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Efeitos de 8 semanas de treino de moderada intensidade em cicloergômetro sob hipoxia intermitente e 8 semanas de follow-up na aptidão física e cardiorrespiratória e na eritropoiese de convalescentes da covid-19: estudo AEROBICOVID

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Área de concentração: Atividade física e esporte.

Orientador: Prof. Dr. Marcelo Papoti

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Efeitos de oito semanas de treinamento em cicloergômetro sob hipóxia intermitente e oito semanas de follow-up na aptidão física e cardiorrespiratória e na eritropoiese de convalescentes da covid-19: estudo AEROBICOVID, 2024. 60 p : il. ; 30 cm

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COSTA, Gabriel Peinado. **Efeitos de 8 semanas de treino de moderada intensidade em cicloergômetro sob hipoxia intermitente e 8 semanas de follow-up na aptidão física e cardiorrespiratória e na eritropoiese de convalescentes da covid-19: estudo AEROBICOVID**. 2024. Dissertação (Mestrado em Educação Física e Esporte) – Escola de Educação Física e Esporte de Ribeirão Preto, Universidade de São Paulo, 2024.

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RESUMO

COSTA, Gabriel Peinado. **Efeitos de 8 semanas de treino de moderada intensidade em cicloergômetro sob hipoxia intermitente e 8 semanas de follow-up na aptidão física e cardiorrespiratória e na eritropoiese de convalescentes da covid-19: estudo AEROBICOVID**. 2024. Dissertação (Mestrado em Educação Física e Esporte) – Escola de Educação Física e Esporte de Ribeirão Preto, Universidade de São Paulo, 2024.

Este estudo teve como objetivo analisar os efeitos de 8 semanas de treinamento de intensidade moderada em cicloergômetro sob hipóxia intermitente e 8 semanas de follow-up na aptidão física e cardiorrespiratória e na eritropoiese de pacientes recuperados da covid-19. Neste sentido, um ensaio clínico controlado e randomizado foi realizado com 84 participantes adultos com aproximadamente 30 dias após a liberação médica da covid-19, que foram submetidos à coleta de sangue, teste cardiorrespiratório e de caminhada antes (T1), após 8 semanas de intervenção (T2) e após 8 semanas de interrupção do treinamento (T3; acompanhamento). Além do grupo de controle (GC, n=25), os participantes foram divididos aleatoriamente em três grupos: normóxia (G_N, n=20), recuperação de hipóxia (G_{HR}, n=20) e hipóxia (G_H, n=19). A intervenção de exercício foi realizada em um cicloergômetro com um aumento gradual da carga de acordo com os períodos estabelecidos, 3 vezes por semana em séries de 5 min (90-110% limiar anaeróbio 2) e uma recuperação de 2,5 min. Durante todas as sessões de treinamento, foram monitorados a saturação periférica de oxi-hemoglobina (SpO_2), a frequência cardíaca (FC), a percepção subjetiva de esforço (RPE), a concentração sanguínea de lactato ($[La^-]$) e os sintomas do mal agudo da montanha. O primeiro estudo concentrou-se no monitoramento das variáveis das sessões de treinamento, analisando a eficiência e a segurança aguda do protocolo de treinamento e hipóxia. A estratégia adotada promoveu efetivamente a simulação de altitude. Essa estratégia demonstrou ser uma abordagem bem tolerada e segura durante as sessões, conforme indicado pelo baixo índice de doença aguda das montanhas e pelos valores estáveis de FC e SpO_2. O segundo estudo concentrou-se nos efeitos da intervenção e do follow-up sobre o consumo de oxigênio pico (VO_{2PICO}), a distância no teste de caminhada de 6 min, a eritropoietina (EPO), reticulócitos (RET), eritrócitos e hemoglobina. Os grupos expostos à hipóxia apresentaram maiores ganhos no VO_{2PICO}, teste de caminhada de seis min, EPO e RET. Além disso, não houve perda significativa de VO_{2PICO} no G_{HR} e houve manutenção do teste de caminhada de 6 min no G_H, ambos após o acompanhamento.

Palavras-chave: Exercício físico. Hipóxia. Altitude. Aptidão física. Covid-19.

ABSTRACT

COSTA, Gabriel Peinado. **Effects of 8 weeks of moderate-intensity cycle ergometer training on intermittent hypoxia and 8 weeks of follow-up on physical and cardiorespiratory fitness and erythropoiesis in COVID-19 convalescents: AEROBICOVID study**. 2024. Dissertação (Mestrado em Educação Física e Esporte) – Escola de Educação Física e Esporte de Ribeirão Preto, Universidade de São Paulo, 2024.

This study aimed to analyze the effects of 8 weeks of moderate-intensity cycle ergometer training on intermittent hypoxia and 8 weeks of follow-up on physical and cardiorespiratory and erythropoiesis in patients recovered from COVID-19. Therefore, a randomized controlled clinical trial was performed with 84 adult participants approximately 30 days after COVID-19 medical release. They underwent blood sampling, cardiorespiratory and walking tests before (T1), after 8 weeks of intervention (T2), and after 8 weeks of training cessation (T3; follow-up). Besides the control group (CG, n = 25), participants were randomly divided into 3 groups: normoxia (G_N, n = 20), hypoxia recovery (G_{HR}, n = 20), and hypoxia (G_H, n = 19). The exercise intervention was carried out on a cycloergometer with a gradual load increase according to the established periods (3-6 sets), 3 times a week in sets of 5-minute (90-110% anaerobic threshold 2), and a 2.5-minute recovery. During all training sessions, peripheral oxyhemoglobin saturation (SpO_2), heart rate (HR), rate of perceived exertion (RPE), blood lactate concentration ($[La^-]$) and symptoms of acute mountain sickness have been monitored. The first study focused on monitoring variables of the training sessions, analyzing the effectiveness and acute safety of the training and hypoxia protocol. The current strategy effectively promoted altitude simulation. This strategy was well-tolerated and acutely safe exposure during the sessions, as indicated by the low acute mountain sickness and the stable HR and SpO_2 values. The second study focused on the intervention and follow-up effects on peak oxygen consumption (VO_{2PEAK}), distance in the six-minute walk test, erythropoietin (EPO), reticulocytes (RET), erythrocytes and hemoglobin. Groups exposed to hypoxia showed greater gains in VO_{2PEAK}, 6-minute walk test, EPO, and RET. Furthermore, there was no significant loss of VO_{2PEAK} in the G_{HR} and maintenance of the 6-minute walk test in the G_H, both post follow-up.

Keywords: Physical exercise. Hypoxia. Altitude. Physical fitness. Covid-19.

SUMÁRIO

1. APRESENTAÇÃO

A presente dissertação seguiu o modelo de conjunto de artigos, composta por dois trabalhos, que fazem parte de um grande projeto denominado AEROBICOVID, liderado pelo Prof. Dr. Átila Alexandre Trapé e conta com a colaboração dos docentes Prof. Dr. Marcelo Papoti e Prof. Dr. Carlos Arterio Sorgi. O primeiro, um trabalho publicado, apresentando os dados de monitoramento da intervenção e o segundo, um trabalho apto para publicação, apresentando os efeitos da mesma.

O estudo 1 (Effectiveness, implementation, and monitoring variables of intermittent hypoxic bicycle training in patients recovered from COVID-19: The AEROBICOVID Study) publicado em 02 de novembro de 2022 na revista Frontiers in Physiology (doi: 10.3389/fphys.2022.977519), teve como objetivo analisar a eficiência, tolerância e segurança aguda de 24 sessões de treino em cicloergômetro realizadas em condições de hipóxia intermitente, por meio da análise da saturação periférica de oxihemoglobina, frequência cardíaca, percepção subjetiva de esforço, concentração de lactato sanguínea ($[La^-]$) e sintomas de doença aguda da montanha em pacientes recuperados da covid-19.

O estudo 2 (Effects of intermittent hypoxia training on blood variables and physical and cardiorespiratory fitness in convalescents from covid-19: the AEROBICOVID study), teve como objetivo analisar os efeitos de oito semanas de treinamento em cicloergômetro seguido por oito semanas de follow-up na aptidão física e cardiorrespiratória, eritropoietina, contagem de reticulócitos, eritrócitos e hemoglobina em convalescentes da covid-19.

1.1. Contextualização

O surto da doença covid-19, causada pelo vírus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), teve início em Wuhan na China em novembro de 2019 e evoluiu para um problema complexo e global. Em março de 2020 a Organização Mundial de Saúde (OMS) elevou o estado da contaminação da covid-19 à pandemia (World Health Organization, 2023).

A covid-19 pode causar problemas de saúde persistentes mesmo em casos mais leves, como tosse, dor no peito, dispneia, distúrbios cognitivos, insônia, falta de ar e fadiga (Batiha, G. E. S. *et al.*, 2022; Townsend, Dowds, O'Brien, Sheill, Dyer, O'Kelly, Hynes, Mooney, Dunne, Cheallaigh, *et al.*, 2021). Esses aspectos podem estar relacionados a dificuldades na manutenção das atividades de vida diária e funcionalidade (Greenhalgh, Knight, A’Court, Buxton e Husain, 2020). As manifestações pós-covid-19 são variadas e sabe-se que perduram por meses a anos após a recuperação da fase aguda (Helmsdal, Hanusson, Kristiansen, Foldbo, Danielsen, Steig, B. á, *et al.*, 2022). Como o mal-estar pós-exercício e a incapacidade funcional também podem ser sintomas da covid longa, a atividade física e os níveis de aptidão física destas pessoas podem ficar comprometidos (Gennaro, Di *et al.*, 2023; Twomey, DeMars, *et al.*, 2022).

Foi observado que pessoas que vivem na altitude, com menores níveis de oxigênio (O_2), apresentam menor prevalência da covid-19 bem como menor gravidade nos casos de infecção (Accinelli e Leon-Abarca, 2020; Arias-Reyes *et al.*, 2020). Os fatores que podem estar envolvidos nesta menor suscetibilidade à covid-19 envolvem as adaptações fisiológicas e anatômicas nos pulmões, com melhora para a perfusão e a capacidade, mas principalmente a ativação do fator induzível por hipóxia 1α (hypoxia-inducible-factor-1α - HIF-1α) (Choquenaira-Quispe, Saldaña-Bobadilla e Ramirez, 2020).

Além da exposição passiva à hipóxia, o treinamento associado à hipóxia tem sido tratado como uma estratégia promissora para a saúde (Behrendt, Bielitzki, Behrens, Herold, *et al.*, 2022; Camacho-Cardenosa *et al.*, 2020), mesmo com a dificuldade de se estabelecer uma dose ideal de hipóxia (Lizamore *et al.*, 2016). Diversos estudos associaram o treinamento físico e a hipóxia normobárica, mostrando como a hipóxia moderada (2500 a 3000 m de altitude simulada) é uma prática segura (Navarrete-Opazo e Mitchell, 2014), que apresenta resultados significativos em comparação com situações normóxicas, como redução da massa gorda, aumento da massa magra e melhora cardiorrespiratória (Camacho-Cardenosa *et al.*, 2018). Além disso, tem sido proposto um menor stress mecânico com alterações fisiológicas similares em pessoas com condições especiais de saúde (Girard, Matic Girard e Peeling, 2021; Pramsohler *et al.*, 2017). Um estudo recente de Carvalho *et al.* (2022) demonstrou que a exposição hipóxica durante os intervalos entre os esforços pode ser um estímulo adicional, representado por alterações na FC e na saturação periférica de oxihemoglobina (SpO_2) ao treinamento, sem prejudicar a carga externa e a qualidade das sessões de treinamento. Esse método permite a manutenção da qualidade do exercício e ainda apresenta os benefícios da exposição à hipóxia intermitente.

Especificamente em relação à covid-19, o fator induzido por hipóxia (HIF-1α) pode aumentar a expressão do gene para produzir eritropoietina (EPO), contrapondo os efeitos negativos da covid-19, já que dentre os benefícios da EPO estão neuroproteção, estimulação da ventilação central, proteção do endotélio e vasodilatação pulmonar, produção de hemácias e efeito anti-inflamatório (Soliz *et al.*, 2020).

Os avanços nesta área mostraram que o treino em hipóxia pode ser benéfico em condições clínicas como a doença coronária e a doença pulmonar obstrutiva crônica (Camacho-Cardenosa *et al.*, 2018, 2020), e proporciona menos stress mecânico (Camacho-Cardenosa *et al.*, 2019; Żebrowska *et al.*, 2019), o que pode ser interessante em algumas condições de saúde específicas em que as pessoas têm mobilidade reduzida, sofrem de fadiga cronica ou têm uma tolerância ao exercício diminuída, como é o caso das pessoas que vivem com covid-19 (Choquenaira-Quispe, Saldaña-Bobadilla e Ramirez, 2020; Intimayta-Escalante, Rojas-Bolivar e Hancco, 2020).

Até setembro de 2022, já foram registrados mais de 380 ensaios em 42 países, a maioria dos quais investigando a reabilitação destes doentes (Fawzy *et al.*, 2023). Assim, com um elevado número de doentes recuperados da covid-19 e sintomas remanescentes, as intervenções não farmacológicas para recuperar a funcionalidade são fundamentais.

Portando, a partir do alto número de pessoas acometidos pela covid-19, com sintomas persistentes, do conhecido benefício do exercício físico e o potencial uso da hipóxia, desenvolveu-se o projeto para investigar os efeitos do exercício físico associado à hipóxia na recuperação de convalescentes da covid-19.

1.2. Objetivos

1.2.1. Objetivo geral

Analisar os efeitos de oito semanas de treinamento de moderada intensidade associada à hipóxia, seguida por oito semanas de follow-up, sob variáveis de aptidão física e cardiorrespiratória e variáveis sanguíneas em pacientes recuperados da covid-19.

1.2.2. Objetivos específicos

Analisar a eficiência, tolerância e segurança aguda de 24 sessões de treino em cicloergômetro realizadas em condições de hipóxia intermitente, por meio da análise da saturação periférica de oxihemoglobina, frequência cardíaca, percepção subjetiva de esforço, concentração de lactato sanguínea ([La-]) e sintomas de doença aguda da montanha em pacientes recuperados da covid-19 em convalescentes da covid-19 (Estudo 1).

Analisar os efeitos de oito semanas de treinamento em cicloergômetro seguido por oito semanas de follow-up na aptidão física e cardiorrespiratória, eritropoietina, contagem de reticulócitos, eritrócitos e hemoglobina em convalescentes da covid-19 (Estudo 2).

2. ESTUDO 1

Effectiveness, implementation, and monitoring variables of intermittent hypoxic bicycle training in patients recovered from COVID-19: The AEROBICOVID Study

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2.1. Introduction

COVID-19, caused by the SARS-CoV-2 coronavirus, was initially characterized as an severe acute respiratory syndrome. However, after 526 million confirmed cases worldwide until June 2022 (World Health Organization, 2022), it is now known that not only during infection but also afterward, harmful effects can occur in the respiratory tract and lungs, as well as in the cardiovascular, nervous, and other systems (Gupta *et al.*, 2020; Higgins *et al.*, 2021).

Physical training, performed three to five days a week, lasting 15 to 45 minutes, continuously or intermittently, at moderate intensity, with heart rate and rate of perceived exertion to prescribe and monitor exercise, has been highly recommended to recover cardiopulmonary function (Bhatia *et al.*, 2020; Laddu *et al.*, 2021; Simpson e Katsanis, 2020) and improve the immune and nervous system, in patients affected by COVID-19 (Calabrese *et al.*, 2021; Zhao, Xie e Wang, 2020). The adequate load results in beneficial adaptations, for example, a 6-week rehabilitation program that consisted of walking or treadmill exercise successfully improved respiratory capacity and endurance (Millet, Gregoire P. *et al.*, 2016a). In contrast, people hospitalized due to COVID-19, who did not undergo exercise or rehabilitation when subjected to cardiopulmonary exercise test after three months of hospital discharge, still had a lower peak oxygen uptake (VO_{2PEAK}) than expected (Skjørten *et al.*, 2021).

It is essential to highlight the need to monitor and adequately control the training load. Proper exercise monitoring ensures that the patient/athlete adapts to the exercise program and minimizes the probability of developing an injury/illness or reaching an overtraining state (Halson, 2014). Training load refers to quantification by training volume and intensity. External load uses external intensity parameters, either resistance, speed, or power. In contrast, internal load quantifies physiological stress, through biomarkers, e.g., heart rate (HR), rate of perceived exertion (RPE), blood lactate concentration ($[La^-]$), ventilation, or oxygen uptake (Halson, 2014).

Hypoxic training has been treated as a promising strategy for health (Behrendt, Bielitzki, Behrens, Herold, *et al.*, 2022; Camacho-Cardenosa *et al.*, 2020), even with the difficulty of establishing an optimal hypoxia dose (Lizamore *et al.*, 2016). Regarding hypoxia as an additional stimulus to exercise and the exercise workload, it is important to control the dose of exposure to hypoxia (Behrendt, Bielitzki, Behrens, Herold, *et al.*, 2022; Millet, Grégoire P. *et al.*, 2016; Soo *et al.*, 2020). Several studies associated physical training and normobaric hypoxia, showing as moderate hypoxia (2,500 to 3,000 m simulated altitude) is a safe practice (Navarrete-Opazo e Mitchell, 2014), which presents significant results compared to normoxic situations, like fat mass reduction (Camacho-Cardenosa *et al.*, 2018), lean mass increase (Matsumoto *et al.*, 1999), cardiorespiratory improvement (Camacho-Cardenosa *et al.*, 2018). In addition, less mechanical stress with similar physiological changes have been shown in the special health condition population (Maggiorini *et al.*, 1998; Pramsohler *et al.*, 2017). A recent study by Carvalho *et al.* (2022) demonstrated that hypoxic exposure during the intervals between efforts can be an additional stimulus, represented by changes in HR and peripheral oxyhemoglobin saturation (SpO_2) to training, without impairing the external load and the quality of the training sessions. This method allows maintenance of exercise quality and still has the benefits of exposure to intermittent hypoxia.

Therefore, hypoxic training could be considered a potential treatment to optimize recovery in COVID-19 convalescents. However, close monitoring is strongly recommended to ensure patient safety and enable its use in healthcare. Training monitoring using HR, SpO_2, and RPE, among other variables, is widely used to identify desired and undesired responses (Chapman *et al.*, 2011; Deb *et al.*, 2018; Maggiorini *et al.*, 1998; Naeije, 2010) while ensuring patient safety. Therefore, this study aimed to evaluate the effectiveness and acute safety of 24 training sessions of bicycle training associated with intermittent hypoxic through the description of SpO_2, HR, RPE, $[La^-]$ analyses, in addition evaluating tolerance of hypoxic training in patients recovered from COVID-19.

2.2. Materials and Methods

2.2.1. Design and participants

The present study follows the work of the AEROBICOVID project, a clinical trial controlled double-blind study were performed between September and December 2020, and details could be found elsewhere (Trapé *et al.*, 2021). Participants aged 30 - 69 years and COVID-19 convalescents (with a positive diagnostic test) who had symptoms approximately 30 days since recovery from clinical signs or medical discharge were included. In addition, exclusion criteria were: individuals exposed to high altitude >1,500 m in the past three months, with significant physical limitations to perform the intervention, acute or chronic medical conditions without medical supervision, having anemia, using immunosuppressive drugs, being pregnant, hormone replacement, smokers, and excessive use of alcohol or drugs.

Participants were divided into three groups according to the combination of effort and recovery in normoxia and hypoxia conditions, i.e., training in normoxia and recovery in normoxia (G_N); training in normoxia and recovery in hypoxia (G_{HR}); and training in hypoxia and recovery in hypoxia (G_H). The randomization was performed by four groups, with participants being directed to the control or one of three training groups. For the participants' allocation to groups, it was taken into consideration the variables, gender, age, participant's fitness level (result in the incremental test), and gravity during the disease (COVID-19).

Blinding was done between the two research teams (evaluation and monitoring teams) and the participants.

The COVID-19 severity has been defined based on National Institutes of Health of United States of America criteria (Galloway *et al.*, 2020; Gude, Riveiro, Rodríguez-Núñez, Ricoy, Lado-Baleato, Lourido, Rábade, Lama, Casal, Abelleira-París, Ferreiro, Suárez-Antelo, Toubes, Pou, Taboada-Muñiz, Calle-Velles, Mayán-Conesa, Molino, M. L. P. del, *et al.*, 2020). For, mild severity: have any symptoms of COVID-19, such as fever, cough, etc., but do not have shortness of breath or dyspnoea; moderate: have any symptoms of COVID-19 and have shortness of breath or dyspnoea; severe: have any symptoms of COVID-19 and need hospitalisation, but not intensive care; or critical: have any symptoms of COVID-19 and need hospitalisation and intensive care.

This study was approved by the Research Ethics Committees from the School of Physical Education and Sport of Ribeirao Preto – University of Sao Paulo (USP) and School of Pharmaceutical Sciences of Ribeirao Preto - USP (CAAE: 33783620.6.0000.5659; CAAE: 33783620.6.3001.5403, respectively) and registered in the Brazilian Registry of Clinical Trials (RBR-5d7hkv).

2.2.2. Instruments

The research experimental setup included two tents (Colorado Altitude Training Tent^{TM}, USA), with 12,000 liters of air capacity, and a hypoxia generator (CAT-430^{TM}, Altitude Control Technologies, USA) for each tent (Trapé *et al.*, 2021). There were participants from all three training groups around the tents and individual hoses directed towards the tent, all being covered by a tarp. Both tents had a tarp around them for blinding, hiding where the hoses (IVPU, vacuum air PU 1.1/2-cm) were attached to the tents. The hoses were located at the lower corners of the tents in all groups. In the GN, participants breathed ambient air because the hoses were on the side of the tents (outside) but covered by the tarp; in the GHR and GH, the participants breathed air with lower oxygen concentration, so the hoses were inside the tents, also covered by the tarp. The bicycles were positioned at a distance, which prevented visualization of the positioning of the hoses. At the end of the intervention, the participants received a questionnaire to answer between two options, whether they believed they belonged to the group in hypoxia or normoxia.

There were three types of bicycles, aiming to meet each participant's limitations and individualities (e.g., joint pain, mobility difficulties, balance insecurity or uncomfortable seating): vertical spinning, vertical ergometric, and horizontal ergometric. In addition, each participant received a kit with a unidirectional mask (Air safety, Brazil) for individual use throughout intervention (Trapé *et al.*, 2021) and a training diary. Each team member supervised up to three participants and was responsible for monitoring, collecting data, timing each training moment, and informing the participant what should be done.

2.2.3. Procedure

The hypoxia tents were initially designed for individual use, with the participant inside. The proposed new strategy, employed two tents and provided 16 participants simultaneously; four under hypoxia and four under normoxia.

Some procedures were carried out to avoid participants' re-infection. Besides maintaining distance between them and each one receiving a unidirectional mask kit for individual use, all connections between the hose and masks remained submerged under hypochlorite for at least thirty minutes after use. These connection materials were washed with alcohol 70% and, after thirty minutes, were wrapped with a plastic paper.

Regarding the protection of the research team, each member received an individual face shield and used a disposable apron and cap beside the surgery mask. In addition, all disposable equipment was changed between sessions, and permanent equipment was sterilized with alcohol 70%.

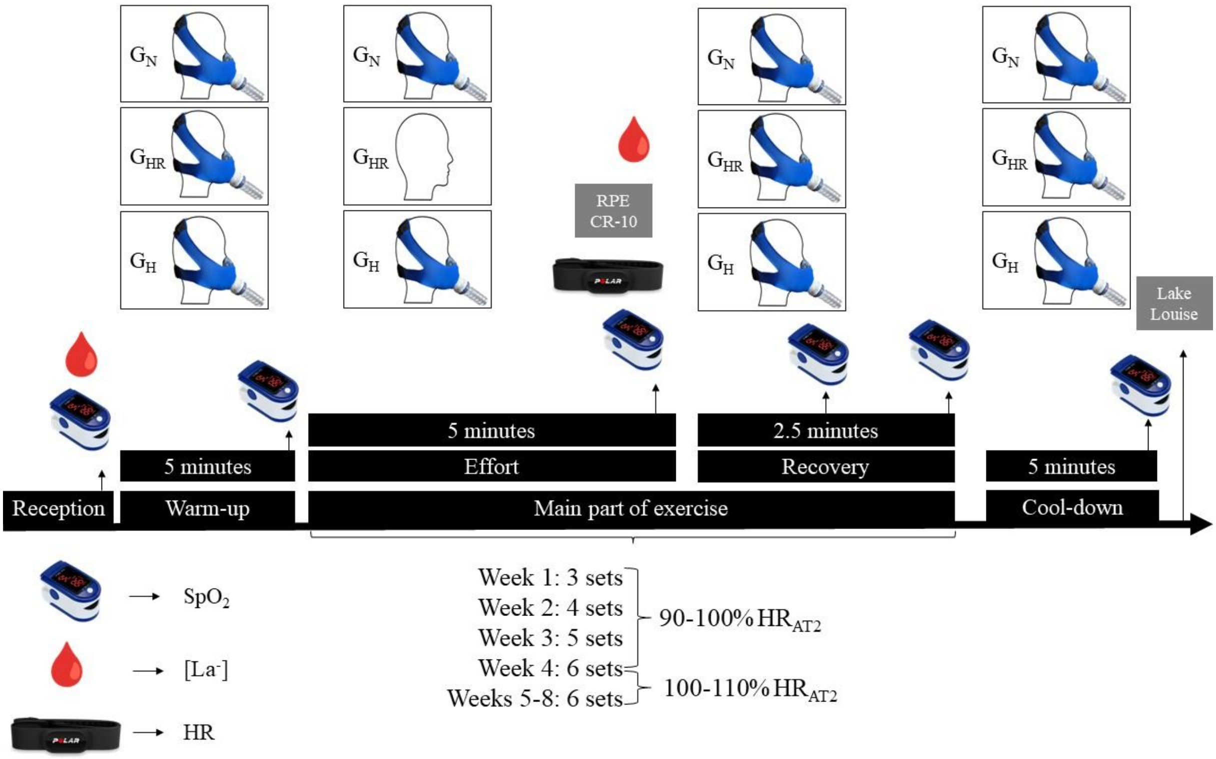
A cardiopulmonary exercise incremental test was performed in a pendular cycle ergometer with mechanical braking (Ergometrica, Monark, Brazil) to determine the training intensity. Initially, the participants started a 5-minute warm-up without any additional load; after that, the intensity was increased by 0.25 kp (~15 watts) every two minutes until the participant did not maintain the 60-rpm cadence or volitional exhaustion. Blood samples (25 µL) were collected from the earlobe at the end of each stage using previously calibrated heparinized capillaries. Blood samples were immediately dispensed and homogenized in microtubes containing 1% sodium fluoride for [La^-] analysis using the YSI 2300 STAT analyzer (Yellow Springs, OH, USA). Concomitantly, HR and RPE were monitored at the end of each stage.

Anaerobic threshold 2 (AT2) was calculated for each subject from a blood lactate plot versus exercise intensity. Initially, the inflection point in the blood [La$^-$] was determined by visual inspection. Subsequently, two linear regressions were plotted (before and after the inflection point), and the intercept of the lines $(y' = y) = \left(\frac{(b'-b)}{(a'-a)}\right)$ was defined as AT2 (Matsumoto *et al.*, 1999; Papoti *et al.*, 2009). Having calculated AT2 intensity, the HR value referring to that intensity was assumed as 100%, and each participant should maintain the HR ranges during the workouts were calculated.

2.2.4. Intervention protocol

The bicycle training was performed three times per week and consisted of three parts (warm-up, main part of exercise, and cool-down). The 5-min warm-up and the 3-min return to rest were performed in RPE 2, considered "easy" in a 0 to 10 scale (Foster *et al.*, 2001). The main part of exercise was composed of three to six sets according to the established periods: each set was composed of 5-min efforts at an HR corresponding to 90-100% (first to the fourth week) and 100-110% (fifth to the eighth week) of the AT2, followed by a pause of 2.5 min to recover between sets (Figure 1).

FIGURE 1 Experimental design of the training session.



G_N = group in normoxia; G_{HR} = group in hypoxia recovery; G_H = group in hypoxia; RPE = rate of perceived exertion; SpO_2 = peripheral oxyhemoglobin saturation; $[La^-]$ = blood lactate concentration; HR = heart rate; HR_{AT2} = heart rate relative to the anaerobic threshold 2.

Source: from (Costa *et al.*, 2022)

The G_N and G_H used the mask during the entire workout (warm-up, effort, recovery and cool-down); G_{HR} used the mask all the time (warm-up, recovery and cool-down) except during efforts in the main part of exercise. At the hypoxic moments, participants were exposed to an inspired fraction of O_2 (FiO_2) of ~13.5% (corresponding to 3,000 m altitude), being monitored inside the tent by an O_2 sensor (Oxygen Sensor R-17MED, Teledyne Analytical Instruments, USA). At normoxic moments, participants breathed ambient air, with a FiO_2 of ~20.9% (a city with 526 m altitude).

2.2.5. *Blood collection for lactate analysis*

Blood collections for quantifying [La^-] were performed at rest and at the end of each main part of exercise effort at weeks 2, 4, 6 and 8. [La^-] was collected and determined according to the procedure previously described.

2.2.6. *Exercise monitoring*

The SpO_2 was monitored by using a pulse oximeter (Portable, G-Tech Solutions, India) at rest (Rest), end of warm-up (W-Up), end of each effort (E-Effort), lowest value during recovery, end of each recovery (E-Recovery), and end of the cool-down (C-Down). For G_N and G_H, the value during recovery was the mean value between the end of the effort and the recovery (M-R); for G_{HR}, the value during recovery was the mean value among the end of the effort, the lowest value during recovery, and the end of recovery (M-R). The value during recovery was calculated individually for all training groups for each set and participant. The SpO_2 value collected at the end of the last effort was used to calculate the average between this point and the end of recovery (M-CD).

The training diary contained the HR interval information to be used during training effort and spaces to annotate HR and RPE at the end of each set. HR was tracked in real-time and individually throughout the training. The SpO_2 was recorded by the work team, positioning the device only when it was close to the moment of collection.

The monitoring of training intensity and the collection of values at the end of each effort of the main part of exercise were carried out by HR and RPE, using the Polar H10 tape and the scale adapted by Foster (1998), respectively.

2.2.7. *Acute Mountain Sickness*

The Lake Louise Scale (Maggiorini *et al.*, 1998; Roach *et al.*, 2018) was used to collect information related to acute mountain sickness and monitor acute responses to hypoxic exposure (headache, nausea/vomiting, fatigue, dizziness/light-headedness, and difficulty sleeping). The participants in the three training groups were asked to answer this questionnaire once a week. This questionnaire data should be used for descriptive purposes only and not to diagnose AMS, since there is a limitation because it is commonly applied in exposures longer than six hours.

2.2.8. Training zones

Using the HR data, the set percentage that participants remained below HR_{AT2} (<90%), within HR_{AT2} (90-100%; 100-110%), and above HR_{AT2} (>110%) were calculated. For RPE, the set percentage that participants remained below RPE_{AT2} ($<RPE_{AT2}$), equal to RPE_{AT2} ($=RPE_{AT2}$), and above RPE_{AT2} ($>RPE_{AT2}$) were quantified. Regarding [La^-], differently from AT2 calculations, the set percentage was evaluated in each training zone based upon fixed [La^-], aiming to reduce potential variations arising from nutritional status. For each zone, it was defined that below 2 mmol refers to zone 1 (Z1); between 2 and 4 mmol, zone 2 (Z2); and above 4 mmol, zone 3 (Z3).

2.2.9. Training load

The internal training load was calculated by training impulse (TRIMP) in arbitrary units (a.u.) using the average of the HR [$TRIMP_{HR}$ = HR * training volume (min)] and the RPE [$TRIMP_{RPE}$ = RPE * training volume (min)] (Foster, 1998) from the main part of exercise.

2.2.10. Statistical analysis

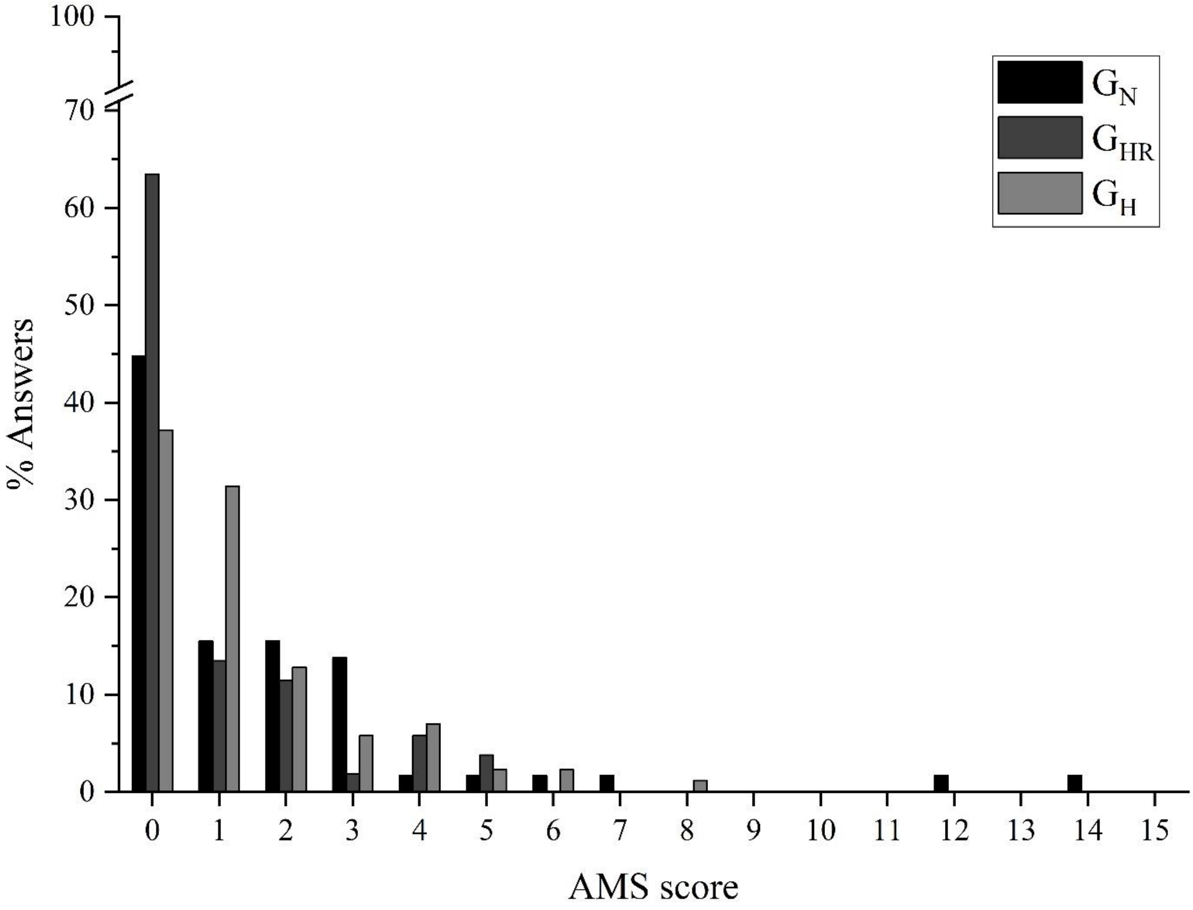
The continuous and ordinal variables were expressed in basic descriptive statistics, mean (standard deviation) and median (minimum-maximum values). The association between categorical variables has been analyzed by chi-square. The Shapiro-Wilk and the Levene test were used to determine data normality and homogeneity, respectively. A two-way ANOVA with Tukey's Post Hoc was performed to analyze and compare the groups and time variances. The significance level was set at 5% ($p < 0.05$) in all analyses, and the program used was JAMOVI version 2.3.

2.3. Results

2.3.1. Safety and effectiveness in the protocol implementation

According to COVID-19 severity, the distribution of participants among the groups was 4 (G_N), 4 (G_{HR}), and 3 (G_H) for mild severity; 11 (G_N), 12 (G_{HR}), and 17 (G_H) for moderate; 3 (G_N), 0 (G_{HR}), and 1 (G_H) for severe; and 3 (G_N), 2 (G_{HR}), and 1 (G_H) for critical. Blinding appears was successful since 52.2% of participants incorrectly answered the perception of their group belonging. Furthermore, no association was observed between the hypoxic exposure models adopted and self-report symptoms of acute mountain sickness ($p = 0.082$). More than 93% of the participants showed no (score up to 2) or mild (score from 3 to 5) acute mountain sickness throughout Lake Louise Scale (Figure 2).

Figure 2 - Acute mountain sickness symptom scores.



AMS = acute mountain sickness; GN = group in normoxia; GHR = group in hypoxia recovery; GH = group in hypoxia.

Source: from (Costa *et al.*, 2022)

Table 1 shows peripheral oxyhemoglobin saturation (SpO_2), heart rate (HR), and lactate concentration ($[La^-]$) training responses to 8 weeks of the intervention. G_{HR} showed significantly lower values of SpO_2 ($p < 0.001$) and percentage of maximum HR ($\%HR_{MAX}$) ($p < 0.001$) compared with G_N. G_H showed significantly lower values of SpO_2 and percentage of reserve HR ($\%HR_{RES}$) compared with G_N (SpO_2, $p < 0.001$; $\%HR_{RES}$, $p < 0.001$) and compared with G_{HR} (SpO_2, $p < 0.001$; $\%HR_{RES}$, $p < 0.001$), and showed significantly higher values of $\%HR_{AT2}$ compared with G_N ($p < 0.001$) and G_{HR} ($p < 0.001$). Still, $\%HR_{MAX}$ was significant higher in G_H compared with G_{HR} ($p < 0.001$).

Table 1 - Training characterization of the main part of exercise through peripheral oxyhemoglobin saturation (SpO_2), heart rate (HR), and the blood lactate concentration ($[La^-]$) over the 8 weeks of intervention.

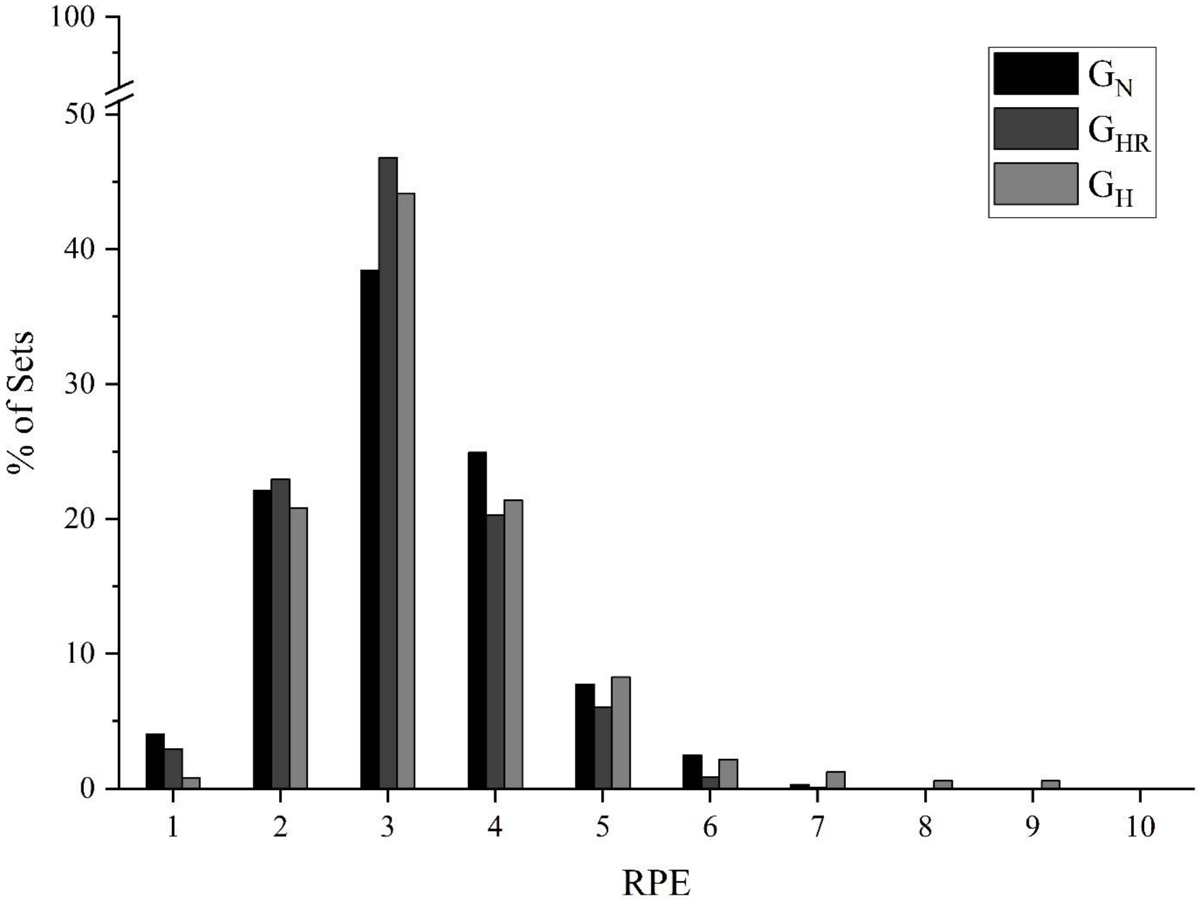
Group	n	SpO_2	$\%HR_{MAX}$	$\%HR_{AT2}$	$\%HR_{RES}$	$[La^-]$ (mM)
G_N	21	96.9(1.6)	88.3(8.0)	98.2(8.3)	175.5(46.3)	4.9(1.8)
G_{HR}	18	95.1(3.1)*	87.0(8.0)*	98.3(8.5)	176.4(41.8)	5.0(2.1)
G_H	22	87.7(6.5)$^{*\#}$	88.6(7.8)$^{\#}$	100.1(7.8)$^{*\#}$	162.0(27.0)$^{*\#}$	4.8(2.0)

G_N = group in normoxia; G_{HR} = group in hypoxia recovery; G_H = group in hypoxia; $\%HR_{MAX}$ = relative maximum heart rate; $\%HR_{AT2}$ = relative heart rate to the anaerobic threshold 2; $\%HR_{RES}$ = relative reserve heart rate; * = $p < 0.05$ compared to G_N; $^{\#}$ = $p < 0.05$ compared to G_{HR}.
Source: from (Costa *et al.*, 2022)

2.3.2. *Monitoring variables*

In all groups, the most frequent RPE was "3" (moderate), and all groups maintained less than 10% above RPE "5" (hard) (Figure 3).

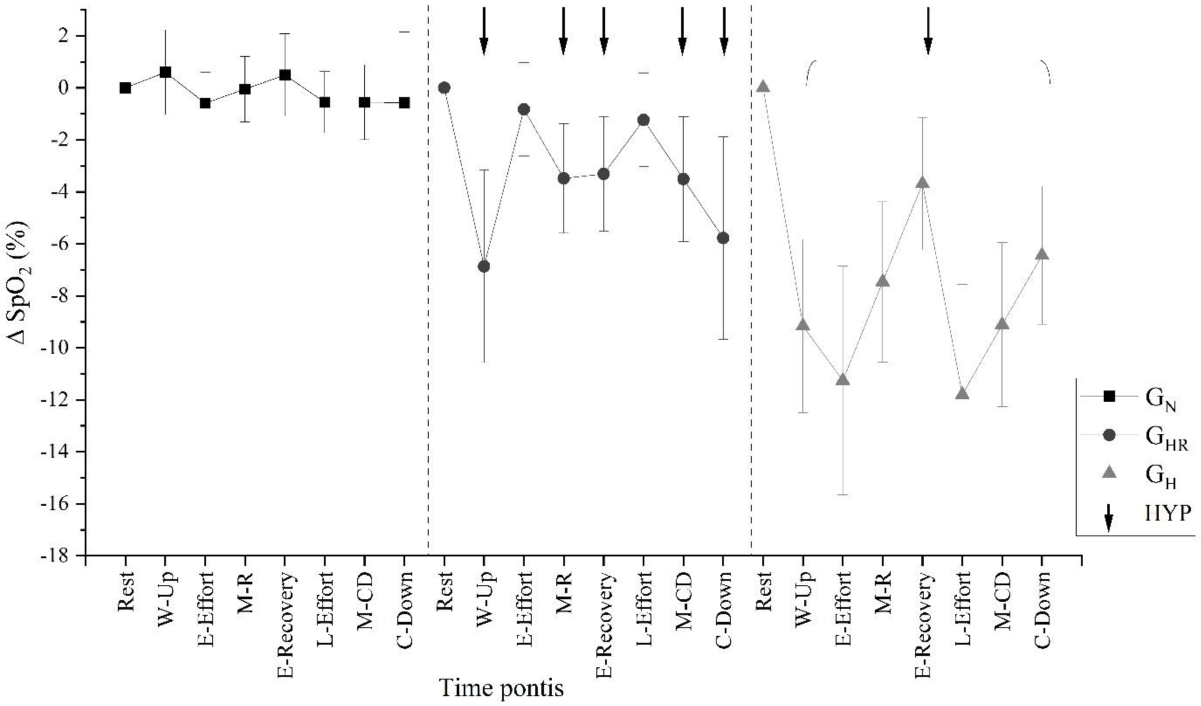
Figure 3 - Frequency and distribution of the rate of perceived exertion (RPE) in the main part of exercise from the eight weeks of intervention.



G_N = group in normoxia; G_{HR} = group in hypoxia recovery; G_H = group in hypoxia.
Source: from (Costa *et al.*, 2022)

The mean FiO_2 inside the tent was 13.42 (0.34) %, while ambient air was stable at 20.9 (0.0) %. Figure 4 shows the kinetics by the mean delta SpO_2 of each set measurement from the first week of training (rest, warm-up, end effort, during recovery, end recovery, last effort, during the return to cool-down, and after cool-down). SpO_2 decreases according to hypoxia exposure in G_{HR}, and a greater SpO_2 reduction magnitude when the hypoxia is associated with the effort in G_H.

Figure 4 - Average peripheral oxyhemoglobin saturation (SpO_2) by delta kinetics of each set measurement from the first week of training.



G_N = group in normoxia; G_{HR} = group in hypoxia recovery; G_H = group in hypoxia; HYP = times in hypoxia. Rest = rest; W-Up = warm-up; E-Effort = end effort; M-R = mean value during recovery; E-Recovery = end recovery; L-Effort = last effort; M-CD = average between "end of last effort" and "cool-down"; C-Down = cool-down.
Source: from (Costa *et al.*, 2022)

Table 2 presents the intra and between groups analysis of the SpO_2 during the first week of training. Regarding the analysis among groups at each time point, the G_{HR} showed a difference ($p < 0.001$) compared to the G_N according to hypoxic exposure in the warm-up, during recovery, end of recovery, and return to and after cool-down. Except at rest, G_H showed significantly lower ($p < 0.001$) SpO_2 values compared with G_N. In addition, SpO_2 at the end of the effort, during recovery, during the last effort, and during cool-down were significantly lower ($p < 0.005$) in G_H compared with G_{HR}. Regarding the intragroup analysis, all normoxic time points were significantly different from hypoxic time points ($p < 0.001$) in G_{HR}. G_H group showed statistically significant differences ($p < 0.001$) in each point compared with the rest (Table 2). G_N showed no difference for SpO_2 at any time point.

Table 2 - Average peripheral oxyhemoglobin saturation (SpO_2) of each measurement of a set from the first week of training.

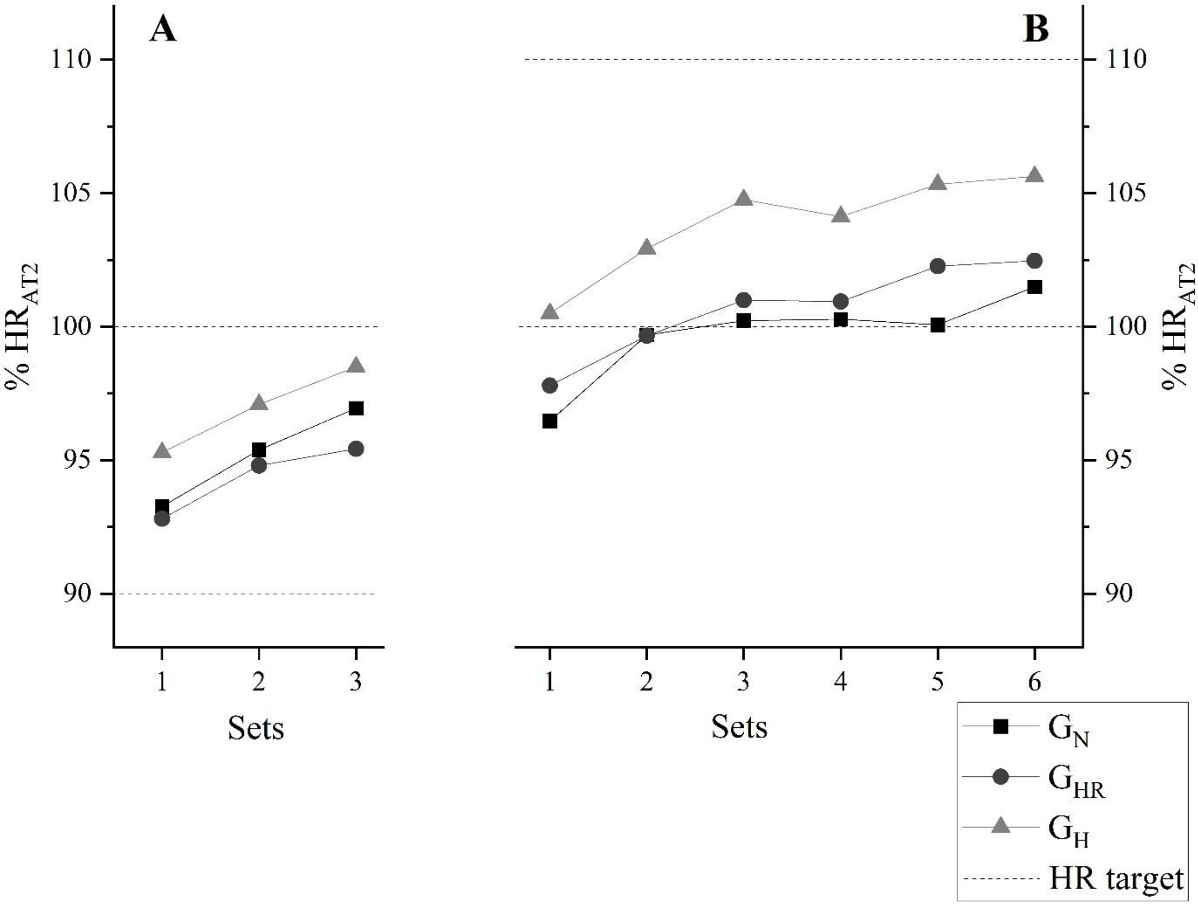
	Time points							
Group	Rest	W-Up	E-Effort	M-R	E-Recovery	L-Effort	M-CD	C-Down
G_N	96.5(2.8)	97.4(0.9)	96.2(1.9)	96.8(1.4)	97.3(1.2)	96.2(1.9)	96.4(1.9)	96.6(2.4)
G_{HR}	96.9(1.6)	88.6(4.6)*^{a}	95.8(2.3)^{b}	93.5(3.0)*^{ac}	93.5(3.6)*^{abc}	95.5(2.3)^{b}	93.3(3.1)*^{ac}	91.2(5.3)*^{acef}
G_H	95.9(3.5)	87.0(5.8)*^{a}	85.1(5.9)*$^{\#a}$	88.8(4.3)*$^{\#ac}$	92.4(3.8)*^{abcd}	84.7(5.4)*$^{\#ade}$	87.2(4.4)*$^{\#acef}$	89.8(4.5)*^{acefg}

G_N = group in normoxia; G_{HR} = group in hypoxia recovery; G_H = group in hypoxia. Rest = rest; W-Up = at the end of warm-up; E-Effort = at the end of end effort; M-R = mean value during recovery; E-Recovery = at the end of end recovery; L-Effort = at the end of last effort; M-CD = mean value during cool-down; C-Down = at the end of cool-down. * = $p < 0.05$ compared to G_N; $^{\#}$ = $p < 0.05$ compared to G_{HR}. a = $p < 0.05$ compared to Rest; b = $p < 0.05$ compared to W-Up; c = $p < 0.05$ compared to E-Effort; d = $p < 0.05$ compared to M-R; e = $p < 0.05$ compared to E-Recovery; f = $p < 0.05$ compared to L-Effort; g = $p < 0.05$ compared to M-CD.

Source: from (Costa *et al.*, 2022)

The participants predominantly stayed within the prescribed target (90-100% of the AT2 in the first four weeks and 100-110% from the fifth to the eighth week) during the sets performed (three sets on week 1 and six sets on week 8). No significant difference was observed among groups in HR values means over the sets neither at week 1 (Figure 5A) nor at week 8 (Figure 5B).

Figure 5 - Average heart rate relative to the anaerobic threshold 2 (HR_{AT2}) kinetics at the end of each set, at week 1, corresponding to 90-100% (**A**), and at week 8, corresponding to 100-110% (**B**).



G_N = group in normoxia; G_{HR} = group in hypoxia recovery; G_H = group in hypoxia.

Source: from (Costa *et al.*, 2022)

Analyzing the average HR of the 8 weeks of intervention, all groups remained predominantly in the HR_{AT2} range (90-100% and 100-110%). In addition, significant differences were found in the set percentage of these ranges compared with "below" (<90%) and "above" (>110%; $p < 0.001$). No differences were observed among groups (Table 3).

Table 3 - Comparison of the set percentage below, within, and above the heart rate relative to the anaerobic threshold 2 (HR_{AT2})

	% of sets below, within, and above the HR_{AT2}			
Group	<90%	90-100%	100-110%	>110%
G_N	16.6(21.7)	41.8(19.5)^a	36.1(25.9)^a	5.5(8.8)^{bc}
G_{HR}	14.7(19.6)	46.7(24.0)^a	32.1(21.9)	6.5(13.2)^{bc}
G_H	9.5(14.2)	43.4(19.4)^a	37.7(18.9)^a	9.5(13.8)^{bc}

G_N = group in normoxia; G_{HR} = group in hypoxia recovery; G_H = group in hypoxia. <90%= below HR_{AT2}; 90-100% = within the proposed HR; >100% = above HR_{AT2}. ^{a} = $p < 0.05$ compared to <90%; ^{b} = $p < 0.05$ compared to 90-100%; ^{c} = $p < 0.05$ compared to 100-110%.
Source: from (Costa *et al.*, 2022)

Analyzing the average HR of the set percentage below, equal to, and above the RPE of the AT2 over the 8 weeks of intervention (Table 4), participants remained predominantly ($p < 0.001$) below the RPE_{AT2} and this percentage was significantly different compared with RPE related to the AT2 ($p < 0.001$) and RPE above AT2 ($p < 0.001$). No significant difference among the groups was observed.

Table 4 - Comparison of the set percentage below, equal to, and above the heart rate relative to the anaerobic threshold 2 (HR_{AT2}) over the 8 weeks of intervention.

	% of sets below, equal to, and above RPE_{AT2}		
Group	$<RPE_{AT2}$	$=REP_{AT2}$	$>RPE_{AT2}$
G_N	63.9(36.1)	21.2(19.5)[a]	14.1(20.7)[a]
G_{HR}	71.8(28.1)	14.2(14.3)[a]	13.6(21.4)[a]
G_H	81.6(20.4)	12.2(15.6)[a]	6.2(11.7)[a]

G_N = group in normoxia; G_{HR} = group in hypoxia recovery; G_H = group in hypoxia; $<RPE_{AT2}$ = below RPE_{AT2}; $=RPE_{AT2}$ = equal to RPE_{AT2}; $>RPE_{AT2}$ = above RPE_{AT2}. a = $p < 0.05$ compared to $<RPE_{AT2}$.
Source: from (Costa *et al.*, 2022)

Regarding absolute [La$^-$], participants of all groups presented significantly higher [La$^-$] in all sets compared with the rest ($p < 0.001$). However, no significant difference among groups was observed (Table 5).

Table 5 - Mean absolute lactate concentration ([La$^-$]) in each set over the 8 weeks of intervention.

	[La$^-$] (mmol)						
Group	Rest	S1	S2	S3	S4	S5	S6
G_N	1.4(0.5)	3.7(1.6)[a]	5.0(1.9)[a]	5.2(2.0)[a]	5.4(2.1)[a]	5.0(2.0)[a]	4.3(1.9)[a]
G_{HR}	1.5(0.6)	4.1(1.8)[a]	5.1(2.3)[a]	5.1(2.1)[a]	5.2(2.1)[a]	5.2(2.3)[a]	5.2(2.3)[a]
G_H	1.4(0.5)	3.9(1.3)[a]	5.0(1.8)[a]	5.2(1.6)[a]	5.3(2.2)[a]	5.4(1.9)[a]	5.1(1.7)[a]

G_N = group in normoxia; G_{HR} = group in hypoxia recovery; G_H = group in hypoxia; S = set. [a] = $p < 0.05$ compared to rest.
Source: from (Costa *et al.*, 2022)

Table 6 shows the set percentage in each training zone according to [La$^-$]. In all of them, the set percentage in Z3 was significantly higher ($p < 0.001$) compared with Z1. No significant difference among groups was observed.

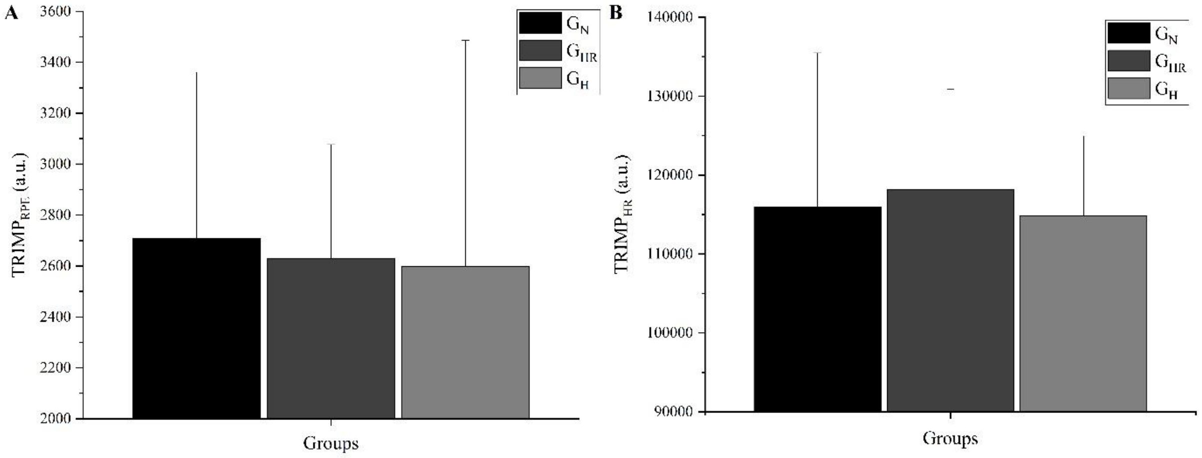
Table 6 - Set percentage in each training zone according to lactate concentration ([La$^-$]) over the 8 weeks of intervention.

	Zones		
Group	%Z1	%Z2	%Z3
G_N	4.2(7.0)	36.7(29.1)	59.1(31.1)^a
G_{HR}	8.8(26.4)	32.8(38.4)	58.4(41.0)^a
G_H	1.6(4.8)	35.4(35.5)	63.0(37.6)^a

G_N = group in normoxia; G_{HR} = group in hypoxia recovery; G_H = group in hypoxia. %Z1 = % of sets below 2 mmol; %Z2 = % of sets between 2 and 4 mmol; %Z3 = % of sets above 4 mmol.
Source: from (Costa *et al.*, 2022)

Regarding the mean TRIMP quantified by RPE (Figure 6A) and HR (Figure 6B) at the end of the 8-week intervention, the groups did not present significant differences in the internal load of training.

Figure 6 - Mean TRIMP quantified by the rate of perceived exertion (RPE) (**A**) and heart rate (HR) (**B**) at the end of the 8-week intervention.



G_N = group in normoxia; G_{HR} = group in hypoxia recovery; G_H = group in hypoxia; $TRIMP_{RPE}$ = TRIMP quantified by RPE; $TRIMP_{HR}$ = TRIMP quantified by HR.
Source: from (Costa *et al.*, 2022)

2.4. Discussion

The present study aimed to describe acute responses of 24 bicycle training sessions combined with intermittent hypoxic through the SpO_2, HR, RPE, $[La^-]$ analyses in patients recovered from COVID-19. Mean SpO_2 was significantly different between groups, but no significant differences in HR_{AT2}, RPE, $[La^-]$ means, and internal load calculated by TRIMP were shown.

2.4.1. Safety and effectiveness in the protocol implementation

The clinical trial was performed with three groups sharing a common training space. Although two groups were exposed to hypoxia, the blinding strategy - composed of tarps and similar individual mask systems - proved effective. It is important to emphasize that the error rate of the participants who incorrectly answered the perception of their group belonging was higher than 50%, even with 2/3 of participants under hypoxia.

Ensuring the safety of the intervention is essential to avoid harmful effects from altitude exposure exceeding 2,500m, such as the increasingly frequent and intense occurrence of anorexia, nausea or vomiting, fatigue or weakness, dizziness or vertigo, or difficulty sleeping (Barry, 2003). A FiO_2 corresponding to 3000m of altitude used in the present study proved to be well tolerated by both groups exposed to hypoxia (G_{HR} and G_H): less than 4% self-reported moderate and 0% severe symptoms indicative of acute mountain sickness, measured by Lake Louise Scale (Roach *et al.*, 2018). Furthermore, the most frequent score related to the acute mountain sickness, measured by Lake Louise Scale, was zero in the present study. It is important to highlight that this result is similar to the result presented by the group not exposed to hypoxia (G_N), showing no or little hypoxic harmful effect.

The method to monitor the safety of hypoxic intervention is through the SpO_2 (Bassovitch e Serebrovskaya, 2011), which must be below 80% (Richalet e Herry, 2003). The SpO_2 average was significantly different between groups in the present study, showing the lowest values in the hypoxia-exposed groups. However, in both groups, the safety values of SpO_2 remained. In a previous study, sedentary individuals exposed to 5,000m simulated hypobaric hypoxia with and without combined bicycle exercise at 30% of VO_{2MAX} for fourteen-day showed a mean of SpO_2 ranged 65.2(9.9)% to 71.5(7.3)% (Ricart *et al.*, 2000). The lowest mean SpO_2 in the present study was 84.7(5.4)%, at the last effort of G_H. Therefore, the system's safety in hypoxic exposure might be verified based on this physiological variable.

Previous literature supports hypoxia in multiple health conditions (Behrendt, Bielitzki, Behrens, Herold, *et al.*, 2022; Millet, Gregoire P. *et al.*, 2016b; Millet e Girard, 2017). More specifically, the safety and efficacy of physical exercises in recovering patients from severe acute respiratory syndrome (SARS) have already been identified but without a detailed description of monitoring variables (Lau *et al.*, 2005). Lau *et al.* (2005) used HR to control that the exercise intensity in different ergometers ranged from 60% to 85% of HR_{MAX}; participants from the three groups of this study maintained the HR mean at nearly 87.9% HR_{MAX}. COVID-19 could carry limit patient functionality due to the generalized infection state and low mobility for a long time (Rooney, Webster e Paul, 2020). Moreover, even with natural fitness evolution through time, scores may remain lower than age-expected for as many as two years after recovery or hospital discharge (Rooney, Webster e Paul, 2020). Therefore, it becomes crucial to have a feasible intervention across all levels of physical function. Because of these aspects, the present research used three different bicycle models to attend to such a demand.

2.4.2. Monitoring variables

Other symptoms that may persist and limit patients in recovery, besides cardiorespiratory impairment, are the blood oxygen content and degree of dyspnea, which have been associated with higher death rates (Deng *et al.*, 2020; Harapan *et al.*, 2020). However, physical exercise must be performed while monitoring variables for safety and the intervention's effectiveness.

For equalizing stimulus between participants, individually calculated internal parameters (HR, RPE, and $[La^-]$) from the incremental test were used to control intensity. In the present study, the prescribed target HR was 90-110% HR_{AT2}, and participants maintained over 77% of the sets as stipulated. Therefore, results of the present study identify the possibility that individuals recovering from COVID-19 can tolerate a relatively high exercise intensity based on target HR or similar.

Among various hypoxia exposure methods (Bassovitch e Serebrovskaya, 2011), this study has used normobaric hypoxia. A concern with this system was the number of participants breathing in the same tent and the air available at the reservoir. Still, the effectiveness of the simulation system used is noted by the mean values of all interventions significantly different between groups and the significant difference between normoxia (G_N: all times; G_{HR}: effort; G_H: none) and hypoxia (G_N: none; G_{HR}: warm-up, recovery, and cool-down; G_H: all times) moments within the group. Although there were differences in SpO_2 between groups, both hypoxic exposure models (G_{HR} and G_H) did not result in severe discomfort. In addition, the most frequently reported RPE was "3" (moderate perceived exertion) in all groups, and 63.9% to 81.6% of the sets were maintained below the RPE_{AT2}, without significant differences between groups. In a study that instructed participants to keep a RPE between "hard" and "very hard" during exercise bicycle ergometer with an intensity of approximately 80% of maximum work rate, lower values were observed in time to exhaustion, VO_2, and minute ventilation when performed under hypoxia ($FiO_2 = 11.4\%$) (Jeffries *et al.*, 2019). This finding reinforces that external load decreases when hypoxic exposure occurs, and the internal load parameter is used to equalize groups.

Beyond the RPE, the $[La^-]$ without difference among sets and between groups demonstrated that intensity 90-110% HR_{AT2} and effort/pause ratio are feasible since nearly 80%

of sets were performed at HR target; and provide a relative physiological steady state. During hypoxia, aerobic energy contribution decreases, which may cause metabolic acidosis and performance reduction (Bowtell *et al.*, 2014). This result could overload already weakened systems of COVID-19 recovery people and result in different responses between groups. However, the non-significant differences could indicate no acidosis, preventing physiological overload. Nam and Park (2020) found a significant increase in $[La^-]$ values (near 9 mmol) and blood pH decrease according to FiO_2 reduction (20.9% to 16.5% and 12.8%), comparing three exposure models associated with 30 minutes of continuous bicycle ergometer exercise at 80% intensity of HR_{MAX}. The interaction between intensity and volume of exercise and FiO_2 results in these reactions: lower FiO_2 decreases VO_2, leading to greater energy contribution from glycolytic pathway and subsequent higher hydrogen ion levels; and, at some point, acid-base balance does occur, and besides lactate not causing metabolic acidosis, it is a biomarker of this phenomenon (Lühker *et al.*, 2017). In the present study, mean HR relative to maximal was between 87% and 88.6%, but with a delta of $[La^-]$ lower than 1 mmol, indicating a relative physiological steady state. Although both values of relative HR and overall effort volume (6 sets of 5 minutes) were similar, we employed intermittent efforts, with an effort/pause ratio of 2:1. The intermittent effort appears to be important to avoid metabolic acidosis.

Deb *et al.* (2018), in a systematic review with meta-regression, demonstrated that continuous or intermittent exercises, with efforts longer than 2 minutes, under simulated or environmental hypoxia (above 1,000m) cause decreased performance and, consequently, lower external load. This reduced performance is also associated with the magnitude of desaturation, showing a VO_{2MAX} reduction of 2% for every SpO_2 reduction of 1% (Chapman *et al.*, 2011). To maintain an equal external load from normoxia to hypoxia, HR tends to increase because of increased pulmonary vascular resistance and maintenance of cardiac output (Naeije, 2010). Zoll *et al.* (2006) showed a significant reduction in external load compared to a normoxic effort during a second ventilatory threshold intensity effort (similar to the AT2) under hypoxia (FiO_2 = 14.5%) at the same relative HR. Based on the magnitude of G_H desaturation, the exposure time to hypoxia, no significant difference in relative HR, and the internal load parameters ($TRIMP_{HR}$ and $TRIMP_{RPE}$), it can be presumed that there was a reduction in external load on G_H. This reduction may negatively affect athletes, but it may benefit individuals with special health conditions because the ergogenic effects would be achieved with less mechanical stress (Girard, Matic Girard e Peeling, 2021). These results demonstrate that this kind of intervention is more suitable for physical limitation or rehabilitation (Maggiorini *et al.*, 1998). Therefore, the G_{HR} arises with the proposal of performing the efforts in normoxia and recovery in hypoxia, obtaining the ergogenic effects of hypoxia exposure without reducing the external load.

Some limitations should be described. First, the load was only quantified through $TRIMP_{HR}$ and $TRIMP_{RPE}$, internal load quantification methods, and without external load quantification. This can be explained through the number and diversity of bicycle ergometers required for the study according to the participant's limitations. The sample size could also be considered a limitation (described in the study protocol) but explained by the health epidemic scenario where the study was carried out and the complexity of the experimental design. Nevertheless, the study had extensive data collection to provide a robust understanding of physiological responses, having 15,826 SpO_2 values, 6,036 HR values, and 7,198 RPE values.

It is important to highlight that the present study is original and provides detailed descriptions, never seen before in severe acute respiratory syndrome disease, of the materials, methods, and physiological responses expected by a protocol that proved safe and effective. These descriptions and results bring the literature closer to clinical and professional practice, enabling the protocol's replication. Furthermore, once the effectiveness, tolerance and acute safety of 24 training sessions have been demonstrated, technologies can be developed to decrease the large costs required by such protocol and a portable device, enabling other exercises to explore other conditioning and coordinative capacities.

2.5. Conclusion

The current strategy effectively promoted altitude simulation. This strategy, it has been shown as well-tolerated and acutely safe exposure during the sessions, as indicated by the low acute mountain sickness and the stable HR and SpO_2 values. Furthermore, it was possible to monitor exercise-induced physiological responses under three different environmental conditions in recovered patients from COVID-19 with persistent symptoms. For future research, an improved quantifying of the external load is suggested. The findings presented in this study may strengthen the tolerance and safety of developing hypoxia combined with physical exercise interventions for COVID-19 convalescents and developing technologies that facilitate accessibility.

3. ESTUDO 2

Effects of intermittent hypoxia training on blood variables and cardiorespiratory and physical fitness in convalescents from covid-19: the AEROBICOVID study

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3.1. Background

Until 2021, seven coronaviruses had been detected (Kesheh *et al.*, 2022). Three have been detected since 2000, being highly pathogenic (SARS-CoV, MERS-CoV, and SARS-CoV2) and responsible for respiratory diseases (Kesheh *et al.*, 2022). SARS-CoV and MERS-

CoV both caused epidemics and SARS-CoV2, the cause of COVID-19 disease, caused a pandemic, leaving more than 771 million people infected (Kesheh *et al.*, 2022; World Health Organization, 2023). The COVID-19 disease can cause persistent health-related problems even in milder cases, such as cough, chest pain, dyspnea, cognitive disorders, insomnia, shortness of breath, and fatigue (Batiha, G. E.-S. *et al.*, 2022; Townsend, Dowds, O'Brien, Sheill, Dyer, O'Kelly, Hynes, Mooney, Dunne, Ni Cheallaigh, *et al.*, 2021). These aspects can be related to difficulties in maintaining daily life activities and increasing vulnerabilities (Greenhalgh, Knight, A'Court, Buxton, Husain, *et al.*, 2020). The post-COVID-19 manifestations vary widely and are known to last for months to years after recovery from the acute phase (Helmsdal, Hanusson, Kristiansen, Foldbo, Danielsen, Steig, B., *et al.*, 2022). As post-exertional malaise and functional impairment may also be symptoms of long COVID, these patients' physical activity and physical fitness levels could be compromised (Gennaro, Di *et al.*, 2022; Twomey, Demars, *et al.*, 2022).

Studies have shown that physical activity can have positive effects on the functional capacity of the heart and lungs (Fletcher *et al.*, 1996), decrease systemic inflammatory levels (Kantorowski *et al.*, 2018), and help the human body protect and defend from viral infections and associated sequelae (Kohut *et al.*, 2009). In addition, higher levels of aerobic capacity can lead to short-term improvements in the immune and respiratory systems (Mohamed e Alawna, 2020). Moreover, physical activity is one of the most effective ways of preventing premature death (Warburton, 2006) and exercise is known to have numerous health benefits (Reiner *et al.*, 2013). In the pandemic context, it has become even more critical to explore the benefits of exercise training in treating long-standing COVID-19 consequences (Arkkukangas *et al.*, 2022).

On the other hand, as a method to improve physical fitness, altitude training has become very popular in recent decades among athletes who want a further increase in performance at sea level or acclimate for competitions at altitude (Millet *et al.*, 2010). High altitude is a natural method of hypoxic exposure, however, in recent decades, other hypoxic exposure techniques have been developed and tested (Bassovitch e Serebrovskaya, 2011). These include hypobaric chambers, normobaric rooms with reduced oxygen levels, and hypoxicators with mask systems that produce hypoxic air.

Advances in this field have shown that hypoxia training could be beneficial in clinical conditions such as chronic and coronary heart disease and chronic obstructive pulmonary disease (Hoppeler, Klossner e Vogt, 2008; Navarrete-Opazo e Mitchell, 2014), and provides less mechanical stress (Camacho-Cardenosa *et al.*, 2018; Girard, Matic Girard e Peeling, 2021), which may be of interest in some particular health conditions in which people have reduced mobility, suffer from chronic fatigue, or have decreased exercise tolerance, such as people living with long COVID-19 (Gennaro, Di *et al.*, 2022; Twomey, Demars, *et al.*, 2022).

With increasing numbers of recovered COVID-19 patients and remaining symptoms, non-pharmacological interventions to recover functionality are fundamental. More than 380 trials have been developed across 42 countries, most of them investigating the rehabilitation of these patients (Fawzy *et al.*, 2023). Hence, the study aims to analyze the effects of 8 weeks of moderate-intensity cycle ergometer training on intermittent hypoxia and 8 weeks of follow-up on physical and cardiorespiratory fitness, erythropoietin, reticulocytes, erythrocytes and hemoglobin.

3.2. Methodological procedures

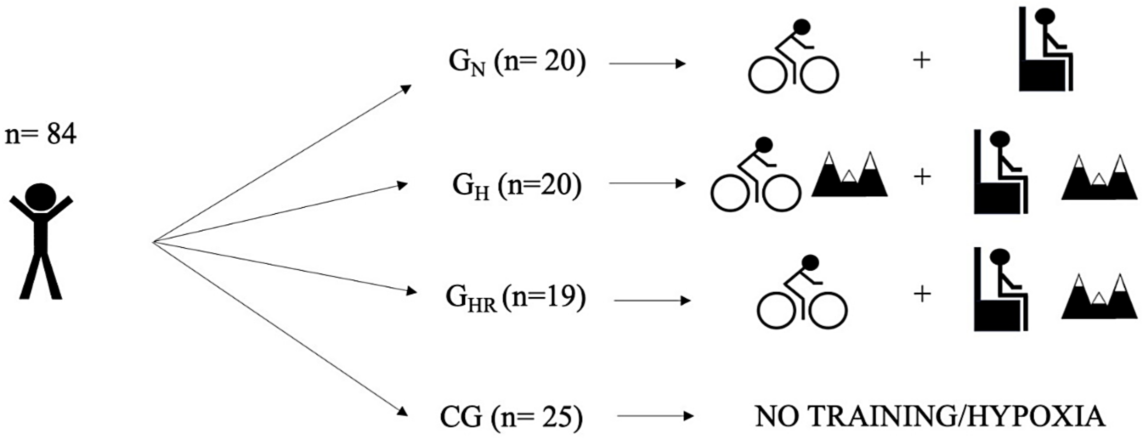
The present study follows the AEROBICOVID protocol, a randomized controlled clinical trial double-blind study, performed between September 2020 and February 2021, conducted during a period when vaccines were being developed and populations were severely restricted in circulation, and more specific details can be found in the published study protocol (Trapé *et al.*, 2021). In the study protocol, it had been planned to complete the project in December, although because of the logistical nature of evaluations at the year end, it was decided to double the follow-up period and carry out the last evaluations in February. This project was approved by the Research Ethics Committees from the School of Physical Education and Sport of Ribeirao Preto - University of Sao Paulo (USP) and School of Pharmaceutical Sciences of Ribeirao Preto - USP (CAAE: 33783620.6.0000.5659; CAAE: 33783620.6.3001.5403, respectively) and registered in the Brazilian Registry of Clinical Trials (RBR-5d7hkv).

The evaluations and intervention took place at the School of Physical Education and Sport of Ribeirao Preto – USP, which included: (1) participants aged between 30 and 69 years old and recovered from COVID-19, (2) having presented mild to critical symptoms, and (3) from 15 to 60 days after recovery from clinical signs or medical discharge (if they had been hospitalized). The exclusion criteria were (1) exposure to high altitude places > 1500 m in the last 3 months, (2) significant physical limitations to carry out evaluations or intervention, (3) acute or chronic clinical illnesses without medical supervision, (4) anemia, (5) use of immunosuppressive drugs, (6) pregnant women, (7) hormone replacement, (8) smokers, (9) excessive use of alcohol or drugs, (10) three absences in a row during the intervention, and (11) taking part in less than 75% of the total sessions planned.

The COVID-19 severity has been defined based on the National Institutes of Health of United States of America criteria (Galloway *et al.*, 2020; Gude, Riveiro, Rodríguez-Núñez, Ricoy, Lado-Baleato, Lourido, Rábade, Lama, Casal, Abelleira-París, Ferreiro, Suárez-Antelo, Toubes, Pou, Taboada-Muñiz, Calle-Velles, Mayán-Conesa, Molino, M. L. P. del, *et al.*, 2020). For mild severity: have any symptoms of COVID-19, such as fever, cough, etc., but do not have shortness of breath or dyspnea; moderate: have any symptoms of COVID-19 and have shortness of breath or dyspnea; severe: have any symptoms of COVID-19 and need hospitalization, but not intensive care; or critical: have any symptoms of COVID-19 and need hospitalization and intensive care.

Participants were divided into 4 groups, where 3 performed sets according to the combination of training (effort) and recovery (break) in normoxia and hypoxia conditions, i.e., training in normoxia and recovery in normoxia (G_N); training in normoxia and recovery in hypoxia (G_{HR}); and training in hypoxia and recovery in hypoxia (G_H). The randomization was performed in four groups, with participants being directed to the control (CG) or one of three training groups (figure 7). For the participants' allocation to groups, it was taken into consideration the variables, gender, age, participant's cardiorespiratory fitness level (result in the incremental test), and severity during the disease (COVID-19). Blinding was done between the two research teams (evaluation and monitoring teams) and the participants.

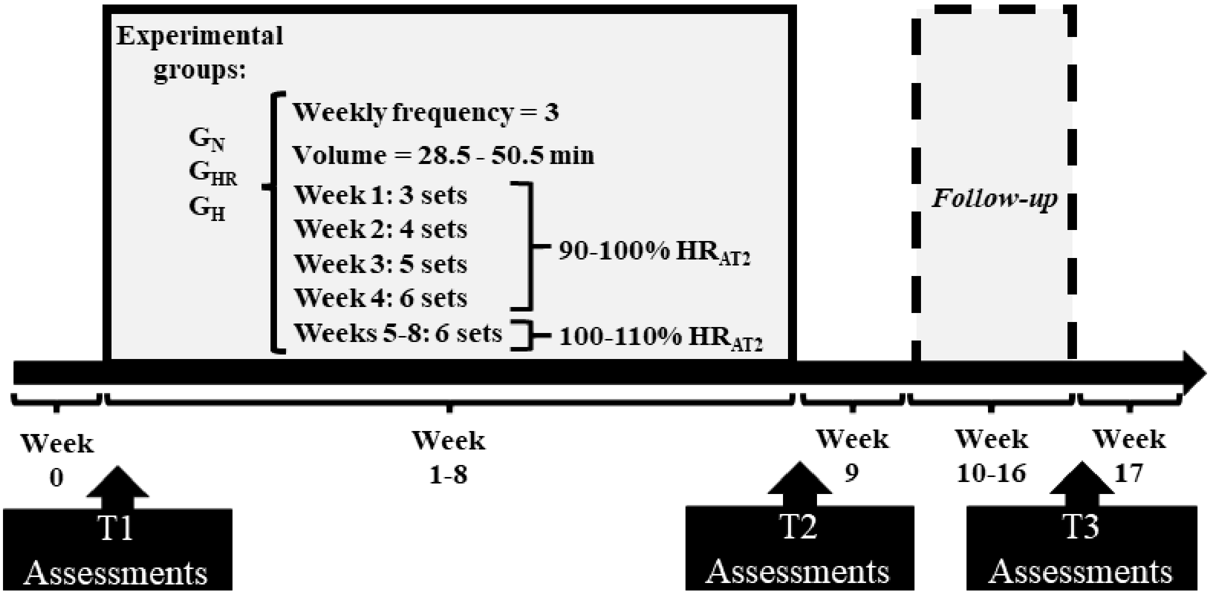
Figure 7 - All four groups included in the study.



G_N: group in normoxia; G_{HR}: group in hypoxia recovery; G_H: group in hypoxia
Source: own authorship

The experimental protocol was composed of 16 weeks in total. The physical training intervention was performed during the first 8 weeks. In weeks 0 (T1 - before the intervention, n = 84), 9 (T2 - after the intervention, n = 65), and 17 (T3 - 8 weeks after the end of the intervention, n = 40), the following evaluations were performed: application of anamnesis, blood sampling, cardiopulmonary exercise testing and motor test to assess aerobic fitness (figure 8). The participants did not receive any intervention during follow-up.

Figure 8 - Experimental design of the evaluations and the training protocol.



G_N: group in normoxia; G_{HR}: group in hypoxia recovery; G_H: group in hypoxia HR_{AT2}: heart rate at anaerobic threshold 2; min = minutes.

Source: own authorship

The bicycle training was performed 3 times per week. The protocol started with 5-minute warm-up and finished with 3-minute return to rest, which were performed in a rate of perceived exertion 2, considered “easy” on a 0 to 10 scale (Foster *et al.*, 2001). The main part of the exercise was composed of 3 to 6 sets according to the established periods: each set was composed of 5-min efforts at a heart rate corresponding to 90% - 100% (first to the fourth week) and 100% - 110% (fifth to the eighth week) of the anaerobic threshold 2 (previously explained (Dellavechia de Carvalho *et al.*, 2023), followed by a break of 2.5 min to recover between sets. The G_N and G_H used the mask during the entire workout (warm-up, effort, recovery, and cool-down); G_{HR} used the mask all the time (warm-up, recovery, and cool-down) except during efforts in the main part of the exercise (figure 8). At the hypoxic moments, participants were exposed to an inspired fraction of O_2 (FiO_2) of ~13.5% (corresponding to 3,000 m altitude). At normoxic moments, participants breathed ambient air, with a FiO_2 of ~20.9% (a city with 526 m altitude). Further detailed information about effectiveness, implementation, and monitoring variables has been published elsewhere (Costa *et al.*, 2022).

Participants answered an anamnesis, which included questions about the birth date, sex, skin color, questions to classify COVID-19 severity, medication use, physical and/or motor injuries or limitations.

Blood collection was performed by peripheral venous access after 8 hours overnight fasting, carried out by a trained and specialized professional. The hemogram parameters were evaluated at the Clinical Analysis Laboratory, Faculty of Pharmaceutical Sciences of Ribeirão Preto according to the technical service's standard routine and methodology. The erythropoietin (EPO) plasma concentration was determined by immunoassay according to the fabricant (EPO ELISA Kit R&D Systems).

A cardiopulmonary exercise testing was used to estimate peak of oxygen consumption (VO_{2PEAK}) and was performed in a pendular cycle ergometer with mechanical braking (Ergométrica, Monark). First, the participants started a 5-minute warm-up without any additional load; after that, the intensity was increased by 15 watts every 2 min until the participant did not maintain the 60-rpm cadence or volitional exhaustion. Oxygen uptake was measured breath by breath by the gas analyzer (K4b2, COSMED), calibrated according to the manufacturer's specifications. The VO_{2PEAK}) was defined as the highest VO_2 average in the last 60 seconds in the test.

The assessment of lower limb strength resistance was performed using the chair and standing test and consisted of the number of repetitions (rep) that the participant was able to sit and stand up from a chair for 30 seconds, with the arms crossed in the chest (Rikli e Jones, 1999).

The assessment of aerobic fitness was performed using the 6-minute walk test and the distance (meters (m)) was noted. The course was a rectangle measuring 4.57 m by 18.28 m, and the participant was instructed and encouraged to walk as fast as possible, without running (Rikli e Jones, 1999).

3.2.1. Statistical analysis

Quantitative continuous variables are expressed as mean (standard deviation) and categorical variables as absolute frequency and percentage.

Time and group effects and their interaction were analyzed by a generalized linear regression with mixed effects with Gamma distribution, with random effect on individuals and covariate, with Bonferroni post-hoc. The Gamma distribution has been chosen after comparing the AIC (akaike information criterion) and residual distribution (by Q-Q plot) between the models.

Regression slope comparisons represent the differences between two groups at two-time points; for this, CG and T1 were assumed as the reference group and time point for the intervention analyses and Inactive and T2 for the follow-up analyses. The values are expressed as mean difference [95%CI].

Covariates have been determined by residual analysis, crossing the dependent variables only of the pre-moment with other variables collected, by Pearson correlation and one-way ANOVA and including variables with $p<0.05$ in the model.

Cohen's *d* effect sizes were computed as the mean difference relative to the pooled standard deviation of baseline scores, where 0.2 was considered a small effect, 0.5 a moderate effect, and 0.8 a large effect (Ferguson, 2009). JAMOVI software (v 2.3) has been used for all analyses assuming a 5% significance level.

3.3. Results

Table 7 shows the mean age of participants in each group, as well the proportion of men and women (predominantly female; 63.0%), and the COVID-19 severity (predominantly moderate severity; 60.7%) in each group. It also shows the sample size of each group throughout the study.

Table 7 - Sample descriptive values. Mean (SD) for age (years) and absolute frequency for sex, COVID-19 severity and sample size at each assessment time point (T1, T2 and T3).

Variables	CG	G_N	G_{HR}	G_H
Age (years)	49.3 (10.4)	48.8 (9.7)	47.7 (9.4)	47.8 (10.2)
Sex				
Female	16	13	12	12
Male	9	7	7	8
COVID-19 severity				
Mild	5	4	4	3
Moderate	13	11	12	15
Severe	5	3	0	1
Critical	2	2	3	1
Sample size				
T1	25	20	19	20
T2	19	14	15	17
T3	6	12	11	11

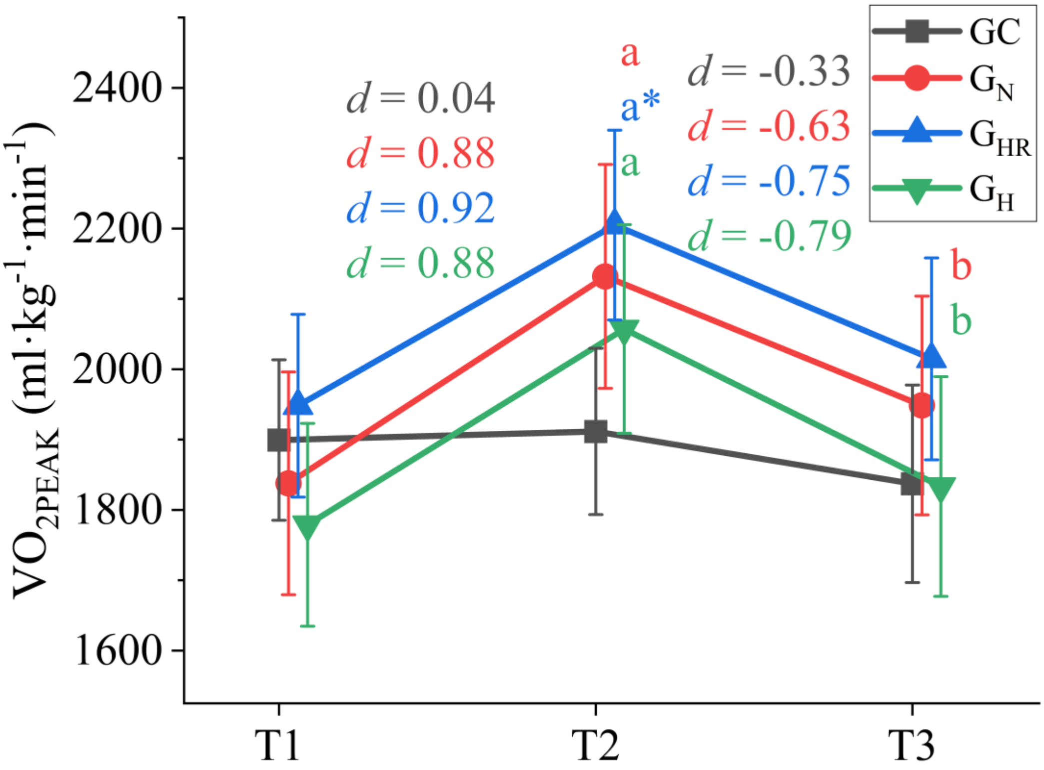
T1: baseline; T2: post-intervention; T3: post-follow-up; G_N: group in normoxia; G_{HR}: group in hypoxia recovery; G_H: group in hypoxia.

Source: own authorship

The groups did not differ significantly in any of the variables analyzed at T1. All three intervention groups showed an increase in VO_{2PEAK} at T2 compared to T1 and G_{HR} showed the largest effect size (*d* = 0.92), being the only one with a significant difference compared to CG in absolute VO_{2PEAK} values. G_{HR} was also the only one with no significant decrease in VO_{2PEAK} after follow-up (figure 9).

All three intervention groups showed an increase in 6-minute walk test distance at T2 compared to T1 and different slope compared to CG (G_N - CG = 49.6 [21.01; 78.2] m; G_{HR} - CG = 48.35 [18.23; 78.46] m; G_H - CG = 60.91 [32.61; 89.2] m), with G_H having the largest effect size (*d* = 70.0) and the only one to maintain the difference at T3 compared to T1 (figure 10).

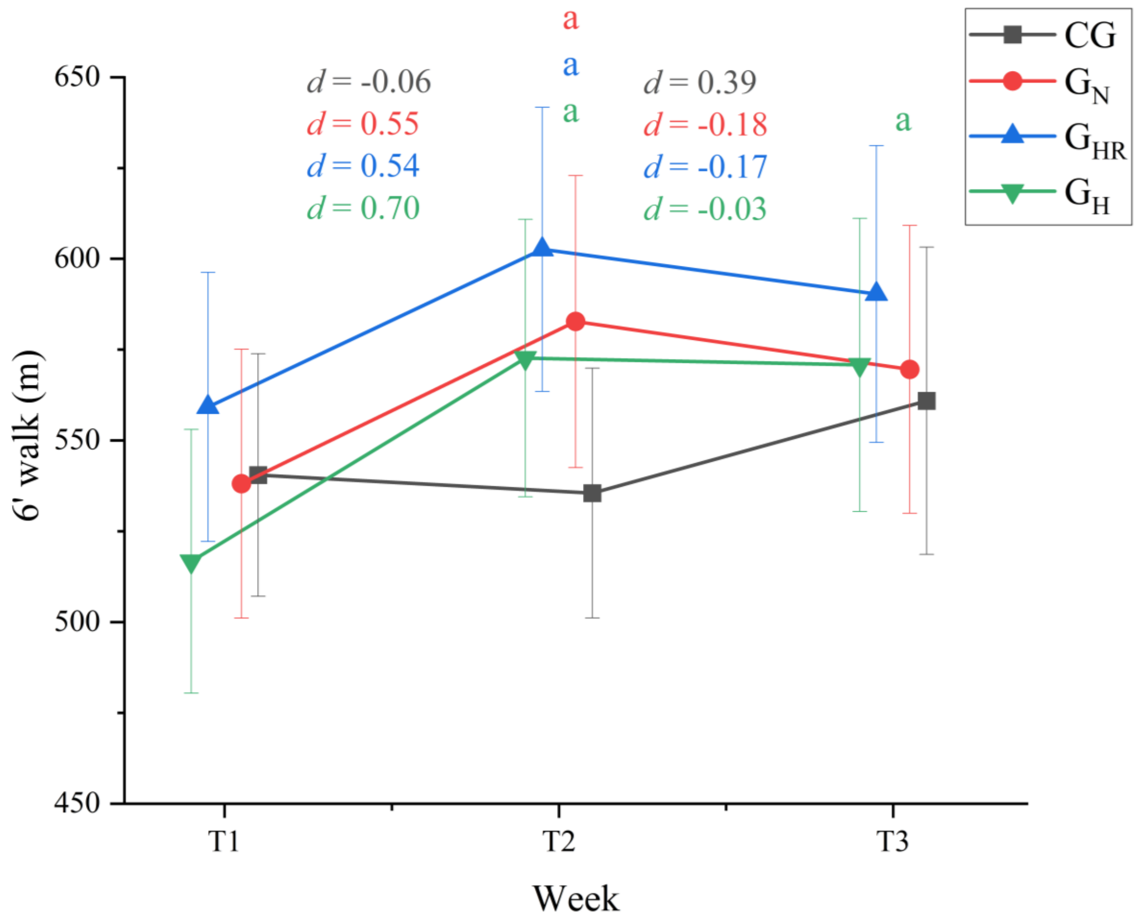
Figure 9 - Intervention effect on VO_{2PEAK}



Mean and 95%CI. VO2PEAK analyzed by a generalized mixed-effects regression model with gamma distribution, covariate by sex, height (m) and age. T1: baseline; T2: post-intervention; T3: post-follow-up; G_N: group in normoxia; G_{HR}: group in hypoxia recovery; G_H: group in hypoxia; *d*: Cohen's d.

Source: own authorship

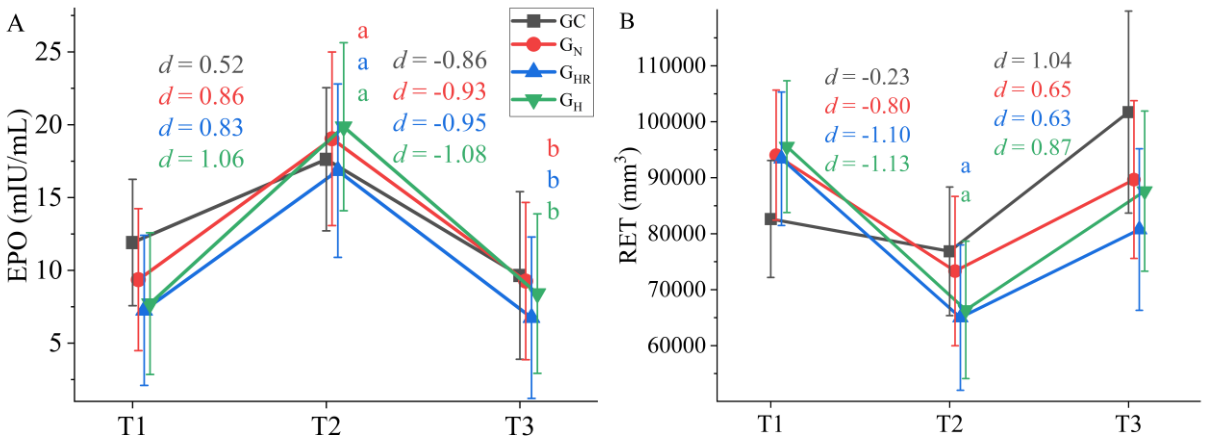
Figure 10 - Intervention effect on 6' walk test



Mean and 95%CI. VO2PEAK analyzed by a generalized mixed-effects regression model with gamma distribution, covariate by sex, COVID-19 severity, body mass index and age. T1: baseline; T2: post-intervention; T3: post-follow-up; G_N: group in normoxia; G_{HR}: group in hypoxia recovery; G_H: group in hypoxia; *d*: Cohen's d.
Source: own authorship

All three training groups showed significant increases in EPO at T2 compared to T1, with G_H showing the largest effect size (*d* = 1.06); all three training groups showed significant losses at T3 compared to T2. Regarding RET, only the groups exposed to hypoxia showed significant reductions, with G_H having the largest effect size (*d* = 1.13).

Figure 11 - Intervention effect on erythropoietin and reticulocytes counts



Mean and 95%CI. EPO and RET analyzed by a generalized mixed-effects regression model with gamma distribution. EPO covariables: COVID-19 severity and age; RET covariables: waist (cm) and sex. T1: baseline; T2: post-intervention; T3: post-follow-up; G_N: group in normoxia; G_{HR}: group in hypoxia recovery; G_H: group in hypoxia; *d*: Cohen's d.

Source: own authorship

Table 8 - Intervention effect on erythrocyte and hemoglobin.

	T1				T2				T3			
	CG	G_N	G_{HR}	G_H	CG	G_N	G_{HR}	G_H	CG	G_N	G_{HR}	G_H
ERI	4,56	4,55	4,63	4,68	4,76	4,73	4,76	4,89	4,67	4,57	4,74	4,78
	(0,40)	(0,40)	(0,40)	(0,41)	(0,38)	(0,37)	(0,38)	(0,38)	(0,29)	(0,36)	(0,35)	(0,35)
HEMO	13,97	13,84	13,91	14,15	14,54	14,18	14,05	14,67	13,86	13,61	13,94	14,42
	(1,04)	(1,06)	(1,06)	(1,08)	(1,01)	(0,98)	(1,00)	(1,02)	(0,81)	(0,96)	(0,93)	(0,93)

Mean (SD). Erythrocytes (ERI) and hemoglobin (HEMO) analyzed by a generalized mixed-effects regression model with gamma distribution. T1: baseline; T2: post-intervention; T3: post-follow-up; G_N: group in normoxia; G_{HR}: group in hypoxia recovery; G_H: group in hypoxia. Covariables ERI and HEMO: age, sex, height (m) and EPO.

Source: own authorship

3.4. Discusssion

The present study aimed to analyze the effects of 8 weeks of moderate-intensity cycle ergometer training on intermittent hypoxia and 8 weeks of follow-up on physical and cardiorespiratory fitness, erythropoietin, reticulocytes, erythrocytes and hemoglobin. The groups exposed to hypoxia showed greater gains in VO_{2PEAK}, 6-minute walk test, EPO and RET. Furthermore, no significant loss of VO_{2PEAK} in the G_{HR} and maintenance of the 6-minute walk test in the G_H, both post follow-up.

Patients recovering from COVID-19 exhibit persistent symptoms and physical limitations even after 6 and 12 months (Seeßle *et al.*, 2022) and, although hypoxia combined with exercise has been highlighted as safe in other debilitated populations (Behrendt, Bielitzki, Behrens, Glazachev, *et al.*, 2022; Burtscher *et al.*, 2004), nevertheless, patient safety is essential, particularly because this is a new disease. For this reason, the safety and effectiveness of the intervention strategy proposed by the AEROBICOVID project have been rigorously monitored, including heart rate, rate of perceived exertion, peripheral oxyhemoglobin saturation, blood lactate concentration, Lake Louise scale, and training load quantification (Costa *et al.*, 2022).

The health benefits of hypoxia in addition to exercise for a variety of special conditions have been highlighted previously (Millet, Gregoire P. *et al.*, 2016a; Navarrete-Opazo e Mitchell, 2014) and physical fitness recovery is essential for patients recovering from COVID-19 to get back their functionalities.

Although the concept of using hypoxia as an additional stimulus for physical fitness recovery has not reflected in significant difference compared to the normoxia group, the hypoxia group had greater improvement magnitudes in VO_{2PEAK} and 6-minute walk tests after intervention; no significant loss of VO_{2PEAK} in the G_{HR} and maintenance of the 6-minute walk test in the G_H, both post follow-up. Besides, the advantage of a probable lower mechanical stress, with less joint and tendon tissue stress, since hypoxia causes a higher pulmonary vascular resistance, increasing heart rate to maintain cardiac output to sustain the same intensity performed in normoxia (Naeije, 2010). The external load has not been quantified in this study, although Zoll *et al.* (2006), showed a significant reduction in external load compared to a normoxic effort during a second ventilatory threshold intensity effort under hypoxia (FiO_2 = 14.5%) at the same relative heart rate.

Besides developing more efficient and effective protocols for functional recovery and quality of life, free and long-term programs that offer physical activity are needed to provide more benefits to patients or at least to maintain benefits (Bull *et al.*, 2020).

The initial hypothesis is that the group that remained only in hypoxia during recovery would benefit from a higher external load in normoxia during the efforts and the physiological benefits of hypoxia during breaks. This hypothesis has not been confirmed, since no difference was observed compared to other training groups, possibly because of the conservative protocol used, with moderate efforts and passive interval bouts. Therefore, future studies could explore protocols with longer effort durations, lower oxygen concentrations, more frequent weekly workouts, or combinations of these. These results could be useful to guide rehabilitation protocols in case a fourth coronavirus occurs, furthermore, based on these findings and the literature (Navarrete-Opazo e Mitchell, 2014), we think this strategy can be promising and deserves to be tested in other health conditions. The slightly smaller sample size than the research protocol's sample size calculation could be considered limitations of this study. However, the study sample size was higher than the median of studies investigating patients recovering from COVID-19, and it is important to highlight the difficulty of access to the participants at the time of the intervention (September 2020 to December 2020) in Brazil, being under mobility restrictions with high infection levels and mortality. So, achieving this number of participants to develop the study was very important, in that pandemic moment, without vaccines available. Although the 6-minute walk test is an indirect measure of physical fitness, was the most frequently used to evaluate the primary outcome (Fawzy *et al.*, 2023). Regarding sampling, considering the current contamination status of COVID-19, future studies could investigate longer durations of persistent symptoms, as well as increase sample size to allow subgroup analysis according to severity or target-specific severity levels.

3.5. Conclusion

Besides the effective hypoxia application in diverse health conditions, the use of hypoxia in recovering patients from COVID-19 has potential, since the groups exposed to hypoxia showed greater gains in VO_{2PEAK}, 6-minute walk test, EPO and RET. Furthermore, no significant loss of VO_{2PEAK} in the G_{HR} and maintenance of the 6-minute walk test in the G_H, both post follow-up.

4. CONSIDERAÇÕES FINAIS

A implementação do protocolo foi efetiva, a partir das análises dos valores de saturação periférica da oxihemoglobina, frequência cardíaca, percepção subjetiva de esforço e concentração sanguínea de lactato.

A ausência de diferença entre os grupos pode ser devido à heterogeneidade da amostra, tempo de intervenção, fração inspirada de oxigênio ou uma combinação destes. Porém, os efeitos da intervenção foram positivos para todos os grupos que treinaram e foi possível observar alternância entre os grupos que foram expostos à hipóxia, sendo os com maiores magnitudes positivas.

Os resultados destes estudos podem balizar e embasar futuras pesquisas com outras doenças causadas por coronavírus, bem como para outras doenças respiratórias.

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