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Oxygen as a Metabolic Modulator: Divergent Physiological Adaptations to Hypoxic and Hyperoxic Tabata Training

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Abstract

Background: Whether inspired oxygen fraction (FiO_2) can modulate the internal metabolic cost of supramaximal high-intensity interval training (HIIT) and thereby direct training adaptation remains unclear. We tested whether hyperoxic versus hypoxic exposure during Tabata-format HIIT induces distinct adaptive phenotypes. **Methods:** Twenty-three physically active men completed 3 weeks of supramaximal Tabata HIIT (3 sessions·week^{-1}; 8 × 20 s with 10 s recovery) under hyperoxia (FiO_2 = 0.60, n = 13) or hypoxia (FiO_2 = 0.16, n = 10). Training intensity was regulated to maintain a comparable internal physiological stimulus rather than an identical external workload. Pre- and post-intervention assessments included maximal oxygen uptake (VO_2max), first and second ventilatory thresholds (VT1, VT2), peak blood lactate, and session rating of perceived exertion (RPE). Post-intervention between-group differences were analysed using ANCOVA adjusted for baseline values; RPE was analysed using a linear mixed-effects model. **Results:** VO_2max improved in both groups but increased more after hyperoxic training than after hypoxic training (+3.69 vs. +1.50 mL·kg^{-1}·min^{-1}; β = 2.18 mL·kg^{-1}·min^{-1}, 95% CI [1.77–2.59], $p < 0.001$). Hyperoxia also produced larger gains in VT1 (β = 29.99 W, 95% CI [17.09–42.89], $p < 0.001$) and VT2 (β = 20.74 W, 95% CI [9.43–32.05], $p = 0.001$). Peak lactate responses diverged bidirectionally, decreasing in hyperoxia (−0.77 mmol·L^{-1}) and increasing slightly in hypoxia (+0.27 mmol·L^{-1}), with a significant adjusted between-group effect (β = −1.02 mmol·L^{-1}, 95% CI [−1.47 to −0.57], $p < 0.001$). RPE declined across sessions in both groups, with a steeper decrease under hyperoxia (Condition × Session: β = −0.36, 95% CI [−0.44 to −0.28], $p < 0.001$). **Conclusions:** Hyperoxic and hypoxic supramaximal HIIT elicited distinct functional adaptive profiles. Hyperoxia induced greater improvements in aerobic capacity and ventilatory thresholds, reduced peak lactate accumulation, and accelerated the decline in perceived exertion, whereas hypoxia was associated with a more glycolytic response pattern. These findings support the interpretation that FiO_2 acts as a modulator of internal physiological load and shapes the metabolic phenotype of adaptation during supramaximal interval training.

Keywords: hyperoxia; hypoxia; Tabata training; VO_2max; Assault-Bike; high intensity interval training; HIIT; human

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1. Introduction

High-intensity interval training (HIIT) is widely recognized as a highly time-efficient strategy for improving maximal oxygen uptake (VO_2max), ventilatory thresholds, and metabolic function across a wide range of populations, from recreationally active individuals to competitive athletes [1–3]. Supramaximal intermittent training protocols such as the Tabata format, repeated 20-s efforts at ~170% VO_2max interspersed with 10 s recovery, are particularly potent. In the original study, six weeks of Tabata training simultaneously improved aerobic and anaerobic capacity despite a total session duration of only four minutes [4]. Nevertheless, under normoxic conditions the magnitude of adaptation remains constrained by the metabolic challenge associated with each training interval. Whether the internal physiological stimulus of supramaximal HIIT can be augmented without modifying the prescribed workload therefore remains an open and practically relevant question. Despite extensive research on hypoxic training and the acute ergogenic effects of hyperoxia, it remains unclear whether inspired oxygen fraction can systematically modulate the internal metabolic cost of supramaximal intervals and thereby direct long-term training adaptation.

Inspired oxygen fraction (FiO_2) represents a potential lever for modulating this internal stimulus. The internal-load framework distinguishes between the external workload imposed on an athlete and the physiological cost required to sustain it [5]. At supramaximal intensities—a distinct exercise domain characterized by rapid oxygen uptake kinetics and substantial anaerobic contribution [6] oxygen delivery and utilization can constrain oxidative ATP resynthesis, thereby determining the glycolytic contribution to each effort bout [7]. Breathing a hyperoxic gas mixture ($FiO_2 > 0.21$) maintains arterial saturation at or near its physiological ceiling, attenuating glycolytic flux and intramuscular metabolic perturbation during supramaximal exercise [8,9]. Conversely, hypoxia ($FiO_2 < 0.21$) amplifies glycolytic reliance at any given external workload [10]. Manipulating FiO_2 therefore provides a means of altering the internal metabolic cost of a fixed training prescription. In line with this concept, previous work from our group demonstrated that even low-intensity exercise performed under moderate hyperoxic conditions was sufficient to induce measurable changes in biomarkers related to metabolic regulation and physical adapatations [11,12].These findings raise the question of whether similar oxygen-mediated modulation may occur during high-intensity interval exercise, where metabolic perturbations are substantially greater.

Evidence that cyclic oxygen exposure may influence training adaptation comes from animal models. Four weeks of exercise training performed under intermittent hyperoxia (cyclic exposure between 21% and 30% O_2) increased maximal exercise capacity and upregulated markers of mitochondrial biogenesis and fatty-acid oxidative capacity compared with normoxic training, whereas continuous hyperoxia produced no such advantage [13]. The superiority of intermittent exposure suggests that transitions between oxygen levels may themselves represent a biologically relevant stimulus. One proposed mechanism is the Normobaric Oxygen Paradox (NOP), whereby a return from hyperoxia to normoxia may be sensed as a relative decrease in oxygen availability and transiently activate hypoxia-responsive signaling pathways despite normoxic conditions [14–17]. Repeated oxygen fluctuations may also modulate redox-sensitive pathways involved in mitochondrial adaptation. Whether such mechanisms operate during supramaximal interval training in humans remains unknown.

If hyperoxia reduces the glycolytic cost of each supramaximal interval, repeated sessions may accumulate a greater oxidative stimulus per unit of prescribed work and thereby accelerate aerobic adaptation relative to normoxic training. This distinction is mechanistically relevant to interval training design. In humans, however, whether breathing a hyperoxic mixture during Tabata-format supramaximal intervals produces aerobic adaptations that are faster or larger than those achieved under normoxic or hypoxic conditions has not been directly examined.

Hyperoxia and hypoxia are rarely examined within the same supramaximal HIIT design, yet such a comparison is informative because the two environments are expected to phenotype-direct adaptation in opposite directions. Hypoxic interval training performed below the exposure threshold

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required for hematological adaptation selectively enhances glycolytic enzymatic capacity by increasing metabolic stress within each effort bout [10,18].

In contrast, hyperoxia may reduce the glycolytic burden of each interval. Across studies that tightly control external workload, however, the aerobic benefits of hyperoxic training have been small or inconsistent [19,20], highlighting the need to frame FiO_2 as a modulator of internal metabolic cost rather than a simple ergogenic aid.

Another limitation of existing research is the reliance on VO_2max as the sole primary outcome. When multiple conditions improve maximal aerobic power, VO_2max alone cannot distinguish between metabolic phenotypes. Complementary markers such as blood lactate kinetics, ventilatory thresholds, and rating of perceived exertion (RPE) provide mechanistically informative indices of the metabolic environment generated during training [21–23].

The present study therefore aimed to compare the effects of three weeks of Tabata-format supramaximal HIIT performed under hyperoxia (FiO_2 0.60) or hypoxia (FiO_2 0.16) on VO_2max, ventilatory thresholds, peak blood lactate, and session RPE in physically active participants. We hypothesized that hyperoxia would augment the oxidative internal stimulus of each interval and produce larger aerobic adaptations than hypoxia, while generating bidirectional lactate responses and a steeper decline in perceived exertion across training sessions.

2. Materials and Methods

2.1. Population

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Academic Ethical Committee of Brussels (Belgium) (approval number: B 200-2022-038). All participants provided written informed consent prior to participation.

Twenty-eight healthy male volunteers were initially recruited for the study. Participants were selected from a population of physiotherapy students and practicing physiotherapists aged between 19 and 35 years. Only male participants were included in order to minimize physiological variability associated with female hormonal fluctuations, particularly those related to the menstrual cycle, which have been reported to influence exercise performance and metabolic responses to exercise [24].

Participants were recreationally active but none were engaged in professional or elite-level sports training. Prior to inclusion, all volunteers underwent medical screening and were considered free from cardiovascular risk according to current exercise testing and sports participation recommendations.

Participants were excluded if they presented any known cardiovascular, pulmonary, metabolic, or neurological disease, or if they were taking medications known to influence cardiovascular or metabolic responses to exercise.

To minimize potential confounding factors, participants were instructed to refrain from strenuous physical activity, alcohol consumption, caffeine intake, and smoking for at least 24 h before each testing session, in accordance with standard exercise testing procedures recommended in exercise physiology guidelines [25,26].

Initially, 28 volunteers were screened for eligibility. Five individuals did not meet the predefined inclusion criteria and were excluded prior to randomization. The remaining 23 participants were randomized and completed the study protocol, and were therefore included in the final analysis (Table 1). The final sample therefore consisted of 23 healthy male participants (mean age 23.5 ± 4.07 years, height 178 ± 5.75 cm, body mass 76.4 ± 9.53 kg). To minimize circadian influences on physiological responses to exercise, all testing sessions were scheduled at the same time of day for each participant, between 08:00 and 11:00 a.m. Allocation was performed by an investigator not involved in data collection to minimize allocation bias, using a computer-generated random number sequence generated using Excel randomization:

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- **Hyperoxia group:** inspired oxygen fraction (FiO_2) = 0.60 (n = 13)
- **Hypoxia group:** inspired oxygen fraction (FiO_2) = 0.16 (n = 10)

2.2. Experimental Protocol

Prior to the start of the 3-week intervention, each participant performed a baseline cardiopulmonary exercise test (CPET) as described below to determine maximal oxygen uptake (VO_2max), maximal heart rate (HRmax), and maximal aerobic power output (Wmax). These parameters were subsequently used to individualize the intensity of the training sessions.

Participants then completed a 3-week supramaximal high-intensity interval training (HIIT) program based on the Tabata protocol, consisting of three sessions per week performed on an air-resistance cycle ergometer (Assault Bike®, Assault Fitness, USA). The Tabata protocol has previously been shown to elicit substantial aerobic and anaerobic adaptations despite its short duration [4,27].

Each training session lasted approximately 20 min and consisted of:

- 10 min of warm-up at 50% of maximal aerobic power output determined during the baseline incremental test
- 8 intervals of 20 s of supramaximal effort performed at approximately 170% of maximal aerobic power output, each separated by 10 s of passive recovery
- 6 min of active recovery at 50% of maximal aerobic power output

Participants were instructed to maintain a cadence above 80 revolutions per minute (rpm) during the high-intensity intervals, with real-time visual feedback provided on the ergometer display. Although the target workload corresponded to approximately 170% of maximal aerobic power output determined at baseline, workload was adjusted when necessary during the intervention in order to maintain the intended cardiovascular strain [27]. Training intensity was therefore monitored using heart rate, and participants were required to reach at least 85% of their individual maximal heart rate during high-intensity intervals. Training intensity was therefore regulated to maintain a comparable internal physiological stimulus rather than an identical external workload. Heart rate was continuously monitored using a chest strap heart rate monitor (Polar H10 Electro, Finland).

The inspired oxygen fraction (FiO_2) administered during the exercise sessions depended on the assigned experimental condition. Oxygen delivery was provided through high-flow nasal cannulas combined with a Double Trunk Mask (DTM) positioned over the participant's face to maintain the target inspired oxygen concentration during exercise.

2.2.1. The Double Trunk Mask

The Double Trunk Mask (DTM) is a device designed to increase the effective inspired oxygen fraction (FiO_2) in subjects receiving oxygen through nasal cannulas by increasing the reservoir volume available for re-inhalation.

The mask contains an internal dead space of approximately 210 mL, while the two lateral tubes ("trunks") provide an additional reservoir volume of approximately 120 mL. During expiration, part of the exhaled gas mixture—containing approximately 16% oxygen—is temporarily retained within this reservoir. During the subsequent inhalation, this oxygen-enriched mixture is partially re-inhaled, thereby increasing the effective inspired oxygen fraction.

The DTM has previously been shown to improve oxygen delivery efficiency in situations characterized by high inspiratory flow rates by limiting dilution of administered oxygen with ambient air [28,29]

In the present study, standard high-flow nasal oxygen alone was not sufficient to ensure a stable inspired oxygen fraction during supramaximal exercise. During intense effort, participants frequently switch to oral breathing and minute ventilation (VE) increases substantially, which can dilute the delivered oxygen fraction with ambient air. The DTM system was therefore used to increase the effective oxygen reservoir and stabilize FiO_2 during exercise.

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Oxygen delivery was provided using an oxygen concentrator (EverFlo™, Philips Respironics, USA) for the hyperoxic condition and a normobaric hypoxic generator (Hypoxico HYP-123, Hypoxico Inc., USA) for the hypoxic condition.

A constant gas flow of 20 L·min^{-1} was delivered through the nasal cannulas during the training sessions in order to maintain the targeted inspired oxygen fraction and reduce dilution by ambient air.

Before each training session, the oxygen fraction delivered at the mask level was verified using a portable oxygen analyzer (Forensics Detectors™, USA) capable of measuring oxygen concentrations between 0–100% O_2. FiO_2 values were periodically verified during exercise sessions at the mask outlet to ensure stability of the inspired oxygen fraction despite high ventilatory rates (FiO_2 ≈ 0.60 for hyperoxia and FiO_2 ≈ 0.16 for hypoxia).

This configuration allowed reliable control of inspired oxygen conditions during high-intensity interval exercise while minimizing dilution effects associated with elevated ventilation rates.

2.2.2. Ergospirometry Test

Before each cardiopulmonary exercise test (CPET), the metabolic analysis system (Ergocap®, MS Medisoft, Sorinnes, Belgium) was calibrated according to the manufacturer's recommendations and current international guidelines for cardiopulmonary exercise testing [30,31]. Gas analyzers were calibrated using a two-step procedure in accordance with international CPET methodological recommendations (ATS/ACCP, 2003). First, calibration was performed using ambient air, assuming an inspired oxygen fraction of 20.93% and a carbon dioxide fraction of approximately 0.03–0.04%. Second, a certified reference gas mixture (16% O_2 and 5% CO_2, balance N_2) was used to calibrate the oxygen and carbon dioxide analyzers. Ventilatory flow was calibrated using a 3-L syringe to ensure accurate measurement of ventilatory volumes as recommended in CPET methodological guidelines [31]. Calibration procedures were conducted before each test session to ensure measurement reliability. All exercise tests were performed under standardized laboratory conditions [32]. Ambient temperature and relative humidity were maintained within a controlled range (approximately 20–22 °C and 40–60% relative humidity) to minimize environmental influences on cardiopulmonary responses. Participants were instructed to avoid strenuous physical activity, caffeine, and alcohol for at least 24 h prior to testing and to arrive at the laboratory in a rested and hydrated state. Testing sessions were scheduled at similar times of day for each participant whenever possible to reduce circadian variability. Participants performed an incremental cardiopulmonary exercise test on an electronically braked cycle ergometer (Ergoline®, Germany) in the Physiology Laboratory of the Haute École Bruxelles-Brabant (HE2B). The protocol consisted of a 3-min warm-up at 50 W followed by a continuous ramp increase in workload of 25 W·min^{-1} until volitional exhaustion. After termination of the incremental phase, a 5-min active recovery period at 50 W was performed. Participants were instructed to maintain a pedaling cadence between 60 and 70 revolutions per minute throughout the test. Standardized verbal encouragement was provided to promote maximal effort. The same testing protocol was used before and after the 3-week training intervention to ensure comparability of physiological responses. Respiratory gas exchange variables were measured breath-by-breath using the metabolic cart and subsequently averaged over 30-s intervals to reduce breath-by-breath variability [33]. Oxygen uptake (VO_2), carbon dioxide production (VCO_2), and minute ventilation (VE) were continuously recorded throughout the test. Participants breathed through a low-resistance facemask connected to the metabolic measurement system. Continuous physiological monitoring included heart rate recording, 12-lead electrocardiography monitoring, and pulse oximetry to ensure participant safety and accurate physiological data collection. The first (VT1) and second (VT2) ventilatory thresholds were determined using a combination of the ventilatory equivalents method and the V-slope method [34]. VT1 was primarily identified by an increase in the ventilatory equivalent for oxygen (VE/VO_2) without a concomitant increase in the ventilatory equivalent for carbon dioxide (VE/VCO_2), reflecting the onset of metabolic acidosis buffering. This determination was cross-validated using the V-slope method [34,35], corresponding to the

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breakpoint at which VCO_2 increases disproportionately relative to VO_2. VT2 was identified by a simultaneous increase in both VE/VO_2 and VE/VCO_2, indicating the onset of ventilatory compensation for metabolic acidosis [36]. Ventilatory thresholds were determined independently by two experienced investigators. In the case of disagreement, the thresholds were re-evaluated jointly until consensus was reached. Maximal oxygen uptake (VO_2max) was defined as the highest 30-s averaged VO_2 value obtained during the incremental test [32]. Achievement of maximal effort was confirmed by the presence of at least two of the following criteria: a respiratory exchange ratio (RER) ≥ 1.10, attainment of ≥ 95% of the age-predicted maximal heart rate, or a high rating of perceived exertion at the end of the test. The ergospirometry protocol used in this study has previously demonstrated good test–retest reliability for maximal and submaximal cardiopulmonary variables when performed under standardized laboratory conditions previously reported coefficients of variation between 3–5% [32,37].

2.2.3. Blood Lactate Measurements

Capillary blood samples were collected from the fingertip to determine blood lactate concentration ($[La^-]$) before and following maximal ergometric test exercise. Prior to sampling, the fingertip was cleaned with an alcohol swab and allowed to dry completely. A sterile single-use lancet was used to puncture the lateral aspect of the fingertip, and the first drop of blood was discarded to avoid contamination with interstitial fluid. A subsequent blood sample (2 µL) was then applied directly onto a Lactate test strip (Roche Diagnostics, Mannheim, Germany) and immediately analyzed using a portable electrochemical analyser (Accutrend® Plus, Roche Diagnostics, Mannheim, Germany). All samples were collected 3 min after the cessation of exercise, in accordance with the recommended post-exercise sampling window that allows for adequate lactate redistribution from the active musculature into the peripheral circulation while minimising the influence of rapid metabolic clearance. All measurements were performed by the same trained operator to ensure procedural consistency, and the ambient temperature was maintained within the manufacturer-specified operating range of 18–35 °C throughout data collection.

2.3. Statistical Analysis

All statistical analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria). Continuous variables are presented as mean ± standard deviation unless otherwise specified. Statistical significance was set at $\alpha = 0.05$ (two-tailed). Between-condition differences in physiological outcomes (VO_2max, VT1, VT2 and peak blood lactate) were assessed using analysis of covariance (ANCOVA). Post-intervention values were entered as dependent variables, experimental condition (Hyperoxia vs. Hypoxia) was included as a fixed factor, and the corresponding baseline value was included as a covariate to control for potential baseline differences and improve statistical power. This analytical approach is recommended for the analysis of pre–post intervention data as it provides greater statistical efficiency and reduces bias compared with analyses based on change scores (Δ) alone [38,39]. Adjusted between-group differences are reported as regression coefficients (β) with corresponding 95% confidence intervals and p-values. The assumption of homogeneity of regression slopes was verified by testing the interaction between baseline values and experimental condition. Model assumptions were further evaluated by inspection of residual plots. Normality of residuals was assessed using the Shapiro–Wilk test and homoscedasticity was verified by examination of residual versus fitted value plots. The incremental variance explained by the experimental condition beyond baseline values was quantified using ΔR^2, defined as the difference between the full model (baseline + condition) and the reduced model (baseline only). Effect magnitude was also expressed using partial R^2. Perceived exertion (Borg scores), collected repeatedly across training sessions, was analyzed using a linear mixed-effects model including condition, session number and their interaction as fixed effects, with participant included as a random intercept to account for repeated measurements within individuals and the hierarchical structure of the data. Associations between individual physiological adaptations were explored using Pearson correlation

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coefficients computed on change scores (Δ = post − pre). To control for type I error inflation due to multiple testing, p-values were adjusted using the Holm correction method. Given the achieved sample size (N = 23), a sensitivity power analysis was conducted for the primary ANCOVA model expressed as a general linear model. With $\alpha = 0.05$ and one predictor of interest (condition), the design provided 80% statistical power to detect an incremental effect size of approximately $f^2 = 0.39$, corresponding to a partial $R^2 \approx 0.28$, which represents a large effect magnitude.

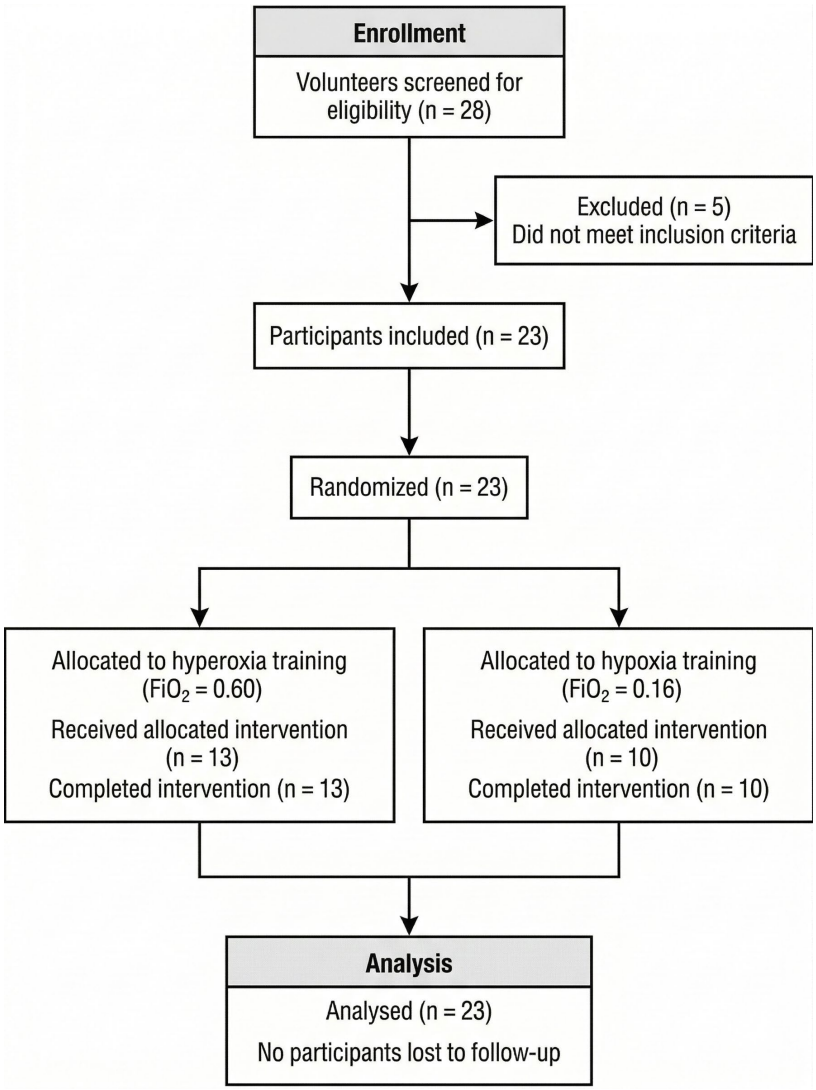

Figure 1. Materials and Methods: CONSORT flow diagram illustrating participant recruitment, allocation to hyperoxia and hypoxia training conditions, and inclusion in the final analysis.

3. Results

3.1. Participant Characteristics

A total of 23 participants were included in the study, with 13 allocated to the hyperoxia group (60% O_2) and 10 to the hypoxia group (16% O_2). All participants were male. Baseline characteristics are presented in Table 1. The two groups exhibited comparable anthropometric and physiological profiles prior to the intervention. Mean age was 24.6 ± 5.4 years in the hyperoxia group and 22.0 ± 1.3 years in the hypoxia group. Body mass and height were similar between groups. Baseline VO_2max, VT1, VT2, and peak lactate concentrations were also comparable, indicating similar aerobic and metabolic status before training. No clinically meaningful baseline imbalance was observed.

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Table 1. Baseline participant characteristics according to training condition. Between-group comparisons are shown for descriptive purposes only.

Variable	Hypoxia (16% O_2) (n = 10)	Hyperoxia (60% O_2) (n = 13)	*p*-value
Sex (Male), n (%)	10 (100%)	13 (100%)	—
Age (years)	22.0 ± 1.3	24.6 ± 5.4	0.143
Body mass (kg)	78.6 ± 11.9	74.8 ± 8.0	0.370
Height (cm)	178.0 ± 7.5	177.2 ± 5.1	0.760
VO_2max (ml·kg^{-1}·min^{-1})	38.30 ± 9.91	37.77 ± 8.67	0.891
VT1 (W)	160 ± 47	175 ± 47	0.472
VT2 (W)	207.5 ± 47.6	232.7 ± 47.6	0.234
Lactate (mmol·L^{-1})	9.22 ± 1.20	9.58 ± 1.20	0.504

3.2. VO2 Max

Baseline VO_2max values were comparable between groups (Table 1). Primary analysis using ANCOVA adjusting post-intervention VO_2max for baseline performance revealed a significant effect of oxygen condition (β = 2.18 mL·kg^{-1}·min^{-1}, 95% CI [1.77–2.59], $p < 0.001$), indicating a greater training-induced improvement under hyperoxic (60% O_2) compared with hypoxic (16% O_2) training. Descriptively, VO_2max increased from 37.77 ± 8.67 to 41.46 ± 8.41 mL·kg^{-1}·min^{-1} in the hyperoxia group (+3.69 mL·kg^{-1}·min^{-1}) and from 38.30 ± 9.91 to 39.80 ± 9.80 mL·kg^{-1}·min^{-1} in the hypoxia group (+1.50 mL·kg^{-1}·min^{-1}). Individual trajectories and relative percentage changes are illustrated in Figure 1.

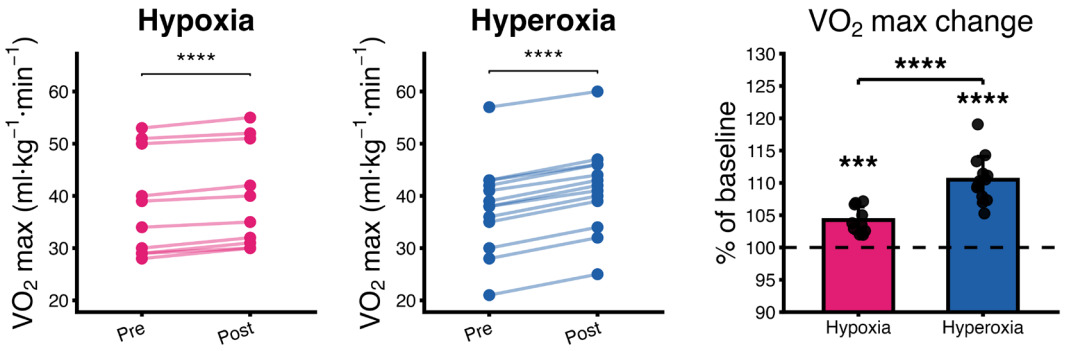

Figure 2. Effects of oxygen availability on VO_2max following 3 weeks of Tabata training. Left and middle panels display individual pre- and post-training VO_2max values (ml·kg^{-1}·min^{-1}) under hyperoxic (60% FiO_2) and hypoxic (16% FiO_2) conditions. The right panel illustrates relative percentage changes from baseline (baseline normalized to 100%). Within-group changes are shown for descriptive purposes. The primary statistical inference was based on ANCOVA adjusting post-intervention values for baseline performance ($p < 0.001$=***; $p < 0.0001$=****).

3.3. First Ventilatory Threshold (VT1)

Baseline VT1 values were comparable between groups (Table 1). Primary analysis using ANCOVA adjusting post-intervention VT1 for baseline VT1 revealed a significant effect of oxygen condition (β = 29.99 W, 95% CI [17.09–42.89], $p < 0.001$), indicating a greater improvement following hyperoxic (60% O_2) compared with hypoxic (16% O_2) training. The additional variance explained by training condition beyond baseline performance was 8.6% (ΔR^2 = 0.086). The assumption of homogeneity of regression slopes was satisfied (p = 0.310), supporting model validity. Descriptively, VT1 increased by +38 W in the hyperoxia group and by +10 W in the hypoxia group. These findings indicate that the upward shift in the first ventilatory threshold was significantly greater following hyperoxic training.

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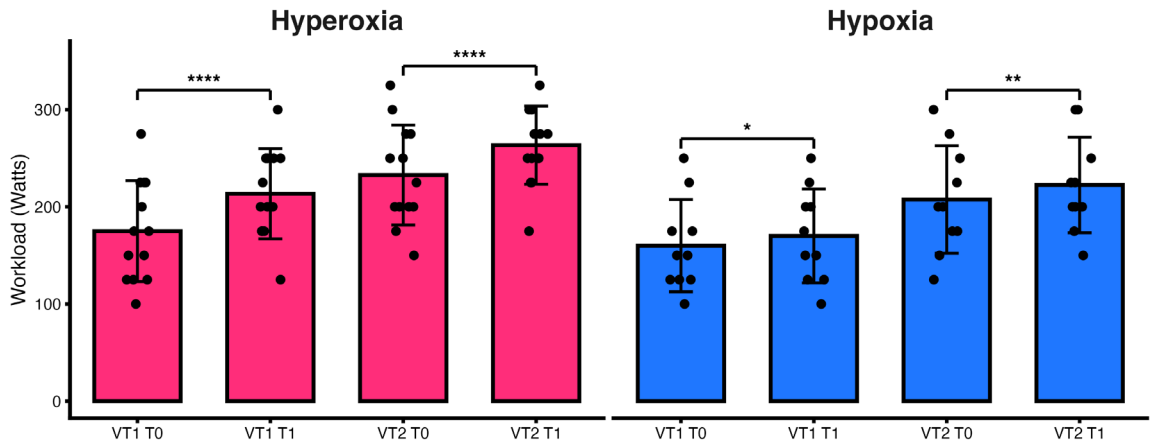

Figure 3. Differential Adaptation of Ventilatory Thresholds (VT1 and VT2) Following Hyperoxic vs. Hypoxic Training. Differential adaptation of ventilatory thresholds (VT1 and VT2) following hyperoxic vs. hypoxic training. Left panel: Hyperoxia group (60% FiO_2, n = 13). Right panel: Hypoxia group (16% FiO_2, n = 10). Workload at the first (VT1) and second (VT2) ventilatory thresholds was assessed before (T0) and after (T1) the 3-week intervention. Bars represent mean ± SD, with individual data points shown. Within-group pre–post comparisons are displayed for descriptive purposes. Primary statistical inference was based on ANCOVA adjusting post-intervention values for baseline performance ($p < 0.05$=*; $p < 0.01$=**; $p < 0.0001$=****).

3.4. Second Ventilatory Threshold (VT2)

Baseline VT2 values were comparable between groups (Table 1). Primary analysis using ANCOVA adjusting post-intervention VT2 for baseline VT2 revealed a significant effect of oxygen condition (β = 20.74 W, SE = 5.42, 95% CI [9.43–32.05], $p = 0.001$), indicating a greater improvement following hyperoxic (60% O_2) compared with hypoxic (16% O_2) training. Training condition explained an additional 4.5% of variance beyond baseline VT2 ($\Delta R^2 = 0.045$). The effect size for the adjusted between-group difference was moderate-to-large (Hedges' g_gain = 0.94). The assumption of homogeneity of regression slopes was satisfied ($p = 0.242$), supporting model validity. Descriptively, VT2 increased by +30.8 W in the hyperoxia group and by +15.0 W in the hypoxia group. Although statistically significant, the magnitude of the adjusted between-group effect for VT2 was smaller than that observed for VT1 and VO_2max**.**

3.5. Lactate

Baseline peak lactate concentrations were comparable between groups (Table 1). Primary analysis using ANCOVA with post-intervention lactate as the dependent variable and baseline lactate as a covariate revealed a significant effect of oxygen condition (β = −1.02 mmol·L^{-1}, SE = 0.22, 95% CI [−1.47 to −0.57], $p < 0.001$), indicating a greater reduction in peak lactate following hyperoxic (60% O_2) compared with hypoxic (16% O_2) training. Training condition explained an additional 15.8% of variance beyond baseline lactate ($\Delta R^2 = 0.158$). The assumption of homogeneity of regression slopes was satisfied ($p = 0.882$), supporting model validity. Descriptively, the hypoxia group exhibited a slight increase in peak lactate concentration (+0.27 mmol·L^{-1}), whereas the hyperoxia group demonstrated a reduction (−0.77 mmol·L^{-1}). This pattern indicates lower post-exercise lactate accumulation following hyperoxic training and suggests a reduced glycolytic contribution at maximal effort.

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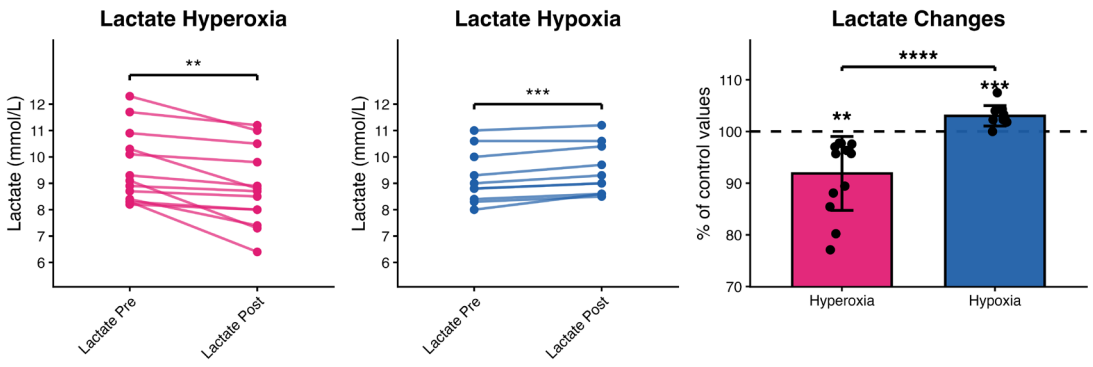

Figure 4. Differential Lactate Response Following Hyperoxic vs. Hypoxic Training. Effects of oxygen availability on peak lactate concentration following 3 weeks of Tabata training. Left and middle panels display individual pre- and post-training peak lactate values (mmol·L^{-1}) under hyperoxic (60% FiO_2) and hypoxic (16% FiO_2) conditions, respectively. The right panel illustrates relative percentage changes from baseline (baseline normalized to 100%). Within-group changes are shown for descriptive purposes. Primary statistical inference was based on ANCOVA adjusting post-intervention values for baseline lactate ($p < 0.001$=***; $p < 0.01$=**).

3.6. Perceived Exertion (Borg Scale)

Perceived exertion during training sessions was analyzed using a linear mixed-effects model to account for repeated measurements across sessions. Borg scores decreased significantly over time (β = −0.13 ± 0.03 per session, 95% CI [−0.19 to −0.08], $p < 0.001$), indicating a progressive reduction in perceived exertion across the intervention period. A significant Condition × Session interaction (β = −0.36 ± 0.04 per session, 95% CI [−0.44 to −0.28], $p < 0.001$) demonstrated a substantially steeper decline in perceived exertion under hyperoxic training compared with hypoxic training. At Session 1 (reference session), Borg scores were modestly higher in the hyperoxia group (β = +1.01, $p = 0.041$). However, the significant interaction indicates that perceived exertion decreased more rapidly across sessions under hyperoxic conditions.

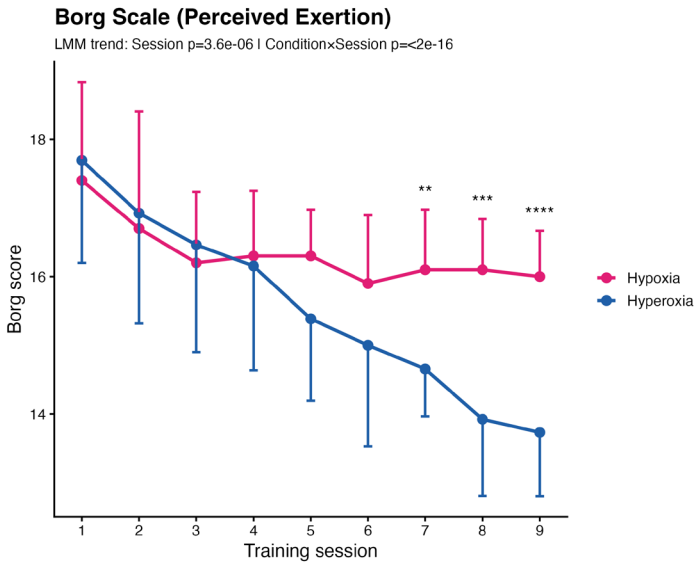

Figure 5. Steeper Reduction in Perceived Exertion During Hyperoxic Compared to Hypoxic Training. Session-by-session changes in Borg scale ratings during the 3-week intervention under hypoxic (16% O_2) and hyperoxic (60% O_2) conditions. Values represent mean ± SD. Linear mixed-effects modeling revealed a significant Condition × Session interaction ($p < 0.001$), indicating a faster decline in perceived exertion over time in the hyperoxic group. Asterisks denote between-condition differences at specific sessions (*= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$; ****= $p < 0.0001$).

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Table 2. Linear mixed-effects model for Borg.

Fixed Effect	Estimate (β)	SE	95% CI	df	t	*p*-value
Intercept (16%—Session 1)	17.00	0.36	[16.29; 17.71]	29.19	47.75	<0.001
Condition (60% vs. 16%)	1.01	0.47	[0.03; 1.99]	29.19	2.14	0.041
Session (per session)	−0.13	0.03	[−0.19; −0.08]	182	−4.78	<0.001
Condition × Session	−0.36	0.04	[−0.44; −0.28]	182	−9.71	<0.001

3.7. Heart Rate Responses

3.7.1. Resting Heart Rate

Baseline resting heart rate was similar between groups. After the intervention, opposite responses were observed according to oxygen condition. In the hypoxia group, resting heart rate increased significantly from 72.5 ± 8.1 to 74.6 ± 8.1 bpm (Δ = +2.1 bpm, $p = 0.0006$), whereas in the hyperoxia group it decreased significantly from 70.7 ± 10.5 to 66.6 ± 8.9 bpm (Δ = −4.1 bpm, $p < 0.001$). Relative changes corresponded to 102.9 ± 1.9% of baseline in hypoxia and 94.5 ± 2.7% in hyperoxia. Baseline-adjusted ANCOVA indicated a marked between-condition difference at post-intervention (β = −6.39 bpm, 95% CI [−7.71 to −5.07], $p < 0.001$). However, the condition × baseline interaction was significant ($p = 0.0258$), suggesting a potential violation of the homogeneity-of-regression-slopes assumption; this adjusted effect should therefore be interpreted with caution. Individual trajectories and percentage changes are shown in Figure 6.

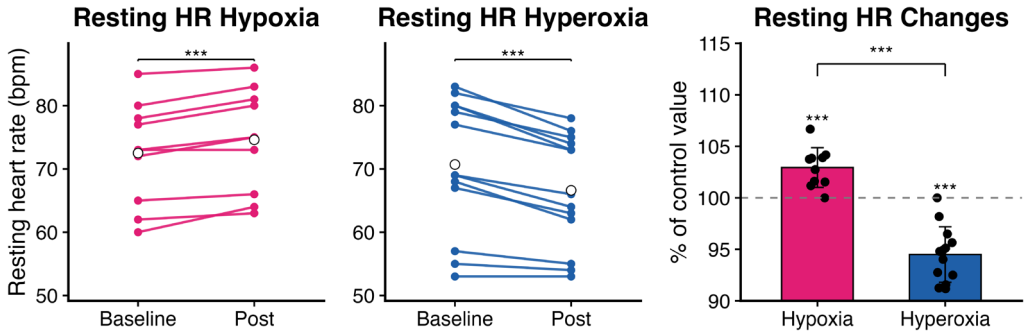

Figure 6. Resting heart rate responses following hyperoxic and hypoxic Tabata training. Left and middle panels show individual pre- and post-intervention resting heart rate values in the hyperoxia and hypoxia groups, respectively. Open circles indicate group means. The right panel shows percentage change relative to baseline (baseline = 100%). Within-group changes are displayed for descriptive purposes. The primary between-group inference was based on ANCOVA adjusting post-intervention values for baseline resting heart rate. (***= $p < 0.001$).

3.7.2. Maximal Heart Rate

Baseline maximal heart rate was also comparable between groups. Following training, maximal heart rate remained relatively stable in both conditions. In the hypoxia group, HRmax decreased from 178.0 ± 18.3 to 174.1 ± 20.6 bpm (Δ = −3.9 bpm, $p = 0.0725$), whereas in the hyperoxia group it changed from 183.8 ± 8.5 to 182.2 ± 8.3 bpm (Δ = −1.6 bpm, $p = 0.0892$). Relative changes corresponded to 97.7 ± 3.7% of baseline in hypoxia and 99.1 ± 1.7% in hyperoxia. ANCOVA adjusting for baseline HRmax revealed no significant between-group difference (β = 2.02 bpm, 95% CI [−2.21 to 6.25], $p = 0.331$; $\Delta R^2 = 0.004$). These findings indicate that oxygen condition did not significantly affect maximal heart rate responses over the intervention period.

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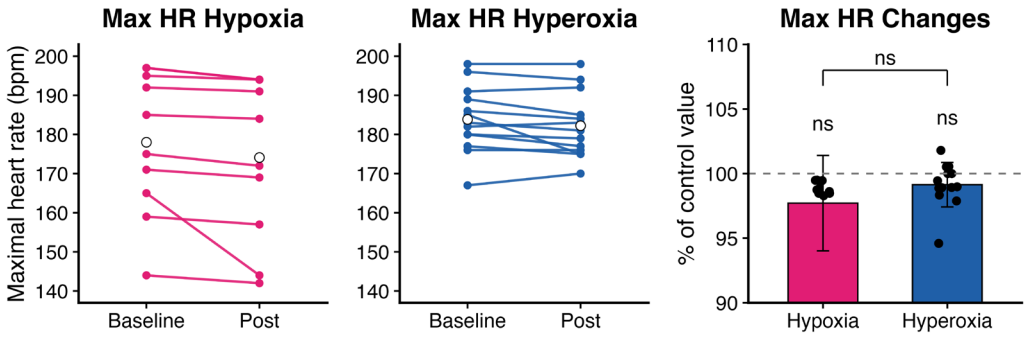

Figure 6. Maximal heart rate responses following hyperoxic and hypoxic Tabata training. Left and middle panels show individual pre- and post-intervention maximal heart rate values in the hyperoxia and hypoxia groups, respectively. Open circles indicate group means. The right panel shows percentage change relative to baseline (baseline = 100%). No significant between-group effect was observed after adjustment for baseline HRmax session.

3.8. Integrated Training Effects

Baseline-adjusted ANCOVA analyses revealed consistent condition-dependent adaptations between training conditions across maximal, submaximal, metabolic, and cardiac domains. A significant effect of oxygen condition was observed for VO_2max ($\beta = 2.18$ mL·kg^{-1}·min^{-1}, 95% CI [1.77–2.59], $p < 0.001$), indicating a greater improvement following hyperoxic training compared with hypoxic training. The additional variance explained by training condition beyond baseline performance was modest ($\Delta R^2 = 0.016$), reflecting the strong predictive influence of baseline VO_2max on post-intervention values. Similar patterns were observed for submaximal performance indices. VT1 increased significantly more following hyperoxic training ($\beta = 29.99$ W, $p < 0.001$), with condition explaining an additional 8.6% of variance ($\Delta R^2 = 0.086$). VT2 also improved significantly in the hyperoxia group ($\beta = 20.74$ W, $p = 0.001$), although the magnitude of the effect was smaller ($\Delta R^2 = 0.045$). Metabolic responses exhibited the opposite directional pattern. Peak lactate concentration decreased significantly more following hyperoxic training ($\beta = -1.02$ mmol·L^{-1}, $p < 0.001$), suggesting a reduced glycolytic contribution or altered metabolic response during maximal exercise. In this case, the training condition explained 15.8% of additional variance beyond baseline values ($\Delta R^2 = 0.158$). Cardiac responses displayed a domain-specific pattern. Resting heart rate diverged markedly between conditions, decreasing following hyperoxic training while increasing under hypoxic training, indicating opposite resting cardiac responses depending on oxygen availability during training. In contrast, maximal heart rate remained largely unchanged in both conditions and did not differ significantly between groups after baseline adjustment, suggesting that oxygen availability during training did not influence maximal chronotropic responses. Standardized effect size estimates derived from gain scores suggested large differences between training conditions for VO_2max and lactate, and moderate-to-large effects for ventilatory thresholds. These estimates should be interpreted cautiously because gain-score standardization may inflate standardized effects when inter-individual variance is small. Correlation analyses further revealed coordinated physiological adaptations across performance domains. Improvements in VO_2max were positively associated with increases in VT1 ($r = 0.65$) and VT2 ($r = 0.57$) and inversely associated with changes in peak lactate concentration ($r = -0.72$). After Holm correction for multiple comparisons, all correlations involving ΔVO_2max remained statistically significant, whereas associations among submaximal and metabolic variables did not survive correction. Collectively, these findings suggest that hyperoxic training promoted coordinated physiological adaptations across multiple domains, including improvements in maximal oxygen uptake, ventilatory thresholds, and metabolic responses to maximal exercise, accompanied by divergent resting cardiac responses between conditions.

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Table 3. Integrated training effects across physiological domains (ANCOVA).

Outcome	β	95% CI	*p*-value	ΔR²	Partial R²	t
VO_2max (ml·kg⁻¹·min⁻¹)	2.18	[1.77; 2.59]	<0.001	0.016	**0.086**	11.05
VT1 (W)	29.99	[17.09; 42.89]	<0.001	0.086	0.540	4.85
VT2 (W)	20.74	[9.43; 32.05]	0.001	0.045	0.423	3.83
Lactate (mmol·L⁻¹)	−1.02	[−1.47; −0.57]	<0.001	0.158	0.529	−4.74

Note. Values represent adjusted between-group differences (Hyperoxia − Hypoxia) estimated using analysis of covariance (ANCOVA), with post-intervention values adjusted for baseline performance. β indicates the adjusted mean difference between training conditions. ΔR² represents the additional proportion of variance explained by training condition beyond baseline performance. Partial R² reflects the proportion of residual variance attributable to the training condition after accounting for baseline values.

Table 4. Correlations between individual adaptations (Δ values).

Comparison	r	95% CI	*p*-value	*p*-Holm
ΔVO_2max − ΔVT1	0.65	[0.32; 0.84]	<0.001	0.0042
ΔVO_2max − ΔVT2	0.57	[0.21; 0.80]	0.0045	0.0182
ΔVO_2max − ΔLactate	−0.72	[−0.87; −0.43]	<0.001	<0.001
ΔVT1 − ΔVT2	0.47	[0.07; 0.74]	0.0229	0.0688
ΔVT1 − ΔLactate	−0.42	[−0.71; −0.01]	0.0452	0.0903
ΔVT2 − ΔLactate	−0.14	[−0.52; 0.29]	0.5352	0.5352

4. Discussion

Oxygen is not merely a substrate; at supramaximal intensities, its availability can reshape the internal metabolic environment of a training session even when the external prescription remains comparable. In the present protocol, both groups performed Tabata intervals targeting 170% of individually determined VO_2max-derived wattage from CPET. However, training intensity was operationally regulated by maintaining participants above ~85% of maximal heart rate, allowing the workload to be individually adjusted across sessions to preserve a comparable internal physiological stimulus. Training intensity was therefore regulated to maintain a comparable internal physiological stimulus rather than an identical external workload. This approach reflects contemporary exercise physiology frameworks emphasising internal physiological load as the primary driver of training adaptation [22,40]. Consequently, the primary experimental variable remained the fraction of inspired oxygen (FiO_2) breathed during the intervals

After three weeks, the groups diverged not only in aerobic capacity (VO_2max: +3.69 vs. +1.50 mL·kg⁻¹·min⁻¹; β = 2.18 [95% CI: 1.54–2.82], $p < 0.001$) but also in the direction of their lactate response: lower under hyperoxia and higher under hypoxia. This bidirectional response is central to the present findings. It suggests that VO_2max, the conventional primary endpoint, is insufficient to fully discriminate the metabolic phenotype induced by each oxygen condition. In contrast, the lactate response more clearly reveals the divergent metabolic adaptations associated with hyperoxic versus hypoxic training environments.

These findings are therefore interpreted within an internal-load framework, acknowledging that external workload was adjusted across sessions to maintain the targeted physiological intensity. As a result, oxygen availability likely influenced the metabolic cost of achieving a similar internal training stimulus rather than acting on a strictly identical external workload. Importantly, realized interval work was not independently recorded, and all mechanistic interpretations remain inferential pending direct molecular and metabolic validation.

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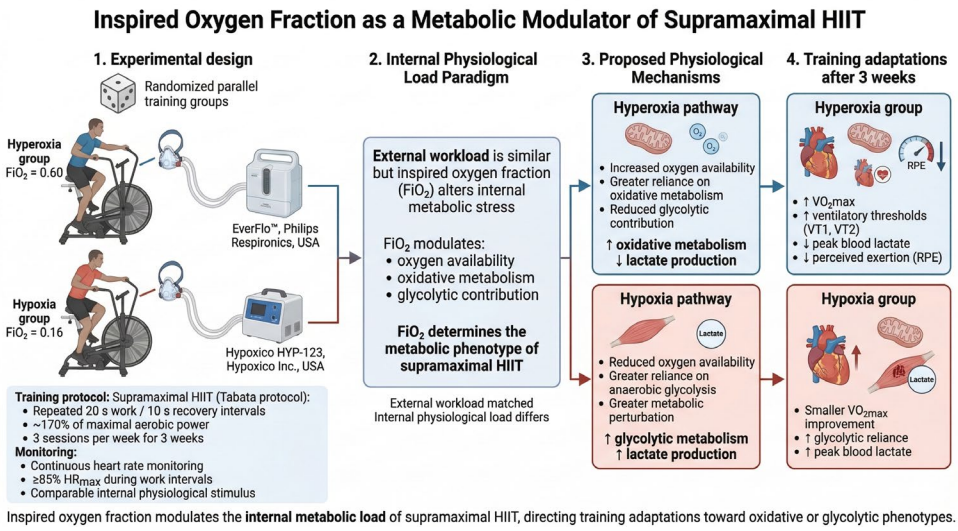

Figure 8. Integrative model of the effects of inspired oxygen fraction on metabolic stress and training adaptations during supramaximal HIIT. Participants performed three weeks of supramaximal high-intensity interval training under hyperoxic (FiO_2 = 0.60) or hypoxic (FiO_2 = 0.16) conditions. Although external workload was comparable between groups, inspired oxygen fraction modulated internal physiological load. Hyperoxia favored oxidative metabolism and resulted in greater improvements in VO_2max and ventilatory thresholds, accompanied by reduced lactate accumulation and perceived exertion. In contrast, hypoxia increased reliance on glycolytic metabolism, leading to higher lactate responses and smaller improvements in aerobic capacity.

4.1. VO_2max: Oxidative Stimulus Quality Under a Fixed Prescription

The VO_2max advantage observed under hyperoxia (β = 2.18 mL·kg^{-1}·min^{-1} [95% CI: 1.54–2.82], p < 0.001) is informative because target power was prescribed at identical relative intensity in both groups. Two considerations nevertheless qualify this comparison. VO_2max-derived wattages were determined on an electromagnetically braked cycle ergometer and transferred to the Assault Bike, an air-resistance device with distinct biomechanics (bilateral arm–leg involvement) and a device-specific power estimation algorithm with limited validation at supramaximal intensities. In addition, cadence variation on air-resistance devices can produce disproportionate differences in realized power. Consequently, potential between-condition cadence drift would not be detectable from the displayed target alone. Adaptive divergence therefore most plausibly reflects differences in internal metabolic cost, while acknowledging that differences in realized external work may also have contributed.

Expressed as a weekly rate ($\Delta \div 3$ weeks; linear approximation; [41], hyperoxia yielded ~+1.23 vs. ~+0.50 mL·kg^{-1}·min^{-1}·wk^{-1} for hypoxia. The hypoxia rate aligns with the HIIT meta-analytic mean [3], whereas the hyperoxia rate approaches the weekly improvement reported in the original Tabata protocol despite half the session frequency [4].

At FiO_2 0.60, prior work suggests arterial O_2 saturation remains near its physiological ceiling during supramaximal exercise [7]. Because SaO_2 was not measured, this inference remains indirect. Under comparable prescribed targets and a similar internal training stimulus, hyperoxia would be expected to reduce the metabolic cost required to sustain the interval workload. Net realized power was not independently recorded, and intracellular metabolites were not measured. Across 9 sessions, even modest per-interval reductions in metabolic perturbation could accumulate into a substantial difference in oxidative stimulus quality.

Across studies tightly controlling external workload, incremental aerobic benefits of hyperoxia are generally smaller or inconsistent [20] supporting the interpretation that FiO_2 primarily modulates the internal metabolic cost of a prescribed target rather than acting as an independent driver of adaptation.

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This effect may also depend on the intermittent nature of oxygen exposure. In mice, training under intermittent hyperoxia (cycling between 21% and 30% O_2) increased maximal exercise capacity compared with normoxic training, whereas continuous hyperoxia produced no advantage [13], consistent with the plausibility of an interval-based hyperoxic exposure design.

The hypoxia group's +1.50 $mL \cdot kg^{-1} \cdot min^{-1}$ improvement ($\beta = 1.50$, $p < 0.001$) is indistinguishable from published HIIT benchmarks [3], consistent with a normotypic aerobic response. Three weekly sessions of FiO_2 0.16 remain far below the sustained exposure required to stimulate EPO-mediated erythropoiesis (≥12 $h \cdot day^{-1}$ for ≥4 weeks;[18]), precluding a haemic contribution. The $\Delta R^2 = 0.016$ attributable to condition further indicates that baseline aerobic fitness, not oxygen fraction, is the primary determinant of adaptive magnitude, with oxygen availability modulating adaptation efficiency around this baseline.

4.2. Enhanced Ventilatory Threshold Adaptations

Hyperoxia shifted both ventilatory thresholds substantially (VT1: $\beta = 29.99$ W, $p < 0.001$; VT2: $\beta = 20.74$ W, $p = 0.001$), corresponding to increases of +22.0% and +13.2%, respectively. In contrast, hypoxia produced smaller changes (+6.2% and +7.2%). For context, Maciejczyk et al. [42], reported improvements of approximately +7–11% following four weeks of intermittent hypoxic training.

The magnitude observed under hyperoxia was therefore substantially larger despite the shorter intervention duration, although differences in protocol and population limit direct comparison.

The asymmetric VT1–VT2 pattern carries mechanistic implications. Under hyperoxia, VT1 improved more than VT2, consistent with preferential improvement in oxidative efficiency at moderate-to-heavy exercise intensities [22]. This pattern is compatible with enhanced mitochondrial coupling and improved submaximal lactate clearance [21], although it does not allow attribution to specific transporter or mitochondrial adaptations. In contrast, the hypoxia condition produced near-symmetric threshold shifts with a marginal VT2 advantage, suggesting modest glycolytic upregulation without substantial improvement in submaximal aerobic efficiency [22].

Ventilatory thresholds reflect the integrated balance between oxidative energy provision, CO_2 buffering, and systemic acid–base regulation [22]. Their differential displacement under an identical prescribed workload therefore provides independent support for the internal-load interpretation: the same external prescription generated qualitatively distinct internal metabolic stimuli.

4.3. Reduced Peak Lactate Accumulation Following Hyperoxic Training

Peak blood lactate changed in opposite directions ($\beta = -1.02$ $mmol \cdot L^{-1}$, $p < 0.001$; −0.77 hyperoxia; +0.27 hypoxia) despite comparable target workload. This bidirectional response provides the clearest mechanistic discrimination between conditions. A study reporting only VO_2max, which increased significantly under both conditions, would characterize both groups as aerobically adaptive. The lactate data reveal that the underlying metabolic remodeling is qualitatively divergent.

The hyperoxia pattern, lower peak lactate with higher VO_2max and elevated thresholds, is consistent with the canonical signature of aerobic training adaptation. At a fixed supramaximal prescription, hyperoxia attenuates net systemic lactate appearance and glycogenolytic flux [8]. Training-induced upregulation of Monocarboxylate transporter 1 (MCT1) and mitochondrial-associated LDH increases the capacity for oxidative lactate clearance. This adaptation can occur independently of major changes in glycolytic enzyme activity [21,43–45]. The observed lactate reduction is therefore compatible with an altered balance between lactate appearance and disposal consistent with, but not diagnostic of, transporter and mitochondrial remodeling. Production versus clearance contributions cannot be resolved without isotope-tracer Ra/Rd measurements[45].

Preclinical support is provided by Suzuki (2024). In mice exposed to intermittent hyperoxia, pyruvate dehydrogenase complex activity increased across oxidative tissues including red gastrocnemius, diaphragm, and left ventricle (BF ≥3). The LDH-LP/LDH-PL ratio also shifted toward enhanced oxidative lactate utilization [13]. Direct murine-to-human extrapolation requires caution, but this molecular profile is coherent with the lactate reduction observed here.

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The lactate increase observed in the hypoxia group should not be interpreted as maladaptation but rather as expression of a distinct metabolic phenotype. Repeated supramaximal efforts under oxygen restriction selectively upregulate glycolytic capacity, including phosphofructokinase activity and LDH-M isoform expression [10]. Under the fixed prescription used here, FiO_2 0.16 increases glycolytic reliance within each interval, thereby providing a systematic training stimulus for anaerobic enzymatic upregulation. This metabolic shift represents the expected physiological consequence of the protocol design.

4.4. Perceived Exertion Adaptations

RPE decreased more steeply across sessions in the hyperoxia condition (Condition × Session interaction, $p < 0.001$) despite comparable target workload. Within the psychobiological model of effort [23] and central regulation frameworks of exercise performance [46], perceived effort is thought to reflect the balance between central motor command and sensory feedback from the working musculature. Group III/IV muscle afferent feedback increases in proportion to intramuscular metabolic perturbation [47–50]. If hyperoxia attenuates that perturbation at a given external workload, as suggested by the lactate and threshold responses observed here, lower perceived effort represents a plausible physiological consequence. Direct causal confirmation would require afferent-level measurements such as muscle oxygenation assessed via near-infrared spectroscopy, arterial oxygen saturation kinetics, or experimental afferent blockade, none of which were recorded in the present study.

The steeper decline in RPE may therefore represent a potential self-reinforcing internal-load loop. Reduced metabolic disturbance would attenuate afferent inhibition, potentially allowing higher-quality motor recruitment at the prescribed target wattage. Over repeated sessions this could increase cumulative oxidative stimulus while lowering the perceived cost of maintaining the prescribed workload. This interpretation is mechanistically coherent and consistent with the parallel divergence observed in RPE and VO_2max, although it remains inferential. The small additional variance explained by condition ($\Delta R^2 = 0.016$) indicates that this mechanism likely amplifies pre-existing aerobic capacity rather than generating adaptation de novo. Hyperoxic HIIT may therefore be most effective in athletes for whom afferent inhibition represents a limiting constraint on training quality during supramaximal interval exercise.

4.5. Molecular Hypotheses: NOP and the ROS–Nrf2–PGC-1α Axis

The cyclic FiO_2 transitions, 0.60 during intervals and ~0.21 during recovery, may engage oxygen-sensitive transcriptional programs beyond those driven by mechanical load alone. Two candidate pathways are considered here as strictly hypothesis-generating; no molecular endpoint was measured in the present study, and neither mechanism is invoked to explain the observed performance outcomes.

The Normobaric Oxygen Paradox (NOP) [15] proposes that the post-interval return to normoxia may be sensed by prolyl hydroxylase domain proteins as a relative decrease in PO_2. This has been proposed to transiently stabilize HIF-1α and elevate erythropoietin production [14,51]. A more widely replicated candidate mechanism involves reactive oxygen species (ROS) acting as redox signaling molecules at sub-toxic concentrations, a process described as oxidative eustress [52]. Hydrogen peroxide promotes dissociation of Nrf2 from its cytosolic inhibitor Keap1, enabling nuclear translocation and activation of antioxidant response element (ARE)-dependent transcription [53,54]. This pathway can promote mitochondrial biogenesis through upregulation of PGC-1α [55], acting additively with the canonical AMPK/CaMKII → PGC-1α signaling cascade induced by exercise [56,57].

Nevertheless, recent human data indicate that pulsed hyperoxia can modulate circulating oxidative stress markers and is accompanied by signals consistent with mitochondrial biogenesis in peripheral blood mononuclear cells, suggesting that intermittent hyperoxic exposure may engage redox-sensitive adaptive pathways in vivo [58].

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Preclinical evidence in an exercise–hyperoxia context is provided by Suzuki [13]. Acute intermittent hyperoxia elevated TFAM (BF ≥10) and PFK (BF ≥10) mRNA expression in red gastrocnemius within three hours. Four weeks of intermittent hyperoxic training increased NT-PGC1α nuclear protein abundance and HAD activity (BF ≥3–7.9), indicating coordinated upregulation of mitochondrial biogenesis and fatty-acid oxidative capacity. Extrapolation to humans requires caution given species differences and the lower FiO_2 used in the present protocol (0.60 vs. 0.30). Nevertheless, a recent study from our group showed a positive increase of some biomarkers showing that a low intensity training with the adjunction of hyperoxia (even moderate) was able to act as a stimulating metabolic modulator [59].

Future trials should therefore incorporate direct molecular endpoints, including serum EPO, systemic oxidative stress markers (e.g., 8-isoprostane), and skeletal muscle biopsy analyses of mitochondrial and metabolic proteins such as citrate synthase, TFAM, and MCT1/MCT4.

4.6. Hypoxia: A Mechanistic Double Constraint

The hypoxia profile observed here, normotypic VO_2max improvement combined with elevated peak lactate and modest threshold shifts, is consistent with the internal-load framework described above. At 170% VO_2max-watts, breathing FiO_2 0.16 increases glycolytic reliance during each interval while simultaneously constraining oxidative flux. However, the hypoxic exposure used in the present protocol is far below the dose required to induce haematological adaptations (≥12 h·day^{-1} over ≥4 weeks; [18,60]. As a result, the intervention provides neither sufficient hypoxic exposure to stimulate erythropoietic adaptation nor a clear additional stimulus for aerobic remodeling beyond conventional HIIT. The resulting phenotype therefore reflects a predictable interaction between supramaximal exercise intensity and limited hypoxic dose rather than a failure of physiological adaptation.

4.7. Limitations and Future Directions

Several methodological and logistical constraints should be considered when interpreting the present findings.

External-Load Metrology.

Three interrelated metrological limitations bound the external-load interpretation. First, VO_2max-derived wattage was determined on an electromagnetically braked ergometer and subsequently transferred to the Assault Bike (Rogue Fitness), an air-resistance device with distinct biomechanics involving bilateral arm–leg coupling and a proprietary power estimation algorithm. Wattage equivalence across ergometer types cannot therefore be assumed. Second, on air-resistance devices, resistance and power scale non-linearly with cadence, meaning that small cadence differences between conditions could produce amplified differences in realized work. Because training intensity was regulated to maintain a comparable internal physiological stimulus rather than an identical external workload, some variability in realized external power between sessions cannot be excluded.

Sample Size and Intervention Duration.

The relatively small sample size limits statistical power and may have masked subtle inter-group differences. In addition, the intervention duration (three weeks with nine sessions) was shorter than classical Tabata-style interventions, which often span approximately six weeks. Such reduced exposure likely attenuated the magnitude of metabolic and physiological adaptations, particularly those associated with cumulative mitochondrial remodeling and enzymatic upregulation.

Training Prescription Anchors.

Exercise intensity in the present protocol was prescribed as a fixed percentage of VO_2max-derived power to remain consistent with the original Tabata design. However, recent methodological work indicates that prescribing intensity based on maximal anchors (e.g., VO_2max, Wmax, HRmax) may not reliably target distinct metabolic domains, whereas submaximal anchors such as ventilatory or lactate thresholds provide more precise physiological workload prescription (Jamnick et al., 2020).

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Continuous lactate measurements during the incremental ramp test would therefore have allowed a more detailed characterization of the metabolic response to different oxygen fractions.

Molecular and biochemical endpoints.

No direct molecular measurements were collected. Muscle biopsy analyses (e.g., citrate synthase activity, TFAM expression, MCT1/MCT4 abundance) and circulating biomarkers such as erythropoietin, total antioxidant capacity, or oxidative stress markers (e.g., 8-isoprostane) would have enabled direct testing of the mechanistic hypotheses proposed here. Similarly, isotope-tracer lactate kinetics (Ra/Rd) would be required to distinguish whether the observed lactate changes primarily reflect altered production, enhanced clearance, or both.

Temporal resolution and generalizability.

The pre–post design provides only two time points and cannot capture the temporal dynamics of training adaptation. Aerobic adaptations are typically non-linear and often front-loaded during the early neural and enzymatic phases of training. Weekly repeated measurements would allow determination of whether divergence between conditions emerges during early metabolic adjustments (weeks 1–2) or later structural adaptations (weeks 2–3). Additionally, the exclusively male trained-athlete sample limits generalisability to female athletes, older populations, and individuals with altered oxygen-delivery kinetics.

Dose–response uncertainty.

The optimal FiO_2 for hyperoxic interval training remains undetermined. Oxygen fractions substantially higher than those used here may increase reactive oxygen species to levels that activate pro-apoptotic rather than adaptive signaling cascades (Sies & Jones, 2020). Systematic dose-response trials across FiO_2 values ranging approximately from 0.30 to 0.80, combined with detailed physiological and molecular instrumentation, are therefore required to define the adaptive window of intermittent hyperoxia.

Despite these limitations, the present study contributes to a growing body of research exploring the interaction between oxygen availability and high-intensity interval training. The hypotheses generated here provide a framework for future investigations aimed at clarifying the mitochondrial and metabolic responses to intermittent hyperoxia in exercise contexts.

5. Conclusions

Three weeks of Tabata-format HIIT performed under comparable prescribed targets produced divergent adaptive phenotypes as a function of inspired O_2 fraction. Hyperoxia induced greater improvements in VO_2max, larger ventilatory threshold shifts, and lower peak lactate, whereas hypoxia produced a normotypic aerobic gain accompanied by a glycolytic phenotype characterized by elevated peak lactate. The bidirectional lactate response, rather than VO_2max alone, emerged as the most mechanistically informative signal, indicating qualitatively distinct metabolic remodeling under the two oxygen conditions. These findings are most consistently interpreted within an internal-load framework in which FiO_2 modulates the metabolic cost required to sustain each training interval. Interpretation must nevertheless consider external-load metrology. VO_2max-derived wattage was transferred from an electromagnetically braked ergometer to an Assault Bike, and interval power was monitored visually rather than recorded. Consequently, a partial contribution of divergent realized workloads cannot be excluded. However, the convergence of four independent physiological signals—VO_2max, ventilatory thresholds, peak lactate, and RPE—supports the internal-load interpretation. More broadly, these findings identify inspired oxygen fraction as a controllable environmental variable capable of shaping the metabolic phenotype of high-intensity interval training.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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Abbreviations

The following abbreviations are used in this manuscript.

ATP	Adenosine triphosphate
DTM	Double trunk mask
FiO_2	Inspired oxygen fraction
FMD	Flow-mediated dilation
GSH	Reduced glutathione
GSSG	Oxidized glutathione
H_2O_2	Hydrogen peroxide
HFNO	High-flow nasal oxygen
HIIT	High-intensity interval training
HIF-1α	Hypoxia-inducible factor-1 alpha
NADH	Nicotinamide adenine dinucleotide (reduced form)
NAD^+	Nicotinamide adenine dinucleotide (oxidized form)
NOP	Normobaric oxygen paradox
NRF2	Nuclear factor erythroid 2–related factor 2
PDH	Pyruvate dehydrogenase
RER	Respiratory exchange ratio
ROS	Reactive oxygen species
RPE	Rating of perceived exertion
VE	Minute ventilation
VCO_2	Carbon dioxide output
VO_2	Oxygen uptake
VO_2max	Maximal oxygen uptake
VT1	First ventilatory threshold
VT2	Second ventilatory threshold

References

1. Gibala, M.J.; Little, J.P.; Macdonald, M.J.; Hawley, J.A. Physiological adaptations to low-volume, high-intensity interval training in health and disease. *J Physiol* **2012**, *590*, 1077-1084, doi:10.1113/jphysiol.2011.224725.
2. MacInnis, M.J.; Gibala, M.J. Physiological adaptations to interval training and the role of exercise intensity. *J Physiol* **2017**, *595*, 2915-2930, doi:10.1113/jp273196.

 doi:10.20944/preprints202603.1074.v1

3. Milanović, Z.; Sporiš, G.; Weston, M. Effectiveness of High-Intensity Interval Training (HIT) and Continuous Endurance Training for VO2max Improvements: A Systematic Review and Meta-Analysis of Controlled Trials. *Sports Med* **2015**, *45*, 1469-1481, doi:10.1007/s40279-015-0365-0.
4. TABATA, I.; NISHIMURA, K.; KOUZAKI, M.; HIRAI, Y.; OGITA, F.; MIYACHI, M.; YAMAMOTO, K. Effects of moderate-intensity endurance and high-intensity intermittent training on anaerobic capacity and ˙VO2max. *Medicine & Science in Sports & Exercise* **1996**, *28*, 1327-1330.
5. Impellizzeri, F.M.; Marcora, S.M.; Coutts, A.J. Internal and External Training Load: 15 Years On. *Int J Sports Physiol Perform* **2019**, *14*, 270-273, doi:10.1123/ijspp.2018-0935.
6. Poole, D.C.; Jones, A.M. Oxygen uptake kinetics. *Compr Physiol* **2012**, *2*, 933-996, doi:10.1002/cphy.c100072.
7. Dempsey, J.A.; Wagner, P.D. Exercise-induced arterial hypoxemia. *J Appl Physiol (1985)* **1999**, *87*, 1997-2006, doi:10.1152/jappl.1999.87.6.1997.
8. Stellingwerff, T.; LeBlanc, P.J.; Hollidge, M.G.; Heigenhauser, G.J.F.; Spriet, L.L. Hyperoxia decreases muscle glycogenolysis, lactate production, and lactate efflux during steady-state exercise. *American Journal of Physiology-Endocrinology and Metabolism* **2006**, *290*, E1180-E1190, doi:10.1152/ajpendo.00499.2005.
9. Cardinale, D.A.; Ekblom, B. Hyperoxia for performance and training. *J Sports Sci* **2018**, *36*, 1515-1522, doi:10.1080/02640414.2017.1398893.
10. Faiss, R.; Léger, B.; Vesin, J.M.; Fournier, P.E.; Eggel, Y.; Dériaz, O.; Millet, G.P. Significant molecular and systemic adaptations after repeated sprint training in hypoxia. *PLoS One* **2013**, *8*, e56522, doi:10.1371/journal.pone.0056522.
11. Balestra, C.; Lambrechts, K.; Mrakic-Sposta, S.; Vezzoli, A.; Levenez, M.; Germonpré, P.; Virgili, F.; Bosco, G.; Lafère, P. Hypoxic and Hyperoxic Breathing as a Complement to Low-Intensity Physical Exercise Programs: A Proof-of-Principle Study. *Int J Mol Sci* **2021**, *22*, doi:10.3390/ijms22179600.
12. Vervloet, G.; Fregosi, L.; Gauthier, A.; Grenot, P.; Balestra, C. Impact of Five Weeks of Strengthening Under Blood Flow Restriction (BFR) or Supplemental Oxygen Breathing (Normobaric Hyperoxia) on the Medial Gastrocnemius. *J Funct Morphol Kinesiol* **2024**, *9*, doi:10.3390/jfmk9040258.
13. Suzuki, J. Effects of exercise training with intermittent hyperoxic intervention on endurance performance and muscle metabolic properties in male mice. *Physiological Reports* **2024**, *12*, e16117, doi:https://doi.org/10.14814/phy2.16117.
14. Balestra, C.; Germonpré, P.; Poortmans, J.R.; Marroni, A. Serum erythropoietin levels in healthy humans after a short period of normobaric and hyperbaric oxygen breathing: the "normobaric oxygen paradox". *J Appl Physiol (1985)* **2006**, *100*, 512-518, doi:10.1152/japplphysiol.00964.2005.
15. Cimino, F.; Balestra, C.; Germonpre, P.; De Bels, D.; Tillmans, F.; Saija, A.; Speciale, A.; Virgili, F. Pulsed high oxygen induces a hypoxic-like response in human umbilical endothelial cells and in humans. *J Appl Physiol (1985)* **2012**, *113*, 1684-1689, doi:10.1152/japplphysiol.00922.2012.
16. Balestra, C.; Baldelli, S.; Virgili, F.; Salvagno, M.; Mrakic-Sposta, S.; Fratantonio, D. Pulsed Hyperoxia Acts on Plasmatic Advanced Glycation End Products and Advanced Oxidation Protein Products and Modulates Mitochondrial Biogenesis in Human Peripheral Blood Mononuclear Cells: A Pilot Study on the « Normobaric Oxygen Paradox ». *International Journal of Molecular Sciences* **2024**, *25*, 2394.
17. Salvagno, M.; Coppalini, G.; Taccone, F.S.; Strapazzon, G.; Mrakic-Sposta, S.; Rocco, M.; Khalife, M.; Balestra, C. The Normobaric Oxygen Paradox-Hyperoxic Hypoxic Paradox: A Novel Expedient Strategy in Hematopoiesis Clinical Issues. *Int J Mol Sci* **2022**, *24*, 82, doi:10.3390/ijms24010082.
18. Chapman, R.F.; Stray-Gundersen, J.; Levine, B.D. Individual variation in response to altitude training. *J Appl Physiol (1985)* **1998**, *85*, 1448-1456, doi:10.1152/jappl.1998.85.4.1448.
19. Sperlich, B.; Zinner, C.; Hauser, A.; Holmberg, H.C.; Wegrzyk, J. The Impact of Hyperoxia on Human Performance and Recovery. *Sports Med* **2017**, *47*, 429-438, doi:10.1007/s40279-016-0590-1.
20. Cyr-Kirk, S.; Billaut, F. Hyperoxia Improves Repeated-Sprint Ability and the Associated Training Load in Athletes. *Front Sports Act Living* **2022**, *4*, 817280, doi:10.3389/fspor.2022.817280.
21. Brooks, G.A. The Science and Translation of Lactate Shuttle Theory. *Cell Metabolism* **2018**, *27*, 757-785, doi:10.1016/j.cmet.2018.03.008.
22. Faude, O.; Kindermann, W.; Meyer, T. Lactate Threshold Concepts. *Sports Medicine* **2009**, *39*, 469-490, doi:10.2165/00007256-200939060-00003.

 doi:10.20944/preprints202603.1074.v1

23. Marcora, S. Perception of effort during exercise is independent of afferent feedback from skeletal muscles, heart, and lungs. *J Appl Physiol (1985)* **2009**, *106*, 2060-2062, doi:10.1152/japplphysiol.90378.2008.
24. McNulty, K.L.; Elliott-Sale, K.J.; Dolan, E.; Swinton, P.A.; Ansdell, P.; Goodall, S.; Thomas, K.; Hicks, K.M. The Effects of Menstrual Cycle Phase on Exercise Performance in Eumenorrheic Women: A Systematic Review and Meta-Analysis. *Sports Med* **2020**, *50*, 1813-1827, doi:10.1007/s40279-020-01319-3.
25. Fletcher, G.F.; Ades, P.A.; Kligfield, P.; Arena, R.; Balady, G.J.; Bittner, V.A.; Coke, L.A.; Fleg, J.L.; Forman, D.E.; Gerber, T.C.; et al. Exercise Standards for Testing and Training. *Circulation* **2013**, *128*, 873-934, doi:doi:10.1161/CIR.0b013e31829b5b44.
26. Forman, D.E.; Myers, J.; Lavie, C.J.; Guazzi, M.; Celli, B.; Arena, R. Cardiopulmonary exercise testing: relevant but underused. *Postgrad Med* **2010**, *122*, 68-86, doi:10.3810/pgm.2010.11.2225.
27. Tabata, I. Tabata training: one of the most energetically effective high-intensity intermittent training methods. *The Journal of Physiological Sciences* **2019**, *69*, 559-572, doi:https://doi.org/10.1007/s12576-019-00676-7.
28. Duprez, F.; Laghmiche, A.; Trimpont, F.V.; Gatera, E.; Bodur, G. Clinical Evaluation of New Ways of Administration of Oxygen: Tusk Mask II and Double Trunk Mask. *Prehospital and Disaster Medicine* **2001**, *16*, S23-S23, doi:10.1017/S1049023 × 00035317.
29. Duprez, F.; Cocu, S.; Legrand, A.; Brimioulle, S.; Mashayekhi, S.; Bodur, G.; Bruyneel, A.; Roeseler, J.; Cuvelier, G.; Reychler, G. Improvement of arterial oxygenation using the double trunk mask above low flow nasal cannula: a pilot study. *Journal of Clinical Monitoring and Computing* **2021**, *35*, 213-216, doi:10.1007/s10877-020-00485-z.
30. ATS/ACCP Statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med* **2003**, *167*, 211-277, doi:10.1164/rccm.167.2.211.
31. Radtke, T.; Crook, S.; Kaltsakas, G.; Louvaris, Z.; Berton, D.; Urquhart, D.S.; Kampouras, A.; Rabinovich, R.A.; Verges, S.; Kontopidis, D.; et al. ERS statement on standardisation of cardiopulmonary exercise testing in chronic lung diseases. *Eur Respir Rev* **2019**, *28*, doi:10.1183/16000617.0101-2018.
32. Poole, D.C.; Jones, A.M. Measurement of the maximum oxygen uptake Vo(2max): Vo(2peak) is no longer acceptable. *J Appl Physiol (1985)* **2017**, *122*, 997-1002, doi:10.1152/japplphysiol.01063.2016.
33. Robergs, R.A.; Dwyer, D.; Astorino, T. Recommendations for Improved Data Processing from Expired Gas Analysis Indirect Calorimetry. *Sports Medicine* **2010**, *40*, 95-111, doi:10.2165/11319670-000000000-00000.
34. Beaver, W.L.; Wasserman, K.; Whipp, B.J. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol (1985)* **1986**, *60*, 2020-2027, doi:10.1152/jappl.1986.60.6.2020.
35. Wasserman, K. Principles in exercise testing and interpretation. *Principles in exercise testing and interpretation* **1994**.
36. Hassanein, Y.E.; Rogers, B.; Ibrahim, D.; Fleitas-Paniagua, P.R.; Murias, J.M.; Townsend, N.E. Do Agreements between HRV and Gas Exchange Thresholds Still Hold under Hypoxic Conditions? *Med Sci Sports Exerc* **2025**, *57*, 2062-2072, doi:10.1249/mss.0000000000003744.
37. Midgley, A.W.; McNaughton, L.R.; Polman, R.; Marchant, D. Criteria for determination of maximal oxygen uptake: a brief critique and recommendations for future research. *Sports Med* **2007**, *37*, 1019-1028, doi:10.2165/00007256-200737120-00002.
38. Vickers, A.J.; Altman, D.G. Statistics notes: Analysing controlled trials with baseline and follow up measurements. *Bmj* **2001**, *323*, 1123-1124, doi:10.1136/bmj.323.7321.1123.
39. Senn, S. Change from baseline and analysis of covariance revisited. *Stat Med* **2006**, *25*, 4334-4344, doi:10.1002/sim.2682.
40. Jamnick, N.A.; Pettitt, R.W.; Granata, C.; Pyne, D.B.; Bishop, D.J. An Examination and Critique of Current Methods to Determine Exercise Intensity. *Sports Med* **2020**, *50*, 1729-1756, doi:10.1007/s40279-020-01322-8.
41. Egan, B.; Zierath, J.R. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metab* **2013**, *17*, 162-184, doi:10.1016/j.cmet.2012.12.012.

 doi:10.20944/preprints202603.1074.v1

42. Maciejczyk, M.; Palka, T.; Wiecek, M.; Szymura, J.; Kusmierczyk, J.; Bawelski, M.; Masel, S.; Szygula, Z. Effects of Intermittent Hypoxic Training on Aerobic Capacity and Second Ventilatory Threshold in Untrained Men. *Applied Sciences* **2023**, *13*, 9954.
43. Brooks, G.A. Lactate as a fulcrum of metabolism. *Redox Biol* **2020**, *35*, 101454, doi:10.1016/j.redox.2020.101454.
44. Dubouchaud, H.; Butterfield, G.E.; Wolfel, E.E.; Bergman, B.C.; Brooks, G.A. Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *Am J Physiol Endocrinol Metab* **2000**, *278*, E571-579, doi:10.1152/ajpendo.2000.278.4.E571.
45. Messonnier, L.A.; Emhoff, C.A.; Fattor, J.A.; Horning, M.A.; Carlson, T.J.; Brooks, G.A. Lactate kinetics at the lactate threshold in trained and untrained men. *J Appl Physiol (1985)* **2013**, *114*, 1593-1602, doi:10.1152/japplphysiol.00043.2013.
46. Noakes, T.D. Time to move beyond a brainless exercise physiology: the evidence for complex regulation of human exercise performance. *Appl Physiol Nutr Metab* **2011**, *36*, 23-35, doi:10.1139/h10-082.
47. Amann, M.; Blain, G.M.; Proctor, L.T.; Sebranek, J.J.; Pegelow, D.F.; Dempsey, J.A. Implications of group III and IV muscle afferents for high-intensity endurance exercise performance in humans. *J Physiol* **2011**, *589*, 5299-5309, doi:10.1113/jphysiol.2011.213769.
48. Gandevia, S.C. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* **2001**, *81*, 1725-1789, doi:10.1152/physrev.2001.81.4.1725.
49. Balestra, C.; Levenez, M.; Lafère, P.; Dachy, B.; Ezquer, M.; Germonpré, P. Respiratory rate can be modulated by long-loop muscular reflexes, a possible factor in involuntary cessation of apnea. *Diving Hyperb Med* **2011**, *41*, 3-8.
50. Duchateau, J.; Balestra, C.; Hainaut, K. Reflex modulation during fatigue of maximal and submaximal contractions. *Muscle & Nerve* **1996**, *suppl. 4*, 550.
51. Fratantonio, D.; Virgili, F.; Zucchi, A.; Lambrechts, K.; Latronico, T.; Lafere, P.; Germonpre, P.; Balestra, C. Increasing Oxygen Partial Pressures Induce a Distinct Transcriptional Response in Human PBMC: A Pilot Study on the "Normobaric Oxygen Paradox". *Int J Mol Sci* **2021**, *22*, doi:10.3390/ijms22010458.
52. Sies, H.; Jones, D.P. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat Rev Mol Cell Biol* **2020**, *21*, 363-383, doi:10.1038/s41580-020-0230-3.
53. Itoh, K.; Chiba, T.; Takahashi, S.; Ishii, T.; Igarashi, K.; Katoh, Y.; Oyake, T.; Hayashi, N.; Satoh, K.; Hatayama, I.; et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun* **1997**, *236*, 313-322, doi:10.1006/bbrc.1997.6943.
54. Motohashi, H.; Yamamoto, M. Nrf2-Keap1 defines a physiologically important stress response mechanism. *Trends Mol Med* **2004**, *10*, 549-557, doi:10.1016/j.molmed.2004.09.003.
55. Aquilano, K.; Baldelli, S.; Pagliei, B.; Cannata, S.M.; Rotilio, G.; Ciriolo, M.R. p53 orchestrates the PGC-1α-mediated antioxidant response upon mild redox and metabolic imbalance. *Antioxid Redox Signal* **2013**, *18*, 386-399, doi:10.1089/ars.2012.4615.
56. Egan, B.; Carson, B.P.; Garcia-Roves, P.M.; Chibalin, A.V.; Sarsfield, F.M.; Barron, N.; McCaffrey, N.; Moyna, N.M.; Zierath, J.R.; O'Gorman, D.J. Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor coactivator-1 mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. *J Physiol* **2010**, *588*, 1779-1790, doi:10.1113/jphysiol.2010.188011.
57. Scarpulla, R.C. Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. *Biochim Biophys Acta* **2011**, *1813*, 1269-1278, doi:10.1016/j.bbamcr.2010.09.019.
58. Balestra, C.; Baldelli, S.; Virgili, F.; Salvagno, M.; Mrakic-Sposta, S.; Fratantonio, D. Pulsed Hyperoxia Acts on Plasmatic Advanced Glycation End Products and Advanced Oxidation Protein Products and Modulates Mitochondrial Biogenesis in Human Peripheral Blood Mononuclear Cells: A Pilot Study on the "Normobaric Oxygen Paradox". *Int J Mol Sci* **2024**, *25*, doi:10.3390/ijms25042394.

 doi:10.20944/preprints202603.1074.v1

59. Balestra, C.; Lambrechts, K.; Mrakic-Sposta, S.; Vezzoli, A.; Levenez, M.; Germonpre, P.; Virgili, F.; Bosco, G.; Lafere, P. Hypoxic and Hyperoxic Breathing as a Complement to Low-Intensity Physical Exercise Programs: A Proof-of-Principle Study. *Int J Mol Sci* **2021**, *22*, 9600, doi:10.3390/ijms22179600.
60. Stray-Gundersen, J.; Chapman, R.F.; Levine, B.D. "Living high-training low" altitude training improves sea level performance in male and female elite runners. *J Appl Physiol (1985)* **2001**, *91*, 1113-1120, doi:10.1152/jappl.2001.91.3.1113.