

# Effects of moderate-intensity intermittent hypoxic training on health outcomes of patients recovered from covid-19: the AEROBICOVID study protocol for a randomised controlled trial

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## Research Article

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# Abstract

**Background:** Recent studies point to a lower number and a reduction in the severity of cases in higher altitude cities, with decreased oxygen concentration. Specific literature has shown several benefits of physical training, so, in this sense, physical training with hypoxic stimulus appears as an alternative that supports the conventional treatments of the COVID-19 patient's recovery. Thus, this study's primary aim is to analyse the effects of moderate-intensity intermittent hypoxic training on health outcomes in COVID-19 recovered patients.

**Methods:** A clinical trial controlled double-blind study was designed. Participants (30-69 years old) will be recruited among those with moderate to severe COVID-19 symptoms, approximately 30 days after recovery. They will be included in groups according to the training (T) and recovery (R) association with hypoxia (H) or normoxia (N): a)  $T_H:R_H$ , b)  $T_N:R_H$ , c)  $T_N:R_N$ , and, last, d) the control group. The 8-week exercise bike intervention will be carried out with a gradual load increase according to the established periods, three times a week in sets of 5 minutes, 90 to 100% of the anaerobic threshold (AT), and a 2,5-minute break. Blood will be collected for genotyping. First, after four weeks (partial), after eight weeks, and later, four weeks after the end of the physical training intervention, participants will perform assessments. The primary outcome is the maximum oxygen consumption ( $VO_2\max$ ). The secondary outcomes include lung function, inflammatory mediators, haematological, autonomic parameters, AT, body composition analysis, quality of life, mental health, anthropometric measurements, and physical fitness. The statistical analysis will be executed using the linear regression model with mixed effects at a 5% significance level.

**Discussion:** This study is designed to provide evidence to support the clinical benefits of moderate-intensity intermittent hypoxic training as a part of the treatment of patients recovered from COVID-19. It may also provide evidence on the efficacy and safety of intermittent hypoxic training in different health conditions. Lastly, this study presents an innovative strategy enabling up to 16 participants in the same training session.

**Trial registration:** Brazilian Clinical Trials Registry, RBR-5d7hkv. Registered after the start of inclusion; 3 November 2020; <https://ensaiosclinicos.gov.br/rg/RBR-5d7hkv>

## Background

In March 2020, the World Health Organization raised the state of COVID-19 contamination to the pandemic. Currently, there are approximately 58 million infected cases and more than 1,3 million deaths. News related to COVID-19 are commented daily in the media and has caused great concern and impact on Global Public Health [1].

Symptoms presented by those infected with COVID-19 are common to other respiratory infections. They may include fever, cough, sore throat, headache, fatigue, muscle pain, loss of smell, and shortness of breath. The clinical condition is diverse, ranging from an asymptomatic state to acute respiratory

syndrome and damage to various body systems, increasing inflammatory markers, cardiovascular alterations, and injuries to the lungs and kidneys. The mortality rate of COVID-19 is between 2 and 3%; therefore, the recovered population could be investigated [2-4].

Recent studies indicate that people who live at altitude with low levels of oxygen ( $O_2$ ) have a lower prevalence of COVID-19 as well as less severity in cases of infection [5-10]. The factors that may be related to lower susceptibility to COVID-19 involve physiological and anatomical adaptations in the lungs, with perfusion and capacity improvements, but mainly hypoxia-inducible-factor-1 $\alpha$  (HIF-1 $\alpha$ ) activation [5-7], which has become scientifically relevant due to its discovery behind the Nobel Prize in Physiology or Medicine in 2019 [11]. According to the phenomenon of hormesis, moderate doses of intermittent hypoxia would lead to the activation of HIF-1 $\alpha$ , triggering positive adaptations in the face of different diseases [12].

Specifically concerning COVID-19, HIF-1 $\alpha$  can decrease the expression of the angiotensin-converting enzyme (ACE)2, which is indicated as a facilitator of the SARS-CoV2 virus into cells, mainly in the lungs [6, 7, 13]. HIF-1 $\alpha$  production increases the expression of ACE1, stimulating the formation of angiotensin II, which regulates angiotensin II type 1 receptor (AGTR1). This process would reduce the expression of ACE2 [5, 6]. The infection with SARS-CoV2 leads to the destruction of epithelial cells, representing an important part of airway immunity. Consequently, a series of changes in immunological and inflammatory markers is triggered [14, 15]. Still, it is important to note that HIF-1 $\alpha$  can increase the gene expression to produce erythropoietin (EPO), countering the harmful effects of COVID-19. Among EPO's benefits are highlighted: neuroprotection, central ventilation stimulation, endothelium protection, and pulmonary vasodilation, red blood cell production, and anti-inflammatory effect [16].

On the other hand, moderate-intensity interval exercise can reduce chronic inflammation and strengthen the immune system [17-20], reducing the severity and mortality in viral diseases [21]. Higher aerobic capacity can produce short-term improvements in the immune and respiratory systems [22], both affected by COVID-19 [23].

Training methods using hypoxia as a resource have existed since the 1960s to increase performance at sea level or generate acclimatisation for athletes to improve their performance in altitude tests [24, 25]. More recent studies have shown that this type of intervention can present good health outcomes and that physical training under normobaric hypoxia is safe and can be performed with different populations. A reduction of fat mass has been reported with a concomitant increase in lean mass [26] and increases in cardiorespiratory fitness [27] in overweight and obese women. These results were better in the groups that performed interval training or sprints in hypoxia than those trained in normoxia after 12 weeks (36 sessions). Also, it has been reported the effects on bone mineral density in older adults, but only the group that underwent training of whole-body vibration in hypoxia showed improvements after 18 weeks (36 sessions), while the group that trained in normoxia did not improve [28]. Additionally, in other study performed along three weeks (9 sessions), the aerobic capacity, HIF-1 $\alpha$ , nitric oxide, and pro-angiogenic factors improved, but only in the group of active young men who performed high-intensity interval

training under hypoxic conditions on a bicycle [29]. Moreover, a systematic review and meta-analysis identified that hypoxic training promoted better responses in reducing triglycerides and increasing muscle mass than the same exercise performed in normoxia [30].

Although the studies previously mentioned [26-29] had shown health benefits for participants right after the intervention, a recent study [31] that performed high-intensity training under hypoxic conditions found effects after four weeks of the end of the intervention. At that moment, the trunk fat mass of overweight and obese women decreased significantly compared with the result after the intervention. This result may indicate that the adaptations promoted by the hypoxic training can last longer since this improvement after four weeks from the end was not found in the group that trained in normoxia.

Even though exposure to physical training and hypoxia can result in several health benefits, the response magnitude can vary considerably between individuals. Genetic characteristics could explain this variation and the different reactions that patients recovered from COVID-19 present in the gradual recovery process. The COVID-19 number and severity of cases in some populations have been associated with ACE gene and the frequency of I allele (insertion) or D allele (deletion) of 287-bp, drawing attention to the activity of this protein, which has a relationship with these genetic variants [32]. However, the results are still preliminary and point to this relationship's complexity, requiring further exploration. Furthermore, since the modulation of ACE2 by HIF-1 $\alpha$  involves the regulation of AGTR1, the investigation of genetic variants related to this receptor may also be necessary to understand this context better [9, 10]. Genetic variation can also influence the response to training in hypoxia, and the DD genotype of ACE may be related to a worse adaptation in altitude. The presence of the I allele may indicate a better possibility of adaptation [33]. There is also evidence about an association between EPO's production and genetic variants of the promoter region of this gene [34].

Thus, we hypothesise that training in normoxia would promote improvements in health status comparing to the control group that will not train. Both groups that will train in hypoxia would present even better results than the normoxia group, helping in the faster and more complete recovery of the organism, restoring immune system homeostasis and cardiorespiratory capacity. Still, it is expected that the studied genetic variants would affect the magnitude of response to the intervention.

## Methods

This protocol was written in accordance with the Standard Protocol Items: Recommendations for Interventional Trials guidelines [35]. We used the SPIRIT checklist when writing our report [36].

### Aims

#### Primary aim

To analyse the effects of moderate-intensity intermittent hypoxic training on the health outcomes of patients recovered from COVID-19.

## Secondary aims

To describe the health status in COVID-19 patients recovered who had presented moderate to severe symptoms through lung function, haematological, immunological, and autonomic parameters, in addition to physical fitness, quality of life, and mental health.

To analyse the effects of moderate-intensity intermittent hypoxic training in patients recovered from COVID-19 who presented moderate to severe symptoms on:

- Haematological parameters, using the blood count and other biochemical analyses; immunological ones through inflammation markers (protein and lipid mediators); and erythropoiesis through EPO analysis;
- Physical fitness through aerobic power, aerobic capacity, strength resistance of lower limbs, agility, and dynamic balance;
- Anthropometric measurements and body composition through the distribution of lean, bone, and adipose tissue;
- Blood pressure and autonomic parameters through heart rate variability (HRV);
- The state of mental health through the assessment of levels of depression, anxiety, and stress, using the Depression, Anxiety and Stress Scale (DASS - 21);

To determine the effects after four weeks of the end of the moderate-intensity intermittent hypoxic training on lung function, haematological, immunological, and autonomic parameters and physical fitness, quality of life, and mental health in COVID-19 recovered patients with previous moderate to severe symptoms.

To analyse the influence of genetic variants of ACE, AGTR1, a promoter region of the EPO gene, and the EPO receptor on the effects of moderate-intensity intermittent hypoxic training in COVID-19 recovered patients.

## Design and setting

The study design is based on a randomised, double-blind controlled clinical trial composed of four groups. The control group being formed by the participants who are not available to participate in the intervention and accept to carry out a follow-up through the evaluations; and the physical training groups randomly divided according to the association of training (T) and recovery (R) with hypoxia (H) or normoxia (N): a)  $T_H:R_H$ , b)  $T_N:R_H$ , c)  $T_N:R_N$ .

As Figure 1 shows, the experimental protocol will consist of:

- i) familiarisation and carrying out the initial assessments (evaluation 1) in the three sessions of week 0;

- ii) 8-week intervention with a partial assessment to adjust the training load on week 5 (half of the intervention - evaluation 2);
- iii) reassessments on week 9, following the end of physical training intervention (evaluation 3);
- iv) reassessments on week 13, four after the end of the intervention (evaluation 4).

### **Characteristics of participants**

The inclusion criteria will be (1) participants aged between 30 and 69 years old and convalescent from COVID-19 (having the test with a positive diagnosis), (2) having moderate to severe symptoms, (3) approximately 30 days since recovery from clinical signs or medical discharge (if they had been hospitalised). The exclusion criteria will be (1) exposure to high-altitude places > 1,500 m in the last three months, (2) significant physical limitations to carry out assessments or intervention, (3) acute or chronic clinical illnesses without medical supervision, (4) anaemia, (5) use of immunosuppressive drugs, (6) pregnant women, (7) hormone replacement, (8) smokers, and (9) excessive use of alcohol or drugs.

### **Instrumentation normobaric hypoxia**

The three training groups, except the control, will carry out the training program in the same way: on bicycles, around the normobaric hypoxia tents (Colorado Altitude Tents – Colorado- USA) using the individual system that includes the face mask. Participants will not be aware of the O<sub>2</sub> concentration in the air contained in the tent in which they are positioned. This procedure will be done to minimise possible psychological influences from exposure to hypoxia.

A system that includes an individual face mask to be worn in the training program has been developed. Each participant will receive a mask for exclusive use in the initial evaluations and be instructed to wear it during the entire intervention aiming to work at the highest level of safety. Figure 2 illustrates the strategy that will be used for the intervention. The normobaric hypoxia tents with O<sub>2</sub> concentration control will be set up at the Multisport Gymnasium at the University. This space has good air circulation, and the bikes will be positioned around the tents, respecting 3m between each bike. Up to eight participants will breathe the air in the normobaric hypoxia tents through hoses and an individual system that includes the mask. Two tents will be used simultaneously, enabling the training of up to 16 participants per period.

Each system will consist of a face mask of personal protective equipment with membranes that will allow only the inspired air to enter the upper cavity (nose) and only the exhaled air to exit the lower cavity (mouth). This membrane system will prevent air from being exhaled through the upper cavity, thus avoiding the possibility of contamination of the air contained in the tent, or else, inspired by the lower cavity, avoiding that the ambient air is inspired in this case, which would decrease the precision of the study. A safety valve will be used on the bottom of resuscitation bags to increase this strategy's efficiency and facilitate the connection of the hose that will take the air from the normobaric hypoxia tent to the

individual system (mask). To provide greater comfort and stability during physical training, the system will be covered with neoprene protection (Figure 3).

## Intervention Protocol

The moderate training sessions in bicycles will be performed with a frequency of 3 times a week, and consist of three parts (warm-up, main part, and cool down) with a total duration of up to 50.5 minutes. The initial stage (warm-up) and the final stage (cool down) will last for 5 and 3 minutes respectively and will be of low intensity, corresponding to "easy" by the rate of perceived exertion (RPE). The training intensity in the main part will be based on the AT values. Blood lactate concentration [La-], target heart rate, and RPE will control the load. The training will consist in sets with efforts lasting 5 minutes with an intensity close to the upper limit of training zone 2 (90 - 100% of AT) with a passive pause of 2.5 minutes between efforts. Training loads will be increased during the first four weeks as it is explained in Table 1.

Additionally, the internal training load (Trimp) will be quantified at each session. It will be assumed as the product between the duration of the session (minutes) by the RPE values, the results being expressed in arbitrary units (AU). The RPE will be presented after the end of each training session [37]. This procedure will monitor possible differences in the absolute internal load among the  $T_H:R_H$ ,  $T_N:R_H$ ,  $T_N:R_N$  groups. An estimative is presented in Table 1 with a fixed RPE value of 5.

**Table 1.** Training dynamics week by week, the progression of the load, the total volume of each session, and internal training load (Trimp)

Training dynamics	W1	W2	W3	W4-W8
Number of sets	3	4	5	6
Efforts (min)	5	5	5	5
Time recovery between efforts (min)	2.5	2.5	2.5	2.5
Total volume of each session (min)	28	35.5	43	50.5
RPE	5	5	5	5
Trimp (AU)	140	177.5	215	252.5

W - week; RPE - rate of perceived exertion; AU - arbitrary units.

It is important to highlight that despite the intensity of the sessions being considered "moderate" and, theoretically allowing participants to carry out the proposed training continuously, we believe that the inclusion of 2.5 minutes between efforts, that is, an effort: pause relationship of 2:1 will allow less discomfort during efforts, without, however, compromising the aerobic "gains" from this training model.

Other necessary adaptations will be made according to the performance and needs of each participant. The final part (cool down) will last for 3 minutes with "easy" intensity by the RPE.

Out of the three training groups, two will be exposed to hypoxia according to the association of training (T) and recovery (R) with hypoxia (H): a)  $T_H:R_H$ , b)  $T_N:R_H$ . These groups will be subjected at an inspired fraction of  $O_2$  ( $FiO_2$ ) to simulate approximately 3,000 m of altitude in the Multisport Gymnasium. The other training group will train in normoxia ( $T_N:R_N$ ) at  $FiO_2$  corresponding to sea level in the Multisport Gymnasium. Hypoxia generators will be used together with the normobaric hypoxia tents. It is important to note that during all training sessions,  $O_2$  saturation will be monitored, which will allow us to verify the response to hypoxia during training, together with the application of the Lake Louise Scale.

All sessions will be monitored by training's acute responses using HR, blood oxygen saturation ( $SpO_2$ ), and RPE. Weekly, the acute responses associated with exposure to hypoxia, using a questionnaire. On weeks 2, 4 and 6, blood  $[La^-]$  will be evaluated to control the training load.

### *ACUTE RESPONSES*

#### *Blood oxygen saturation ( $SpO_2$ )*

Blood  $O_2$  saturation will be monitored using a pulse oximeter (G-Tech Portable). The equipment will be positioned on the participants' distal phalanx during training, and the measurements will be recorded in four moments, id. est., rest, end of each stimulus, lowest result during the break, and end of the pause.

#### *Rate of Perceived Exertion (RPE)*

The training intensity will be controlled by RPE[38].

#### *Acute responses associated with exposure to hypoxia*

Both training groups (normoxia and hypoxia) will answer this questionnaire to maintain the impartiality of the study. The Lake Louise Score [39] will be used. There is no intention to diagnose acute mountain disease but verify and monitor the acute responses to hypoxia exposure.

#### *Blood Lactate concentration $[La^-]$*

Blood  $[La^-]$  will be determined using the YSI 2300 STAT analyser (Yellow Springs, OH, USA). For this, 25  $\mu$ L of whole blood will be collected from the earlobe, using previously calibrated heparinised capillaries. Blood samples will be immediately homogenised in microtubes containing 1% sodium fluoride.

### **Outcomes**

The primary outcome is the maximum oxygen consumption ( $VO_{2max}$ ). The secondary outcomes include lung function, inflammatory mediators, haematological, autonomic parameters, AT, and body

composition analysis, quality of life, mental health, anthropometric measurements, and physical fitness. All outcomes will be assessed at baseline, immediately post-intervention, and later, four weeks after the end of the intervention. After four weeks, the primary and some secondary outcomes will be assessed (partial evaluation).

### **Participants timeline**

This study is ongoing and planned to take place from September 2020 until December 2021. The schedule for enrolment, interventions and assessments is presented in Table 2.

**Table 2.** Schedule of enrolment, interventions, and assessments.

TIMEPOINT	STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			
	$-t_1$	0	Baseline	Partial evaluation (week 5)	Post-training (week 9)	After 4 weeks of training end (week 13)
<b>ENROLMENT:</b>						
Eligibility screen	X					
Informed consent	X					
Allocation		X				
<b>INTERVENTIONS</b>						
(Training):						
<i>Normoxia (N)</i>				X		
<i>Hypoxia (H)</i>				X		
<i>Hypoxia recovery (HR)</i>				X		
<i>Control group</i>			X			
<b>ASSESSMENTS:</b>						
<i>Lung function (spirometry)</i>			X	X	X	X
<i>Autonomic parameters (HRV)</i>			X	X	X	X
<i>Max Aerobic power (VO<sub>2</sub>max)</i>			X	X	X	X
<i>Anaerobic threshold</i>			X	X	X	X
<i>Inflammatory mediators<sup>1</sup></i>			X		X	X
<i>Haematological parameters<sup>2</sup></i>			X		X	X
<i>Body composition (iDXA)</i>			X		X	X
<i>Quality of life (SF - 12)</i>			X		X	X
<i>Mental Health (DASS - 21)</i>			X		X	X
<i>Anthropometric (BMI, WC)</i>			X		X	X
<i>Physical fitness (motor tests)</i>			X		X	X

HRV - heart rate variability; max - maximum; BMI - body mass index; WC - waist circumference. <sup>1</sup>Inflammatory mediators include quantification of cytokines and profile of eicosanoids. <sup>2</sup>Haematological parameters include hemogram, total cholesterol, triglycerides, HDL-c, LDL-c, lactic dehydrogenase, liver enzymes (TGO and TGP), and EPO.

## Sample size

For sample size estimation for clinical trial study design, using sample size estimation as comparing parallels groups (such as three training groups: a)  $T_H:R_H$ , b)  $T_N:R_H$ , c)  $T_N:R_N$ ), and control group. A power function of 80% will be used, a level of significance of 5% and an average difference between groups of 10% will be estimated. Based on the formula described previously[40], the sample size required per group is 20. Hence, the total sample size required is 80 for the four groups, considering a drop-out rate of 10%, so the total sample size required is 88, that is, 22 individuals in each group [41].

## Recruitment

The invitation will be made to the Health Institutions to offer the possibility of participating in this research to patients who have had moderate to severe symptoms approximately 30 days after the recovery of clinical signs or medical discharge (hospitalised people). The disclosure will happen through the local television channels, USP's local radio and news from the internet, social networks, among other media outlets. Participants will be asked to complete the evaluations on week 0. After that, they will be assigned randomly to each group. This study will develop every effort to control confounding variables. Randomisation and allocation will be performed by the research coordinator. Considering special randomisation techniques, in this project, we will be using the randomisation of matched pairs [42], considering a baseline pairing strategy regarding confounding variables such as sex, age, and level of physical conditioning of the participants for their selection and random assignment in the research groups. As there are three training groups, a)  $T_H:R_H$ , b)  $T_N:R_H$ , c)  $T_N:R_N$ , pairing groups will be organised consisting of participants based on the matching characteristics above, which will be drawn randomly using Microsoft Excel for allocation to groups. This method is satisfactory and suitable for intervention studies in which there is not a large number of participants. In terms of blinding, only the research coordinator is aware of the allocation, so different researchers will work on the intervention and collect information from the participants as evaluations, who will be blinded to avoid potential bias. To maintain the overall quality and legitimacy of the clinical trial, unblinding should occur only in exceptional circumstances when knowledge of the actual treatment is essential for further management of the patient.

## Data collection

*PAR-Q and Anamnesis*

Firstly, following what is put for people aged between 15 and 69 in the State of São Paulo Law No. 16724 of 22 May, 2018, participants will answer the Physical Activity Readiness Questionnaire (PAR-Q) and those who respond positively to any of the questions, they will sign the Term of Responsibility.

Sociodemographic data, general and specific (COVID-19) health status, related to lifestyle, information on comorbidity, and drug treatment will be assessed through the Anamnesis. Answers about the symptoms associated with COVID-19 will allow assessing the intensity of the disease's manifestation.

## *LONGITUDINAL RESPONSES*

### *Questionnaires*

The International Physical Activity Questionnaire (IPAQ) - short version, validated in Brazil [43], will measure the usual level of physical activity and the Food Consumption Markers Form of the Ministry of Health [44], to assess food consumption. Participants will be instructed to maintain similar physical activity and eating habits throughout the study. The objective is to certify that the possible differences between the groups after the intervention in the variables of interest are related to the physical training intervention's effect, not to other environmental factors.

To assess the perception of the quality of life, the 12-Item Health Survey (SF-12) [45] will be used. Mental health status will be assessed using the Depression, Anxiety and Stress Scale (DASS - 21) [46], and the calculation of the score will be based on a previous study [47].

### *Autonomic parameters*

HR variability (HRV) will be analysed using the RR intervals (RR) from the Polar Team<sup>2</sup> HR transmitter, (Polar Electro Oy, Kempele, Finland) and recorded at a sampling frequency of 1000Hz. The data will be recorded and stored for further analysis. The HRV analyses will be performed using custom computer software (CardioSeries v2.0, <http://sites.google.com/site/cardioseries>) developed by Dias, DPM of the University of São Paulo, Brazil. The interpolated RR series will be divided into half-overlapping sets of 256 data points, overlapping 50% (Welch Protocol). The stationary segment will be visually inspected, and those with artefacts or transients will be excluded. Each RR static segment will be submitted to the spectral analysis by Fast Fourier Transform (FFT), after Hanning window [48]. The analysis of HRV will be performed by linear methods, analysed in the domains of time (SDNN and RMSSD) and frequency (LF, HF, and LF/HF ratio) [49].

### *Anthropometric measurements*

The body mass index (BMI) will be used to measure nutritional status recommended by WHO [50] and waist circumference (WC) [51] will also be measured.

### *Body composition*

For the analysis of body composition and the distribution of lean, bone, and adipose tissue, the dual-energy X-ray absorptiometry technique (iDXA - GE Lunar - DPX-NT) will be used. The radiation dose that the participants will receive will be less than 0.05 [52], equivalent to 50 times less than an X-ray exam. When positioned on the device, the subjects will remain immobile in the supine position during the exam (about 15 minutes). The method will estimate body composition by dividing the body into three anatomical compartments: fat-free mass, fat mass, and bone mineral content.

### *Lung function*

For pulmonary function evaluation, a portable spirometer (Micro Medical, Rochester, United Kingdom) will be used, following the current standards [53] and guidelines for pulmonary function tests [54]. Participants will be instructed to perform the manoeuvres to assess forced vital capacity (FVC) and forced expiratory volume (FEV).

### *Genotyping*

Blood collection will be performed by peripheral venous access after an eight-hour fast, and the DNA extraction process adopted will be Salting Out. The ECA I/D genetic variants (rs1799752) will be amplified by polymerase chain reaction (PCR) (Applied Biosystems StepOnePlus), according to a previous study [55]. The genetic variants of AGTR1 A1166C (rs5186), T>G in the EPO gene promoter region (rs1617640), and G6002A at the EPO receptor (rs121918116) will be determined by real-time polymerase chain reaction (qPCR). The reaction will be performed using an allelic discrimination assay TaqMan (CAH5I790 Thermo Fisher, USA) and TaqMan genotyping master mix (Applied Biosystems, USA).

### *Haematological parameters*

Hemogram parameters, such as total erythrocyte count, haematocrit, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total leukocyte, platelet, and reticulocyte count will be evaluated. Total cholesterol, triglycerides, HDL-c, LDL-c, lactic dehydrogenase, and liver enzymes (TGO and TGP) will also be analysed. The collection will be carried out at the School of Physical Education and Sport of Ribeirão Preto by a trained and specialised professional. Later, the samples will be sent for analysis at the Clinical Analysis Laboratory of Faculty of Pharmaceutical Sciences of Ribeirão Preto, according to the technical service's standard routine and methodology. Aiming to determine the EPO concentration in the plasma samples, the EPO Immunoassay ELISA Kit (R&D Systems, Billings, USA) will be used.

### *Dosage of inflammatory soluble protein mediators*

The quantification of cytokines IL-6, IL-8, IL-10, and TNF- $\alpha$  will be performed by the ELISA - Multiplex method according to the manufacturer's specifications (R&D Systems, Billings, USA).

### *Inflammatory lipid mediators*

The profile of eicosanoids will be evaluated in plasma by mass spectrometry (LC-MS/MS) in samples collected with EDTA anticoagulant at the facility available from Faculty of Pharmaceutical Sciences of Ribeirao Preto - USP. The standard protocol requires the use of lipid solid-phase extraction and high-resolution multiple-reaction monitoring to develop a target bioanalytical method for eicosanoid quantification. Data acquisition and processing will be performed using PeakView™ and MultiQuant™ software (Sciex, Foster, CA, USA) (Sorgi CA, et al, Scientific Data, 2018).

### *Blood pressure*

Blood pressure will be assessed using an automatic digital arm pressure meter (OMRON, model HEM-7113), following the "VII Brazilian Guidelines on Hypertension" [56].

### *Physical Fitness*

*Maximum aerobic power (VO<sub>2</sub>max), anaerobic threshold (AT), and exercise intensity corresponding to VO<sub>2</sub>max (iVO<sub>2</sub>max)*

An incremental test will be used to estimate AT, VO<sub>2</sub>max, and iVO<sub>2</sub>max. The warm-up will be 5 minutes in cycle ergometer (E200, COSMED, Italy) without load (0 Kp). At each 2-minute stage, there will be an increase of 0.25 Kp (approximately 13 W) until voluntary exhaustion [27]. O<sub>2</sub> consumption will be measured with each breath, using the gas analyser (K4b<sup>2</sup>, COSMED, Italy), which will be calibrated according to the manufacturer's specifications. Blood samples (25 µL) will be collected from the earlobe at each stage's end to analyse the blood [La-]. Concomitantly, HR and PSE will be monitored at the end of each stage. VO<sub>2</sub>max will be defined as the highest mean VO<sub>2</sub> in the last 60 s in the test, considering at least three of the criteria: volitional exhaustion, blood [La-] ≥ 8.0 mmol, > 90% of the maximum HR predicted for age (HRmax = 220 - age), PSE ≥ 9, respiratory quotient ≥ 1.10, inability to maintain a frequency of at least 60 rpm. The iVO<sub>2</sub>max will be the lowest intensity at which the individual reaches VO<sub>2</sub>max during the test. If the individual doesn't sustain the intensity until the stage's end, the iVO<sub>2</sub>max will be assumed like peak power (PP), estimated by the equation [57]:

$$PP = \text{Last full intensity} + \left( \frac{\text{Dwell time at last intensity}}{\text{Time of each stage}} \right) * \text{Increment}$$

The points obtained between blood [La-] and intensities will be subjected to two linear adjustments so that the intersection will be assumed as the intensity of AT [58].

A partial evaluation containing the incremental test will be carried out at week 5 (half of the intervention) to adjust the training load (evaluation 2). Therefore, the incremental test will be performed in four moments: evaluation 1 (week 0 - pre), evaluation 2 (week 5 - half of the intervention), evaluation 3 (week 9 - post), and evaluation 4 (week 13 - four after the intervention's end).

## *Strength resistance of lower limbs and agility and dynamic balance*

Two motor tests will be performed to assess: (i) Strength resistance of lower limbs [59]; (ii) Agility and Dynamic Balance [60].

### **Statistical Analysis**

After double data entry, an exploratory analysis of the data will be carried out, with the primary objective of summarising the values, organising, and describing the data through tables with descriptive measures and graphs. Continuous variables will be expressed in terms of basic descriptive statistics (mean, median, and standard deviation), whereas categorical variables will be described in terms of frequency and percentage. The effects of the different types of training (hypoxia and normoxia), before, after eight weeks, and after four weeks after the intervention, for continuous outcomes will be compared using a linear regression model with mixed effects (fixed and random). If there are binary outcomes, a cox proportional hazards model will be used to estimate the incidence rates and their respective confidence intervals, which can include potential confounders as covariates. The possible associations between haematological, immunological, autonomic, aerobic, and anaerobic variables will be verified with hypothesis testing and the use of other regression models. In the case of handling missing data, we will use statistical methods as multiple imputation or Bayesian methods. The level of significance will be set at 5% in all analyses.

### **Ethics**

This study was approved by the School of Physical Education and Sport of Ribeirão Preto– University of São Paulo (USP) and Faculty of Pharmaceutical Sciences of Ribeirao Preto – USP Research Ethics Committees. Activities will start after signing the free and informed consent term presented by trained researchers of the work team. Biological samples can be stored in repositories at EEFERP-USP for use in future studies depending of the participants consent.

All care for the safety of the participants and the work team will be taken. First, an evaluation of the health status will be carried out. The participants who do not have limitations or discomfort that may prevent the evaluations' performance or the intervention will participate in the proposed intervention. Data will be added to a confidential dataset, and an alphanumeric code will be assigned to each participant. All local databases will be secured with password protected access systems.

Any modifications to the protocol which may impact on the conduct of the study, potential benefit of the participant or may affect safety, including changes of study objectives, study design, participant population, sample sizes, study procedures, or significant administrative aspects will require a formal amendment to the protocol.

Authors will create scientific reports to be submitted to peer-reviewed journals. Also, information about the different outcomes will be shared in different scientific events.

## Discussion

It is plausible that the convalescent people of COVID-19 who showed moderate to severe symptoms are in the process of gradual recovery and still have changes in their health status, even after approximately 30 days of recovery from clinical signs or medical discharge. The monitoring control group that will not train will describe the effects of COVID-19 on lung function, haematological, immunological, autonomic parameters, physical fitness, and mental health.

The gradual recovery process of the participants will be observed according to the association of training (T) and recovery (R) with hypoxia (H) or normoxia (N). It is expected that the normoxia training and recovery group ( $T_N:R_N$ ) can show better results compared to the control group. Besides, both groups that will train in hypoxia may demonstrate even better results than the  $T_N:R_N$  group, with a higher result for the group that will perform the training in normoxia and the recovery in hypoxia ( $T_N:R_H$ ), comparing the group that will perform training and recovery in hypoxia ( $T_H:R_H$ ). The justification for these two groups to train differently associated with hypoxia is that exercise intensity can be reduced with hypoxia exposure. In this way, to minimise this possibility, the group that will breathe hypoxia air only during the pause could perform the training stimulus in greater intensity (normoxia).

The expectation is that physical activity can be used to face the COVID-19 pandemic and other health problems as a complementary treatment. The consolidated evidence on the benefits of physical activity for physical, physiological, social, and mental health and the knowledge generated by this research project may contribute to solving priority health problems in Brazil. This project's results may contribute to the qualification of health care practices and promote scientific, technological, and innovation development in the Health area, aiming at strengthening the Unified Health System of Brazil.

Still, it is expected that the genetic variants studied may be associated with the recovery process of the convalescents of COVID-19 and with the effect of moderate training combined with intermittent normobaric hypoxia. The study of genetic variants can bring important advances in understanding the variation in responses related to the triggering and progression of some diseases and the effect of physical training.

To improve retention, we will provide: (i) written feedback to all participants about the assessments results at the beginning, after four, eight and 12 weeks of the intervention; (ii) periodic communications and presentations to inform the current status of the study, plans for the next phase, and motivating the participation; (ii) flexible as possible with training sessions and evaluations schedule.

Accessing participants can be difficult due to the social isolation imposed by the pandemic. This limitation can be minimised by emphasising the benefits that the intervention proposed by the present study can promote and emphasising that all safety-related care will be taken.

The pandemic caused by the SARS-CoV2 virus is not yet under control, and the state of global emergency in Public Health remains declared. The number of deaths and new cases remains high. Even though the

recovery rate is high, changes are observed in recovered patients even after 30 days of the interruption of clinical signs or medical discharge, in addition to some undeclared sequelae. Thus, a moderate-intensity interval training program performed in intermittent normobaric hypoxia could be an efficient alternative in rehabilitating convalescents who presented moderate to severe symptoms to recover lung function, physical fitness, quality of life, and mental health, as well as immunological homeostasis, preventing chronic and autoimmune inflammatory diseases and preventing new infections. And the study of genetic variants can provide important advances in a better understanding of disease progression variability and proposed treatments.

## **Strengths and limitations**

Some points strengthen and support this proposal:

- consolidated evidence on the benefits of physical training for health promotion, prevention, and control of various diseases;
- decreased number and reduction in the severity of COVID-19 cases in cities with higher altitude;
- use of an innovative hypoxia utilisation protocol as an additional training stimulus, applicable to the participants' real-life context.
- increased EPO's production, stimulated by HIF-1 $\alpha$ , recent scientific relevance in Physiology and Medicine, with benefits that can counter the harmful effects of COVID-19;

Regarding limitations, it may be challenging to have access to participants due to social isolation in the pandemic context.

## **Trial status**

Recruiting started in September 2020 and is expected to be completed in December 2021. The current protocol is version 4 approved by the ethical committee on 14 August 2020. Currently (11 December 2020) we included 84 participants.

## **Abbreviations**

ACE: angiotensin-converting enzyme

AGTR1: angiotensin II type 1 receptor

AT: anaerobic threshold

AU: arbitrary units

BMI: body mass index

DASS – 21: Depression, Anxiety and Stress Scale

EPO: erythropoietin

FEV: forced expiratory volume

FiO<sub>2</sub>: inspired fraction of O<sub>2</sub>

FVC: forced vital capacity

H: hypoxia

HIF-1 $\alpha$ : hypoxia-inducible-factor-1 $\alpha$

HRV: heart rate variability

IPAQ: International Physical Activity Questionnaire

iVO<sub>2</sub>max: exercise intensity corresponding to VO<sub>2</sub>max

[La-]: blood lactate concentration

N: normoxia

O<sub>2</sub>: oxygen

PAR-Q: Physical Activity Readiness Questionnaire

PCR: polymerase chain reaction

PP: peak power

qPCR: real-time polymerase chain reaction

R: recovery

RPE: the rate of perceived exertion

SF-12: 12-Item Health Survey

SPO<sub>2</sub>: blood oxygen saturation

T: training

Trimp: internal training load

USP: University of Sao Paulo

VO<sub>2</sub>max: maximum oxygen consumption

WC: waist circumference

## **Declarations**

### **Availability of data and materials**

The datasets used and/or analyzed during the current study will be made available from the corresponding author upon reasonable request.

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### **Authors contributions**

AAT, MCC, ACC, EMN, and JBS made substantial contributions to the conception of and contributed to drafting the work and revising important intellectual content. AAT, JALR, EASL, CAS, and MP made significant contributions to the work design. All authors gave final approval of the version to be published.

### **Ethics approval**

The entire protocol was reviewed and approved by the School of Physical Education and Sport of Ribeirão Preto – University of São Paulo (USP) (CAAE: 33783620.6.0000.5659) and Faculty of Pharmaceutical Sciences of Ribeirão Preto –USP (CAAE: 33783620.6.3001.5403) Research Ethics Committees. All participating patients will provide written informed consent.

### **Consent for publication**

This manuscript does not contain individual personal data from patients.

### **Competing interests**

The authors declare that they have no competing interests.

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# Figures

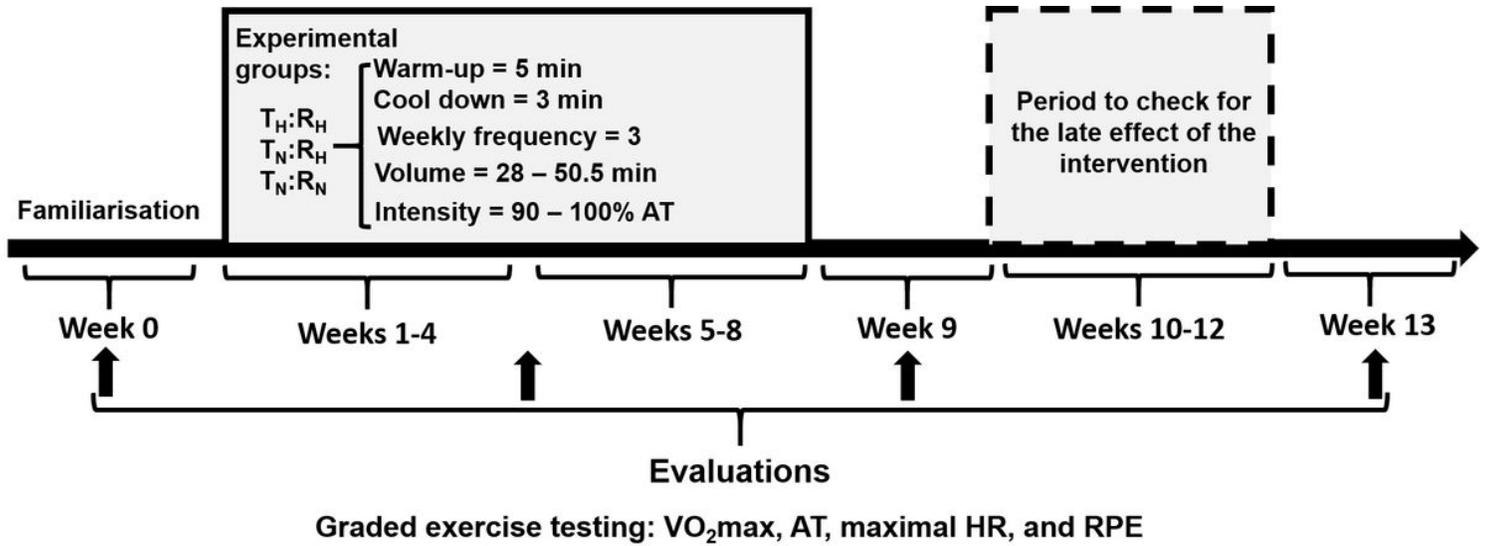


Figure 1

Experimental design.  $VO_2\text{max}$  – maximal oxygen uptake; AT - anaerobic threshold; HR - heart rate; RPE - rate of perceived exertion.

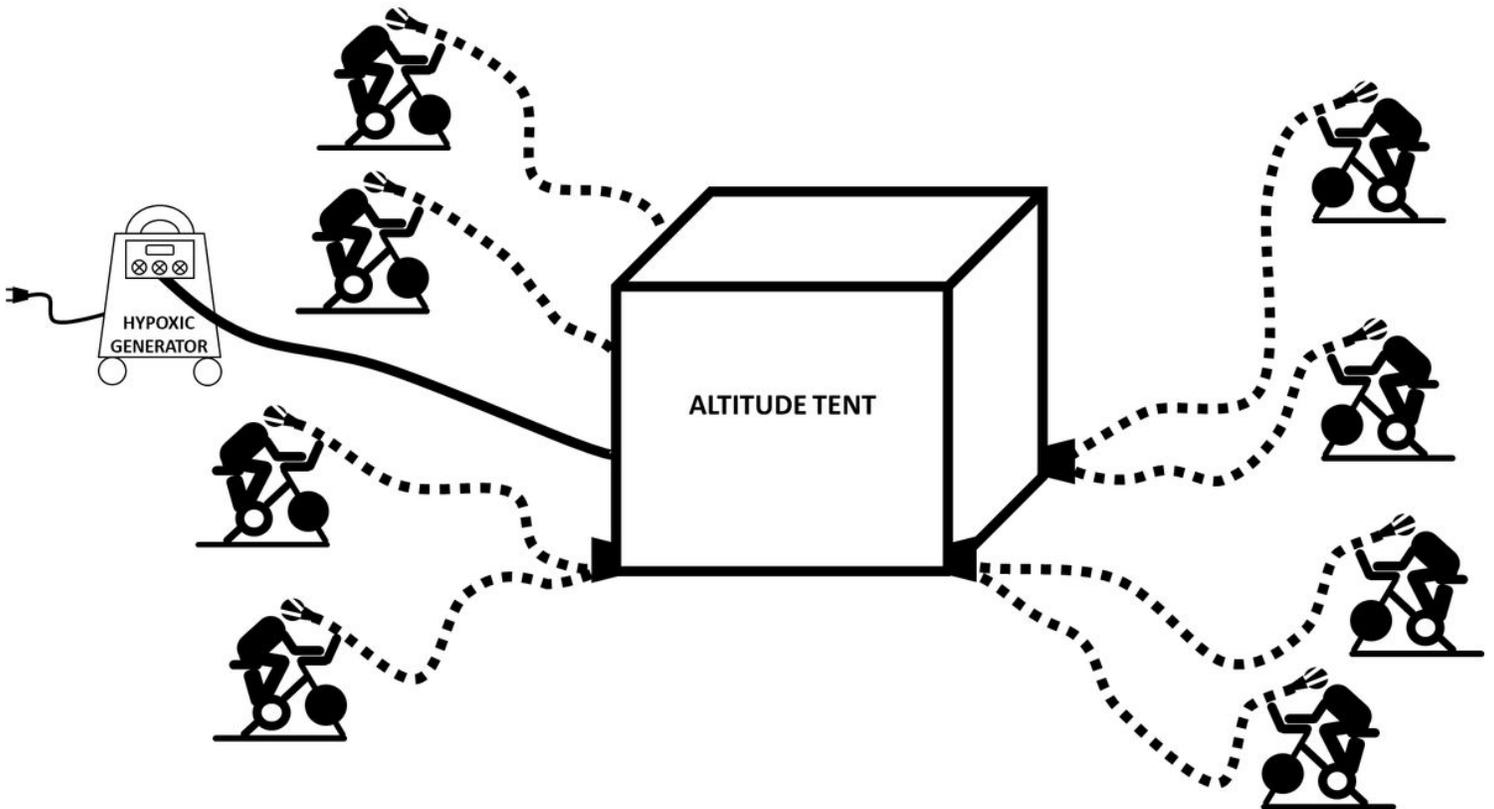
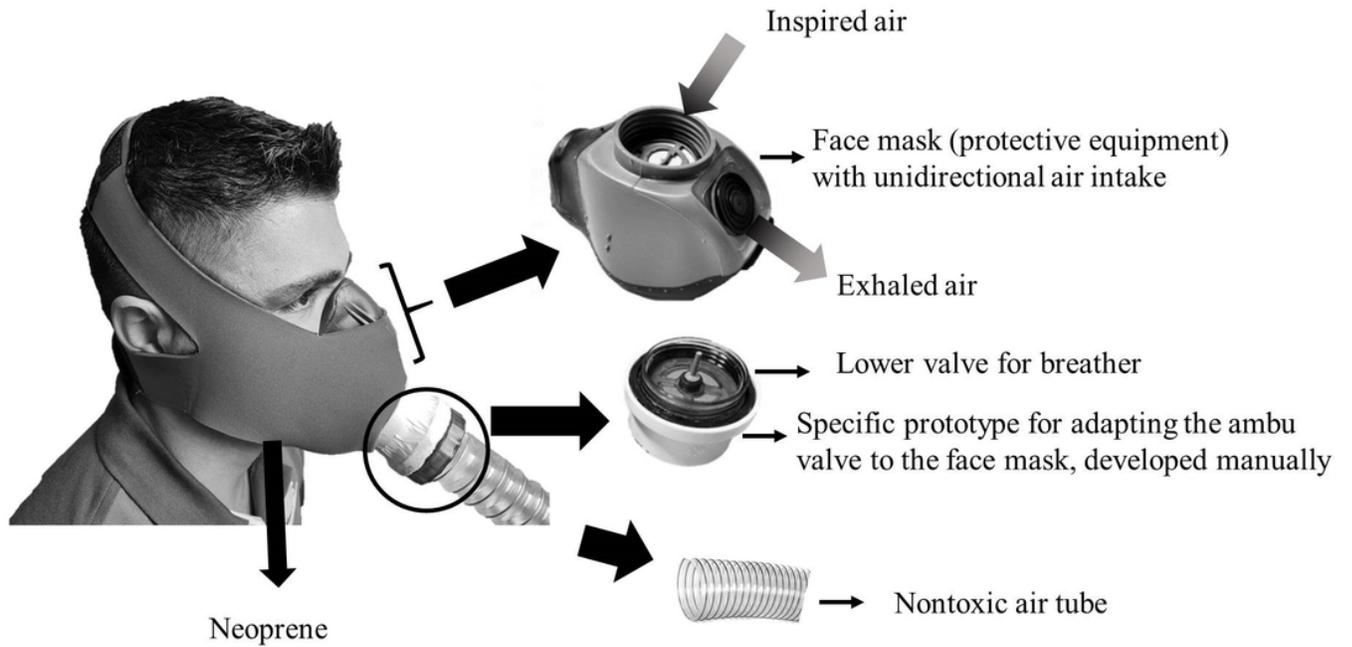


Figure 2

Strategy for training participants.



**Figure 3**

Individual mask for training participants.

## Supplementary Files

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