Study protocols in physical activity and health

RBAFS Revista Brasileira de Atividade Física & Saúde
SOCIEDADE BRASILEIRA DE ATIVIDADE FÍSICA & SAÚDE

rbafs.org.br

High-intensity interval training under hypoxia for individuals with type 2 diabetes: the study protocol

Treinamento intervalado de alta intensidade sob hipóxia para pessoas com diabetes tipo 2: protocolo de estudo

AUTHORS

Matheus Simionato Firmino[1]
Tiago Rezende Figueira[1]
Matheus Silva Norberto[2]
Francisco José Albuquerque de Paula[3]
Olivier Girard[4]
Marcelo Papoti[1,5]

1 Universidade de São Paulo, Ribeirão Preto, Escola de Educação Física e Esportes de Ribeirão Preto, São Paulo, Brazil.

2 Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil.

3 Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto, Departamento de Clínica Médica, Ribeirão Preto, São Paulo, Brazil.

4 The University of Western Australia, School of Human Sciences (Exercise and Sport Science), Perth, Austrália Ocidental, Austrália.

5 Universidade Estadual de Campinas, Escola de Ciências Aplicadas, Hypoxia, Sport and Health Team, Limeira, São Paulo, Brazil.

CORRESPONDING

Marcelo Papoti
mpapoti@usp.br
Av. Bandeirantes, 3900, Bairro Monte Alegre, Ribeirão Preto, São Paulo, Brazil.
Zip code: 14.040-907.

DOI

10.12820/rbafs.31e0458

Trial Registration: Protocol version 1. Registered on 30 August 2024 with the Brazilian Clinical Trials Registry (under the code RBR-9gmbt9v; https://ensaiosclinicos.gov.br/). Universal trial number: U1111-1311-8523.

ABSTRACT

Background: High-intensity interval training (HIIT) is known to improve insulin sensitivity, glycemic control, and body composition in people with type 2 diabetes mellitus (T2DM). Intermittent hypoxia (i.e., reduced oxygen availability) combined with HIIT may enhance glycemic control through convergent insulin-independent molecular pathways, including AMP-activated protein kinase activation and GLUT-4 translocation. Objective: To evaluate whether 8 weeks of intermittent hypoxia and HIIT, both isolated and combined, improve glucose homeostasis and related health outcomes in individuals with T2DM. Methods: This single-blind, randomized controlled trial will recruit 64 individuals (18-65 years) with T2DM. Participants will be randomly assigned to one of four groups: 1) HIIT with inter-effort recovery hypoxia (inspired oxygen fraction, FiO_2, of 0.135), 2) HIIT in normoxia (FiO_2 = 0.209), 3) passive intermittent hypoxia (FiO_2 = 0.135) or 4) control. Interventions will last 8 weeks, with three sessions per week. HIIT will be performed on a cycle ergometer at intensities based on the heart rate corresponding to the respiratory compensation point and peak power output, as determined by baseline cardiopulmonary exercise testing. The primary outcome is glycated hemoglobin, while secondary measures include glucose homeostasis, cardiorespiratory fitness, cardiovascular health, body composition, and hematological, metabolic, and behavioral outcomes. Generalized linear mixed models (α = 0.05) will be used for statistical analysis. Discussion: This study explores a novel non-pharmacological approach to T2DM management by applying intermittent hypoxia only during recovery periods of HIIT sessions. This method aims to elicit adaptations comparable to conventional intermittent hypoxia, while preserving training quality and offering a more practical, ecologically valid intervention.

Keywords: High-intensity interval training; Hypoxia; Type 2 diabetes mellitus; Glycated hemoglobin; Physical fitness.

RESUMO

Introdução: O treinamento intervalado de alta intensidade (HIIT) pode melhorar a sensibilidade à insulina, o controle glicêmico e a composição corporal em indivíduos com diabetes mellitus tipo 2 (DM2). A hipóxia intermitente (redução da disponibilidade de oxigênio), quando combinada ao HIIT, parece melhorar o controle glicêmico através de vias moleculares convergentes independentes da insulina. Objetivo: Avaliar se oito semanas de hipóxia intermitente e HIIT, isolados ou combinados, melhoram a homeostase glicêmica e desfechos relacionados à saúde em indivíduos com DM2. Métodos: Este ensaio clínico randomizado, controlado e simples-cego recrutará 64 indivíduos (18–65 anos) com DM2, randomizados em 4 grupos: 1) HIIT com hipóxia durante a recuperação dos esforços (fração inspirada de oxigênio, FiO_2 = 0,135); 2) HIIT em normóxia (FiO_2 = 0,209); 3) hipóxia intermitente passiva (FiO_2 = 0,135); ou 4) controle. As intervenções terão duração de 8 semanas (3 sessões semanais). O HIIT será realizado em cicloergômetro, em intensidades correspondentes à frequência cardíaca do ponto de compensação respiratória e à potência pico. O desfecho primário inclui a hemoglobina glicada, as medidas secundárias incluem homeostase glicêmica, aptidão cardiorrespiratória, saúde cardiovascular, composição corporal e desfechos hematológicos, metabólicos e comportamentais. A análise estatística será conduzida por meio de modelos lineares generalizados mistos (α = 0,05). Discussão: Este estudo investiga uma abordagem não farmacológica inovadora para o manejo do DM2, aplicando hipóxia exclusivamente durante os períodos de recuperação do HIIT. Essa estratégia busca induzir adaptações fisiológicas comparáveis às da hipóxia intermitente convencional, preservando a qualidade do treinamento e oferecendo uma intervenção mais prática e ecologicamente válida.

Palavras-chave: *Treinamento intervalado de alta intensidade; Hipóxia; Diabetes mellitus tipo 2; Hemoglobina glicada; Aptidão física.*

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic, lifestyle-related metabolic disease marked by rapidly increasing global prevalence and substantial mortality burden. Driven primarily by insulin resistance in adipose tissue, skeletal muscle, and liver, T2DM disrupts energy metabolism and results in chronic hyperglycemia[1]. Persistent hyperglycemia contributes to microvascular and macrovascular complications, including retinopathy, neuropathy, nephropathy, hypertension, and endothelial dysfunction, which markedly elevate cardiovascular risk and premature mortality[1]. As no cure currently exists and key aspects of T2DM pathophysiology remain unresolved, identifying effective management strategies is essential, with particular emphasis on non-pharmacological interventions. Among these, exercise and environmental hypoxia represent two promising physiological modulators, whose relevance to T2DM is explored in the following subsections.

High intensity interval training as a therapy for T2DM

Regular physical activity is widely recognized as a key strategy for preventing and managing T2DM[2]. In individuals with T2DM, regular exercise improves glycemic control and significantly lowers the burden of T2DM comorbidities and mortality[2]. High-intensity interval training (HIIT) has emerged as an exercise strategy that produces greater benefits than continuous aerobic training (CAT) for individuals with metabolic diseases[3,4]. HIIT is characterized by a lower time commitment and is associated with reduced systemic inflammation, improved insulin sensitivity, and favorable changes in body composition[3,4] and provides longer-lasting benefits after training cessation[5]. In T2DM specifically, HIIT may outperform CAT in improving fasting blood glucose concentration (FBG)[6], insulin resistance[7], body weight[8], and aerobic fitness[9]. Moreover, a recent meta-analysis has shown that HIIT, compared to control (no exercise) and other types of physical activities, has the greatest probability of being the best in promoting reductions in glycated hemoglobin (HbA1c) levels in individuals with T2DM[10].

At the molecular level, HIIT causes rapid fluctuations in ATP turnover, intracellular calcium concentration, and redox balance, activating key signaling pathways such as AMP-activated protein kinase (AMPK) and calcium/calmodulin-dependent protein kinase (CaMK)[3]. These pathways promote mitochondrial biogenesis, enhance oxidative capacity, and facilitate GLUT-4 translocation, thereby improving glucose uptake in skeletal muscle[3].

Intermittent hypoxia as a physiological modulator and its application in therapeutics

In addition to exercise, environmental hypoxia also induces metabolic and cardiorespiratory strain. Both acute exercise and acute hypoxia elicit rapid systemic responses to maintain the balance between systemic oxygen delivery and peripheral oxygen demand. Interestingly, populations living at high altitudes exhibit reduced risk of metabolic syndrome[11], and decreased cardiovascular mortality[12]. These findings support the usefulness of low oxygen exposure as a non-pharmacological therapy in various metabolic diseases[13].

In patients with T2DM, 14 consecutive nights of continuous hypoxia exposure (inspired oxygen fraction – FiO_2 = 0.150) improved oral glucose tolerance[14]. However, a shorter intervention of 10 consecutive nights (FiO_2 = 0.155) showed no improvement in glycemic control, gut microbiome, appetite, and inflammation in T2DM patients[15]. Hypoxia enhances skeletal muscle glucose uptake through insulin-independent mechanisms[16], primarily mediated by AMPK activation, increased production of reactive oxygen species, and calcium-dependent signaling pathways[17]. These molecular changes activate transmembrane glucose transporter type 1 (GLUT-1) and stimulate the translocation of GLUT-1 and GLUT-4 to the sarcolemma. Based on this rationale, D'Hulst et al.[16] showed that 4 hours of acute hypoxia increased sarcolemmal GLUT-4 by 30% and lowered postprandial glucose despite no activation of insulin-dependent signaling, indicating an insulin-independent mechanism.

The dose of hypoxic exposure is a key factor in determining whether physiological responses will be adaptive and beneficial or lead to pathophysiological effects[13]. While conditions such as obstructive sleep apnea, characterized by a high frequency of hypoxic cycles and severe oxygen desaturation, are associated with inflammatory, cardiovascular, and cognitive impairments[18], intermittent hypoxia applied in mild to moderate doses, with or without exercise, has well-established clinical applications and is associated with improvements in health and performance[13]. However, the optimal dose remains unclear, particularly given the substantial interindividual variability in physiological responses to the same external hypoxic stimulus[19]. Evi-

dence suggests that higher levels of normobaric hypoxia (e.g., ~0.12 to 0.15 FiO_2) and/or longer cumulative exposures promote improvements in glycemic control[14,20], whereas milder hypoxia (~0.15 to 0.165 FiO_2) or short interventions may be insufficient to elicit additional benefits[21,22]. Thus, both normobaric hypoxic level and exposure duration across sessions and weeks appear critical.

The combination of intermittent hypoxia with exercise

Considering that the mechanisms promoting GLUT-4 translocation during exercise and hypoxic exposure are insulin-independent and may act synergistically[17], it is reasonable to test these interventions in combination. The effects of continuous, lower-intensity exercise in hypoxia have been studied in patients with T2DM and other chronic diseases[23]. Acutely, a single bout of continuous or intermittent exercise under hypoxic conditions (FiO_2 = 0.147) increased insulin sensitivity, whereas the same exercise in normoxia did not[24]. A systematic review confirmed that acute hypoxic exercise lowers blood glucose and enhances insulin sensitivity[23]. However, findings from chronic interventions remain scarce and inconsistent with respect to glucose regulation[23]. Furthermore, some studies report additional benefits of intermittent hypoxic training in individuals with T2DM compared to normoxia, including improvements in aerobic capacity[25,26], functional capacity[25], bone mineral content[25], and the activation of nitric oxide synthase in erythrocytes[26].

Few studies have examined the therapeutic effects of combining HIIT with intermittent hypoxia in community-dwelling individuals. An 8-week program conducted under hypoxic conditions (FiO_2 = 0.120) produced physical fitness gains comparable to normoxia despite a 20% lower external workload, as physiological load was matched between conditions[27]. Additionally, only participants undergoing a 12-week hypoxic HIIT protocol (FiO_2 = 0.170) exhibited meaningful reductions in waist circumference and trunk fat[28]. Although this combined approach has not yet been investigated in individuals with T2DM, its rationale is strengthened by the independent metabolic benefits demonstrated for both HIIT and hypoxia.

The use of HIIT with intermittent hypoxia only during recovery phases: the inter-effort hypoxic recovery model

In sports training, hypoxia poses a key drawback by reducing tolerance for high-intensity aerobic exercise and compromising overall performance. Thus, the training stimulus may be reduced under hypoxia, particularly during high-intensity aerobic sessions[29]. While hypoxic exercise offers potential benefits[13], however, the reduced exercise tolerance often limits its practical application. It is widely argued that the benefits of adding hypoxia are only realized when the absolute intensity of the session is preserved[30].

Using intermittent hypoxia only during the recovery periods of a HIIT protocol (the inter-effort hypoxic recovery model – IEH_{HIIT}) may overcome the limitations of exercising solely in hypoxia[30]. This protocol involves performing high-intensity efforts under normoxia, while breathing normobaric hypoxic air during the recovery intervals. Importantly, this model preserves the prescribed external workload, thereby maintaining the intended training stimulus while inducing systemic hypoxic stress[31,32].

Thus, if an IEH_{HIIT} protocol delivers an effective hypoxic dose, it may combine the benefits of both high-intensity exercise and hypoxia, without compromising high-intensity performance. In a practical context, IEH_{HIIT} represents a more ecologically valid intervention, allowing individuals to exercise freely in different sports or physical activities while breathing normobaric hypoxic air only during recovery periods[30], delivered through hypoxic air storage systems[33].

Although the benefits of HIIT for individuals who are overweight, obese, or living with T2DM are well demonstrated[4,7], few studies have addressed whether exercise in hypoxia is beneficial in this population (see review by Kindlovits et al.[23]). To our knowledge, no study has investigated the combination of intermittent hypoxia and HIIT as a therapeutic intervention for T2DM. We hypothesize that both intermittent hypoxia and HIIT independently improve health-related outcomes in T2DM, and that combining HIIT with breathing hypoxic air intermittently during recovery periods will generate even greater benefits. This model may therefore represent an innovative, practical, and physiologically meaningful approach for improving glucose regulation in individuals with T2DM.

The general aim is to evaluate the effects of intermittent hypoxia and HIIT, both isolated and combined, on glucose homeostasis, cardiorespiratory fitness, cardiovascular health, body composition and hematological, metabolic and behavioral outcomes in

individuals with T2DM.

Specific aims

The specific aims are to test whether 8 weeks of three interventions in adults with T2DM, namely i) passive intermittent hypoxia, ii) HIIT sessions under inter-effort hypoxic recovery, and iii) HIIT sessions under normoxia, improve the selected outcomes listed in Table 1.

Table 1 – Selected outcomes related to health and disease control in T2DM

Selected outcomes assessed before and after the intervention	
Primary	Glycated hemoglobin (HbA1c)
Secondary	Other glucose homeostasis outcomes (triglyceride glucose index, area under the curve for glucose in an oral glucose tolerance test, fasting blood glucose, and homeostasis model assessment)
	Cardiorespiratory fitness (peak oxygen uptake, respiratory thresholds, heart rate, and blood lactate concentration during ramp exercise test)
	Cardiovascular health (flow mediated dilation, resting arterial blood pressure)
	Body composition (fat percentage, lean mass, and bone mineral density)
	Hematological outcomes (fasting plasma lipid profile, biomarkers of erythropoiesis, and inflammatory status)
	Metabolic outcomes (resting energy expenditure via indirect calorimetry)
	Behavioral outcomes (dietary intake of energy, macro and micronutrients, physical activity questionnaire)

Methods

This protocol was designed following the Consolidated Standards of Reporting Trials 2010 statement, the 2022 extension, and the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013. The detailed information about blinding, randomization and allocation concealment, safety procedures, strategies for participant retention and adherence, data management, participants orientations, dissemination policy and sample size calculation, is available in Supplementary document. The SPIRIT Checklist 2025 are available as Supplementary document.

Ethics

This study was approved by the Research Ethics Committee of the School of Physical Education and Sport of Ribeirão Preto, University of São Paulo (CAAE: 79919924.8.0000.5659). It was also authorized by the Research Project Evaluation Commission of the Municipal Health Department of Ribeirão Preto, São Paulo, Brazil (PMRP process 2024/130663, under the code SMQWATZE) to recruit participants from the city's public primary health care centers. Activities will only begin after participants provide written informed consent. Participation is voluntary, with no financial compensation, individuals may withdraw at any time.

At any time, if necessary, the changes to the protocol, such as modifications to the experimental design, procedures, target population, or sample size, will require formal authorization from the Research Ethics Committee. If approved, the changes will be reported to the Brazilian Clinical Trials Registry (RBR-9gmbt9v) and will be implemented only after the approval from both aithorities.

Recruitment of participants

Study announcements will be disseminated to the population of Ribeirão Preto, São Paulo, Brazil, and neighboring region through local television channels, online news platforms from the university, as well as radio, social media, and other communication means. Additionally, patients from 12 authorized public primary health care clinics will be invited to participate. The announcements will highlight key eligibility criteria, including age and diagnosed T2DM.

Elegibility, inclusion, and exclusion criteria for participants

Eligible participants will be men and women aged 18-65 years with an established diagnosis of T2DM (defined according to established clinical criteria [FBG] ≥126 $mg{\cdot}dL^{-1}$, or >200 $mg{\cdot}dL^{-1}$ at 120 minutes (OGTT), or HbA1c ≥ 6.5%) who are not under insulin therapy.

Inclusion criteria, assessed after the initial interview, include the absence of: unstable body mass (changes > 5% over the last three months), exposure to altitudes above 1500 m in the past three months, angina, uncontrolled arterial hypertension, lung diseases, severe heart valvular diseases, decompensated heart failure, renal failure, neurological and orthopedic issues that prevent exercise, scheduled surgery during the study period. In addition, all participants will undergo a retinal assessment by an ophthalmologist specialized in evaluating individuals diagnosed with diabetes mellitus. Those with evidence of severe or proliferative retinopathy will not be included.

The exclusion criteria include experiencing any of the following aspects during the study period: traveling to locations above 1500 meters of altitude, or having any metabolic, cardiac, neurological, orthopedic disease, or musculoskeletal injury that prevents physical activity.

After signing the informed consent form, participants will complete and submit the Physical Activity Readiness Questionnaire (PAR-Q, in accordance with São Paulo State Law No. 16724 of May 22, 2018) and provide information on comorbidities, medication treatments, and overall health during an initial anamnesis. If any positive response is recorded on the PAR-Q, the participant will be required to sign a responsibility waiver for engaging in physical activity. This information will be used to determine eligibility based on the criteria outlined above. The participant timeline is summarized in Table 2.

Experimental design

This study is a blinded, randomized controlled clinical trial designed to evaluate the independent and combined effects of intermittent hypoxia and HIIT (Figure 1). Two of the four experimental groups will undergo a HIIT program: i) under normoxic condition (N_{HIIT}; FiO_2 = 0.209) or ii) IEH_{HIIT} (FiO_2 = 0.135). The IEH_{HIIT} involves exercising in normoxia with inter-effort recovery phases conducted in normobaric

Table 2 – Time schedule of enrolment, interventions, assessments and visits for participants.

	Study Period									
	Enrolment	Allocation	Post-allocation							Closeout
Time point	t_{-1}	0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_9
Enrolment:										
Eligibility screen	X									
Informed consent	X									
Medications	X									X
Health history	X									
Allocation		X								
Interventions:										
N_{HIIT}			◆	—	—	—	—	—	—	◆	
IEH_{HIIT}			◆	—	—	—	—	—	—	◆	
PIH			◆	—	—	—	—	—	—	◆	
CON			◆	—	—	—	—	—	—	◆	
Familiarization:	X	X									
Assessments:										
Aerobic fitness (VO_{2peak}, RCP, PPO, and RCP intensity)	X										X
Glycemic control (HbA1c, AUC OGTT and [FBG])	X										X
Insulin resistance (TyG and HOMA)	X										X
Blood outcomes (erythropoiesis and inflammation)	X										X
Body composition (lean mass, fat mass, and bone mineral content, visceral fat mass)	X										X
Cardiovascular measurements (Arterial Pressure, endothelial function, maximal HR)	X										X
Resting metabolic rate	X										X
Behavioral outcomes (food intake and outside physical activity)	X										X
Hypoxia dose			◆	—	—	—	—	—	—	◆	
Training load (RPE and TRIMP)			◆	—	—	—	—	—	—	◆	

AUC OGTT: area under curve for glucose in an oral glucose tolerance test N_{HIIT}: high intensity interval training (HIIT) under normoxia group; IEH_{HIIT}: HIIT under inter-effort hypoxia recovery group; PIH: passive intermittent hypoxia group; CON: control group; VO_{2peak}: peak oxygen uptake; RCP: respiratory compensation point; PPO: peak power output; [FBG]: fasting blood glucose concentration; HbA1c: glycated hemoglobin; TyG: triglyceride glucose index; HOMA: Homeostasis model assessment; HR: heart rate; RPE: rating of perceived exertion; TRIMP: training impulse.

hypoxia. The other two groups will serve as controls: iii) passive intermittent hypoxia exposure (PIH; FiO_2 = 0.135) and iv) absolute control group (CON; FiO_2 = 0.209), which involves neither exercise training nor hypoxia exposure.

The interventions will last eight weeks, preceded by a one-week familiarization period. This duration is consistent with previous research demonstrating significant physiological changes within this timeframe for intermittent hypoxia combined with exercise[25] and without exercise[14,15]. The duration was also selected to balance the likelihood of observing meaningful adaptations with the risk of participant withdrawal (See Participant retention and adherence section in Supplementary document 1).

The primary (HbA1c) and secondary (glucose homeostasis, cardiorespiratory fitness, cardiovascular health, body composition, and hematological, metabolic, and behavioral) outcomes, as detailed in Table 1, will be measured at baseline and post-intervention (Table 2). The planned timing for these assessments is the week before the familiarization period and the week immediately following the intervention. An allowable window of up to two weeks before and after these dates will be permitted to accommodate participant scheduling. HIIT sessions will involve cycling at intensities between the heart rate (HR) at the respiratory compensation point (RCP) and peak power output (PPO), obtained from a standardized ramp test at baseline. Normobaric hypoxia will be administered at an FiO_2 of 0.135, corresponding to a simulated altitude of approximately 3600 m.

Assessments of outcomes at pre- and post-intervention time points

Aerobic fitness

Participants will perform a graded exercise test (GXT) on a cycle ergometer (Cg-08, Inbramed, Brazil) with continuous monitoring of HR (polar H10, Polar Electro Oy, Finland) and breath-by-breath pulmonary gas exchange (Quark CPET, Cosmed, Italy), calibrated according to the manufacturer's specifications. The initial warm-up consists of a 5-minute effort at 5W, followed by a ramp exercise protocol to voluntary exhaustion. The increment rate in power output will be individu-

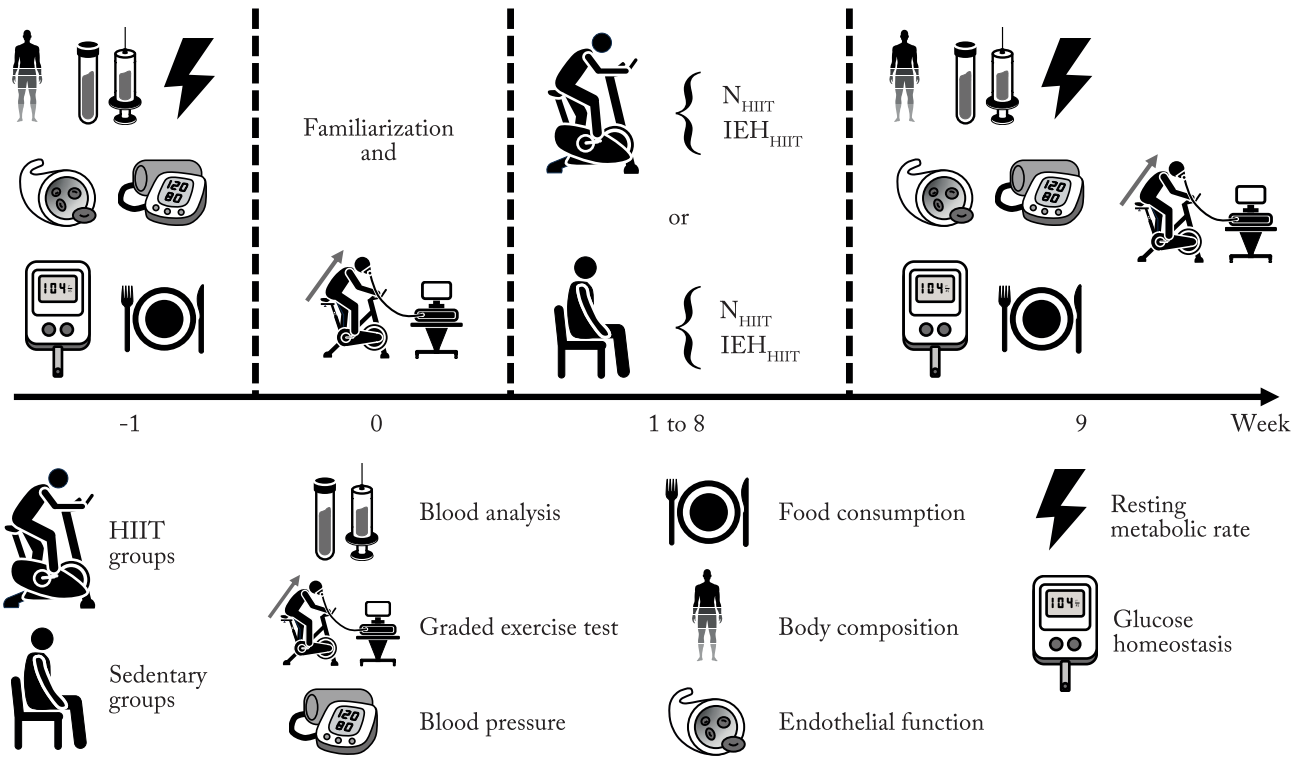


Figure 1 – Overview of experimental design. The entire study will span 11 consecutive weeks, including 1 week of familiarization, 8 weeks of interventions, and 2 weeks for pre-and post-assessments. At specified time points, participants will undergo venous blood sampling, ramp exercise tests to voluntary exhaustion with respiratory and hemodynamic monitoring, arterial blood pressure measurement, and indirect colorimetry during resting, and dual-energy X-ray absorptiometry exams to determine body composition (see Table 1 for outcomes). The four experimental groups are: non-exercised absolute normoxic control (CON), HIIT with inter-effort hypoxic recovery (IEH_{HIIT}), passive intermittent hypoxia exposure (PIH), and HIIT under normoxia (N_{HIIT}).

ally determined to promote exhaustion within 10 ± 2 min, according to the following equation[34]:

Cycling work rate increment ($W \cdot min^{-1}$) = (estimated VO_{2peak} – estimated $VO_{2\text{-unloaded}}$)/92.5

where: estimated $VO_{2\text{-unloaded}}$ (oxygen uptake in an unloaded effort; $L \cdot min^{-1}$) = 150 + (6 · body mass); estimated VO_{2peak} (peak oxygen uptake; $L \cdot min^{-1}$) = [(0.046 · height · 100) – (0.021 · age) – (0.62 · sex) – 4.31] · 1000; and, sex = 0 for man or 1 for woman.

Participants will undergo the first GXT for familiarization during the week before baseline assessments. If the exhaustion time falls outside the target range of 10 ± 2 min, the increment rate will be adjusted for the subsequent GXT. This adjustment will be set at one-tenth of the PPO observed in the GXT familiarization. Participants will abstain from smoking for at least 8 hours, be tested under their optimal medication regimen, refrain from vigorous exercise for 24 hours, and consume only a light meal at least 2 hours before testing. All tests will be performed with appropriate clothing and scheduled at the same time of day for both pre- and post-intervention assessments.

Raw breath-by-breath data will be processed using 20-second time-averaging windows aligned to the center of the interval (i.e., 10s). When aligning mechanical power output or HR to respiratory data, the same 20-s centered averaging will be applied. The processed respiratory data, namely oxygen uptake, minute ventilation (VE), carbon dioxide production (VCO_2), partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$), partial pressure of end-tidal oxygen, along with power output and HR, will be used to determine key aerobic fitness indices: PPO, VO_{2peak}, and RCP. RCP will be determined according to Keir et al.[35], excluding the first 60 seconds of the test. For RCP identification, VCO_2, $P_{ET}CO_2$, VE/VCO_2, and respiratory exchange ratio will be plotted against VO_2, and RCP will be determined as the point of marked inflection in the VE/VCO_2 curve. Two experienced researchers will independently assess RCP; in case of disagreement, a third evaluator will be consulted. RCP will be expressed as the corresponding power output, VO_2, and HR, with power output and HR derived via linear regressions between VO_2 and each variable.

Furthermore, blood lactate concentration will be measured pre- and post-GXT using a YSI 2300 STAT analyzer (Yellow Springs, OH, USA). For this analysis, 25 μL blood samples will be collected from the earlobe at baseline and at 3 and 5 minutes following the GXT using pre-calibrated heparinized capillary tubes. Samples will be immediately dispensed and homogenized in Eppendorf tubes containing 1% sodium fluoride.

Assessment of glycemic control and other dependent variables in blood

General procedures

Participants will be instructed to avoid vigorous physical activity and maintain a normal intake of carbohydrates. Venous blood samples will be collected after a 12-hour fasting to measure glycemia, fasting insulin concentration ([FI]), HbA1c, plasma lipid profile (total triglycerides, total cholesterol and its fraction), C-reactive protein and hemogram [plus reticulocyte count (Ret)].

An experienced professional will collect venous blood samples using strict hygiene and antisepsis protocols. Samples will be immediately placed in tubes containing EDTA or heparin and sent to the Clinical Analysis Service at the Faculty of Pharmaceutical Sciences of Ribeirão Preto for the measurement. The variables will include complete blood count analysis [total erythrocyte count, hematocrit, hemoglobin concentration (Hb), mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular Hb, total leukocyte, platelet, and Ret], and serum C-reactive protein levels. To determine EPO concentration, specific enzyme-linked immunosorbent assay kits will be used, following the manufacturer's instructions.

Subsequently, an oral glucose tolerance test (OGTT) will be conducted by ingesting 75g of anhydrous glucose diluted in 300 mL water. Additional blood samplings will be collected at 30, 60, 90, and 120 min post-ingestion to determine glycemic and insulinemic excursions. The OGTT will be expressed as the area under the glycemic curve (AUC OGTT).

Calculation of indexes from blood measured variables

The hematological stimulation index (off-score) will be determined by measuring Hb ($g \cdot L^{-1}$) and Ret (%)[36]: off-score = Hb · 60 · √Ret.

Insulin resistance will be assessed using the homeostasis model assessment of insulin resistance (HOMA-IR), calculated as: HOMA-IR = [FBG] · [FI] / 22.5. Additionally, the function of pancreatic β-cells will be evaluated with the homeostasis model assessment of pancreatic β-cells function (HOMA-%β) using the formula: HOMA-%β = 20 · [FI] / ([FBG] - 3.5). Where [FBG] is expressed in mmol/l, and FI

in μU/mL.

The triglyceride glucose index (TyG) will be calculated by[37]: TyG = ln[fasting triglyceride (mg/dl) · [FBG] (mg/dl) / 2]

Body composition

Dual-energy X-ray absorptiometry (GE Lunar – DPX-NT) will be employed to analyze body composition. When positioned in the equipment, participants will remain in a supine position throughout the examination (approximately 15 minutes). This method partitions the body into lean mass, fat mass, and bone mineral content in total body and across anatomical compartments: trunk, lower limbs, and upper limbs. It also enables estimation of visceral fat mass. Results will be expressed in both absolute and relative forms. To ensure reliability, participants should adhere to the standardized conditions: they will arrive in the morning after an overnight fast of at least 8 hours, having refrained from vigorous exercise and alcohol consumption for 24 hours. They will be instructed to wear light clothing without metal and to empty their bladder immediately before assessment.

Cardiovascular responses

Systolic and diastolic blood pressure will be measured using a calibrated automatic arm device, which operates through the oscillometric method (OMRON, model HEM-7113). Initially, blood pressure will be measured in both arms, first the left, then the right. Subsequently, two additional measurements will be taken exclusively on the right arm, with a two-minute rest interval between readings. The mean of the right arm measurements will be calculated, and the difference between the right and left arms will be determined. All measurements will be taken after the participant has rested for five minutes in a seated position.

Endothelial function will be evaluated through flow-mediated dilation (FMD), following established standardized guidelines. A 2D bidirectional ultrasound device with color and spectral Doppler and a 14 MHz linear probe transducer (FP-102, SAEVO, Brazil) will be employed. On the test day, participants will lie in a supine position and rest completely for 20 minutes before the procedure. The assessment will be conducted on the right arm, positioned with 90° abduction. The probe will be placed on the medial section of the upper arm to capture a longitudinal B-mode image of the brachial artery, located 5–10 cm above the antecubital fossa. After measuring the baseline diameter ($D_{baseline}$), the transducer location will be marked with an anthropometric marker to ensure a consistent location for post-occlusion measurements. Subsequently, the brachial artery will be occluded for 5 minutes using a pressure cuff applied to the arm and inflated to 200 mmHg. Following cuff deflation, the artery diameter will be manually and continuously measured for 240 seconds to identify the peak diameter (D_{peak}). FMD will be calculated as: FMD (%) = (D_{peak} – $D_{baseline}$) / $D_{baseline}$ · 100. Intra- and inter-rater reliability will be assessed in a subsample of participants.

For both blood pressure and FMD assessments, participants will be instructed to avoid vigorous exercise for at least 24 hours and to undergo an overnight fast of 8 hours. Medications will remain consistent between pre- and post-intervention assessments, which will also be conducted at the same time of day. The testing room will be controlled for both lighting and temperature.

Resting metabolic rate

Indirect calorimetry will be used to analyze the Resting metabolic rate (RMR) through the Quark CPET gas analyzer (Cosmed, Rome, Italy) operating in basal metabolism mode. The equipment will be calibrated prior to each test according to the manufacturer's recommendations. The participant will undergo the test in a fasted state of at least 5 hours, abstaining from alcohol and nicotine for a minimum of 2 hours and from caffeine for 4 hours. They will be instructed not to engage in moderate or vigorous activity for 14 hours before the test. Upon arrival, the participant will rest in a supine position for 10 to 20 minutes before data acquisition begins, which will last for 10 minutes. The first 5 minutes of recording will be discarded, and the average of the final 5 minutes will be used, provided the coefficient of variation (standard deviation / mean of the measurements) for VO_2 and VCO_2 is < 0.10. RMR will be calculated via the abbreviated Weir equation: RMR = [3.9 · VO_2 + 1.1· VCO_2] · 1.44.

Behavioral outcomes

To assess food intake, 24-hour dietary recalls will be collected on two nonconsecutive weekdays during both pre- and post-intervention periods. An experienced nutritionist will interview the participants using the multiple-pass method. The NutraBem pro software (NutraBem Institute, Santos, Brazil), which contains Brazilian food composition tables, will be used to cal-

culate energy and the contents of macronutrients and micronutrients. Usual intake will be estimated after adjusting for intra-individual variability using the Multiple Source Method. Participants will be instructed to maintain their usual dietary habits throughout the study. Supplement intake will be recorded at both pre- and post-intervention assessments.

To monitor external physical activity, participants will complete the short version of the International Physical Activity Questionnaire both pre- and post-intervention. They will also be instructed to maintain their usual physical activity levels throughout the study, regardless of group assignment.

Intervention protocols

Normobaric hypoxia exposure device

The hypoxia device, illustrated in Figure 2, consists of an air generator that reduces the oxygen fraction through a filtration mechanism (CAT 430 TM, Altitude Control Technologies, USA). This device was validated by Norberto et al.[33] and will be set to generate air with FiO_2 of 0.135. Through a hose connected to a face mask equipped with a unidirectional nonrebreathing valve (allowing only influx from the storage system), the participant will inhale the stored hypoxic air, with a FiO_2 of 0.135, thus inducing systemic normobaric hypoxia. Participants will only breathe through the mask during the planned periods of hypoxia exposure or during the respective sham conditions, when the masks will deliver atmospheric normoxic air (FiO_2 of 0.209). Participant blinding regarding the FiO_2 will be maintained using a hidden system of valves that allows ambient air to flow into the hose, while the generator motor remains under operation in all conditions (i.e., crucial for blinding). This design enables individuals to breathe either hypoxic or ambient air via the same device in a fully blinded fashion.

Passive intermittent hypoxia (PIH) and control (CON) groups

To ensure participant blinding and to align expectations, the PIH and CON groups will undergo identical procedures. Participants will attend laboratory sessions at the same frequency (three times per week) and for the same duration (55-70 minutes) as the HIIT groups.

During these sessions, participants in both the PIH and CON groups will remain seated at rest while wearing the same mask connected to the hypoxia device. The

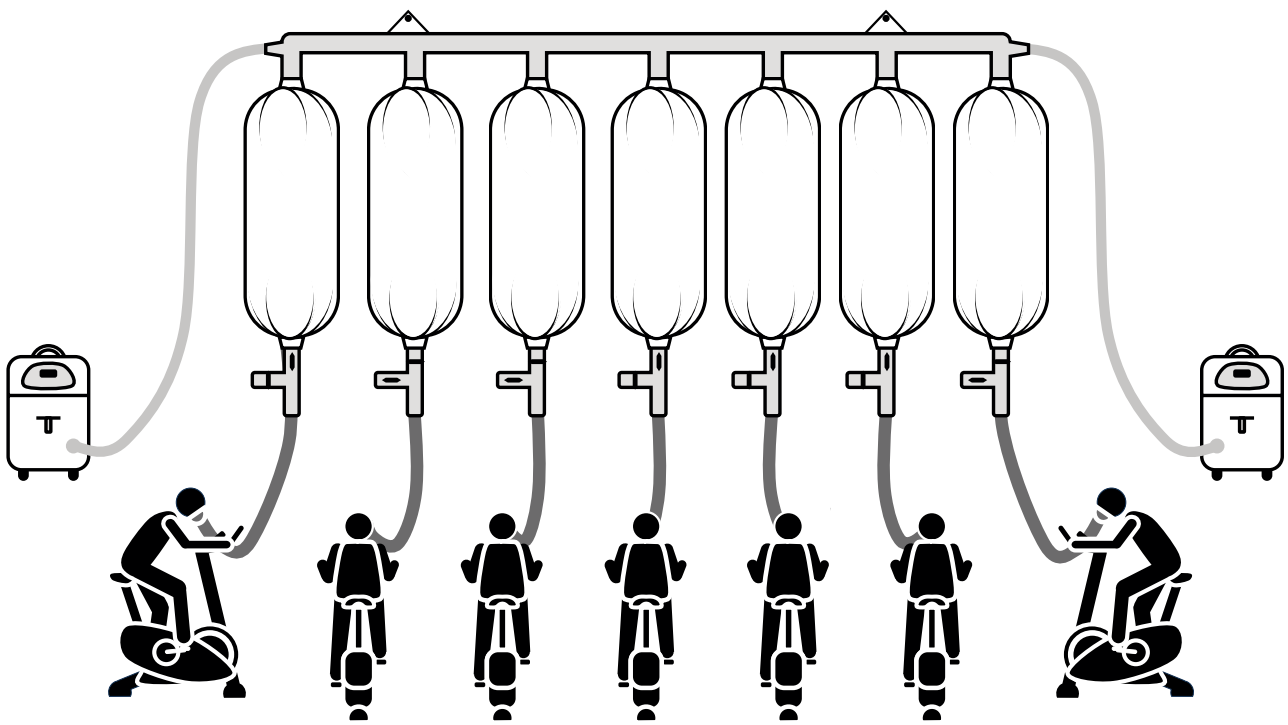


Figure 2 – Schematic of the device used to deliver normobaric hypoxia to participants. Hypoxia generators partially filter O_2 from atmospheric air and pump it to a multi-bag storage system. Participants inhale the stored hypoxic air (O_2 fraction of ~0.135) through a hose connected to a mask with an in-line unidirectional valve; exhalation occurs through a separate unidirectional valve on the mask. Concealed T-valves at the hose-bag junction allow switching between hypoxic (from the bag) or normoxic (from the atmosphere) air. The hypoxia generator remains on in all conditions to allow for blinding of the participants.

PIH group will follow a time-matched protocol (Figure 3) replicating the intermittent hypoxia exposure of the IEH_{HIIT} group, receiving intermittent hypoxic air (FiO_2 = 0.135). Conversely, the CON group will receive a time-matched sham-hypoxia protocol, in which intermittent normoxic air (FiO_2 = 0.209) is delivered for the same duration and with the same sensory experience.

The exercise training program

Participants in the N_{HIIT} and IEH_{HIIT} groups will begin the monitored exercise training program on stationary bicycles (magnetically-braked, model 364GX, Embreex, Brazil) after pre-assessments.

Each session will start with a 5-min warm-up at 50% of PPO under normoxia. Intermittent exercise will follow, with intensities based on HR at RCP and PPO, as determined from the GXT and detailed in Table 3. RCP-intensity HIIT sessions will consist of six 5-min effort with 3-min recovery intervals (Figure 3). On the first three training days, effort durations will be progressively increased (3:20, 4:00, and 4:30 minutes, respectively). PPO-intensity sessions will consist of five 3-min efforts, also with 3 min of recovery. The N_{HIIT} group will perform both effort and recovery phases while breathing ambient air (FiO_2 = 0.209) through the mask. The IEH_{HIIT} will inhale hypoxic air (FiO_2 = 0.135) only during the recovery phases (not during efforts). Additionally, IEH_{HIIT} will breathe in hypoxia for three 5-min periods, before and after the warm-up, and after the cool-down to accumulate a total exposure of approximately 30 minutes. The cool-down will consist of 3 min of low-intensity exercise (50% of PPO) under normoxia for both HIIT groups.

All training sessions will be conducted by a qualified team composed of one physical education professional and one nutritionist specialized in exercise. The interventions will take place at the School of Physical Education and Sport of Ribeirão Preto, University of São Paulo, Brazil. Training sessions will be performed groups consisting of 4 to 12 participants per session.

The determination of exercise intensities on the training stationary bicycles:

Each participant will exercise on the same stationary bicycle throughout the training program. During a familiarization session, they will perform four graded 5-min efforts with progressively increasing power outputs – set at 45, 30, 15, and 0 W below the power output corresponding to RCP (as measured in the previous GXT) – each separated by a 3-min passive recovery. The mean HR during the last 30 s of each stage will be subjected to a linear regression against

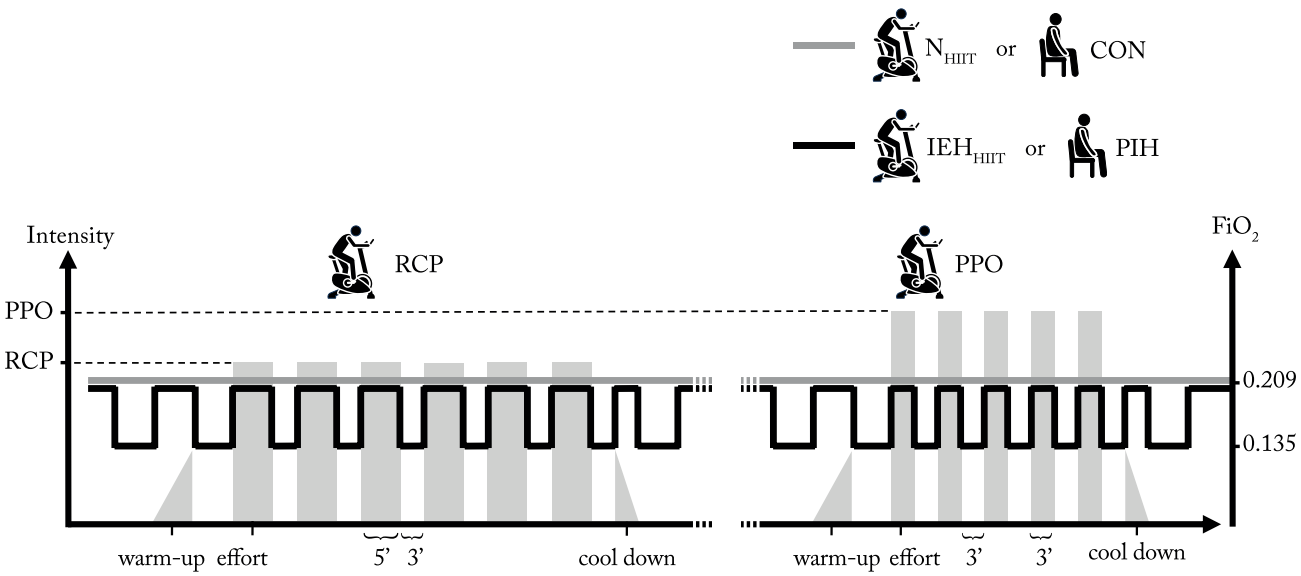


Figure 3 – Training and intermittent hypoxia exposure programs for high-intensity interval training (HIIT) and sedentary groups. The lines represent the inspired oxygen fraction (FiO_2) of all groups throughout the intervention. The bars correspond to the effort intensity only for HIIT under the inter-effort recovery hypoxia group (IEH_{HIIT}) and normoxia group (N_{HIIT}). When training is prescribed at the heart rate (HR) corresponding to the respiratory compensation point (RCP), participants will complete six 5-min efforts with 3-min recovery intervals. When training is prescribed at HR in peak power output (PPO), there will be five 3-min efforts with 3-min recovery intervals. For both, all efforts, the warm-up and cool down will be performed in normoxia (FiO_2 of 0.209). The IEH_{HIIT} will breathe in hypoxia during all recoveries (FiO_2 of 0.135), prior to the warm-up and after the warm-up, and during the cool down. The PIH group will receive the same hypoxia protocol as IEH_{HIIT}, without performing exercise. CON: control.

the power output displayed on the stationary bicycle screen. Using this regression, the HR values at RCP and maximal effort (from the GXT) will be used to estimate the device-specific power outputs to elicit intensities corresponding to RCP and 100% of VO_{2peak}. These calculated power outputs will be applied in the HIIT sessions (Table 3 and Figure 3).

Table 3 – Training program proposed.

Week	Prescribed exercise intensities		
	Monday	Wednesday	Friday
-1	Pre-assessments		
0	Familiarization	Familiarization	Familiarization
1	RCP	RCP	RCP
2	RCP	RCP	RCP
3	RCP	PPO	RCP
4	PPO	RCP	PPO
5	PPO	RCP	PPO
6	PPO	PPO	PPO
7	PPO	PPO	PPO
8	PPO	PPO	PPO
9	Post-assessments		

RCP: Intensity corresponding to the respiratory compensation point (6 efforts of 5' with 3' recovery); PPO: peak power output (5 efforts of 3' with 3' recovery).

Physiological monitoring during the interventions

Hypoxia dose determination

The external hypoxic dose will be quantified as the product of equivalent altitude (in kilometers) and exposure duration (in hours), expressed as km·h. For this protocol, a cumulative dose of approximately 45 km·h is expected. Additionally, the internal hypoxic dose (iHD) will be estimated based on peripheral oxygen saturation (SpO_2) and according to Millet et al.[38] suggestion, thereby accounting for inter-individual variability in physiological responses.

$$iHD\ (\%\cdot h) = (98/\text{measured } SpO_2 - 1) \cdot t \cdot 100$$

where t is the hypoxia exposure time (in hours).

SpO_2 will be monitored using a pulse oximeter (Multilaser, Brazil). Measurements will be taken during at least one intervention session (out of three) per week across all experimental groups. The oximeter will be placed on the distal phalanx of participants and readings will be taken at the end of each HIIT effort and every 30 seconds during recovery periods. In non-HIIT groups, SpO_2 will occur at corresponding time points.

Training loads

Training will be monitored by the 10-point ratings of perceived exertion scale. Internal load will be assessed by the training impulse (TRIMP), using HR and exercise time in the equation bellow[39]:

$$TRIMP = \text{duration of training (min)} \cdot \Delta HR \text{ ratio (bpm)} \cdot Y$$

where $\Delta HR = \frac{HR_{exercise} - HR_{rest}}{HR_{max} - HR_{rest}}$, $Y = 0.64e^{1.92x}$ for males, $Y = 0.86e^{1.67x}$ for females, e = 2.712 and x = ΔHR ratio.

Statistical procedures

Sample size

The sample size was determined by an *a priori* power analysis conducted via Monte Carlo simulation, targeting 80% power at a 5% significance level. The detailed methodology can be assessed in the Supplementary document. Simulations were performed in the R software environment (v. 4.4.1, R core team) using the *lme4* package for linear mixed-effects modeling and *simr* for power analysis. The design accounted for four groups with repeated measures (pre- and post-intervention) for the primary outcome: HbA1c. Simulations (1000 iterations per sample size) tested total sample sizes from 16 to 100 participants, with power defined as the proportion of models in which the Group × Time interaction reached $p < 0.05$.

Simulated power curves indicated that 52 participants achieved a power of 0.82 (95% CI: 0.80; 0.84) for HbA1c. Considering the largest estimated sample and an anticipated 22% participant withdraw, the final recruitment target was set at 64 participants (16 per group) to ensure adequate power across all primary outcomes.

Baseline participants characteristics

Baseline characteristics will be reported for the overall sample and stratified by intervention groups, including age, sex, body mass index, lean mass, fat mass percentage, smoking status, time since T2DM diagnosis, [FBG], pharmacotherapy, VO_{2peak}, peak HR, FMD, physical activity outside the intervention, and usual energy and carbohydrate intake. Categorical variables will be summarized as absolute and relative frequencies, while continuous variables will be presented as mean ± SD. No hypothesis testing will be conducted to compare baseline differences between groups. Clinically relevant imbalances will be identified and considered

as covariates in subsequent analyses.

Statistical analysis

Generalized linear mixed models with an identity link function will be used for statistical analysis. For the pre- and post-intervention outcome variables, Time (pre- vs. post-intervention), Group (N_{HIIT} vs. IEH_{HIIT} vs. HIP vs. CON), and their interaction (Time×Group) will be included as fixed effects. Covariates will be selected for each dependent variable. The models will be tested using a gamma or gaussian distribution and with different combinations of covariates, ensuring no multicollinearity.

The primary analytical model (continuous variable) will be specified as follows:

$$HbA1c \sim Time*Group + Covariates + (1|ID)$$

For this outcome, covariates will be: age, sex, body composition, daily antidiabetic pharmacotherapy, dietary intake, physical fitness, physical activity outside intervention, any variable that shows meaningful baseline imbalance, and, if applicable, pharmacotherapy used before the measurement. All covariates in the model will respect multicollinearity.

All secondary outcomes (glucose homeostasis, cardiorespiratory fitness, cardiovascular health, body composition, hematological, metabolic, and behavioral measures) will be treated as continuous variables. The same covariates as for the primary analysis will be included, except when a covariate constitutes the dependent variable under investigation. Furthermore, exploratory mediational analyses will be conducted to investigate whether changes in secondary outcomes mediate the effect of the interventions on glucose homeostasis.

The tested models will be evaluated to ensure they meet the assumptions of homoscedasticity and normality of residuals. Therefore, to determine the best-fitting statistical model for the data, Akaike's information criterion and the following plots will be analyzed: i. residuals vs. predicted values; ii. residuals vs. observed values; iii. observed vs. predicted values; iv. QQ plot of residuals with a 95% confidence band; v. histogram and box plot of the residuals; and vi. Cook's distance. When multiple models provide a comparable fit, the most parsimonious will be selected.

Statistical inference will be conducted using the selected model with a predefined significance level of $\alpha < 0.05$. When significant fixed effects are identified, Bonferroni corrections will be applied for post hoc comparisons. The analyses will be conducted in the R programming environment (v 4.4.1, R Core Team) using the *emmeans* and *lme4* packages.

Statistical inference will be based on the 95% confidence interval of the difference between means, where evidence of a difference will be considered when the range does not contain zero. Finally, the effect size for the differences between means will be calculated as Cohen's d and interpreted as follows: 0.01 = very small; 0.2 = small; 0.5 = medium; 0.8 = large; 1.2 = very large; and 2.0 = huge.

Analyses will primarily follow the intention-to-treat principle, including all randomized participants in the groups to which they were originally assigned, regardless of adherence. In addition, a sensitivity analysis will be conducted using a per-protocol approach, excluding participants who complete less than 87.5% of the planned intervention sessions.

Discussion

Our study aims to evaluate the independent and combined effects of intermittent hypoxia and HIIT on health-related outcomes in individuals with T2DM. We are employing a robust design with a wide variety of assessment techniques to enhance the validity and originality of the study and address our aims. Our study involves a detailed evaluation of multiple parameters that assess the individual's overall health status, with HbA1c as the primary outcome. We will also evaluate glucose homeostasis related variables, cardiorespiratory fitness, cardiovascular health, body composition, and hematological metabolic, and behavioral outcomes. Collectively, these measures provide a multidimensional and transdisciplinary framework that enhances the clinical and scientific relevance of the study.

This study is the first to evaluate the combined effects of intermittent hypoxia and HIIT in individuals with T2DM. While both interventions have shown independent benefits in this population[6–8,14], their combination may provide additional advantages, as previously demonstrated in individuals with obesity[28]. Furthermore, applying intermittent hypoxia exclusively during recovery periods preserves training quality[31,32], while still inducing systemic hypoxia[31] and, chronically, promoting greater improvements in physical fitness in healthy individuals[40]. The inter-effort hypoxia model is also more practical and accessible, as it enables intermittent exercise without requiring extensive infrastructure to deliver normobaric hypoxia.

We carefully designed this study to minimize biases through the use of control groups, randomization, blinding, and data treatment with covariates, thereby enhancing the internal validity and strengthening causal inference. Although few studies involve HIIT, the N_{HIIT} group serves as a positive control, as physical activity is well-established to improve the health parameters under investigation. The CON group, on the other hand, is the negative control group, representing the absence of intervention. This design allows us to compare intermittent hypoxia, whether combined with exercise or applied passively, not only against both control groups but also between hypoxia conditions themselves. To further reduce group variability, we will conduct randomization using the paired clustering technique based on variables that could become confounding factors if not well-distributed across the groups. Finally, the statistical analysis will treat variables that cannot be fully controlled as covariates. However, the controlled nature of the protocol may limit external validity and the immediate generalizability of the findings to real-world clinical settings.

This study is expected to contribute to the understanding of combined hypoxic and exercise-based interventions in T2DM. Our long-term goal is to establish new clinical guidelines for non-pharmacological therapy in T2DM treatment, aiming at improving patients' quality of life through more efficient glycemic control and cardiovascular health methods. In this sense, our aim is aligned with the Sustainable Development Goal of the World Health Organization, particularly Good Health and Well-Being. The intervention emphasizes innovative, non-pharmacological strategies that may reduce disease burden, enhance quality of life, and inform future applications within primary healthcare settings and the Brazilian Unified Health System, although its immediate implementation may be limited by the need for specialized equipment and supervision. The findings may also optimize exercise prescription for individuals with T2DM, particularly regarding training intensity, structure, and metabolic adaptations. Lastly, the results from this study protocol will be submitted for publication in high-impact academic journals.

Strengths and limitations

Our protocol offers several potential strengths:

- To our knowledge, this is the first randomized controlled trial to investigate the combined effects of HIIT and intermittent hypoxia in individuals with T2DM, a highly prevalent and increasingly common disease worldwide.
- Primary outcomes focus on HbA1c, while secondary outcomes encompass glucose homeostasis, cardiorespiratory fitness, cardiovascular health, body composition, and hematological, metabolic, and behavioral measures. All these aspects bring a high level of interdisciplinarity to the protocol.
- The use of hypoxia during recovery periods is an innovative and ecologically valid approach [30]. Although hypoxia generators are required, the individual is free to perform the exercise and only needs to wear a mask during recovery periods.

Despite its strengths, this study protocol has some limitations:

- The high training frequency (allowing a maximum of three absences) and extended intervention duration may lead to high dropout rate. Additionally, the use of hypoxia may cause discomfort due to the need to wear a mask after performing intense exercises. To promote adherence, measures such as creating health reports, close monitoring, and encouragement will be taken.
- The absence of direct measurements of the molecular mechanisms underlying the intervention, despite the detailed mechanistic rationale.
- Lastly, our study does not evaluate other forms of hypoxia exposure, such as continuous hypoxia throughout sessions or intermittent hypoxia during exercise efforts only.

Trial status

The study is currently in the data collection phase for the main experiment, following the initiation of participant recruitment in December 2025.

Conflict of interest

The authors declare no conflict of interest.

Funding

This work was supported by the São Paulo Research Foundation (process number: 2023/02728-3 and 2024/04856-1) and Coordination for the Improvement of Higher Level Personnel – Brazil (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* - CAPES).

Author's contributions

Firmino MS: Conceptualization; Methodology; Validation; Formal analysis; Investigation; Data curation; Project administration; Visualization; Funding acquisition; Writing – original draft; Writing – review & editing; Approval of the final version. Figueira TR, Paula FJA and Girard O: Methodology; Writing – review & editing; Approval of the final version. Norberto MS: Investigation; Writing – review & editing; Approval of the final version. Papoti M: Conceptualization; Methodology; Formal analysis; Investigation; Resources; Supervision; Project administration; Funding acquisition; Writing – original draft; Writing – review & editing; Approval of the final version.

Declaration regarding the use of artificial intelligence tools in the article writing process

The artificial intelligence tools ChatGPT and Gemini were used to perform the following activities: text revision, text translation, and/or text organization. The authors declare that all material derived from this process has been reviewed and assume full responsibility for all the content of the manuscript.

Availability of research data and other materials

The data cannot be made publicly available. Since this is a study protocol, the article does not contain data; any program code can be shared upon request from the reviewers.

References

1. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. The Lancet. 2005;365(9467):1333–46. doi: https://doi.org/10.1016/s0140-6736(05)61032-x
2. Sampath Kumar A, Maiya AG, Shastry BA, Vaishali K, Ravishankar N, Hazari A, et al. Exercise and insulin resistance in type 2 diabetes mellitus: A systematic review and meta-analysis. Ann Phys Rehabil Med. 2019;62(2):98–103. doi: https://doi.org/10.1016/j.rehab.2018.11.001
3. Jiménez-Maldonado A, García-Suárez PC, Rentería I, Moncada-Jiménez J, Plaisance EP. Impact of high-intensity interval training and sprint interval training on peripheral markers of glycemic control in metabolic syndrome and type 2 diabetes. Biochim Biophys Acta Mol Basis Dis. 2020;1866(8):165820 doi: https://doi.org/10.1016/j.bbadis.2020.165820
4. Khalafi M, Symonds ME. The impact of high-intensity interval training on inflammatory markers in metabolic disorders: A meta-analysis. Scand J Med Sci Sports. 2020;30(11):2020–36. doi: https://doi.org/10.1111/sms.13754
5. Gripp F, Nava RC, Cassilhas RC, Esteves EA, Magalhães COD, Dias-Peixoto MF, et al. HIIT is superior than MICT on cardiometabolic health during training and detraining. Eur J Appl Physiol. 2021;121(1):159–72. doi: https://doi.org/10.1007/s00421-020-04502-6
6. Arrieta-Leandro MC, Moncada-Jiménez J, Morales-Scholz MG, Hernández-Elizondo J. The effect of chronic high-intensity interval training programs on glycaemic control, aerobic resistance, and body composition in type 2 diabetic patients: a meta-analysis. J Endocrinol Invest. 2023;46(12):2423–43. doi: https://doi.org/10.1007/s40618-023-02144-x
7. Jelleyman C, Yates T, O'Donovan G, Gray LJ, King JA, Khunti K, et al. The effects of high-intensity interval training on glucose regulation and insulin resistance: A meta-analysis. Obes Rev. 2015;16(11):942–61. doi: https://doi.org/10.1111/obr.12317
8. Liu J xin, Zhu L, Li P jun, Li N, Xu Y bing. Effectiveness of high-intensity interval training on glycemic con-trol and cardiorespiratory fitness in patients with type 2 diabetes: a systematic review and meta-analysis. Ag-ing Clin Exp Res. 2019;31(5):575–93. doi: https://doi.org/10.1007/s40520-018-1012-z
9. De Nardi AT, Tolves T, Lenzi TL, Signori LU, Silva AMV da. High-intensity interval training versus contin-uous training on physiological and metabolic variables in prediabetes and type 2 diabetes: A meta-analysis. Di-abetes Res Clin Pract. 2018;137:149–59. doi: https://doi.org/10.1016/j.diabres.2017.12.017
10. Garcia SP, Cureau FV, Iorra F de Q, Bottino LG, Laura LE, Leivas G, et al. Effects of exercise training and physical activity advice on HbA1c in people with type 2 diabetes: A network meta-analysis of randomized con-trolled trials. Diabetes Res Clin Pract. 2025;221:112027. doi: https://doi.org/10.1016/j.diabres.2025.112027
11. Lopez-Pascual A, Bes-Rastrollo M, Sayón-Orea C, Perez-Cornago A, Díaz-Gutiérrez J, Pons JJ, et al. Living at a geographically higher elevation is associated with lower risk of metabolic syndrome: Prospective analysis of the SUN cohort. Front Physiol. 2017;7(658):1–9. doi: https://doi.org/10.3389/fphys.2016.00658
12. Ortiz-Prado E, Espinosa PS, Borrero A, Cordovez SP, Vasconez JE, Barreto-Grimales A, et al. Stroke-Related Mortality at Different Altitudes: A 17-Year Nationwide Population-Based Analysis From Ecuador. Front Physiol. 2021;12: 733928. doi: https://doi.org/10.3389/fphys.2021.733928
13. Navarrete-Opazo A, Mitchell GS. Therapeutic potential of intermittent hypoxia: a matter of dose. Am J Physiol Regul Integr Comp Physiol. 2014;307:118197. doi: https://doi.org/10.1152/ajpregu.00208.2014
14. Marlatt KL, Greenway FL, Kyle Schwab J, Ravussin E. Two weeks of moderate hypoxia improves glucose tol-erance in individuals with type 2 diabetes. Int J Obes. 2019;44(3):744–7. doi: https://doi.org/10.1038/s41366-019-0422-0
15. Shepherd AI, James TJ, Gould AAM, Mayes H, Neal R, Shute J, et al. Impact of nocturnal hypoxia on gly-caemic control, appetite, gut microbiota and inflammation in adults with type 2 diabetes mellitus: A single-blind cross-over trial Handling Editors. J Physiol. 2024;602(21):5835-58541–20. doi: https://doi.org/10.1113/JP285322#support-information-section
16. D'Hulst G, Sylow L, Hespel P, Deldicque L. Acute systemic insulin intolerance does not alter the response of the Akt/GSK-3 pathway to environmental hypoxia in human skeletal muscle. Eur J Appl Physiol. 2015;115(6):1219–31. doi: https://doi.org/10.1007/s00421-015-3103-2
17. Soo J, Raman A, Lawler NG, Goods PSR, Deldicque L, Girard O, et al. The role of exercise and hypoxia on glucose transport and regulation. Eur J Appl Physiol. 2023;123(6):1147–65. doi: https://doi.org/10.1007/s00421-023-05135-1
18. Lavalle S, Masiello E, Iannella G, Magliulo G, Pace A, Lechien JR, et al. Unraveling the Complexities of Oxi-dative Stress and Inflammation Biomarkers in Obstructive Sleep Apnea Syndrome: A Comprehensive Review. Life (Basel). 2024;14(4):425. doi: https://doi.org/10.3390/life14040425
19. Soo J, Girard O, Ihsan M, Fairchild T. The Use of the SpO2 to FiO_2 Ratio to Individualize the Hypoxic Dose in Sport Science, Exercise, and Health Settings. Front Physiol. 2020;11:570472.. doi: https://doi.org/10.3389/fphys.2020.570472

20. Serebrovska T V., Portnychenko AG, Drevytska TI, Portnichenko VI, Xi L, Egorov E, et al. Intermittent hypoxia training in prediabetes patients: Beneficial effects on glucose homeostasis, hypoxia tolerance and gene expression. Exp Biol Med. 2017;242(15):1542–52. doi: https://doi.org/10.1177/1535370217723578

21. Schreuder THA, Nyakayiru J, Houben J, Thijssen DHJ, Hopman MTE. Impact of hypoxic versus normoxic training on physical fitness and vasculature in diabetes. High Alt Med Biol. 2014;15(3):349–55. doi: https://doi.org/10.1089/ham.2013.1144

22. van Meijel RLJ, Vogel MAA, Jocken JWE, Vliex LMM, Smeets JSJ, Hoebers N, et al. Mild intermittent hy-poxia exposure induces metabolic and molecular adaptations in men with obesity. Mol Metab. 2021;53:101287. doi: https://doi.org/10.1016/j.molmet.2021.101287

23. Kindlovits R, Pereira AMDS, Sousa AC, Viana JL, Teixeira VH. Effects of Acute and Chronic Exercise in Hypoxia on Cardiovascular and Glycemic Parameters in Patients with Type 2 Diabetes: A Systematic Review. High Alt Med Biol. 2022;23(4):301–12. doi: https://doi.org/10.1089/ham.2022.0029

24. Mackenzie R, Maxwell N, Castle P, Elliott B, Brickley G, Watt P. Intermittent exercise with and without hypoxia improves insulin sensitivity in individuals with type 2 diabetes. J Clin Endocrinol Metab. 2012;97(4). doi: https://doi.org/10.1210/jc.2011-2829

25. Kindlovits R, Sousa AC, Viana JL, Milheiro J, Oliveira BMPM, Marques F, et al. Eight Weeks of Intermit-tent Exercise in Hypoxia, with or without a Low-Carbohydrate Diet, Improves Bone Mass and Functional and Physiological Capacity in Older Adults with Type 2 Diabetes. Nutrients. 2024;16(11). doi: https://doi.org/10.3390/nu16111624

26. Ladage D, Braunroth C, Lenzen E, Berghöfer S, Graf C, Bloch W, et al. Influence of intermittent hypoxia in-terval training on exercise-dependent erythrocyte nos activation and blood pressure in diabetic patients. Can J Physiol Pharmacol. 2012;90(12):1591–8. doi: https://doi.org/10.1139/y2012-138

27. Ghaith A, Chacaroun S, Borowik A, Chatel L, Doutreleau S, Wuyam B, et al. Hypoxic high-intensity interval training in individuals with overweight and obesity. Am J Physiol Regul Integr Comp Physiol. 2022;323(5):R700–9. doi: https://doi.org/10.1152/ajpregu.00049.2022

28. Camacho-Cardenosa A, Camacho-Cardenosa M, Brazo-Sayavera J, Burtscher M, Timón R, Olcina G. Effects of high-intensity interval training under normobaric hypoxia on cardiometabolic risk markers in over-weight/obese women. High Alt Med Biol. 2018;19(4):356–66. doi: https://doi.org/10.1089/ham.2018.0059

29. Wehrlin JP, Hallén J. Linear decrease in VO2max and performance with increasing altitude in endurance ath-letes. Eur J Appl Physiol. 2006;96(4):404–12. doi: https://doi.org/10.1007/s00421-005-0081-9

30. Papoti M, Manchado-Gobatto FB, Gobatto CA. Inter-effort recovery hypoxia: a new paradigm in sport sci-ence? BMJ Open Sport Exerc Med. 2023;9(3):e001520. doi: https://doi.org/10.1136/bmjsem-2022-001520

31. Foresti YF, Carvalho CD De, Ribeiro FA, Andreossi JC, Luches-Pereira G, Bertucci DR, et al. Acute physio-logical responses to "Recovery Intermittent Hypoxia" in HIIT. Rev Bras Med Esporte. 2024;30:e2021_0499. doi: https://doi.org/10.1590/1517-8692202430022021_0499i

32. Dellavechia de Carvalho C, Marcolino Putti G, Figueiredo Foresti Y, Alves Ribeiro F, Causin Andreossi J, Ferraz de Campos G, et al. Recovery in normobaric hypoxia as an additional stimulus for high-intensity inter-mittent training. Sci Sports. 2023;38(2):189–96. doi: https://doi.org/10.1016/j.scispo.2021.12.007

33. Norberto MS, Torini JVG, Firmino MS, Papoti M. Validation of Air Storage System for Hypoxia Exposure During Exercise. High Alt Med Biol. 2024; 25(2):122-8. doi: https://doi.org/10.1089/ham.2023.0122

34. Radtke T, Crook S, Kaltsakas G, Louvaris Z, Berton D, Urquhart DS, et al. ERS statement on standardisation of cardiopulmonary exercise testing in chronic lung diseases. European Respiratory Review. 2019;28(154). doi: https://doi.org/10.1183/16000617.0101-2018

35. Keir DA, Iannetta D, Mattioni Maturana F, Kowalchuk JM, Murias JM. Identification of Non-Invasive Exer-cise Thresholds: Methods, Strategies, and an Online App. Sports Med. 2022;52(2):237–55. doi: https://doi.org/10.1007/s40279-021-01581-z

36. Sanchis-Gomar F, Martinez-Bello VE, Domenech E, Nascimento AL, Pallardo F V., Gomez-Cabrera MC, et al. Effect of intermittent hypoxia on hematological parameters after recombinant human erythropoietin admin-istration. Eur J Appl Physiol. 2009;107(4):429–36. doi: https://doi.org/10.1007/s00421-009-1141-3

37. Simental-Mendía LE, Rodríguez-Morán M, Guerrero-Romero F. The product of fasting glucose and triglycer-ides as surrogate for identifying insulin resistance in apparently healthy subjects. Metab Syndr Relat Disord. 2008;6(4):299–304. doi: https://doi.org/10.1089/met.2008.0034

38. Millet GP, Debevec T, Brocherie F, Girard O, Pialoux V. Commentaries on Viewpoint: Human skeletal mus-cle wasting in hypoxia: a matter of hypoxic dose? J Appl Physiol. 2017;122(2):409. doi: https://doi.org/10.1152/japplphysiol.01084.2016

39. Borresen J, Lambert MI. The Quantification of Training Load, the Training Response and the Effect on Per-formance. Sports Med. 2009;39(9):779–95. doi: https://doi.org/10.2165/11317780-000000000-00000

40. Putti GM, Costa GP, Norberto MS, Carvalho CD de, Bertuzzi RC de M, Papoti M. Use of Inter-Effort Re-covery Hypoxia as a New Approach to Improve Anaerobic Capacity and Time to Exhaustion. High Alt Med Biol. 2024;25(1):68–76. doi: https://doi.org/10.1089/ham.2023.0096

Received: 03/04/2026
Reviewed: 04/15/2026
Approved: 06/09/2026

Cite this article as:
Firmino MS, Figueira TR, Norberto MS, Paula FJA, Girard O, Papoti M. High-intensity interval training under hypoxia for individuals with type 2 diabetes: the protocol. Rev. Bras. Ativ. Fis. Saúde. 2026;31:e0458. doi: 10.12820/rbafs.31e0458

Supplementary document

Blinding, randomization and allocation concealment

The primary and secondary outcomes are delineated in Table 2 of the article, with the timing of each measurement outlined in Table 3. All assessments will be conducted with participants blinded to group allocation. However, both asessors and therapist administrating the training protocol will not be blinded. To assess the integrity of the blinding process, participants will complete a treatment-guess questionnaire at the end of the study. A standardized procedure for emergency unblinding will be in place. Unblinding of an individual participant will only be permitted in the case of a medical emergency, where the treating physician deems it essential for clinical management (see Safety procedures section).

Participants will be randomly assigned to one of four groups using matched-pair clustering based only on sex. After clustering, random allocation in blocks will be performed using Microsoft Excel (version 16.98). The randomization sequence will be generated by a researcher with no involvement in data collection or in administrating the training protocol. To ensure allocation concealment, group assignments will be placed in individual, sequentially numbered, opaque, and sealed envelopes prepared by the same researcher. After a participant is formally enrolled and completes baseline assessments, the next envelope in sequence will be delivered directly to the therapist responsible for administering the intervention.

Safety procedures

Capillary blood glucose will be assessed before exercise and at the end of exercise bouts during training sessions using an enzymatic glucose dehydrogenase-based glucometer (Freestyle Freedom Lite, Abbott). Blood pressure will be measured at the same time points with an automated oscillometric device (OMRON, model HEM-7113).

Prior to exercise, acceptable blood glucose values will range from 90-250 mg/dL. If pre-exercise glucose is <90 mg/dL, participants will ingest 15-30 g of fast-acting carbohydrate, and additional carbohydrate will be administered if glucose falls below 90 mg/dL during exercise[1]. Exercise will be interrupted if glucose decreases to <70 mg/dL, and participants will be immediately treated with carbohydrate. For blood pressure, exercise initiation will be initiated only when resting systolic pressure is <160 mmHg and diastolic pressure is <100 mmHg. Exercise sessions will be discontinued if systolic pressure reaches 230 mmHg or diastolic pressure exceeds 115 mmHg[2].

The SpO_2 will be continuously monitored during hypoxic exposure using a pulse oximeter. Exposure to intermittent hypoxia has been shown to be safe[3], including for elderly patients with cardiovascular diseases[4]. Considering that a target SpO_2 around 80% can acutely improve glucose homeostasis[5], a safety cutoff of 75% will be adopted. If participants remain asymptomatic despite reaching this threshold, the unblinded therapist administrating the training protocol will adjust the valve to deliver normoxic air (as illustrated in Figure 2) until SpO_2 rise above 75%. Conversely, if symptoms of hypoxemia arise, participants will be requested to discontinue intermittent hypoxia immediately, irrespective of whether it occurs during exercise or at rest.

Given the non-pharmacological and minimal-risk nature of the intervention, a formal independent Data Monitoring Committee will not be established. Participant safety will be continuously monitored by the research team, which includes investigators trained in advanced life support and exercise testing supervision. All adverse events will be systematically monitored and documented throughout the study. In the case of a serious adverse event, the study will be immediately paused and unblinding will be permissible, and the local research ethics committee will be notified within 24 hours in accordance with regulatory guidelines. A comprehensive investigation will be conducted with the committee to determine the causes, the potential relationship to the study interventions, and other relevant factors. The study will only resume after a formal review and receipt of a favorable opinion from the ethics committee.

Participants who experience any adverse event or clinical complication during study procedures will receive immediate assistance from trained staff, and, when necessary, will be referred to appropriate medical care within the university healthcare system. The research team will provide guidance and clinical referral for any relevant abnormal findings identified during assessments. After study completion, participants will receive their clinical results and general recommendations for continued health care and diabetes management. In the event of harm directly related to trial

participation, participants will be entitled to medical care and support according to institutional and national ethical regulations, with treatment provided at no cost to the participant.

Participant retention and adherence

To promote retention and adherence, a multi-faceted approach will be adopted. During the familiarization week, each participant will undergo a personalized onboarding session to align expectations and address any initial concerns, such as the HIIT sessions and the exposure to intermittent hypoxia. Throughout the intervention, the research team will maintain regular contact to reinforce motivation and provide flexible scheduling, including opportunities to make up missed sessions. To further support engagement, participants will receive easy-to-understand health reports after baseline and post-intervention assessments, summarizing their individual results across all health-related outcomes.

Data management and sharing

All original research data, including digitized physical forms and electronic files, will be securely stored at the research laboratory, with all digital files backed up daily to a secure, university-provided cloud server. To ensure participant confidentiality, all data will be de-identified prior to analysis. In accordance with open science principles, the final de-identified dataset will be made publicly available on the Open Science Framework (https://osf.io/) repository following the publication of the main results, and the data will be preserved for a minimum of ten years.

Orientations for participants

The participants will be instructed to maintain their common habits for physical activity and dietetic consumption throughout the protocol. Also, they will be instructed to not initiate any nutritional supplementation during the study and to modify their current medication only under medical supervision. Medication use prior to each assessment, supplementation, habitual dietary intake, and physical activity performed outside the protocol will be assessed before and after the intervention, as described in the main protocol.

Dissemination Policy

At the end of the study, all participants will individually receive a report containing the results of all assessments, presented in clear and accessible language, accompanied by a plain-language summary explaining the main findings and their health implications.

The trial results will be disseminated regardless of the direction or magnitude of the observed effects. Findings will be submitted for publication in peer-reviewed scientific journals and presented at national and international scientific conferences targeting both the academic community and healthcare professionals.

In addition, results will be communicated to the general public through science communication materials, including graphical summaries and explanatory texts shared via the institutional social media channels of the university and research group. Whenever possible, findings will also be disseminated through local media outlets, such as television interviews and news reports. The clinical trial registry will be updated with the final results after study completion to ensure transparency and public access to information.

Detailed sample size calculation method

The sample size was determined through an a priori power analysis conducted via Monte Carlo simulation, targeting 80% power at a 5% significance level. Simulations were performed in the R software environment (v 4.4.1, R Core Team) using the lme4[6] package for linear mixed-effects modeling and simr[7] for power estimation. The design accounted for four groups with repeated measures (pre- and post-intervention) across the primary outcome: glycated hemoglobin (HbA1c). Statistical power was calculated for total sample sizes ranging from 16 to 100 participants, increasing in increments of four, with 1000 iterations per sample size. Power was defined as the proportion of simulated datasets in which the fixed interaction term (Group × Time) reached statistical significance ($p < 0.05$). The model structure was specified as lmer(VD ~ Group * Time + (1|ID)), assuming a Gaussian distribution with an identity link, and included random intercepts to account for within-subject repeated measures.

Glycated hemoglobin (HbA1c)

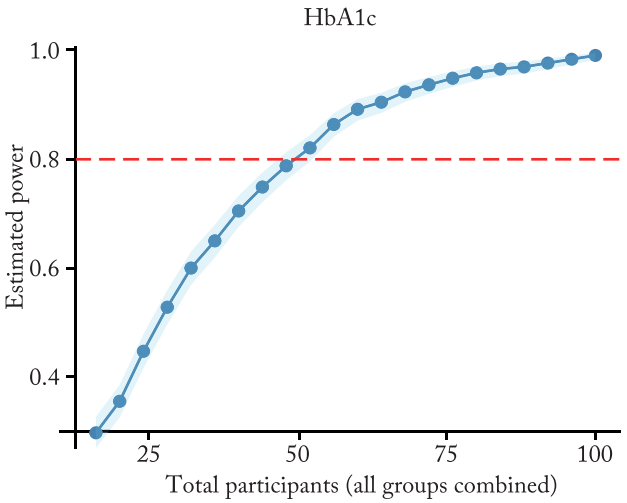
The mean baseline HbA1c value for Brazilian adults with type 2 diabetes mellitus (T2DM) was assumed to be 7.5%, based on epidemiological data from the de Almeida-Pititto et al.[8]. The total standard deviation (SD_{total}) was 1.5%, derived from the same source. For post-intervention estimates, the control group (CON) was assumed to remain unchanged. A decrease of 0.1%

was assigned to the passive hypoxia group (PIH), based on findings from Lippl et al.[9], who observed reductions in HbA1c following 14 days of hypoxic exposure in obese individuals. For both exercise groups, normoxia high intensity interval training (N_{HIIT}) and HIIT under inter-effort hypoxia (IEH_{HIIT}), a decrease of 0.9% was adopted, consistent with the mean effect reported in a meta-analysis by Opazo-Díaz et al.[10]. The within-subject coefficient of variation (CVi) for HbA1c was 0.083, obtained from the meta-analysis of Gough et al.[11]. The residual standard deviation (σ_{resid}) was computed as CVi × mean baseline HbA1c, i.e. 0.62%, and the between-subject standard deviation (σ_{id}) was estimated as the formula below:

$$\sigma_{id} = \sqrt{(SD_{total}^2 - \sigma_{resid}^2)} = 1.80.$$

Final sample size result

The power curve for primary outcome can be viewed in Supplementary Figure 1. A total of 52 participants provided a power of 0.82 (95% CI: 0.80 ; 0.84). Considering the estimated sample size and anticipating a 22% rate of participant withdraw, the final recruitment target was set at 64 participants (16 per group) to ensure adequate statistical power for the analyses.



Supplementary Figure 1 – Power curve for primary outcome. HbA1c: glycated hemoglobin.

Supplementary References

1. Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, et al. Physical activity/exercise and diabetes: A position statement of the American Diabetes Association. Diabetes Care. 2016;39(11):2065–79. doi: https://doi.org/10.2337/dc16-1728
2. Pescatello LS, Franklin BA, Fagard R, Farquhar WB, Kelley GA, Ray CA. Exercise and Hypertension: Position Stand of American College of Sports Medicine. Med Sci Sports Exerc. 2004;36:533–53. doi: https://doi.org/10.1249/01.mss.0000115224.88514.3a
3. Smith CM, Salmon OF. Safety and effectiveness of acute intermittent hypoxia during a single treatment at different hypoxic severities. Respir Physiol Neurobiol. 2025;331:104358. doi: https://doi.org/10.1016/j.resp.2024.104358
4. Glazachev OS, Kryzhanovskaya SY, Zapara MA, Dudnik EN, Samartseva VG, Susta D (2021) Safety and Efficacy of Intermittent Hypoxia Conditioning as a New Rehabilitation/Secondary Prevention Strategy for Patients with Cardiovascular Diseases: A Systematic Review and Meta-analysis. Curr Cardiol Rev. 2021;17(6):e051121193317. doi: https://doi.org/10.2174/1573403x17666210514005235
5. Zhao J, Massoudian SD, Stray-Gundersen S, Wojan F, Lalande S Short bouts of hypoxia improve insulin sensitivity in adults with type 2 diabetes. J Appl Physiol. 2025;138:873–80. doi: https://doi.org/10.1152/japplphysiol.00932.2024
6. Bates D, Mächler M, Bolker BM, Walker SC. Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw. 2015;67(1):1–48. doi: https://doi.org/10.18637/jss.v067.i01
7. Green P, Macleod CJ (2016) SIMR: An R package for power analysis of generalized linear mixed models by simulation. Methods Ecol Evol. 2015; 7(4):493–8. doi: https://doi.org/10.1111/2041-210X.12504
8. de Almeida-Pititto B, Eliaschewitz FG, de Paula MA, Ferreira GC (2022) BrazIliaN Type 1 & 2 Diabetes Disease Registry (BINDER): longitudinal, real-world study of diabetes mellitus control in Brazil. Front Clin Diabetes Healthc. 2022;3:934629. doi: https://doi.org/10.3389/fcdhc.2022.934629
9. Lippl FJ, Neubauer S, Schipfer S, Lichter N, Tufman A, Otto B, Fischer R (2010) Hypobaric hypoxia causes body weight reduction in obese subjects. Obesity (Silver Spring). 2010;18(4):675-81. doi: https://doi.org/10.1038/oby.2009.509
10. Opazo-Díaz E, Montes-de-Oca-García A, Galán-Mercant A, Marín-Galindo A, Corral-Pérez J, Ponce-González JG (2024) Characteristics of High-Intensity Interval Training Influence Anthropometrics, Glycemic Control, and Cardiorespiratory Fitness in Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Sports Med. 2024;54(12):3127–49. doi: https://doi.org/10.1007/s40279-024-02114-0
11. Gough A, Sitch A, Ferris E, Marshall T (2023) Within-subject variation of HbA1c: A systematic review and meta-analysis. PLoS One. 2023;18(8):e0289085. doi: https://doi.org/10.1371/journal.pone.0289085

Reviewers' assessment

The reviews of this article were originally conducted in Portuguese. This version has been translated using ChatGPT and subsequently reviewed by the Chief Editors.

Reviewer A

Rodrigo Sudatti Delevatti
Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

Format

- Does the article comply with the manuscript preparation guidelines for submission to the Revista Brasileira de Atividade Física e Saúde?
  Yes
- Regarding formal aspects, is the manuscript well structured, containing the sections: introduction, methods, results, and discussion (with conclusion as part of the discussion)?
  Yes
- Is the language appropriate, and is the text clear, precise, and objective?
  Yes
- Were any signs of plagiarism observed in the manuscript?
  No

Suggestions/comments:

- In the title, I suggest including the study design. For example: randomized clinical trial protocol.

Abstract

- Are the abstract and abstract (in English) appropriate (including: objective, information about participants, studied variables, main results, and a conclusion) and do they reflect the manuscript content?
  Yes

Suggestions/comments:

- Line 21: remove "da" in "curva da glicêmica".

Introduction

- Was the research problem clearly stated and delimited?
  Yes
- Is the research problem adequately contextualized in relation to existing knowledge, moving from general to specific?
  Yes
- Are the reasons justifying the need for the study (including the authors' assumptions about the problem) well established?
  Yes
- Are the references used to support the presentation of the research problem current and relevant?
  Partially
- Was the objective clearly presented?
  Partially

Suggestions/comments:

- There is current evidence, such as the meta-analysis by Piccoli-Garcia et al. (2025), showing HIIT superiority even compared to combined training, not only CAT.
- https://pubmed.ncbi.nlm.nih.gov/39904457/
- In section 1.4, insert "of" after "The use".

Objectives

- In the objectives, I suggest using something more specific than "health-related outcomes".
- In the general objective, I suggest revising the wording, since having a group with isolated HIIT means the objective is not only to assess the effect of hypoxia with and without HIIT, but rather to analyze the effects of hypoxia and HIIT, both isolated and combined, on the intended outcomes.

Methods

- Are the methodological procedures, in general, appropriate to address the research problem?
  Yes
- Are the methodological procedures described in sufficient detail?
  Yes
- Was the participant selection/recruitment procedure appropriate and clearly described?
  Yes
- Were details about data collection instruments, their psychometric properties (e.g., reproducibility, internal consistency, validity), and operational definitions of variables provided when relevant?
  Yes
- Is the data analysis plan appropriate and adequately described?
  Yes
- Are the inclusion/exclusion criteria described and appropriate?

Yes
- Did the authors provide clarification about ethical procedures?
  Yes

Suggestions/comments:
- Page 11, lines 1–2: "The announcements will highlight key eligibility criteria, including age and diagnosed T2DM without the need of insulin therapy."
- Could individuals using insulin participate? The sentence ending makes the criterion somewhat unclear.
- Page 12 (design description): revise wording, as it does not account for the HIIT-only group.
- Page 13, line 5: remove "The" before "Assessments…".
- In the interventions, include the number and training of professionals involved, location, and whether sessions were individual or group-based. Although a figure provides a general overview, the text lacks sufficient detail for reproducibility.
- Page 22, line 24: replace "frequencies and percentages" with "absolute and relative frequencies".
- Why was 87.5% adherence chosen for the per-protocol analysis?

Results

- Are tables and figures used appropriately?
  Not applicable
- Is the number of illustrations adequate according to journal guidelines?
  Yes
- Are participant numbers at each stage, including losses and refusals, reported?
  Yes
- Are participant characteristics sufficiently described?
  Not applicable
- Are results presented appropriately?
  Not applicable

Suggestions/comments:
- Not applicable (protocol article).

Discussion

- Are the main findings presented?
  Not applicable
- Are limitations and strengths presented and discussed?
  Yes
- Are results discussed considering limitations and existing knowledge?
  Not applicable
- Are potential contributions discussed?
  Not applicable

Suggestions/comments:
- Add a period at the end of the first paragraph.
- Expand discussion on internal and especially external validity. The study appears more physiological than clinical, yet the authors suggest application in Primary Health Care. How would this be feasible given the number of required devices?
- Regarding strengths: authors state glycemic homeostasis as the primary outcome, but HOMA appears as secondary in the outcomes table. Table 1 lists three primary outcomes—why? While all are glycemic markers, they are distinct. Why are TG-Gly index and OGTT primary, while HOMA is secondary? I suggest using HbA1c as the sole primary outcome, with HOMA, TG-Gly, and OGTT grouped as secondary outcomes, separate from cardiorespiratory ones.

Conclusion

- Was the conclusion appropriately presented and aligned with the objective?
  Not applicable
- Is the conclusion original?
  Not applicable

Suggestions/comments:
- Not applicable.

References

- Are references up to date and sufficient?
  Yes
- Are most references original articles?
  Yes
- Do references comply with journal standards?
  Yes
- Are in-text citations appropriate?
  Yes

Suggestions/comments:
- Appropriate.

Comments to the author

- Congratulations on the excellent work, with strong innovation, challenges, and well-structured training design.

Final decision

- Minor revisions required

Reviewer B

Ricardo José da Palma Minhalma
University of Algarve, Portugal

Article Title

- The title could be improved to indicate the authors' methodological approach. See the attached document for suggestions.

Format

- Does the article comply with the manuscript preparation guidelines for submission to the Revista Brasileira de Atividade Física e Saúde?
  Yes
- Regarding formal aspects, is the manuscript well structured, containing the sections: introduction, methods, results, and discussion (with conclusion as part of the discussion)?
  Yes
- Is the language appropriate, and is the text clear, precise, and objective?
  Yes
- Were any signs of plagiarism observed in the manuscript?
  No
  Suggestions/comments:
- Very well-structured and organized text.

Abstract

- Are the abstract and abstract (in English) appropriate (including: objective, participant information, studied variables, main results, and a conclusion) and do they reflect the manuscript content?
  Yes
  Suggestions/comments:
- There are some imprecisions in the writing, which are indicated in the attached document.

Introduction

- Was the research problem clearly stated and delimited?
  Yes
- Is the research problem adequately contextualized in relation to existing knowledge, moving from general to specific?
  Yes
- Are the reasons justifying the need for the study (including the authors' assumptions) well established?
  Yes
- Are the references current and relevant?
  Yes
- Was the objective clearly presented?
  Yes
  Suggestions/comments:
- Some issues related to grammar and clarity of ideas are noted in the attached document. It would also be important to include discussion on the dose–response relationship, particularly to support the rationale for the selected training dose in these populations.

Methods

- Are the methodological procedures appropriate to address the research problem?
  Yes
- Are they sufficiently detailed?
  Yes
- Was participant recruitment appropriate and clearly described?
  Yes
- Were instruments and their psychometric properties described?
  Yes
- Is the data analysis plan appropriate?
  Yes
- Are inclusion/exclusion criteria adequate?
  Partially
- Were ethical procedures described?
  Yes
  Suggestions/comments:
- Some issues related to English language usage.
- Review aspects related to experimental control of variables that may introduce bias (see attached document).

Results

- Are tables and figures appropriate?
  Not applicable
- Is the number of illustrations adequate?
  Not applicable
- Are participant flow and losses described?
  Not applicable
- Are participant characteristics described?
  Not applicable
- Are results appropriately presented?
  Not applicable

Suggestions/comments:
Not applicable.

Discussion

- Are the main findings presented?
  Yes
- Are strengths and limitations discussed?
  Yes
- Are results discussed considering limitations and existing knowledge?
  Yes
- Are potential contributions discussed?
  Yes

Suggestions/comments:

- Some issues related to English language usage.

Conclusion

- Is the conclusion appropriate and aligned with the study objective?
  Yes
- Is the conclusion original?
  Yes

Suggestions/comments:

- Not applicable.

References

- Are references up to date and sufficient?
  Yes
- Are most references original articles?
  Yes
- Do references follow journal standards?
  Yes
- Are citations appropriate?
  Yes

General comments

- The manuscript demonstrates high scientific quality and a robust methodological design, with relevant innovation in proposing a model of intermittent hypoxia during recovery in HIIT (IEHHIIT), not yet explored in type 2 diabetes.
- However, important limitations remain that justify major revision:
- Insufficient justification of the hypoxic dose (FiO_2 = 0.135)
- Limited control of variables that may introduce bias (medication, diet, physical activity)
- Issues with clarity and quality of scientific writing
- The study has strong publication potential after substantial revision. Its innovation and potential for positive results may favor acceptance within the scientific community.

Comments to the author

- This protocol presents an innovative and clinically relevant approach to the non-pharmacological treatment of type 2 diabetes mellitus (T2DM), proposing the combination of high-intensity interval training (HIIT) with intermittent hypoxia in a methodologically consistent and well-structured model. The use of hypoxia during recovery periods (inter-effort hypoxic recovery) represents an important conceptual advance, as it allows dissociation between hypoxia-induced mechanical performance limitations and its potential role as an additional metabolic stimulus.
- The theoretical foundation is solid, integrating physiological mechanisms with clinical application. The manuscript clearly articulates the converging effects of high-intensity exercise and hypoxia on insulin-independent glucose uptake pathways (e.g., AMPK activation, CaMK, and GLUT-4 translocation), providing biological plausibility for a synergistic effect. This integration between molecular physiology and applied intervention is a major strength, with strong translational potential for exercise prescription in clinical populations.
- Additionally, the experimental design—randomized, controlled, and including multiple comparison groups—enables the isolation of independent and combined intervention effects, strengthening internal validity. The inclusion of a broad set of outcomes (metabolic, cardiovascular, functional, and behavioral) supports a multidimensional evaluation of intervention impact.
- Despite these strengths, the manuscript still presents limitations that affect its overall scientific quality and readiness for publication. Substantial improvements are needed in three key areas:
- (i) clarity and precision of scientific writing, with persistent issues in sentence structure and terminology consistency;
- (ii) methodological justification, particularly regarding the choice of hypoxic dose;
- (iii) control and discussion of important confounding variables, such as antidiabetic medication, dietary intake, and habitual physical activity, which may significantly influence results.

- In its current form, the manuscript shows strong scientific potential but requires revisions to ensure methodological rigor, transparency, and alignment with publication standards in the field.

Final decision

- Major revisions required