Protocol for a physical exercise and nutritional program to improve the determinants of health in a population working under intermittent hypobaric hypoxia conditions

(Last review by the scientific ethics committee of the Catholic University of Maule, 26-1-2026)

This is a randomized, longitudinal, single-blind clinical trial with two parallel groups (Figure 1). Design: Workers from the ALMA Observatory will be randomly assigned to two intervention groups (G1 and G2). Both interventions will last 14 days, with the physical exercise intervention taking place three times a week during the seven-day work week, while the nutritional intervention will be conducted during all seven days of the work week. All work will be carried out at the ALMA Observatory facilities, in the Operation Support Facility (OSF) at an altitude of 2,950 meters above sea level.

Intervention Groups

Physical Activity and Nutrition Intervention:

(Phase 1)

During this first phase, general nutritional assessments (surveys), physical fitness assessments, dietary intake surveys, fecal DNA collection, and the installation of a heart rate monitor, accelerometer, and continuous glucose analyzer patch will be carried out. In addition, surveys will be administered to determine the quality of life of the study participants (45,46).

For the physical exercise intervention in the group, the first step will be to measure the blood pressure and pulse of each individual. Upon arrival at the gym, each participant will be asked to sit for 5 minutes to reach a state of rest. After this time, their blood pressure will be measured using an OMROM HEM-7142 sphygmomanometer, following the manufacturer's protocol. The data obtained will be recorded on each participant's data sheet. (This initial blood pressure measurement will be taken during the 3 exercise sessions, during the work shift).

1. Similarly, once the blood pressure measurement protocol is completed, two breathing techniques will be performed to determine the respiratory variability of each subject: Technique 1: Inhale for four seconds; hold the inhaled volume for four seconds, without Valsalva maneuver; exhale for four seconds. Repeat for 10 cycles. (Repeat blood pressure measurement).

2. Technique 2: Inhale for four seconds; hold the inhaled volume for seven seconds, without Valsalva maneuver; exhale for eight seconds. Repeat for 10 cycles. (Repeat blood pressure measurement). (This assessment of breathing technique will be considered as secondary data).

Following the blood pressure measurement, the warm-up process will begin on the indoor multi-sports court with a 15-minute continuous jog at 60% of the maximum heart rate estimated using the Karvonen formula.

During the first exercise session, the one repetition maximum (1RM) will be calculated for four muscle groups: pectorals, quadriceps, biceps, and gastrocnemius. This will be done by increasing the weight by 10% with each repetition, resting for 3 minutes between repetitions, until the subject can only perform one repetition with the maximum weight they can lift. Once these values are determined, the following exercise sessions (days 3 and 5) will be performed with 50% of the maximum weight, following the physical exercise intervention program described in Table 1, which was designed by a co-investigator, a physical education teacher on the research team.

Exercise schedule

Table 1: Physical exercise intervention program.

Session Objetive	Medium (Equipment)	Method	Volume	Intesity
Warm-up	Multi-court jogging	Continuous or interval	15 minutes	60% of maximum heart rate
Development of muscle strength	Weights and bars	Eccentric contractions.	3 sets of 10 repetitions	Omni-Res Perception Scale
Back to calm	Mat / stretching	Series and repetitions	3 repetitions: 10 seconds	Omni-Res Perception Scale

For the nutritional intervention, this will be carried out during the seven days of the work shift, for which each individual in Group 1 will be given (through the food concessionaire of the observatory, in order to mask the intervention), a quantified supplementation selected by the research team and which will consist of ingest a supplement capsule rich in lactobacillus,

In addition, the daily intake of subjects in this group will be quantified, and nutritional counseling will be provided to each participant in G1 on days 1, 3, 5, and the last day of their shift, reinforcing healthy eating habits during their days off.

If any individual presents with an anomaly that could be associated with the intake of nutritional supplements, they will be immediately transferred to the clinical care center at the ALMA Observatory for evaluation by the on-duty healthcare staff. These staff

members will decide whether to keep the individual under observation, provide medical treatment, or transfer them to a more specialized care center. The emergence of any complication in a study subject will be sufficient grounds for removing that individual from the study data. Furthermore, this potential anomaly will be promptly reported to the UCM Clinical Research Center (CEC).

Physical Activity Intervention (G2)

This group will undergo the same exercise program proposed for G1, but in a different gym, using the same 1RM calculation program and muscle training plan. This group, G2, does not have nutritional support.

(Phase 2)

A second physical fitness assessment, nutritional and body composition evaluation, fecal DNA collection, and quality of life surveys will be performed on each subject in both groups.

(Phase 3)

Physical fitness and nutritional assessments, fecal DNA collection, and quality of life surveys will be performed on each subject in both groups once they return from their 7-day rest period.

Sample description:

Sample Selection

The population of ALMA observatory employees consists mostly of Chilean men, aged 30 to 55, with higher education in some branch of engineering and/or electronics. To be employed at this astronomical observatory, they must meet the contractual requirement of being "in good health for the work to be performed" and possessing qualifications in areas necessary for the research center. Furthermore, most of the observatory's employees live in low-lying areas distributed throughout the different regions of Chile.

The sample will be selected by convenience sampling. To this end, a call for participation will be made during the different shifts of ALMA observatory staff (120 subjects per shift) through an advertising campaign for this research on closed-circuit television at the observatory during February. An invitation to the first informational meeting will be sent via mass email from the Observatory's Safety Department one week prior to sample collection (which is expected to begin in March if the UCM's CEC approves this project). Subjects may register freely during February and March 2026 by contacting the principal investigator or the Observatory's Safety staff via email; the email address will appear in the closed-circuit television advertising and mass emails described above. The signing and free acceptance of the informed consent of each participant will be done in person in the breastfeeding room, (a place that is not currently in use and that has already been proposed by the management of the observatory to install the provisional laboratory for the collection of samples for this research).

Those who agree to participate in the study will be randomly assigned to one of two intervention groups:

- G1: Physical activity and nutrition intervention
- G2: Physical activity intervention

The sample size calculation will have a 95% confidence level, with a 5% margin of error, using a variance of 0.632 (a value found in a pilot study of this same population to determine differences in phase angle). An additional 20% was added to account for possible

$$= \frac{2 \cdot s^2 (z_{1-\alpha/2} + z_{1-\beta})^2}{d^2}$$

$$n' \geq \frac{n}{1 - L}$$

sample losses during the study.

Sample size calculation (n): 22, (11 subjects per intervention group).

Inclusion and Exclusion Criteria:

(These criteria will be implemented upon contact with potential participants, during the personal interview, in the lactation room).

The following inclusion criteria will be used:

- Be a Chilean employee, contracted by the ALMA Observatory, with more than one year of seniority.
- Remain in the AOS (5,000 m above sea level) or OSF (2,950 m above sea level) sectors during the workday.
- Not suffer from non-communicable diseases (Diabetes mellitus (Type 1 or 2), Hypertension), diagnosed by a primary care physician or by the workers' compensation insurance provider that performed the pre-employment medical examinations. (A brief health interview will be conducted with each interviewee during the initial contact with the study participants.)
- Participants must be adult men or women.

Exclusion Criteria:

- Individuals with pacemakers, metal implants, or who are pregnant.
- Individuals who are only temporarily residing in the observatory areas located in the Antofagasta region.
- Individuals who live in areas above 1,600 meters above sea level.

Protocol for approaching, recruiting, and selecting participants:

Approach: Following the acceptance of this research project by the CEC-UCM, an online meeting will be held with the Director of Health of the astronomical center to communicate the start of the work, the transfer of our scientific team and the start of sample collection at the observatory itself.

Recruitment: One week before the start of sample collection and participant recruitment, an informational meeting will be held in the observatory's auditorium, open to the entire ALMA community. This meeting will discuss the results of the previous pilot project and how this type of study can help improve participants' quality of life. The Observatory's administration will also discuss the importance of conducting this type of work and intervention in their community, offering the necessary support for this research.

Finally, following the informational campaign via closed-circuit television and mass email, all potential new study participants will be registered at this same meeting (their name, phone number, and email address will be recorded). These individuals will then be contacted for a personal meeting in the designated area (the breastfeeding office). During this meeting, key questions will be asked to determine eligibility based on the inclusion criteria. If these criteria are met and the interviewee agrees to sign the informed consent form on-site, they will be included as a study subject and added to the final analysis list.

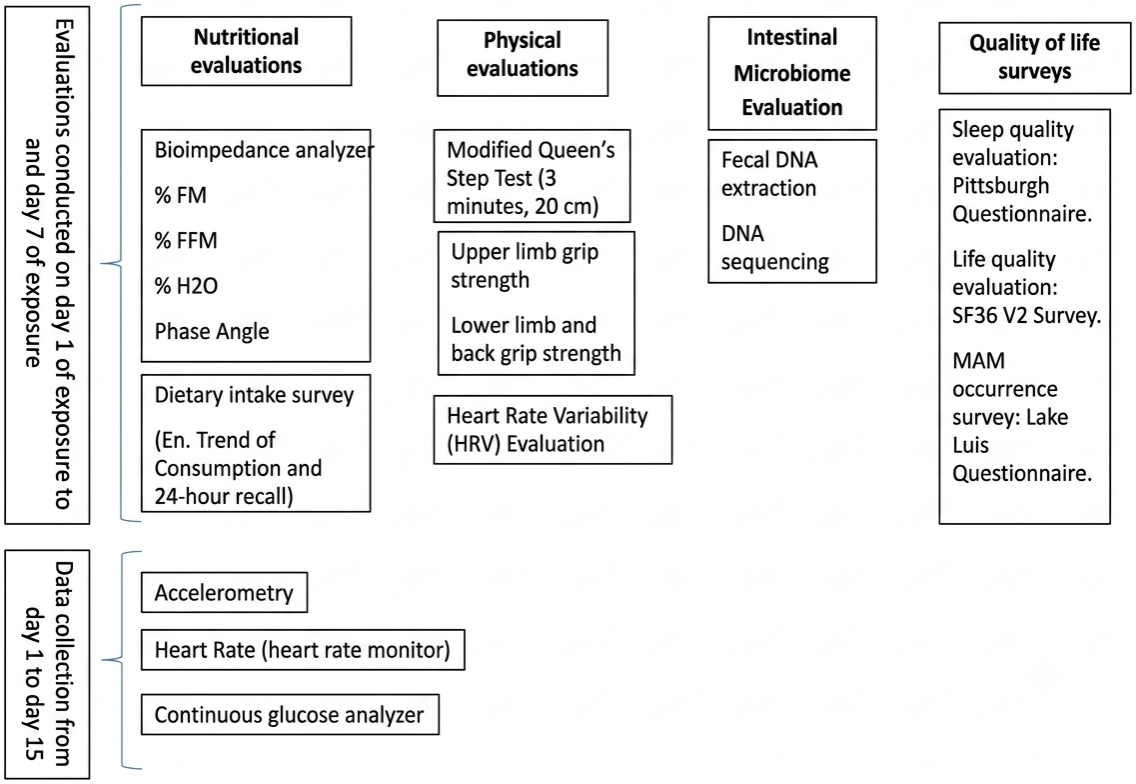
Selection: Once the minimum number of participants for the study has been reached, those who freely agreed to participate by signing the informed consent form in the breastfeeding room of the ALMA observatory and who meet the inclusion criteria as

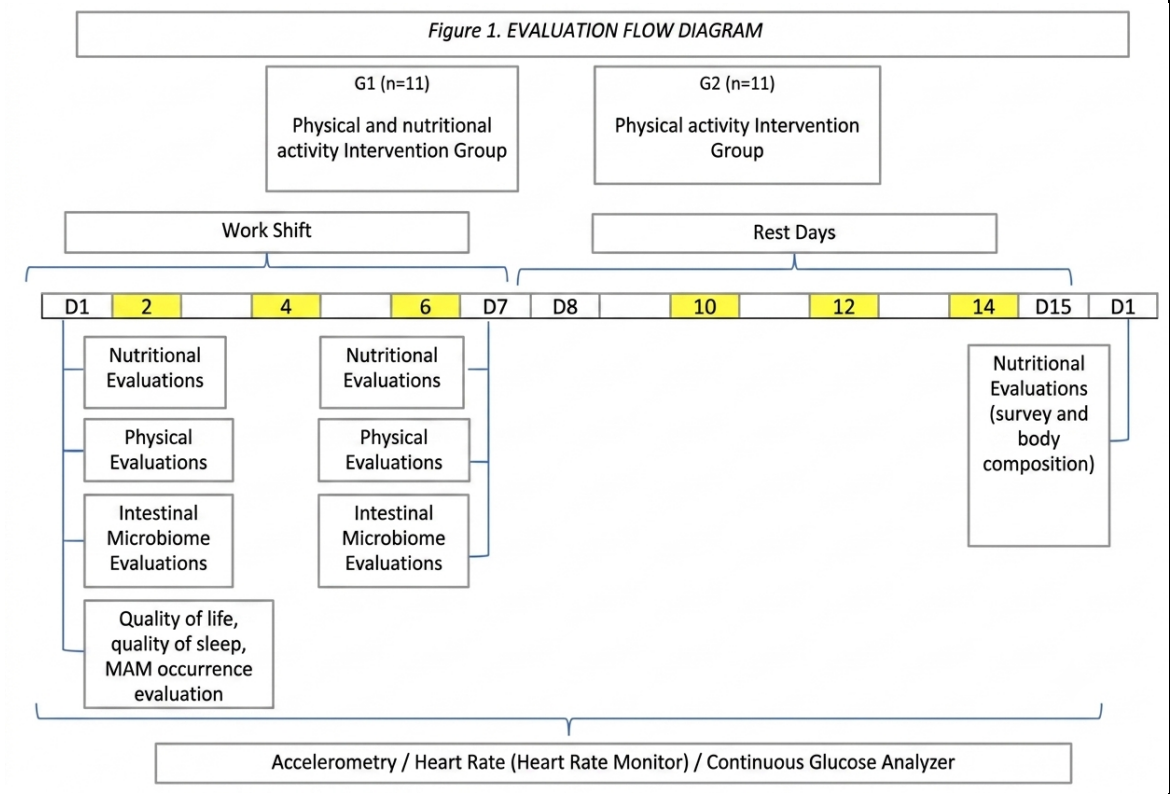
determined by a prior interview and by reading and signing the consent form, their information will be recorded on a data sheet using a code for each registered individual (corresponding to the first three letters of their first name with the first three letters of their last name, example: JORTOR for Jorge Torres), to later make a random distribution of these subjects into the different intervention groups, whereby the research team staff will give them the guidelines for their intervention group, without mentioning the information or intervention of the opposite group, thus achieving bias in the intervention of the research groups.

Techniques and/or instruments and procedures for data collection and handling (who, where, when, how)

Initial phase: All initial and control assessments will be carried out in the OSF sector of the observatory, at 2,950 masl, where the barometric pressure oscillates at 529 + 2 mmHg, starting on day 1 of exposure to hypobaric hypoxia by the study subjects, the seventh day of stay in HH conditions and finally on day 15, once they return from their respective week of rest following the schedules of figures 1 and 2.

Figure 2. SCHEDULE OF EVALUATIONS





Anthropometry and Nutritional Status:

The assessments will be performed on all subjects enrolled in the study. This will begin with the determination of body weight and height, which will be measured using a SECA brand scale and stadiometer, model 700, with 10-gram increments for weight and 1-millimeter increments for height. The protocol used will be that described by the WHO (1995), which will ultimately determine the nutritional status of each participant.

Subjects will be asked to step onto the measuring platform with their backs to the measuring arm, barefoot and wearing as little clothing as possible. The assessments will be performed in triplicate, and the average of these assessments will be used to improve the validity and reliability of the measurements. Subsequently, a dietary trend survey, along with a 24-hour recall, will be administered by a registered dietitian on days 1, 7, and 16.

Body Composition and Phase Angle:

Body composition analysis will consist of measuring subjects while they are fasting from both food and water, on the first and last days of their work shift. Subjects must remove all metal objects from their bodies and lie down on a table, extending their arms and legs to their sides. Two electrodes will be placed on the right foot and two on the right hand, one on the metatarsal area and the other on the ankle and wrist, respectively. The

evaluation will be carried out using a Bodystat brand multifrequency bioimpedance meter, model Quascan 4000, with frequency intensities of 5, 50, 100 and 200 kHz and with resistance, reactance and phase angle calculated from the frequency of 50 kHz, using the formula for its calculation: Arctan (reactance / resistance) * (180º / Pi), this because this technique has been used previously in subjects exposed to hypobaric hypoxia conditions, giving a strong correlation for deuterium oxide, (the latter being the gold standard for the determination of body water and the determination of body composition in two compartments); ($p < 0.001$ $r2 = 0.84$) (49).

Modified Queens Drawer and VO2max Estimation:

This test will be performed on the same day the subject arrives at the observatory. This test will estimate VO2max. It will be carried out using a modified 20 cm step and will last for 3 minutes (28). The subject will wear athletic clothing for the test, which will begin with taking their blood pressure after 10 minutes of sitting with their heart rate monitor connected to the Polar Beat app to continuously monitor their heart rate. First, a step-up and step-down test will be performed on a 20 cm step for 3 minutes at a pace of 90 steps per minute (bpm). The box step technique will be performed with both arms at the sides of the body, stepping onto the dominant foot first and then the second foot until both are fully extended, in time with the pulse determined by a metronome using the "Soundbrenner" app, available for iPhone and Android, according to the protocol used in laboratory conditions by Hermand et al. (2024). The test will end once the 3-minute duration has elapsed or when the subject reaches 90% of their maximum heart rate estimated using the Karvonen formula.

Glucose Curve:

To determine glucose metabolic status, an Abbott FreeStyle Libre continuous glucose monitor will be used. This device consists of a skin patch applied to the right triceps of each participant. The chosen area will first be cleaned with 75% alcohol using a cotton swab, which will be discarded after each glucose meter application. Measurements will be taken daily for the 7 days the subject remains at the observatory at 8:00 PM during dinner time. The final measurement will be taken on day 14, upon the subject's return from their rest days, at which time the patch will also be removed and disposed of in a sharps container. (50).

Determining quality of life:

For this analysis, the WHOQOL survey will be used (See Annex 3), which defines quality of life as an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns (36). Sleep quality: Subsequently, a characterization of the sleep quality condition will be carried out using the Pittsburgh test (See Annex 4).

Dietary Habits Survey: To determine the intake and quantity of macronutrients, a consumption trend survey (see Appendix 5) will be conducted, along with a 24-hour food intake record (see Appendix 6). These will be administered by a registered dietitian.

To determine the potential impact of barometric pressure, the Lake Louise scale (see Appendix 7) will be applied on days 1, 7, and 15 to all participants in both intervention groups.

Gut microbiota analysis:

The gut microbiota will be determined by extracting bacterial DNA from the feces of each study participant. This extraction will be performed on day 1 of exposure to hypobaric hypoxia and on the last day of stay at altitude. The determination will be carried out using the extraction protocol of the manufacturer, QiaGen QIAamp PowerFecal Pro DNA Kit. The procedure will begin by providing each person in groups G1 and G2 with two sealed, sterile containers. Participants will be asked to deposit a small fecal sample in each container on day 1 of their work shift and another sample on day 7 of their shift. (Subsequently, on day 15, upon returning from their rest days, a new fecal sample will be requested using the same procedure described above.) In all cases, the samples will be processed as soon as possible in a laboratory located at the ALMA observatory facilities at 2,950 meters above sea level. First, fecal microbiota DNA will be extracted in the appropriate laboratory at the ALMA Observatory. This procedure will be performed by a biotechnology engineer, a co-investigator on the research team (see co-investigator letters in the appendix). 200 mg of feces will be processed according to the manufacturer's protocol, QiaGen, using the QIAamp PowerFecal Pro DNA analysis kit. These DNA samples will be transported in a refrigerated container to the facilities of the Catholic University of Maule, specifically to the GEMA Laboratory. There, they will be stored in the buffer included with the aforementioned extraction kit and frozen in a special -20°C refrigerator at the GEMA Laboratory for future analysis. The samples will then be analyzed using an Invitrogen Qubit fluorometer to quantify the concentration of extracted DNA. Subsequently, they will be studied by capillary electrophoresis using a Bioptic Fragment Analyzer™ (QSep 1). Following these processes, The libraries will be prepared according to the sequencer to be used, and finally, sequencing will begin on an Illumina Miseq and/or MinION nanopore platform (depending on the DNA samples extracted and the amount of sequencing data). All these molecular procedures, except for DNA extraction, will be carried out in the Applied Genomics and Microbial Ecology Laboratory at the Catholic University of Maule.

Manual Grip Strength:

This will be quantified through the use of a hand-held dynamometer, Baseline brand, with a capacity of 200 lbs. Where the subject will stand, taking the dynamometer in his hand and adjusting his palm on the handle of the dynamometer to the second phalanx of the last four fingers under the other branch of the handle. The subject will stand in a straight line with the forearm letting it hang freely but without touching the thigh of his own leg. When the order is given, the subject under study will squeeze his hand as tightly as

possible, without allowing the hand or arm to touch the body or any other object, otherwise the test must be invalidated and repeated. This test will be performed in three repetitions for each hand, leaving a rest time of 3 minutes between each test, after which the highest value achieved with each hand will be recorded (51).

Leg Strength:

This parameter will be quantified using a leg dynamometer, Baseline brand with a standard platform, with a capacity of 660 lbs. The subject will begin standing holding the center of the bar, with palms facing down, at the level of the pubic bone. Subsequently, with your head erect and your back straight, you will bend your knees at an angle of 120 degrees and the length of the chain will be adjusted (before connecting it to the dynamometer scale), so that the bar is in the crease that is formed between the thigh and the trunk. Subsequently, the subject will place his hands in the middle of the bar and will be asked to try to extend his knees, applying force continuously and vigorously. Upon completion of the test, the subject's knees should be fully extended to ensure that maximum effort has been applied. This procedure will be repeated three times, resting 30 seconds between each attempt, and the highest value obtained on the dynamometer (48) will be recorded.

Energy Expenditure:

Energy expenditure will be determined using an Actigraph WGT3X-BT accelerometer, calibrated for a sensitivity of 100 Hz. This accelerometer will be used by the study participants from the first day of their work shift until the seventh day, at the end of the shift. Along with determining movement intensity, this instrument will estimate each individual's daily energy expenditure in METs/hour, which has been compared to polysomnography, considered the gold standard ($p = 0.000$; $r => 0.92$) (31). In addition to the accelerometer, each participant will wear a Polar H10 chest strap heart rate monitor. This will be used to adjust the intensity of the movements performed to simulate physical activity under intermittent hypobaric hypoxia. It should be noted that both devices must be linked to each other via Bluetooth technology and also constantly connected to the Polar Beat application, where the person's pulse will be continuously monitored for 15 days.

Cardiac Variability Assessment:

(Secondary data)

Finally, secondary data will be collected, assessing heart rate variability using a Polar H10 heart rate monitor in all participants. This test will be performed on a mutually agreed upon date and time during the work shift. Participants will lie on a clinical examination table with the heart rate monitor attached, activated, and connected to the Polar Flow app, which will record RR intervals at a sampling rate of 1000 Hz. Participants will be asked to remain still, without talking or sleeping, for 10 minutes. During this time, blood pressure will be measured on the left arm at 1, 3, 5, and 10 minutes, and respiratory rate will be recorded at 3, 5, and 8 minutes.

Once the evaluation session is recorded, the file will be downloaded from the Polar Flow platform in .txt format. This file will be analyzed using the KUBIOS HRV STANDARD software, which will determine the following variables: high frequency (HF), low frequency (LF), low frequency/high frequency ratio (LF/HF), standard deviation between beats (SDNN), and root mean square deviation (RMSSD). These variables will be considered from the 5 continuous minutes that do not show artifacts through visual analysis, as previously recommended (52).

Hypobaric hypoxia will be verified daily using data obtained from the observatory's own weather station, which will be expressed in millimeters of mercury to describe atmospheric pressure and partial humidity.

Ethics Committee:

This research project will be submitted to the Scientific Ethics Committee of the Catholic University of Maule for approval and registered with the Clinical Trials Committee. In addition, an informed consent form will be prepared, based on the principles expressed in the Declaration of Helsinki (53), which must be accepted and signed by each participant who agrees to take part in this research.

Data analysis techniques

After verifying that randomization has been effective, mixed regression models will be used in which each outcome variable will be the dependent variable, the interventions will be treated as fixed effects (1=G1 and 0=G2), and the models will be adjusted for baseline values of age, sex, and weight. Results will be expressed as absolute differences in changes in variables between baseline and final measurement. When the dependent variable is dichotomous (e.g., prevalence of overweight/obesity), odds ratios will be calculated.

Sensitivity analyses will be performed using multiple imputation techniques, imputing missing measurement values and creating five databases that will be analyzed in parallel. If preliminary analyses detect imbalances that threaten the comparability of the groups, propensity scores will be used to control for covariate imbalances.

Analyses will be conducted from an intention-to-treat perspective, whereby subjects will remain in the analysis in the G1 or G2 group to which they were originally assigned, regardless of their adherence to the intervention. Results will be considered statistically significant at $p < 0.05$.