, IN). Images are stored digitally for subsequent analyses. To insure accuracy, images from at least three cardiac cycles during inspiration is independently analyzed by two experienced sonographers. Stroke volume (annulus diameter x velocity time integral) and cardiac output (stroke volume × heart rate) are calculated.

Maximal exercise and medications are not permitted for a 24-hour period prior to testing. Caffeine, nicotine, or alcohol shall not be consumed within 12 hours prior to testing. Heavy meals should be avoided 4 hours before

testing. However, a light snack of complex carbohydrates 2 hours before testing is permitted.

9.2.2. Maximal Aerobic Capacity (VO^2 max)

Maximum aerobic capacity is assessed using a graded exercise protocol on an electronically braked cycle ergometer , and a metabolic cart for gas exchange determination. For accurate collection of gases, subjects wear a nose clip and breathe through a respiratory valve. The graded exercise protocol provides an individualized approach to achieve subjects' maximum aerobic capacity using small increments in workload. Using this protocol, maximal exercise is achieved in approximately 8 to 15 minutes.

Subjects warm up cycling at a light workload (0 to 75 Watts) for 1 to 3 minutes. During testing, subjects maintain a pedalling cadence of 70 to 75 revolutions per minute (rpm). Workload begins at 50 Watts for 3 minutes and is then increased by 25 W every minute.

Subjects who are small (women of 156 cm or less and men of 169 cm or less) or have low initial aerobic fitness (Women: 22 ml/min/kg < VO^2 max < 25 ml/min/kg, Men: 30 ml/min/kg < VO^2max < 34 ml/min/kg) may use an alternative light protocol starting at 45 W for 3 minutes followed by 15 W increases each minute. Increasing workload in small increments with each minute of exercise allows for optimal evaluation of ventilatory threshold and VO^2max (Amann et al., 2004).

Testing is completed when subjects:

1) are no longer able to maintain a pedalling rate of 70 rpm,
2) reach a plateau in oxygen uptake (VO^2) despite further increases in workload,
3) achieve a heart rate greater than 90% of age predicted maximum accompanied by a respiratory exchange ratio (RER) (VCO^2/ VO^2) greater than 1.10, or
4) indicate a desire to stop the test.

9.3. Muscle standard measures

9.3.1. Muscle strength

These assessments can be completed using any isokinetic dynamometer (such as the Biodex Dynamometer, Biodex Medical Systems, Inc., Shirley, NY). Isometric maximum voluntary contractions will be completed for muscles of the knee, and ankle.

As a warm-up, subjects complete 5 minutes of light exercise (50 Watts) on a cycle ergometer. For each isokinetic test, muscles are warmed up prior to testing by completing 5 contractions with increasing force levels. A 2 to 3 minute rest period is provided between testing of each muscle group. For standardization purposes, knee and ankle testing are performed on the right lower extremity.

No large meals should be eaten within 1.5 hours of testing. However, a light complex carbohydrate meal is recommended before testing and should be eaten within 1.5 hours to 30 minutes prior to testing. Subjects should not undergo maximum exercise within 18 hours before testing. Regular exercise (submaximal exercise) should not occur within 8 hours prior to testing.

1) Knee:

To test isometric knee strength, the seated subject is positioned in the dynamometer with the knee flexed at 60°. The subject then performs three sets of maximum contractions alternating between flexion and extension muscle contractions. Each muscle contraction should last 5 to 7 seconds with 30 seconds of rest between flexion/extension contractions.

For isokinetic testing of the knee:

The subject is placed in a seated position. Knee range of motion on the dynamometer is set for 20° to 95°. The weight of the limb is assessed with the knee positioned at 30°. Isokinetic peak torque (Nm) is assessed at a speed of 60°/s. The subject performs 3 repetitions of continuous concentric extension and flexion motion at maximal effort.

Endurance testing of the knee is assessed at a speed of 180°/s. The subject performs 20 repetitions of

continuous flexion and extension motion at maximal effort. Total work in Nm is recorded.

Table 9.3-1 Isokinetic protocol for the knee.

Speed	**Warm-up**	**Test Repetitions**	**Rest**
Knee (Concentric)			
0°/s (Isometric Mode)	None	Practice Test (2 Sub, 1 Near Max)	60 seconds
0°/s (Isometric Mode)	None	3 Max	60 seconds
60°/s	2 Submax	3 Max	60 seconds
Knee (Endurance)			
180°/s	2 Submax	20 Max	Set-up for next test

2) Ankle

For testing isometric ankle strength, the subject is placed in the prone position with the ankle positioned at 90°. Three sets of maximum contractions are performed alternating between plantar flexion and dorsiflexion muscle contractions. Muscle contractions should last 5 to 7 seconds with 30 seconds of rest between plantar and dorsiflexion contractions.

Isokinetic testing of the ankle:

The subject is placed in the prone position. The range of motion of the dynamometer is adjusted to 5° less than the subject's maximum position of dorsiflexion

and 5° less than the subject's maximum position of plantar flexion. Weight of the limb is assessed with the ankle positioned at 15°. Isokinetic peak torque (Nm) is assessed at a speed of 30°/s. Three repetitions of continuous concentric dorso- and plantar flexion contractions are performed at maximal effort.

Table 9.3-2 The isokinetic protocol for the ankle.

Speed	**Warm-up**	**Test Repetitions**	**Rest**
Ankle (Concentric)			
30°/s	2 Submax	3 Max	60 seconds
Ankle (Eccentric)			
30°/s (Passive Mode)	None	Practice Test (3 Submax)	60 seconds
30°/s (Passive Mode)	None	3 Max	60 seconds
30°/s (Passive Mode)	None	Practice Test (3 Submax)	60 seconds
30°/s (Passive Mode)	None	3 Max	During set-up for next test

9.3.2. Vertical jump

A ground-reaction platform (Kistler Instrument Corp., Amherst, NY) is used for data collection. Data are collected with a custom LabVIEW software program (National Instruments Corp., Austin TX) and sampled at 1000 Hz using a National Instruments data acquisition system (National Instruments Corp., Austin TX). Prior to the jumping task the subject performs a warm-up session and is instructed to perform 3 warm-up squats. The subject is then instructed to perform 2 to 3 practice countermovement jumps at 50% of maximum effort to ensure that proper technique is understood.

Countermovement jump begins by anchoring the hands on the waist while standing with erect posture. The movement is performed by the subject quickly dropping into a squat then reversing the direction by pressing into the platform with maximum force with the aim to jump as high as possible. This motion does not allow pause during the movement and requires the hands to be anchored to the hips at all times to remove moments of inertia contributed by the arms swinging. Once the subject warms up and proper technique is demonstrated, the operator allows the subject to attempt 3 maximum effort jumps. Prior to jumping, body mass is measured during quiet stance assuming the acceleration due to Earth's gravity to be 9.8 m/s^2, then the subject is instructed to jump and encouraged to elevate the head as high as possible. The subject rests 60 to 90 seconds in

between each jump or longer if the subject desires more time between tests. The sum of the vertical ground reaction forces collected from the force plate are divided by body mass to determine the subject's acceleration profile. To calculate the subject's jump height a double integration of the acceleration profile is computed. Instantaneous power is calculated as the product of acceleration and velocity. Variables of peak acceleration in m/s2 (Apeak), peak velocity in m/s (Vpeak), jump height in cm (Hpeak), and peak power in kW (Ppeak) are then assessed as the maximum of these respective curves.

No large meals should be eaten within 1.5 hours of testing. However, a light complex carbohydrate meal is recommended before testing and should be eaten within 1.5 hours to 30 minutes prior to testing. Subjects should not undergo maximum exercise within 18 hours before testing.

9.4. Bone standard measures

9.4.1. Bone Mineral Density

Measures of 2-dimensional bone mineral density (BMD) is obtained by dual-energy X-ray absorptiometry (DEXA).

Calibration shall be completed to ensure accuracy of the data using a phantom scan. Subjects shall not have any radioisotopes or radiopaque contrast agents within one week prior to testing. It is highly desirable to use the same operator to collect all scans for a study to further ensure data accuracy.

Prior to testing, the operator shall ensure that the test subject is free of external metal objects such as metal buttons, jewelry, zippers and belts. Subjects shall not have any radioisotopes or radiopaque contrast agents within one week prior to testing.

For repeated measures such as pre- to post-bed rest, scans should be completed using the same densitometer to allow for accurate comparisons. Variables of BMD and bone mineral content (BMC) are derived from the scans and percent change from pre- to post bed rest is calculated at each site.

The subject is positioned by the operator for each scan. The subject is instructed to lie still during the scan, but may move between scans. Scans are obtained from the whole body, lumbar spine and hip. Scans will be replicated if the participants has moved during the scans in order to get good data.

9.4.2. Bone markers

The following bone markers are measured in the blood or urine to assess the impact of the bed rest on bone degradation and formation. Any measures overlapping with nutrition and hematology can be shared.

Table 9.4-1 Bone standard measures and suggested methodology

Blood/Serum Chemistry		
Calcium Homeostasis	Whole-blood ionized calcium (and pH) is determined electrochemically using a portable analyzer (Smith et al.,	Total Calcium, Whole-blood Ionized Calcium

	1997; Smith et al., 2004). Serum total calcium are determined using atomic absorption spectrophotometry (Smith et al., 2005a; Smith et al., 2008)	
Gonadal Hormones	Liquid chromatography with tandem mass spectrometry, ELISA, or RIA procedures are used for these analyses.	Testosterone, Estradiol
Calcitropic Hormones	PTH are assessed in serum. It is assayed for the intact peptide by radioimmunoassay (RIA). Serum 1, 25 dihydroxyvitamin D is measured by RIA after extraction of samples with acetonitrile and purification on C18OH cartridges, and serum 25 hydroxyvitamin D determined by RIA after acetonitrile extraction, as reported previously (Smith et al., 2005a; Smith et al., 2005b; Zwart et al., 2011b; Zwart et al., 2009).	25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, intact Parathyroid Hormone (PTH)
Endocrine Regulators	Liquid chromatography with tandem mass spectrometry, ELISA, or RIA procedures are used for these analyses.	Thyroxine (Free T4), Thyroid Stimulating Hormone (hTSH III), Cortisol
Bone Turnover Markers (Bone Formation)	Bone specific alkaline phosphatase [BSAP], and osteocalcin are assessed in serum. Serum osteocalcinis also determined by RIA.Serum BSAP activity and N-terminal propeptide of type I procollagen (P1NP) is determined by enzyme linked immunoassay (ELISA) (Spector et al., 2009; Smith et al., 2005a).	Osteocalcin, Bone Specific Alkaline Phosphatase (BSAP), N-terminal propeptide of type I procollagen (P1NP)
Urinary Measures		
Bone Turnover Markers (Bone Resorption)	Urine samples are analyzed for collagen cross links, including N-telopeptide, C-telopeptide, and deoxypyridinoline, using	N-telopeptide, C-telopeptide, Deoxypyridinoline

	commercially available kits (Osteomark® ELISA kit, Ostex International, Inc., Seattle, WA; Urinary CrossLaps ELISA, Nordic Bioscience Diagnostics, Herlev, Denmark; Pyrilinks-D, Metra Biosystems, Palo Alto, CA).	
Minerals	Urinary calcium is determined using atomic absorption spectrophotometry (Smith et al., 2005a; Smith et al., 2008).	Calcium

9.5. Nutrition and hematology standard measures

The of blood drawn from bed rest participants during any study is of concern and is typically regulated by an ethics committee. Note that there is savings of blood based on blood tube sizes when all analyses are combined. Serum chemistry and hematology analyses are conducted using standard clinical techniques.

Total blood volume for required measures with vitamins: 2 × 6 ml SST; 1 × 6 ml blue mineral tube; 2 × 4 ml EDTA; 1 × 1.2 ml LiHep Monovette; 1 × 1.8 ml Na, Citrate tube = 29.0 ml

Total blood volume for required measures without vitamins: 2 × 6 ml SST; 1 × 6 ml blue mineral tube; 1 × 4 ml EDTA; 1 × 1.2 ml LiHep Monovette; 1 × 1.8 ml Na Citrate tube = 25.0 ml

Table 9.5-1 Required Nutrition and hematology standard measures

Required Blood Measures	
Serum Chemistry	Carbon Dioxide, Blood Urea Nitrogen, Phosphorous, Magnesium, Bilirubin, Glutamyltransferase, Alkaline Phosphatase, Lactate Dehydrogenase, Creatine Kinase, Uric Acid, C Reactive Protein, Sodium, Potassium, Chloride, Creatinine, Aspartate Transaminase (AST), Alanine Transaminase (ALT), Cholesterol, Triglyceride, Glucose, Calcium
Whole Blood Analysis (CBC/differential/Platelets)	White Blood Count and differential, Red Blood Count, Hemoglobin, Mean Corpuscular Volume (MCV), Platelet Count, Reticulocyte Count Calculated values: Relative (Red Cell) Distributive Width (RDW), Hematocrit, Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC)
Coagulation Test	Fibrinogen
Finger-Stick Tests (whole blood)	iCa, pH, PCO_2, PO_2(optional tests, if included in analysis: Na, K, glucose, hematocrit) Calculated Values: TCO_2, HCO_3, BE, sO_2, Hgb
Hematologic and Iron Status Indicators	Transferrin Receptors, Transferrin, Ferritin, Ferritin Iron, RBC Folate, Iron, Total Iron Binding Capacity (TIBC) Calculated values: Ferritin Iron % Saturation, Transferrin Saturation
Ionized Calcium Profile†	Whole-blood Ionized Calcium, pH-Whole-blood Calculated value: Ionized Calcium at pH 7.40
Hormones	Thyroxine (Free T4), Thyroid Stimulating Hormone (hTSH III), Testosterone, Estradiol, Dehydroepiandrosterone (DHEA), Dehydroepiandrosterone Sulfate (DHEA-S), Cortisol, Cytokines: TNF alpha, IL-6
Mineral Status	Zinc, Selenium, Iodine, Copper, Ceruloplasmin
Protein Status	Retinol Binding Protein, Transthyretin, Total Protein, Albumin
Nutritional Assessment (if blood volume allows)	

Water Soluble Vitamin Status‡	Erythrocyte Transketolase Stimulation, Erythrocyte Glutathione Reductase Activity, Erythrocyte nicotinamide adenosine dinucleotide and nicotinamide adenosine dinucleotide phosphate (NAD/NADP), Erythrocyte Transaminase Activity, Red Cell Folate, Folate, Homocysteine, Vitamin C, Pyridoxal 5-phosphate (PLP)
Fat Soluble Vitamin Status‡	Retinol, Retinyl palmitate, β-carotene, α –carotene, Serum Phylloquinone, α-tocopherol, γtocopherol, Tocopherol : lipid ratio, vitamin D binding protein and plasma heme, 25-hydroxyvitamin D
Required Urinary Measures	
Urine analysis	Specific Gravity, pH, Color, Appearance, Protein, Glucose, Bilirubin, Urobilinogen, Ketone, Nitrite, Blood, Leukocyte Esterase, Total volume, pH, Creatinine
Minerals	Calcium, Phosphorus, Magnesium, Copper, Selenium, Zinc, Iodine
Protein Status	3-methyl histidine, Nitrogen
Renal Stone Risk	Sodium, Potassium, Uric Acid, Citrate, Oxalate, Sulfate, Suspersaturation of Brushite, Struvite and Calcium Oxalate
Nutritional Assessment (if blood volume allows for full-blood testing complement, then the following urine tests shall be included as well)‡	
Water Soluble Vitamins‡	N-methyl nicotinamide, 2-pyridone, 4-pyridoxic acid

9.6. Immunology standard measures

The following required immunological measures are a minimal set of measures that are limited to assays readily available, and do not require access to specialized equipment or reagents.

- Basic leukocyte subsets (T cells, B cells, NK cells, CD4+/CD8+ T cells, memory/naïve T cells, a constitutively activated T cells)

- Stress hormone levels from saliva and or plasma measures (cortisol and catecholamines)
- Plasma immunoglobulin G (IgG) levels
- Viral antibody levels
- Alpha 1 globulin, alpha 2 globulin, beta globulin, gamma globulin

9.7. Psychology standard measures

9.7.1. Positive and Negative Affect Scale (PANAS)

The PANAS (Watson, 1988; Watson et al., 1988) is a 20-item self-evaluation questionnaire that measures affect, or indicators of emotional states. Emotional states are important to study during situations of isolation and confinement like bed rest experiments because they reflect the general state of the person at one step of the experiment.

Participants are provided either a paper or electronic version to fill. They are asked to indicate to what extent they have felt each positive affect item and each negative affect item in the past week. Items are rated on a scale of 1 to 5 where 1 indicates not at all, and 5 indicates extremely. Scores for each affect are totalled and recorded.

Each assessment (see schedule table 9.1) should be completed at about the same time of day; within ±2 hours of the initial baseline assessment. The PANAS should not be administered immediately after awakening, or following maximal exercise.

9.7.2. General Health Questionnaire (GHQ)

GHQ is a self-evaluation questionnaire measuring the current mental health of the individual. GHQ was first developed as a 60-item tool to screen for non-specific psychiatric morbidity (Goldberg, 1972; Goldberg and Williams, 1988).

For bed rest studies, it is used to measure minor mental health problems and can be useful to prevent more important mental disorders during isolation and confinement situations.

GHQ-28 provides an overall total score and scores on 4 subscales of somatic symptoms, anxiety, insomnia, social dysfunction and severe depression. The advantage of having these 4 subscales makes the GHQ-28 a useful measure for bed rest studies.

The GHQ is available in many languages and can be purchased at:http://www.mapitrust.org/services/questionnairelicensing/cataloguequestionnaires/52-GHQ.

Each assessment (see schedule table 9.1) should be completed about the same time of day; within ±2 hours of the initial baseline assessment. GHQ should not be administered immediately after awakening, or following maximal exercise.

10. Countermeasure procedures

During the inactivity study, half of the research participants will participate in a countermeasure procedure while the others will serve as controls. The standardized countermeasure procedure that will be used for all of the funded projects is a total of 60 minutes of physical exercise per day.

This will involve 3 sessions per day that will include high-intensity interval training (HIIT), lower-intensity aerobic exercise (Aer) and strength exercises (Str). All exercises will be completed in a head-down tilt position or horizontal position. The work rates will be established during the pre-bed rest test of maximal aerobic power and set for each person based on the heart rate reserve (HRR = difference between observed maximum heart rate and resting heart rate).

The series of exercise to be used are described in table 10.1.

Table 10.1 Descriptions of individual **exercise types**

Exercise Type	**Description**
HIIT* – 32'	High Intensity Interval Training (32 min) cycle ergometer: 5 min warmup (40% HRR), 11 intervals (30s @ 80 to 90% HRR, 1,5 min relaxed cycling), 5 min cooldown
Cont Aer – 30'	Cycle ergometer (30 min): 5 min warmup (40 to 50% HRR), 20 min steady state(60-70% HRR), 5 min cooldown (40-50% HRR)
Cont Aer – 15'	cycle ergometer (15 min): 3 min warm-up (40% HRR), 9 min steady state (60 to 70% HRR), 3 min warm-up (40% HRR)

Progressive Aerobic– 15’	Cycle ergometer : 3 min stages at 30%, 40%, 50%, 60%, 40% HRR
Str, Lower – 25’	Strength exercise using resistance bands, cables, body weight : 3 sets (1 warm up) of 10-12 repetitions Exercises: Hip raise, leg press, ankle pump, leg curls
Str, Upper - 25’	Strength exercise using resistance bands, cables, body weight : 3 sets (1 warm up) of 10-12 repetitions Exercises: external and internal shoulder rotation, chest fly, lateral pull-down, dead bug.

Notes:*HIIT: the High Intensity Interval Training protocol is based on published data from Cassidy et al. (2016).**HRR=Heart Rate Reserve=Heart rate max – heart rate min.

The countermeasure exercises will be provided following the schedule included in Table 10.3. The intensity of the exercise countermeasures will be individually adjusted based on individual fitness assessment (see section 4.4.9). The exercise program supervisor can adjust work rate depending on the perceived exertion so the individual maintains an exercise intensity of “very hard” using the Borg scale from https://www.hsph.harvard.edu/nutritionsource/borg-scale/ (Table 10.2).

Table 10.2 The Borg Scale

How you might describe your exertion	Borg rating of your exertion	Examples (for most adults <65 years old)
None	6	Reading a book, watching television
Very, very light	7 to 8	Tying shoes
Very light	9 to 10	Chores like folding clothes that seem to take little effort
Fairly light	11 to 12	Walking through the grocery store or other activities that require some effort but not enough to speed up your breathing.
Somewhat hard	13 to 14	Brisk walking or other activities that require moderate effort and speed your heart rate and breathing but don't make you out of breath.

Hard	15 to 16	Bicycling, swimming, or other activities that take vigorous effort and get the heart pounding and make breathing very fast
Very hard	17 to 18	The highest level of activity you can sustain
Very, very hard	19 to 20	A finishing kick in a race or other burst of activity that you can't maintain for long.

Table 10.3 Exercise schedule for the head down tilt bed rest condition.

Week °1							
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Session 1	Str, Upper 25	Cont Aer – 30"	Progressive Aerobic– 15'	Cont Aer – 30"	HIIT – 32'	Cont Aer – 30"	HIIT – 32'
Session 2	Progressive Aerobic– 15'	Str, Lower – 25'	Cont Aer – 15"	Str, Upper 25	Progressive Aerobic– 15'	Str, Upper 25	Cont Aer – 15"
Session 3	HIIT – 32'	Progressive Aerobic– 15'	HIIT – 32'	Progressive Aerobic– 15'	Str, Lower – 25'	Progressive Aerobic– 15'	Progressive Aerobic– 15'

Week 2							
	Day 8	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**
Session 1	Cont Aer – 15''	HIIT – 32'	Cont Aer – 30''	HIIT – 32'	Cont Aer – 30''	HIIT – 32'	Cont Aer – 15''
Session 2	Str, Upper 25	Cont Aer – 15'''	Str, Lower – 25'	Cont Aer – 15''	Str, Lower – 25'	Progressive Aerobic– 15''	Cont Aer – 30''
Session 3	Progressive Aerobic– 15'	Progressive Aerobic– 15''	Progressive Aerobic– 15'	Progressive Aerobic– 15''	Progressive Aerobic– 15''	Str, Upper 25	Progressive Aerobic– 15'

11. Summary reporting

11.1. Summary report for the cohort

The objective is to provide a summary report of one or two pages after the end of the first recovery period for each cohort. This summary will include the number of participant, the number that abandoned if necessary, a preliminary assessment of the quality of the standardized data collected, lessons learned and solutions required before starting the next cohort as well as an assessment

of how many more participants will be required to complete the study.

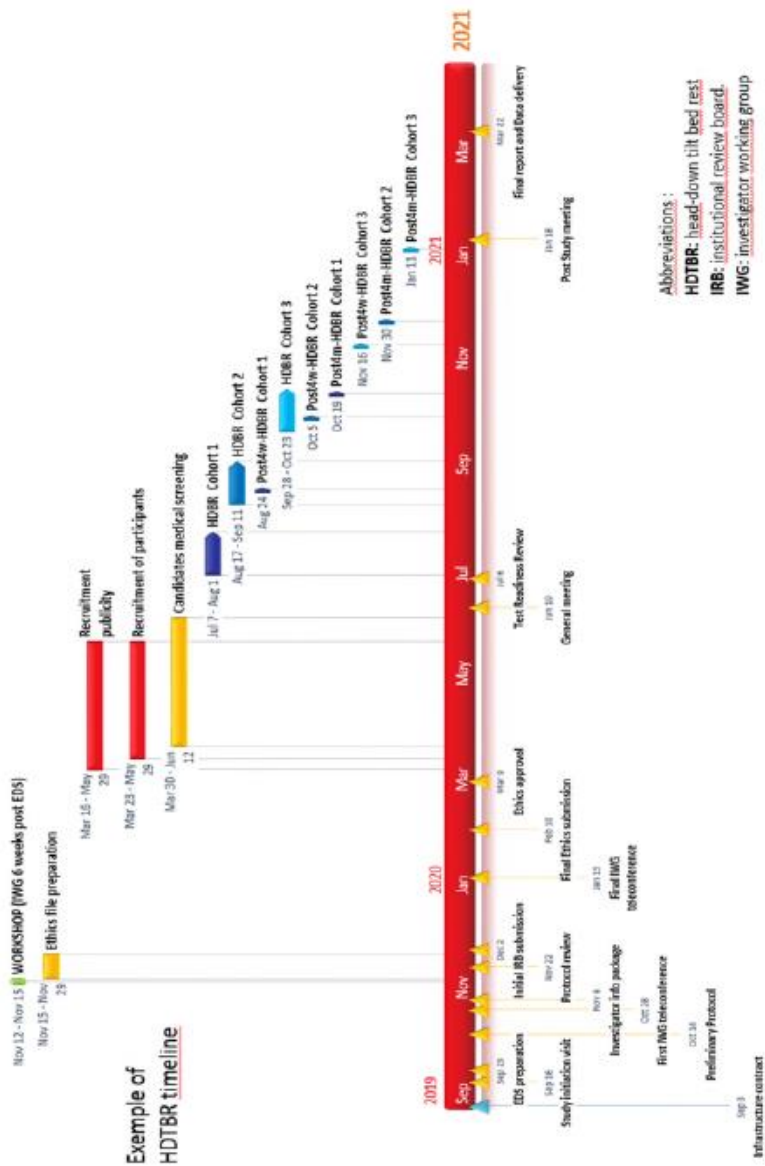
11.2. Mid-term assessment summary report

The objective is to provide a summary report including a preliminary assessment on the effectiveness of the exercise countermeasure using the standard measure data collected and analyzed on the first 12 to 16 subjects. This summary report will include data tables, figures, and a discussion of the preliminary results.

12. Final report

Provide a final report on the effectiveness of the exercise countermeasure using the standard measure data collected and analyzed and integrating the preliminary reports received from the science teams. The final report should include an executive summary, introduction, materials and methods results, discussion, and recommendations. Delivery of the final report is expected 4 weeks after receiving preliminary reports from the CIHR-selected scientific teams.

APPENDIX 2


Exemple of
HDTBR timeline
2019
2020
2021
Sep
Nov
Jan
Mar
May
Jul
Sep
Nov
Jan
Mar
Nov 12 - Nov 15 WORKSHOP (IWG 6 weeks post EOS)
Nov 15 - Nov 25 Ethics file preparation
Mar 15 - May 29 Recruitment publicity
Mar 23 - May 29 Recruitment of participants
Mar 30 - Jun 12 Candidates medical screening
Jul 7 - Aug 1 HDBR Cohort 1
Aug 17 - Sep 11 HDBR Cohort 2
Aug 24 Post4w-HDBR Cohort 1
Sep 28 - Oct 23 HDBR Cohort 3
Oct 5 Post4w-HDBR Cohort 2
Oct 19 Post4m-HDBR Cohort 1
Nov 16 Post4w-HDBR Cohort 3
Nov 30 Post4m-HDBR Cohort 2
Jan 11 Post4m-HDBR Cohort 3
Sep 3 Infrastructure contract
Sep 16 Study initiation visit
Sep 25 EOS preparation
Oct 16 Preliminary Protocol
Oct 28 First IWG teleconference
Nov 4 Investigator info. package
Nov 22 Protocol review
Dec 2 Initial IRB submission
Jan 15 Final IWG teleconference
Feb 10 Final Ethics submission
Mar 9 Ethics approval
Jun 8 Test Readiness Review
Jun 10 General meeting
Jun 18 Post Study meeting
Mar 22 Final report and Data delivery
Abbreviations :
HDTBR: head-down tilt bed rest
IRB: institutional review board.
IWG: investigator working group