

Title: High-intensity interval training combined with blood-flow restriction enhances anaerobic and aerobic power in endurance athletes

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ABSTRACT

High-intensity interval training (HIIT) can improve endurance performance. We investigated the concurrent impact of HIIT and blood-flow restriction (BFR) as a novel approach to further enhance maximal aerobic and anaerobic physiology and performances in trained athletes. In a randomized controlled trial, eighteen endurance-trained males ($\dot{V}O_{2\text{peak}}$ $65.6 \pm 5.1 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) included three sessions of HIIT per week (sets of 15-s efforts at 100% maximal aerobic power, interspersed by 15-s recovery) into their usual training for three weeks, either with restriction imposed on both lower limbs at 50-70% of arterial occlusion pressure (BFR group, $n=10$) or without (CTL group, $n=8$), and were tested for aerobic and anaerobic exercise performance. The total mechanical work developed during a 30-sec Wingate test increased only in BFR (3.6%, $P=0.02$). During the Wingate, changes in near-infrared spectroscopy-derived vastus lateralis muscle oxygenation ($\Delta(\text{deoxy}[\text{Hb}+\text{Mb}]$, % arterial occlusion) were attenuated after BFR training (-8.8%, $P=0.04$). The maximal aerobic power measured during an incremental cycling test also increased only in BFR (4.8%, $P=0.0004$), but there was no change in $\dot{V}O_{2\text{peak}}$ among groups. The improvement in time to complete a 5-km cycling time trial and associated changes in key blood variables (e.g., pH, lactate, bicarbonate and potassium ion concentration, hemoglobin) were not different between groups. Combining short-duration HIIT at 100% MAP with BFR elicited greater improvements in anaerobic performance and maximal aerobic power in endurance-trained athletes, associated with locomotor muscle metabolic adaptations but no meaningful effect on cardiorespiratory fitness.

KEYWORDS: local hypoxia; hypoxic training; vascular occlusion; anaerobic capacity; intermittent training; muscle oxygenation

INTRODUCTION

Training with intentional blood flow restriction (BFR) to active skeletal muscles has gained practical and scientific attention in recent years due to its ability to generate high metabolic, shear and cardiac stress and, thereby, greater physiological adaptations than training with intact O₂ delivery (Bennett & Slattery, 2019; Pignanelli et al., 2021; Smith et al., 2022). Both central and peripheral mechanisms have been demonstrated to contribute to its ergogenicity, such as increased stroke volume, higher angiogenesis and mitochondrial biogenesis, enhanced buffering capacity, as well as greater muscle fiber activation (Ferguson et al., 2021; Pignanelli et al., 2021). Although initially used during low-load resistance exercise, BFR has also been studied in combination with low- and moderate-intensity aerobic exercise, but data on trained athletes (i.e., peak O₂ uptake ($\dot{V}O_{2peak}$) > 60 ml.min⁻¹kg⁻¹) is unfortunately very scarce. Notably, increases of ~3% and ~12% in mean power output developed during a Wingate test and in $\dot{V}O_{2peak}$, respectively, have been reported in basketball players after two weeks of walk training on a treadmill wearing BFR cuffs at a pressure of 160-220 mmHg (Park et al., 2010). In elite rowers, BFR increased $\dot{V}O_{2max}$ by ~9% after 5 weeks of low-intensity row training (Held et al., 2020).

More scarce and mitigated observations have been reported in the moderate-to-heavy intensity domain (Bennett & Slattery, 2019; Pignanelli et al., 2021). For example, 180-mmHg BFR during interval training (one-leg cycling bouts of 2 minutes at an intensity of 60-80% of maximal workload for 6 weeks) improved both muscle glucose extraction and potassium (K⁺) ion regulation (i.e., attenuated thigh K⁺ release) which were associated with a greater endurance performance improvement (Christiansen, Eibye, Rasmussen, et al., 2019). However, in another study, BFR superimposed to eight treadmill interval training sessions at 80% of maximal aerobic speed failed to enhance submaximal and maximal aerobic performance more than a control group using the same training regimen (Paton et al., 2017). These discrepancies may be due to the different training regimens used and non-uniform protocols for setting the occlusion pressure.

To our best knowledge, there is very little evidence documenting the feasibility and potential efficacy of BFR combined with aerobic exercises in the severe-intensity domain (~90-120% maximal aerobic power), although endurance athletes do perform a significant and important portion of high-intensity interval training (HIIT) to target desired adaptations (Buchheit & Laursen, 2013; Rosenblat et al., 2020). This may probably be explained by the fact that BFR induces a severely high hypoxic muscular environment and increased anaerobic glycolic rate that may be counter-productive with an aerobically-oriented HIIT. Furthermore, the high perceptual responses to high-intensity exercise combined with BFR may limit the exercise bout duration and the total session volume that can be sustained, which in turn, could also alter the session training load and physiological adaptations. However, with proper manipulation of key HIIT variables, one may argue that an adapted HIIT program could potentially help generate superior or faster adaptations compared to traditional HIIT. Therefore, the aims of the current study were to examine the feasibility and the effects of combining HIIT and BFR on anaerobic and aerobic maximal performances in endurance-trained athletes, and to clarify some physiological mechanisms involved. The tested hypotheses were: (i) HIIT with BFR elicits greater improvements in anaerobic and aerobic performances compared to HIIT alone, and (ii) performance improvements are associated with central ($\dot{V}O_{2peak}$) and peripheral (muscle oxygenation, regulation of blood lactate, pH and potassium ion concentration) adaptations.

METHODS

Ethical approval

This study was approved by the University Laval ethics committee (#2020-156). Participants were fully informed of the risks and discomforts associated with all experimental trials before providing written, informed consent.

Participants

Nineteen endurance-trained males volunteered for this study, but only eighteen completed the entire study due to schedule conflict (mean \pm SE; age, 24.9 ± 3.5 yr; body mass, 72.4 ± 8.8 kg; stature, 1.77 ± 0.06 m; percent body fat, 9.2 ± 3.0 %; $\dot{V}O_{2\text{peak}}$, 65.6 ± 5.1 ml.min⁻¹kg⁻¹, maximal aerobic power 372.5 ± 55.6 W). All participants were nonsmokers, free of health problems, and did not use any medication or any other tobacco/nicotine products. They trained on average 11.1 ± 3.4 h/week in an endurance sport (cycling, running, swimming) at the time of the study, had a competitive training history of >2 years in their respective sport, and had good cycling experience to undertake all training and testing procedures.

Study design

Participants visited the laboratory for a total of thirteen sessions, including nine training sessions spread over three weeks and four testing days for pre- (2) and post-training (2) evaluations. Pre-training evaluations were divided as follows (see *Experimental procedures* section for details): maximal incremental step test, arterial occlusion pressure (AOP) evaluation, anthropometric measures, and familiarization of main testing on day 1, and 30-s Wingate and 5-km TT tests on day 2. Using a between-group parallel design, participants were pair-matched based on age, $\dot{V}O_{2\text{peak}}$, MAP, and TT performance, as well as on their relative peak power (PPO) and mean power output (MPO) obtained during the Wingate test, and then randomly assigned to BFR or CTL (coin toss) to obtain equivalent groups for every pre-testing variable. Both groups had a similar training intervention. Post-training evaluation included the same testing as in pre-training, without the familiarization part. These evaluations were separated by 2 to 4 days and were executed 2 to 4 days after the ninth training session.

All evaluation sessions were performed at the same time of the day for every participant to avoid potentially confounding circadian rhythm effects. Temperature ($21.2 \pm 0.3^\circ\text{C}$) and humidity ($29.0 \pm 0.4\%$) were kept constant. Prior to each evaluation day, vigorous exercise was avoided for 48 h and alcohol and caffeine were refrained from for 24 h. To control for diet and activity patterns prior to these sessions, participants were asked to record and replicate their dietary intake and physical activity respectively for 24 and 72 h before testing.

During all exercise protocols and training sessions, participants were instructed to remain seated and were strongly verbally encouraged. The handlebars and seat settings of each exercise device were self-chosen by each participant and replicated throughout the study.

Experimental procedures

Maximal incremental step test

This session began with the measurement of resting heart rate (HR) and blood pressure (inclusion criteria <100 beats per minute and <140/90 mmHg) in a seated position, followed by anthropometric measures : body height, body mass, left (BFR: 53.6 ± 3.7 cm; CON: 53.6 ± 3.2 cm) and right (BFR: 54.5 ± 3.3 cm; CON: 54.3 ± 3.2 cm) thigh circumference and thigh skinfold thicknesses (BFR: 5.3 ± 0.7 mm; CON: 6.7 ± 0.6 mm). The body fat percentage was measured by bioelectrical impedance (Tanita TBF-310; Tanita Corp. of America Inc., Arlington Heights, IL) at arrival at the laboratory, pre- and post-training.

The participant was then positioned on a Emonda ALR road bicycle (aluminum alloy frame with carbon-fiber fork; Trek Bicycle Corporation, Waterloo, WI) attached to a direct-drive smart trainer (Tacx NEO smart, Tacx B.V., Wassenaar, The Netherlands). A 2-min baseline was observed, followed by a 5-min warm-up at 100 W, before starting the maximal step test at 30-watts increment per minute until volitional exhaustion. Expired gases were analyzed breath-by-breath throughout the test with a Breezesuite device (MedGraphics Corp., Saint Paul, MN) to assess $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$) and the respiratory exchange ratio. $\dot{V}O_{2peak}$ was taken as the highest 5-s average recorded during the test, and $\dot{V}CO_{2peak}$, peak respiratory exchange ratio, and peak respiratory rate were averaged at the same time. The MAP was taken as the highest 60-s average recorded during the test.

30-s Wingate and 5-km time-trial tests

For pre- and post-training evaluations, the steps were always as follows: 5-min rest in a supine position (allowing for NIRS baseline recording), 6-min standardized warm-up (self-selected pace and three 5-s efforts at 85, 95 and 100% of their maximal effort), 2-min rest in a seated position, 30-s Wingate test, 3-min cool down, 40-min passive rest, 5-min re-warm-up at self-chosen pace, 2-min rest in a seated position, 5-km time trial, and 5-min cool down. The warm-up data was noted by the experimenter and strictly replicated in the subsequent visit. After the time trial, a non-elastic nylon blood pressure cuff with a width of 21cm (WelchAllyn, Skaneateles Falls, NY, USA) was positioned around the upper thigh of the right leg and rapidly inflated to 220 mmHg for 5 min for physiological calibration of the NIRS signals.

The 30-s Wingate test and the 5-km time trial were executed on a computer-controlled electrically braked Velotron Elite cycle ergometer (RacerMate, Seattle, WA). The Wingate test was initiated with a 20-s progressively increase in pedaling rate to reach 100 W, followed by a 5-s acceleration phase to attain a peak power, and then a 30-s maximal effort with a constant resistance equivalent to 7.5% of each participant's body mass (Wingate Software Version 1.11, Lode BV). The peak (PPO) and minimum power output were measured as the highest and lowest power outputs over 1 s. The mean power output (MPO) and total mechanical work were computed over 30 s. The fatigue index was also calculated ($FI = [PPO - \text{lowest 1 s power output}] / PPO \times 100$). For the time trial, participants were instructed to complete the 5 km as quickly as possible, with the distance traveled as the only available information. The mean power output and completion time were recorded.

Training intervention

All training sessions were performed in the same controlled laboratory on an Emonda ALR road bike (Trek Bicycle Corporation, Waterloo, WI) mounted to a Tacx Trainer. Every session was supervised by an experienced investigator. For both groups, the training program consisted of three HIIT sessions per week for 3 weeks. Participants were asked to maintain their usual

low- and moderate-intensity training volume, but to refrain from doing non-prescribed high-intensity training throughout the entire study.

Both groups performed 15-s exercise bouts at a fixed power corresponding to 100% of the MAP determined during the incremental test, interspersed with 15-s active recovery at a power of 75 W. Our pilot testing indicated that cuffs could not be tolerated during an entire HIIT session performed in the severe domain and had to be deflated during the recovery periods between sets for athletes to complete the entire session. Furthermore, a typical HIIT session of 15 s on / 15 s off, usually including 2-4 sets of >10 repetitions, could not be completed in BFR due to the rapid development of muscle fatigue, so we also adjusted the number of repetitions per set and the number of sets in the BFR group, so the total number of repetitions performed was the same between groups and was also deemed to be sufficient to induce cardiometabolic stress and training adaptations (Buchheit & Laursen, 2013). Thus, the BFR group performed 6 sets of 4 repetitions, while the CTL group performed 2 sets of 12 repetitions. In both groups, one set was added per session every week, so that in the third week athletes performed 8 sets of 4 repetitions in BFR and 4 sets of 12 repetitions in CTL. Between sets, participants in both groups had a 90-s period of cycling at 60 W at a chosen cadence, followed by a 120-s passive rest seated on a chair.

All other parameters (cadence, power output, indoor cycle model, handlebars, and seat settings) were replicated for all training sessions, and every session was preceded and followed by a self-paced 10-min warm-up and 2-min cool-down.

Blood-flow restriction method

Arterial occlusion pressure (AOP) was measured on the participant's right leg during the first visit to the laboratory. After 5 minutes in the supine position, a pressure cuff (9 cm in width; AirBands, Newstead, Queensland, Australia) was applied to the most proximal portion of the leg and a hand-held MD6 bidirectional Doppler probe (Hokanson, Washington, US) was placed on the posterior tibial artery to detect the pulse, indicated by both an auditory and visual signals. The cuff was then rapidly inflated to a pressure of 250 mmHg, then gradually deflated in 10-mmHg increments until arterial flow was detected again. AOP was recorded to the nearest 10 mmHg as the lowest cuff pressure at which there was absence of a pulse. If a participant still had a detectable pulse at a pressure of 250 mmHg, the arterial occlusion pressure was set as 250 mmHg.

During training, participants in the BFR group wore a pneumatic cuff (9 cm in width; AirBands, Newstead, Queensland, Australia) on the most proximal part of both thighs. Cuffs were inflated about 30 s before the beginning of each set and deflated immediately after the set for a total occlusion duration of 2 min. Target occlusion pressures were 50% of the AOP of the participant in the first week (average 112 ± 14.4 mmHg), 60% AOP in the second week (average 134 ± 17.3 mmHg) and 70% AOP in the third week (average 157 ± 20.1 mmHg). This was done to compensate for the adaptive effect to blood flow restriction that may occur after a few sessions and in order to maximize the adaptive response to the method, while remaining in an occlusion range considered safe (Patterson et al., 2019).

Instrumentation and measurements

Heart rate

The HR was recorded with a pulse oximeter (Nellcor Bedside, Nellcor Inc. Hayward, CA) and an adhesive forehead sensor secured with a headband at the end of the incremental cycling step test, every 500 m during the 5-km time trial, and after every set in all training sessions.

Near-infrared spectroscopy (NIRS) measurements

A portable spatially-resolved, dual wavelength NIRS apparatus (PortaMon, Artinis Medical Systems BV, The Netherlands) was installed on the distal part of the right vastus lateralis muscle (~15 cm above the proximal border of the patella), parallel to muscle fibers, to quantify changes in the absorption of near-infrared light by oxygenated hemoglobin (Hb) + myoglobin (Mb) ([oxy(Hb+Mb)]) and deoxygenated Hb+Mb ([deoxy(Hb+Mb)]). The skinfold thickness was measured at the site of the application of the NIRS using a Harpenden skinfold caliper (British Indicators Ltd, West Sussex, Great Britain) during the first session, and was less than half the distance between the emitter and the detector (i.e., 20 mm). This thickness allows for adequate penetration of near-infrared light into muscle tissue for valid measurements (McCully and Hamaoka, 2000). The device was packed in transparent plastic wrap to protect it from sweat and fixed with tape. Black bandages were used to cover the device from interfering background light. A picture was taken for a better replacement of the apparatus on the thigh between different sessions. The pressure cuff used to induce BFR was positioned above the NIRS device and did not affect the placement of the device.

A modified form of the Beer-Lambert law, using two continuous wavelengths (760 and 850 nm) and a differential optical path length factor of 4.95 was used to calculate micromolar concentration changes in oxygenated Hb+Mb ($\Delta[\text{oxy}(\text{Hb}+\text{Mb})]$) and deoxygenated Hb+Mb ($\Delta[\text{deoxy}(\text{Hb}+\text{Mb})]$), with respect to a baseline value. In the present study, $\Delta[\text{deoxy}(\text{Hb}+\text{Mb})]$ was taken as an oxygenation index as this variable is relatively insensitive to changes in blood volume (Van Beekvelt et al., 2001; Ferrari et al., 2004). $\Delta[\text{deoxy}(\text{Hb}+\text{Mb})]$ data were expressed as a percentage of the values determined after the exercises by obtaining the maximal deoxygenation of the muscle by cuff inflation at 250 mmHg at the root of the thigh for 5 min.

NIRS data were acquired continuously at 10 Hz during the time trial and the Wingate tests. A 10th order zero-lag low-pass Butterworth filter was applied to smooth NIRS signal (Paradis-Deschênes et al., 2018). Data were averaged over 10 s leading up to every 250 m of the time trial and over 5 s at the end of the Wingate test to determine the peak values.

Blood measurements

Blood samples (92- μL) were drawn from fingertips using disposable lancets (SafetyLancet Neonatal, Sarstedt, Germany) 2 min after the time trial. Samples were collected into a capillary tube (Epoc® Care-fillTM, Siemens Healthinners, Germany) and immediately analysed with a portable blood analyser (Epoc® Blood Analysis System, Siemens Healthinners, Germany). This device measured pH, oxygen and carbon dioxide partial pressure (PCO_2 , PO_2), concentrations of sodium ($[\text{Na}^+]$), potassium ($[\text{K}^+]$), glucose ($[\text{Glu}]$), lactate ($[\text{Lac}^-]$) and hematocrit (Hct). The device also calculated the concentrations of hemoglobin ($[\text{Hgb}]$) and bicarbonate ($[\text{HCO}_3^-]$). Prior to data collection, the analyzer was calibrated according to the manufacturer's specifications (i.e., thermal quality calibration with a buffered aqueous solution).

Perceptual measures

The rate of perceived exertion (RPE) was recorded at the end of the incremental cycling step test and every 500 m during the time trial using the Borg 10-point scale. Furthermore, the breathlessness and lower-limb muscular fatigue were assessed after all training sessions with the Borg 10-point scale (Borg et al., 2010).

The perception of the training efficacy was also evaluated at the end of the 3-week training by asking the following questions: “How do you rate the efficacy of this training intervention?” and “How did you like this training intervention?” with a 10-point Likert scale, ranging from 1 (not at all) to 10 (very, very much).

Statistical Analysis

Statistical analyses were performed on GraphPad Prism 8.42 (GraphPad Software, San Diego, CA). Data were tested for homogeneity of variance using Levene’s test and for normality using Shapiro–Wilk test. When both conditions were met, a two-way repeated measures ANOVA [group (CTL vs. BFR) x time (pre vs. post)] was performed with pairwise multiple comparison procedures (Fisher’s LSD *post hoc*). A mixed-effects analysis was used if values were missing. When either homogeneity of variance or normality were not satisfied, differences between the groups were tested using a Mann–Whitney rank sum test. The Pearson product moment correlation was applied to analyze the relationships of interest. Statistical significance was established *a priori* at $P < 0.05$.

For performance measures, we also evaluated the practical significance of the percentage difference between change in CTL to BFR using Cohen's effect sizes (ES) \pm 90% confidence limits, and compared to the smallest worthwhile change that was calculated as the standardized mean difference of 0.2 between-subject standard deviation (Batterham & Hopkins, 2006; Hopkins et al., 2009). All variables were log-transformed before analysis (Hopkins et al., 2009). Standardized effects were classified as trivial (< 0.2), small (0.2–0.5), moderate (> 0.5 –0.8), or large (> 0.8). Data are reported as mean and standard deviation (SD).

RESULTS

Participants completed 99% of assigned training sessions (two participants missed one training session) and tolerated the BFR procedure without complications. Baseline characteristics of age, height and body mass were not different between groups and were not altered by the intervention. Prior to training, there was also no difference in any physiological and performance variables between groups ($\dot{V}O_{2peak}$, MAP, time-trial completion time, and Wingate peak and mean power outputs).

Performance and muscle oxygenation during the Wingate anaerobic test

Several parameters measured during the Wingate test differed between the two training modalities (Figures 1 and 2). There was a significant group x time interaction for the total mechanical work produced ($P < 0.05$). Post-hoc analyses demonstrated that mechanical work improved from pre- to post-training in BFR (pre: 22.9 ± 2.4 vs post: 23.7 ± 3.1 kJ, $P = 0.02$) but not in CTL (pre: 23.4 ± 2.1 vs post: 23.4 ± 2.0 kJ, $P = 0.84$), with a clear 3.9% group difference

(ES 0.34). There was also a significant group x time interaction for the mean power output ($P < 0.05$). BFR training improved mean power output (pre: 763.2 ± 80.8 W vs post: 792.0 ± 104.8 W, $P = 0.02$) but no change occurred in CTL (pre: 782.1 ± 69.9 W vs post: 779.8 ± 68.8 W, $P = 0.83$). This resulted in a clear group difference of 3.8% (ES 0.35). There was no significant change in peak power output among groups, but there was a significant group x time interaction ($P < 0.05$) for the minimum power developed at the end of the Wingate test (BFR: 6.34%, $P = 0.04$ vs PLA: 4.41%, $P = 0.24$). The fatigue index improved from pre- to post-training in both CTL and BFR (~11.4%, main effect of time: $P < 0.01$) with no group or interaction effect.

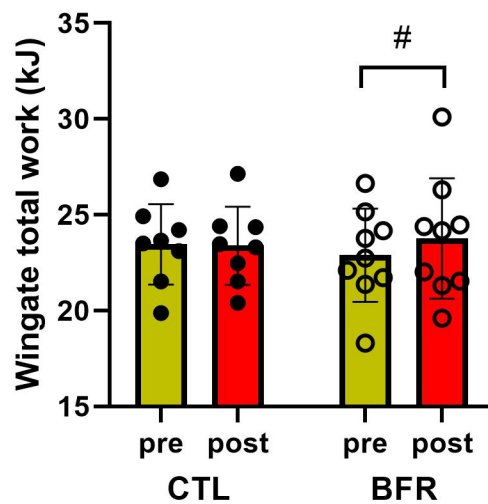


Figure 1. Average and individual values of total mechanical work (J) developed during the Wingate test before and after 3 weeks of high-intensity interval training with (BFR) or without blood-flow restriction (CTL). # Group x time interaction: pre-to-post difference in BFR (3.6%, $P = 0.02$). Clear difference in pre-to-post changes between CTL and BFR (3.9%, ES 0.34).

Changes in muscle oxygenation during the Wingate test are displayed in Figure 2. There was a significant group x time interaction for the changes in muscle O_2 extraction ($P < 0.05$). From pre- to post-training, BFR attenuated the maximal deoxygenation of the vastus lateralis muscle (pre: 94.1 ± 7.8 %AO vs post: 87.1 ± 7.9 %AO, $P = 0.04$). In contrast, it remained unaltered in CTL (pre: 74.0 ± 8.0 %AO vs post: 75.9 ± 8.2 %AO, $P = 0.87$).

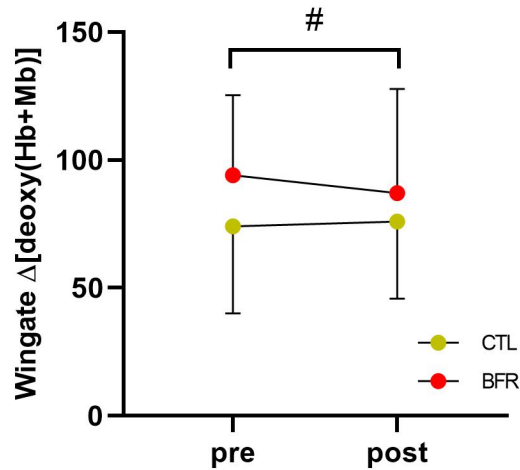


Figure 2. Changes in muscle oxygenation ($\Delta[\text{deoxy}(\text{Hb}+\text{Mb})]$, % of arterial occlusion) during the Wingate anaerobic test before and after 3 weeks of high-intensity interval training with (BFR) or without blood-flow restriction (CTL). # Group x time interaction: pre-to-post difference in BFR (-8.8%, $P=0.04$).

Performance and oxygen uptake during the incremental step test

The effect of training on the maximal aerobic power measured during the incremental step test is displayed in Figure 3. There was a significant group x time interaction ($P<0.001$). The MAP increased in BFR (368.3 ± 58.3 vs 385.8 ± 58.4 W, $P=0.0004$), but there was only a tendency in CTL (377.5 ± 55.4 vs 385.5 ± 55.6 W, $P=0.09$). This resulted in a group difference of 2.8% (ES 0.17) in favor of BFR. The same trend appeared for relative values; MAP improved in BFR only (pre: 5.07 ± 0.7 W.kg⁻¹ vs post: 5.29 ± 0.6 W.kg⁻¹, $P=0.002$).

There was no time, group or interaction effect for $\dot{V}O_{2\text{peak}}$ in both BFR (pre: 64.5 ± 5.4 ml.min⁻¹.kg⁻¹ vs post: 65.8 ± 6.3 ml.min⁻¹.kg⁻¹, $P=0.07$) and CTL (pre: 62.1 ± 7.8 ml.min⁻¹.kg⁻¹ vs post: 63.2 ± 7.3 ml.min⁻¹.kg⁻¹, $P=0.16$).

The effect of training on the aerobic threshold and the respiratory compensation point are displayed in Table 1. Overall, there was only a significant main time effect ($P<0.05$) for the power developed at the RC.

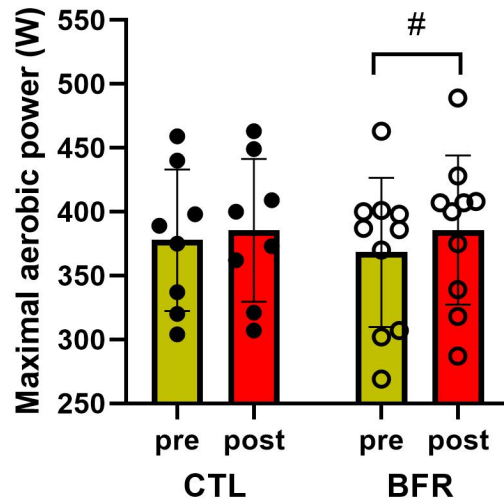


Figure 3. Average and individual values of maximal aerobic power (watts) developed during the incremental cycling test to exhaustion before and after 3 weeks of high-intensity interval training with (BFR) or without blood-flow restriction (CTL). # Group x time interaction: pre-to-post difference in BFR (4.7%, $P=0.0004$).

		CTL		BFR		Interaction P value
		pre	post	pre	post	
AT	Power (W)	217.4±46.2	229.1±36.5	223.4±49.8	234.4±30.5	P=0.87
	Power (%MAP)	58.7±8.4	60.8±3.8	61.6±7.0	62.3±7.0	P=0.69
	VO ₂ (%VO _{2peak})	68.5±8.9	70.3±7.8	66.2±7.7	67.5±7.9	P=0.83
	Power/VO ₂ ratio	5.1±0.6	5.1±0.4	5.1±0.9	5.3±0.8	P=0.63
RC	Power (W)	276.0±65.3	286.6±51.2*	271.1±52.1	285.3±42.7*	P=0.95
	Power (%MAP)	74.1±9.2	76.1±8.2	75.0±7.1	75.6±7.7	P=0.87
	VO ₂ (%VO _{2peak})	78.9±9.6	79.8±9.2	75.8±7.0	79.0±8.9	P=0.75
	Power/VO ₂ ratio	5.4±0.7	5.4±0.4	5.5±0.9	5.6±0.9	P=0.68

Table 1: Power and O₂ uptake at the aerobic threshold (AT) and respiratory compensation point (RC) during the maximal cycling test before and after 3 weeks of high-intensity interval training with (BFR) or without blood-flow restriction (CTL). * Main time effect: $P<0.05$.

Performance, muscle oxygenation and blood profile during the 5-km time trial

The training-induced change in completion time of the 5-km cycling time trial is reported in Figure 4. There was a significant main time effect ($P<0.05$) but no group or interaction effect for this variable (BFR pre: 470.2 ± 38.9 sec vs post: 466.2 ± 38.0 sec; CTL pre: 465.0 ± 33.1 sec vs post: 459.3 ± 28.3 sec). There was no change in muscle oxygenation patterns during the time trial.

The blood variables are presented in Table 2. Overall, there was no significant change in any of the groups.

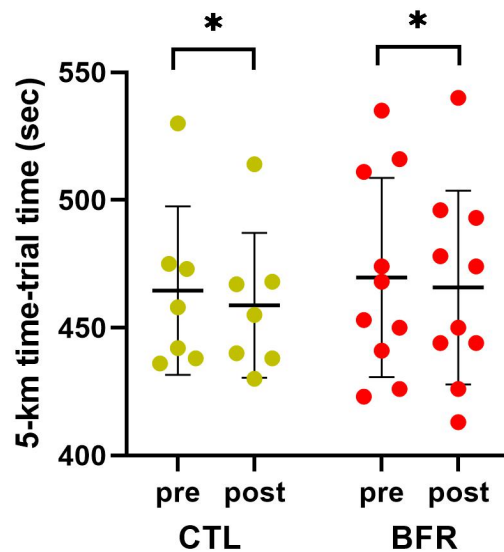


Figure 4. Average and individual values of completion time (sec) of the 5-km cycling time trial before and after 3 weeks of high-intensity interval training with (BFR) or without blood-flow restriction (CTL). * Main time effect: $P<0.05$. No interaction effect.

	CTL				BFR				Interaction P value
	pre		post		pre		post		
	baseline	end TT	baseline	end TT	baseline	end TT	baseline	end TT	
pH	7.41±0.02	7.27±0.05	7.43±0.02	7.28±0.04	7.41±0.03	7.29±0.06	7.41±0.05	7.24±0.05	P=0.87
PO ₂ (mmol·L ⁻¹)	75.68±13.90	93.94±7.85	77.36±8.90	94.34±10.86	77.28±11.11	89.70±7.60	77.37±13.54	93.90±7.98	P=0.65
PCO ₂ (mmol·L ⁻¹)	38.63±6.63	88.85±5.04	37.42±2.19	27.86±5.00	39.64±2.90	26.78±2.08	38.64±6.60	28.60±2.44	P=0.67
[Na ⁺] (mmol·L ⁻¹)	143.40±3.67	142.00±1.58	141.60±2.30	142.60±2.97	141.50±1.05	144.00±2.53	141.50±2.35	145.50±5.77	P=0.71
[K ⁺] (mmol·L ⁻¹)	5.92±0.3	6.34±1.33	6.08±0.73	7.02±1.85	5.32±0.86	6.38±1.72	5.45±1.49	5.95±1.41	P=0.34
[Glu] (mmol·L ⁻¹)	5.76±0.55	5.98±0.54	5.62±0.58	6.54±0.58	5.57±0.48	6.54±0.58	6.12±0.48	7.15±1.05	P=0.77
[Lac] (mmol·L ⁻¹)	2.92±0.34	17.75±1.65	2.81±0.74	17.01±2.82	1.80±0.54	15.92±3.20	2.00±0.64	15.66±3.15	P=0.58
Hct (%)	43.40±2.51	45.60±2.70	42.60±2.19	45.00±2.00	42.33±3.98	45.67±3.01	40.50±4.32	46.50±3.62	P=0.91
cHgb (g·dL ⁻¹)	14.76±0.90	15.50±1.30	14.48±0.78	15.38±0.70	14.37±1.41	15.62±0.96	13.85±1.45	15.87±1.24	P=0.80
[eHCO ₃ ⁻] (mmol·L ⁻¹)	25.09±1.99	13.03±2.45	27.54±0.06	12.05±3.03	24.50±0.99	12.83±1.83	24.34±1.92	12.77±2.72	P=0.49

Table 2: Mean changes in blood parameters during the 5-km cycling time trial before and after 3 weeks of high-intensity interval training with (BFR) or without blood-flow restriction (CTL).
* Main time effect: $P < 0.05$.

Heart rate and perceived exertion during training

There was no group difference in the maximal heart rates measured over the nine training sessions (BFR 91.4% vs CTL 90.0% of maximal heart rate, $P > 0.05$).

The RPE scores recorded during training are displayed in Figure 5. The lower-limb muscle discomfort was higher in BFR than CTL (8.80 vs 6.89 AU, $P = 0.02$), but there was no significant difference for feeling of breathlessness (7.13 vs 6.51 AU, $P > 0.05$).

The perception of training efficacy was not statistically different between groups (BFR 8.2 vs CTL 7.5 AU, $P > 0.05$).

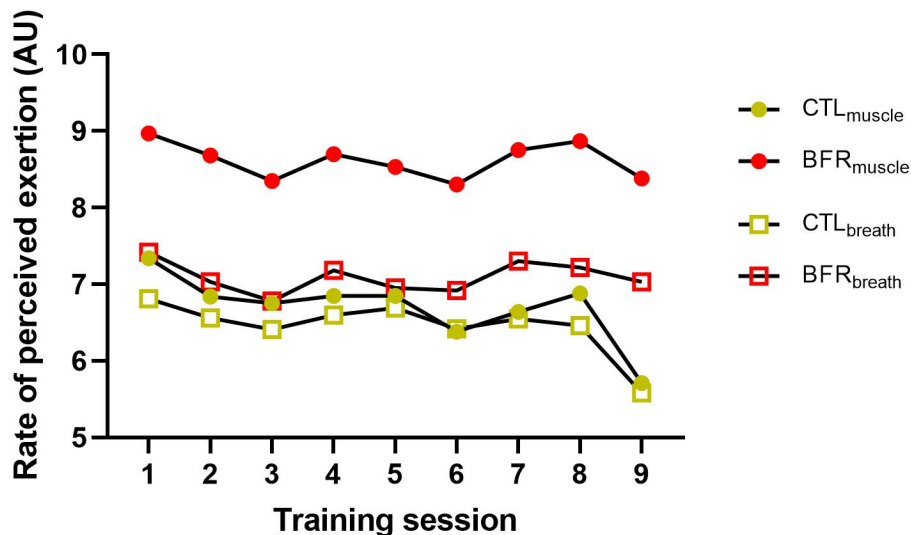


Figure 5. Rate of perceived exertion for lower-limb muscles and difficulty breathing recorded over the nine training sessions during the 3 weeks of high-intensity interval training with (BFR) or without blood-flow restriction (CTL). Main group effect for limb muscle discomfort: $P < 0.05$. Error bars not displayed for clarity.

DISCUSSION

This study demonstrated that BFR can be successfully implemented with athletes during HIIT performed at 100% of maximal aerobic power on both lower limbs simultaneously when using effort bouts of short duration. Procedures and training were well tolerated with no adverse effect or drop-out. Furthermore, such HIIT-BFR combination elicited greater improvements in anaerobic capacity and maximal aerobic power after three weeks, which were associated with changes in muscle metabolic profile in a direction facilitating anaerobic metabolism. This could therefore represent a relevant strategy to boost key factors of endurance performance in a

relatively short time in endurance athletes without compromising maximal cardiorespiratory fitness.

Several laboratories have successfully applied BFR during aerobic exercise from light to heavy intensities (i.e., up to 80% of maximal aerobic power or speed) in young adults with occlusion pressures >130 mmHg (Bennett & Slattery, 2019). More recently, some have also used restriction (40-60% AOP) during all-out sprint (Wizenberg et al., 2023) and repeated-sprint training (Giovanna et al., 2022; Valenzuela et al., 2019), reporting significant disruptions in physiological responses accompanied by larger performance benefits than comparable training without restriction. The current study adds to this literature that HIIT performed at 100% MAP can be done with both legs restricted simultaneously at pressures around 110-154 mmHg induced with a 9-cm wide cuff with relatively modest changes to the actual training session. Importantly, despite higher perceived skeletal muscle exertion during BFR training, which has been reported elsewhere, feeling of breathlessness and heart rate were similar between groups. This may be due to the relatively short duration of efforts (15 s) used in the present study, as a significantly greater dyspnea was observed during repeated 60-s efforts at 90% MAP in BFR vs control (McClellan et al., 2023). Nonetheless, athletes using BFR in our study reported a high perceived efficacy of the occlusion training modality (albeit non-statistically different than CTL), suggesting they did perceive this harder training as beneficial. Thus, a HIIT-BFR combination may represent an additional practical modality for the endurance athlete's toolbox.

The association of physical exercise and blood-flow restriction leads to profound oxygenation, ionic and metabolic disturbances within skeletal muscles due to impeded O₂ delivery, favoring a more anaerobic milieu (Christiansen, 2019). The present study showed that only the group that trained with BFR displayed greater performance improvements in the 30-sec Wingate anaerobic test, despite this group having a 22% lower training volume over 3 weeks (BFR: 3780 sec vs CTL 4860 sec). A lower external training load associated with greater performance gains is not surprising in this field and has been reported by Abe and collaborators (Abe et al., 2010) when a BFR group training 66% less than a control group over 8 weeks still improved time to exhaustion more than control. This finding is very interesting as short-duration HIIT is not a training strategy that targets anaerobic metabolism and performance (Buchheit & Laursen, 2013). In agreement, the CTL group had no improvement in markers of anaerobic capacity. Thus, based on these results, one may argue that BFR can be used to target both anaerobic and aerobic performance components in an *a priori* aerobically-oriented training session. These findings extend the scarce set of published data demonstrating that light- to severe-intensity aerobic exercise may upregulate the anaerobic metabolism when combined with BFR (Bennett & Slattery, 2019; Pignatelli et al., 2021). For examples, the anaerobic capacity, as evaluated from a Wingate test, improved by ~2.5% after walk training with progressive 160-220 mmHg occlusion (in team-sport athletes (Park et al., 2010) and by ~10.5% after 2 min-bouts interval training at 60-85% maximal aerobic speed combined with 160-240 mmHg pressures in active collegiate females (Amani-Shalamzari et al., 2019). Adding BFR to submaximal sprint training also elicited significantly greater improvements in maximal 100-m running sprint speed (~3%) than control (Behringer et al., 2017).

The vascular occlusion-derived chronic physiological changes are consistent with compromised O₂ delivery. In the current study, the greater Wingate performance after BFR training was concomitant with an ~9% lower $\Delta[\text{deoxy}(\text{Hb}+\text{Mb})]$, which can be interpreted as lower O₂ extraction. Taken together, this suggests that the energy to produce the greater power output post-training was probably derived from the anaerobic metabolism. This interpretation is supported by several robust findings highlighting changes in muscle metabolic profile in a

direction facilitating anaerobic metabolism. Increased anaerobic capacity may be, at least partly, attributed to increased muscle glycogen content following training with BFR (Burgomaster et al., 2003), which could be attributed to enhanced glucose uptake consecutive to transient increases in the translocation of glucose transporter isoform 4 (GLUT4) to the sarcolemma in response to hypoxic conditions (Cartee et al., 1991). In agreement, Christiansen et al. (Christiansen, Eibye, Hostrup, et al., 2019) demonstrated a greater muscle glucose extraction after BFR training due to greater abundance of GLUT4 favouring transmembrane glucose diffusion and to nitric oxide (NO) bioavailability. In addition, the same research group further showed that BFR training increases skeletal muscle density of Na⁺,K⁺-pump isoforms (α 1, β 1, and FXYD1) and re-uptake of K⁺ (Christiansen, Eibye, Rasmussen, et al., 2019), adaptations that aid the maintenance of pH homeostasis by promoting hydrogen ions (H⁺) efflux from muscle fibres in exchange of Na⁺ via the Na⁺/H⁺ exchanger 1. In a follow-up study, they demonstrated that BFR interval training also increases the capacity for pH regulation during dynamic exercise mainly via enhancement of muscle lactate-dependent H⁺ transport function and blood H⁺ buffering capacity through arterial bicarbonate (Christiansen et al., 2021). Finally, adaptative changes in muscle morphology occur after BFR due to post-translation regulation of AKT/mTOR pathways, reduced expression of myostatin, and increased protein synthesis (Fry et al., 2010; Laurentino et al., 2012). Nineteen days of BFR resistance training enhances the proliferation of myogenic satellite cells in exercised myofibers (Nielsen et al., 2012). Although these chronic changes were reported after resistance training, they may also be present after high-intensity cycling exercise and thereby contribute to greater sprint performance.

Applying BFR during aerobic exercise, at least up to the heavy domain of intensity, may also improve aerobic fitness and performance in healthy volunteers, but data in trained athletes is very scarce (Bennett & Slattery, 2019; Pignanelli et al., 2021; Smith et al., 2022). In the present study, which was conducted with training bouts in the severe- to supramaximal-intensity domain, however, maximal aerobic power was significantly improved in BFR despite no change $\dot{V}O_{2peak}$. This improvement occurred concomitantly with the enhancement in anaerobic capacity. This later parameter being a factor contributing to aerobic performance (Buchheit & Laursen, 2013), it is therefore likely that it accounted for the greater power output developed in the incremental test instead of a direct contribution from oxidative phosphorylation. A greater cardiorespiratory fitness after BFR training compared to control has been reported in trained rowers ($\dot{V}O_{2peak}$: 63.0 ml.min⁻¹kg⁻¹) after 5 weeks of training at low intensity ([lactate] <2 mmol.l⁻¹) (Held et al., 2020) and in basketball players ($\dot{V}O_{2peak}$: 48.0 ml.min⁻¹kg⁻¹) after two weeks of walk training (Park et al., 2010). This is consistent with locomotor muscle findings of elevated citrate synthase activity (a marker of oxidative capacity), lower activity of lactate dehydrogenase enzyme, and greater number of capillaries per fiber after low-intensity BFR training (45 min at ~55% MAP) (Esbjörnsson et al., 1993). However, BFR does not consistently enhance cardiorespiratory fitness in athletes (Paton et al., 2017). The outcome probably depends on the prescription of exercise training intensity. In the present study, even though intensity was set at the same absolute power measured in non-occluded condition in both groups, which should lead to a higher heart rate (hence, cardiovascular stress) to compensate for the lower stroke volume (Ozaki et al., 2010; Park et al., 2010), the heart rate reached during training was similar in BFR and CTL (reaching ~90% maximal heart rate), indicating a similar high cardiovascular stress in both training regimen. Thus, this data confirms that performing HIIT with the addition of BFR provides little additional benefit to heart rate and $\dot{V}O_2$ when exercise intensity is already high enough to substantially tax the cardiovascular system. Taken together, we could reason that BFR combined with low-intensity exercise training tends to favor aerobic adaptations since the O₂ requirements to sustain exercise are

only impeded which forces muscle fibers to increase extraction and use of available O₂ to maintain ATP synthesis (Calbet & Lundby, 2009; Hochachka et al., 1998), a metabolic scenario similar to what is observed in hypoxic training paradigms (Hoppeler et al., 2008; Vogt & Hoppeler, 2010). On the other hand, when occlusion is superimposed to high-intensity training, O₂ requirements to sustain intensity are probably not met and muscle fibers must revert more significantly to anaerobic sources of energy, which increases by-product accumulation and tends to predominantly upregulate the anaerobic metabolic profile of the skeletal muscle, a scenario that is commonly observed in the “repeated-sprint in hypoxia” training paradigm (Brocherie et al., 2017). Other HIIT training sessions with different characteristics (e.g., longer effort bouts in the severe-intensity domain, effort-recovery ratios) will have to be investigated to ascertain these observations as well as feasibility and safety for athletes to use this modality within the full spectrum of their training.

In conclusion, this study demonstrated that BFR can be successfully implemented with athletes during HIIT at 100% of maximal aerobic power on both legs simultaneously. Such HIIT-BFR combination improved anaerobic capacity and maximal aerobic power in three weeks without compromising maximal cardiorespiratory fitness.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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