applied sciences

MDPI

Article

Effects of Intermittent Hypoxic Training on Aerobic Capacity and Second Ventilatory Threshold in Untrained Men

Marcin Maciejczyk [1,*], Tomasz Palka [1], Magdalena Wiecek [1], Jadwiga Szymura [2], Justyna Kusmierczyk [1], Marek Bawelski [1], Sebastian Masel [1] and Zbigniew Szygula [3]

[1] Department of Physiology and Biochemistry, University of Physical Education, 31-571 Krakow, Poland
[2] Department of Clinical Rehabilitation, University of Physical Education, 31-571 Krakow, Poland
[3] Department of Nutrition and Sport Medicine, University of Physical Education, 31-571 Krakow, Poland
* Correspondence: marcin.maciejczyk@awf.krakow.pl

Featured Application: The effects of IHT reported in this study have great practical applications for coaches. Short IHT can be useful in sports, where the goal of training is to increase maximal power output or power output at second ventilatory threshold.

Abstract: The aim of study was to evaluate the effects of interval training performed in hypoxia on aerobic capacity and second ventilatory threshold in young, untrained men. Participants (n = 48) were randomly divided into a control group and two groups performing the same interval training (three times a week for 4 weeks) in normoxia (200 m asl) (NT) and in hypoxia (IHT) (3000 m asl, FIO_2 = 14.4%). In the incremental test, maximal oxygen uptake (VO_2max) was measured and the first (VT1) and second (VT2) ventilatory thresholds and the maximal power output (Pmax) were determined for each participant. The training workloads of the efforts corresponded to the workload at VT2 (effort) and VT1 (active recovery). Training in both normoxia and hypoxia significantly increased absolute VO_2max (p = 0.02, ES = 0.51 and p = 0.002, ES = 0.47, respectively). In comparison to NT, only IHT significantly (p < 0.001; ES = 0.80) improved Pmax, as well as power at VT2 (p = 0.02; ES = 0.78). The applied IHT was effective in improving Pmax and power at VT2, which was not observed after training in normoxia.

Keywords: hypoxia; interval training; maximal oxygen uptake; anaerobic threshold; training; performance

Citation: 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. *Appl. Sci.* **2023**, *13*, 9954. https://doi.org/10.3390/app13179954

Academic Editor: Alessandro de Sire

Received: 8 August 2023
Revised: 26 August 2023
Accepted: 1 September 2023
Published: 3 September 2023

1. Introduction

Physiological determinants of performance in endurance sports are maximal oxygen uptake (VO_2max), oxygen consumption at lactate thresholds (ventilatory thresholds), velocity (or power output) at metabolic thresholds, submaximal economy and percentage of slow-twitch muscle fibers [1,2]. As exercise intensity increases up to maximal (VO_2max), exercise metabolism changes. When the intensity exceeds the first ventilatory threshold (VT1), metabolic acidosis develops and this threshold appears to be a sensitive indicator of the development of metabolic acidosis [3], and at VT1, there is the greatest intensity of aerobic metabolism. The second ventilatory threshold (VT2) (respiratory compensation point) is useful in the evaluation of aerobic performance [4,5] because exceeding intensity at VT2 induces lactate accumulation in the blood and hyperventilation, leading to fatigue caused by decompensated metabolic acidosis [6].

In sports, special attention is given to VO_2max and VT2 regarding training methods, supplementation or environmental conditions that increase VO_2max and oxygen uptake and power (or speed) at VT2. Recently, one of the most popular methods of supporting physical training is the inclusion of hypoxia in training [7,8]. Physiological adaptations in response to hypoxia are well documented and include increases in maximal aerobic capacity, capillary density, hemoglobin mass and erythrocyte count due to increased erythropoietin

production under hypoxia. A number of hypoxic training protocols have been developed with varying effectiveness by using different combinations of training at varying altitudes and utilizing both natural and artificial altitudes [9].

Training under hypobaric hypoxia is often difficult to implement due to travel time, cost and athlete engagement [10]. For this reason, the live low–train high (LL-TH) training method is gaining popularity and becoming an important method of hypoxic training in modern sports. Intermittent hypoxic training (IHT) is a method in which athletes live at low altitudes, but train under hypoxic conditions (LL-TH), and it is believed that IHT can enhance performance improvements compared to similar training at sea level [11]. In this method, the athlete stays in hypoxia only for training but spends most of the time during the day in normoxia. IHT causes only minor disruptions to athletes' daily routines, allowing them to maintain their normal lifestyles [12]. Importantly, the quality of sleep and recovery is well preserved, as athletes do not sleep in hypoxic conditions [10]. However, published data reported inconclusive effectiveness of IHT in improving VO_2max and hematological parameters [13,14]. After IHT, improvements in aerobic and anaerobic capacity are reported [15,16], no effects [17] or even negative effects of this training are indicated (reduced speeds and power output, reduced oxygen flux in athletes) [18]. Treff et al. [19] recently emphasized that, despite the extensive literature, clear evidence and practical guidance on how hypoxic training allows to increase endurance at sea level is still lacking.

The two main methods of endurance training are moderate-intensity continuous training and interval training. Interval exercise typically involves repeated bouts of relatively intense exercise interspersed by short periods of recovery. These two methods have different efficiencies in improving endurance performance and still not shown what is the optimal model of interval training in terms of intensity, duration and frequency to induce improvements in endurance [20]. The choice of training workload in interval training is usually made in relation to VO_2max, maximal heart rate (HRmax) or maximal workload [20], which does not take into account the exercise metabolism (metabolic thresholds). In this study, to improve endurance performance, we proposed a novel interval training method based on power at VT1 and VT2, which is additionally supported by normobaric hypoxia (IHT). We hypothesized that such a training model would be more effective in the improvement of endurance performance than in interval training performed in normoxia. The purpose of this study was to determine the effectiveness of interval training performed in hypoxia on maximal oxygen uptake and second ventilatory threshold.

2. Methods

Only healthy men (without medical contraindications to physical exercise) were recruited for the study. To confirm the absence of contraindications to physical training, before intervention, participants underwent a medical qualification procedure (e.g., electrocardiogram at rest and during exercise, complete blood count, hemoglobin and iron concentration). Exercise tests were performed under the supervision of a sports medicine physician. Participants were not allowed to participate in regular physical training for 6 months before the intervention.

The sample size was estimated using G*Power 3.1 (Dusseldorf, Germany) and the required sample size was 16 participants per group. Participants (n = 48) were randomly divided into three groups: the control group (ctrl) and 2 groups performing the same interval training (3 times a week for 4 weeks): in normoxia (NT) (200 m asl) and in hypoxia (IHT) (3000 m asl, FIO_2 = 14.4%). Before and 7–10 days after the training, the participants performed the exercise incremental test to volitional exhaustion. The incremental test was conducted in normoxia.

Prior to the intervention, participants were familiarized with the laboratory, testing equipment and procedures, as well as the purpose of the study. They were also instructed on how to prepare for the graded test and training, i.e., avoid heavy physical exertion and dehydration. They should also sleep for at least 6–8 h the night before the test/training and eat a light meal at least 2 h before the test/training. Participants were recommended not to

change their habitual diet and not to engage in any additional physical activity during the intervention.

Participation in the study was voluntary, and participants at any stage of the project were allowed to opt out of further participation. All study participants signed a consent form to participate in the project. The project was approved by the Bioethical Commission of the Regional Medical Chamber in Krakow, Poland (opinion No. 47/KB/OIL/2022). All test procedures were conducted in accordance with the principles adopted in the Declaration of Helsinki.

2.1. Participants

The study involved healthy, moderately physically active men, but not ones who regularly practiced any sport. Their physical activity was varied and spontaneous. The participants were between 19 and 26 years old and the groups did not differ in body composition. Body mass and body composition did not change significantly after training compared to baseline (Table 1).

Table 1. Age and anthropometric measurements of the participants (data are presented as mean ± SD).

Variable	Group	Pre	Post	Effect: Group F p ηp^2	Effect: Time F p ηp^2	Interaction F p ηp^2
Age (yrs)	ctrl	22.9 ± 2.9	-	-	-	-
	NT	20.5 ± 1.0	-			
	IHT	21.5 ± 1.5	-			
BH (cm)	ctrl	178.9 ± 5.9	-	-	-	-
	NT	179.7 ± 5.6	-			
	IHT	182.0 ± 5.5	-			
BM (kg)	ctrl	75.8 ± 11.5	75.9 ± 11.7	0.55 0.58 0.02	0.07 0.80 0.01	0.71 0.49 0.03
	NT	76.7 ± 8.3	76.9 ± 8.2			
	IHT	79.6 ± 10.4	79.2 ± 9.6			
LBM (kg)	ctrl	61.5 ± 7.3	61.9 ± 7.7	1.45 0.24 0.05	2.49 0.12 0.05	0.55 0.57 0.02
	NT	63.1 ± 6.0	63.1 ± 6.1			
	IHT	65.6 ± 7.5	66.1 ± 7.9			
FAT (%)	ctrl	18.3 ± 5.3	18.0 ± 5.0	0.35 0.70 0.01	3.27 0.08 0.06	2.25 0.11 0.09
	NT	17.6 ± 3.9	17.7 ± 4.4			
	IHT	17.4 ± 3.9	16.3 ± 3.8			
FM (kg)	ctrl	14.3 ± 5.4	14.0 ± 5.2	0.07 0.92 0.003	3.20 0.08 0.06	2.44 0.1 0.09
	NT	13.7 ± 3.9	13.8 ± 4.4			
	IHT	14.1 ± 4.5	13.0 ± 4.1			
BMI	ctrl	23.6 ± 2.9	23.7 ± 2.9	0.04 0.95 0.001	0.12 0.72 0.002	0.55 0.57 0.02
	NT	23.7 ± 2.2	23.8 ± 2.2			
	IHT	24.0 ± 2.3	23.9 ± 2.1			

BH: body height; BM: body mass; LBM: lean body mass; FM: fat mass; BMI: body mass index; ctrl: control group; NT: normoxia training; IHT: intermittent hypoxia training.

2.2. Somatic Measurements

Prior to the first and second graded test, participants underwent somatic measurements including body height, body mass (BM) and body composition. Fat percentage (%FAT), fat mass (FM), lean body mass (LBM) and body mass index (BMI) were deter-

mined. Body height was measured with a stadiometer (Seca 217, Hamburg, Germany), whereas body mass and body composition were measured with a body composition analyzer using the bioelectrical impedance method (Jawon Medical, IOI-353, Seoul, Republic of Korea).

2.3. Physical Activity and Diet

A seven-day physical activity recall (PAR) questionnaire was used to assess participants' physical activity [21,22]. Participants were instructed on the purpose of the study and how to complete the questionnaire. The interview was conducted by the researcher, who clarified all the participants' doubts about the answers to the questions in real time. The data presented physical activity before the intervention.

The caloric intake of the diet was estimated using the Fitatu (version 3.37, Poznan, Poland) application [23]. After familiarizing with application, participants kept dietary diaries for 7 days, entering all foods consumed into the application. Caloric consumption is presented as caloric intake per week (kcal/week).

2.4. Aerobic Capacity and Ventilatory Thresholds

Aerobic capacity was examined by direct method (cardiopulmonary exercise test) using a ramp protocol in a graded test. Participants performed the test on an eBike Comfort bicycle ergometer (GE Health Care, Chicago, IL, USA) until volitional exhaustion. The test began with a 4-min warm-up at 60 watts and then power was systematically increased by 15 watts/min. In the incremental test, oxygen uptake (VO_2), heart rate (HR), respiratory exchange ratio (RER), pulmonary ventilation (V_E), carbon dioxide output (VCO_2), fractional concentrations of expired CO_2 ($\%F_ECO_2$) and O_2 ($\%F_EO_2$), ventilatory equivalent ratio for oxygen (V_E/VO_2) and carbon dioxide (V_E/VCO_2) were measured breath by breath using an ergospirometer (MetaLyzer 3R, Cortex, Germany). The first and second ventilatory threshold, maximal power output (Pmax), power output at ventilatory thresholds, maximal oxygen uptake and maximal heart rate were determined for each participant.

The results were analyzed by an experienced exercise physiologist who evaluated the changes of measured parameters with increasing work load. The ventilatory thresholds were determined using the method of respiratory equivalents [24,25]. The first threshold was detected by the power output at which the V_E/VO_2 ratio and F_EO_2 reached a minimum (nadir or first increase of V_E/VO_2 versus work load without a simultaneous increase in V_E/VCO_2 versus work load). The second ventilatory threshold was detected by the power output at which the V_E/VCO_2 ratio reached a minimum and the F_ECO_2 reached a maximum (nadir or nonlinear increase of V_E/VCO_2 versus work load). To determine VO_2max, the following criteria were applied: a plateau in VO_2, RER > 1.1 and HR within 10 bpm of the age-predicted maximum. If no plateau was observed, but the rest of the criteria were met, VO_2peak was taken as the VO_2max [26].

The ergospirometer was calibrated according to the manufacturer's requirements (gas and volume calibration).

2.5. Training

Interval training was performed in a hypoxic, thermoclimatic chamber (Hypoxico, Germany), or in normoxia, on bicycle ergometers (Wattbike, UK) and lasted 60 min. The training consisted of a warm-up (6 min) with power output at VT1 followed by 6 series of efforts lasting 6 min with active recovery of 3 min (2:1 ratio). The work load of the efforts was set individually and corresponded to the work load at VT2 (effort) and VT1 (active recovery). All training sessions took place at the same temperature of 21 $\pm$ 0.5 °C and humidity of 40 $\pm$ 1%.

2.6. Statistical Analysis

Data are presented as mean and standard deviation. Data distribution was checked using the Shapiro–Wilk test. Homogeneity of variance within the groups was tested via

Levene's test. ANOVA with repeated measures or one-way ANOVA was used to assess the effects of training, effect size (partial eta-squared (ηp^2)) and inter-group difference. If a significant ($p < 0.05$) effect of the main factor (group, time or interaction group-time) was found in the ANOVA, post-hoc analysis was performed using the Tukey test. In post hoc analysis (if significant), the effect size (ES: Cohen's d) between baseline and post training was calculated. The effect size was interpreted as small (0.20), medium (0.50) or large (0.80) [ES], or as small (0.01), medium (0.06) or large (0.14) [ηp^2] [27]. The STATISTICA 13 package (StatSoft, Inc., Tulsa, OK, USA) was used for calculations.

3. Results

3.1. Physical Activity and Diet

The weekly caloric intake was similar ($f = 0.16$, $p = 0.85$, $\eta p^2 = 0.01$) in all groups: NT: 21,233 ± 3352 kcal/week, IHT: 20,707 ± 3656 kcal/week and 21,291 ± 2434 kcal/week in the control group. Physical activity also did not differ significantly for the groups ($f = 0.43$, $p = 0.65$, $\eta p^2 = 0.02$). The average weekly energy expenditure in each group was: 21,108 ± 2348 kcal/week (NT), 21,970 ± 3062 kcal/week (IHT) and 21,229 ± 3014 kcal/week (ctrl).

3.2. Aerobic Capacity and Ventilatory Thresholds

Training in both normoxia and hypoxia significantly increased absolute VO_2max ($p = 0.02$, ES = 0.51 and $p = 0.002$, ES = 0.47, respectively; Figure 1). Relative oxygen uptake increased ($p = 0.008$) after training only in the IHT group (Table 2). In comparison to NT, only IHT significantly ($p < 0.001$; ES = 0.80) improved maximal power output (Table 2, Figure 2), as well as power output at VT2 ($p = 0.02$; ES = 0.78) (Table 3). Moreover, IHT also increased oxygen uptake at VT2, which was not observed after training in normoxia (Table 3).

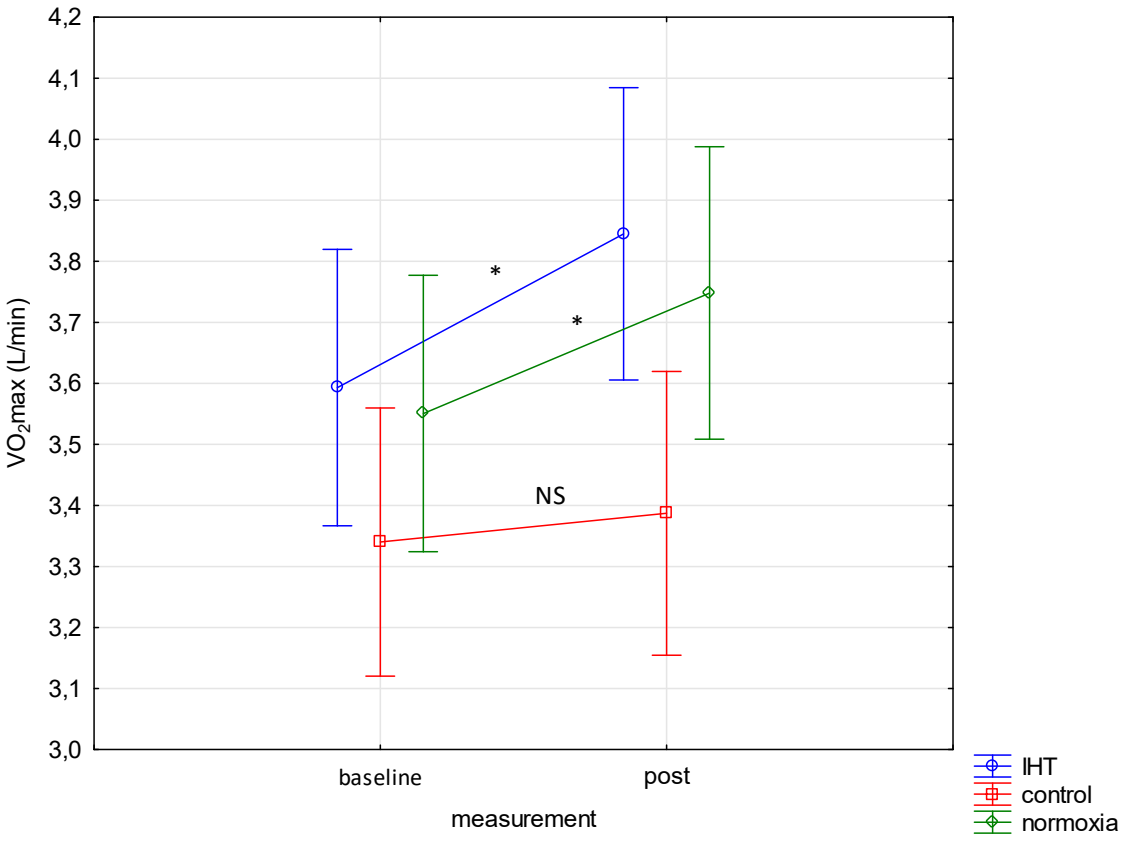

Figure 1. Effect of interval training in normoxia (NT) and in hypoxia (IHT) on absolute maximal oxygen uptake (VO_2max) (*: $p < 0.05$; NS: non-significant; vertical bars indicate 0.95 confidence interval).

Table 2. Effects of interval training performed in normoxia and hypoxia on parameters noted at maximal intensity (data are presented as mean ± SD).

Variable	Group	Pre	Post	Effect: Group F *p* ηp^2	Effect: Time F *p* ηp^2	Interaction F *p* ηp^2	Post Hoc Pre vs. Post *p*	Pre vs. Post ES
Pmax (W)	ctrl	273 ± 11.5	286 ± 43.4	1.44 0.25 0.06	57.15 <0.001 0.55	6.67 0.002 0.22	0.06	0.58
	NT	285 ± 33.1	297 ± 35.5				0.08	0.35
	IHT	286 ± 41.6	319 ± 40.4				<0.001	0.80
rel_Pmax (W/kg)	ctrl	3.65 ± 0.59	3.80 ± 0.62	0.15 0.85 0.02	49.50 <0.001 0.51	6.45 0.003 0.21	0.07	0.25
	NT	3.74 ± 0.47	3.89 ± 0.44				0.17	0.32
	IHT	3.62 ± 0.60	4.04 ± 0.49				<0.001	0.77
HRmax (bpm)	ctrl	183 ± 11	181 ± 11	0.16 0.85 0.006	0.60 0.44 0.01	1.66 0.20 0.07	-	-
	NT	184 ± 11	184 ± 14				-	-
	IHT	181 ± 7	184 ± 7				-	-
RER	ctrl	1.08 ± 0.06	1.12 ± 0.08	1.42 0.25 0.06	2.14 0.15 0.04	4.97 0.01 0.18	0.30	-
	NT	1.12 ± 0.04	1.09 ± 0.07				0.52	-
	IHT	1.11 ± 0.06	1.15 ± 0.08				0.25	-
VO_2max (L/min)	ctrl	3.34 ± 0.43	3.38 ± 0.43	2.96 0.06 0.11	21.90 <0.001 0.32	3.05 0.05 0.11	0.96	0.09
	NT	3.55 ± 0.37	3.74 ± 0.38				0.02	0.51
	IHT	3.59 ± 0.50	3.85 ± 0.60				0.002	0.47
VO_2max (mL/kg/min)	ctrl	44.6 ± 6.0	45.1 ± 5.6	1.97 0.31 0.05	16.67 0.26 <0.001	2.57 0.08 0.10	0.99	0.08
	NT	46.7 ± 5.8	49.1 ± 6.1				0.07	0.40
	IHT	45.5 ± 7.2	48.7 ± 6.1				0.008	0.48

Pmax: maximal power output; HRmax: maximal heart rate; RER: respiratory exchange ratio; VO_2max: maximal oxygen uptake; ctrl: control group; NT: normoxia training; IHT: intermittent hypoxia training.

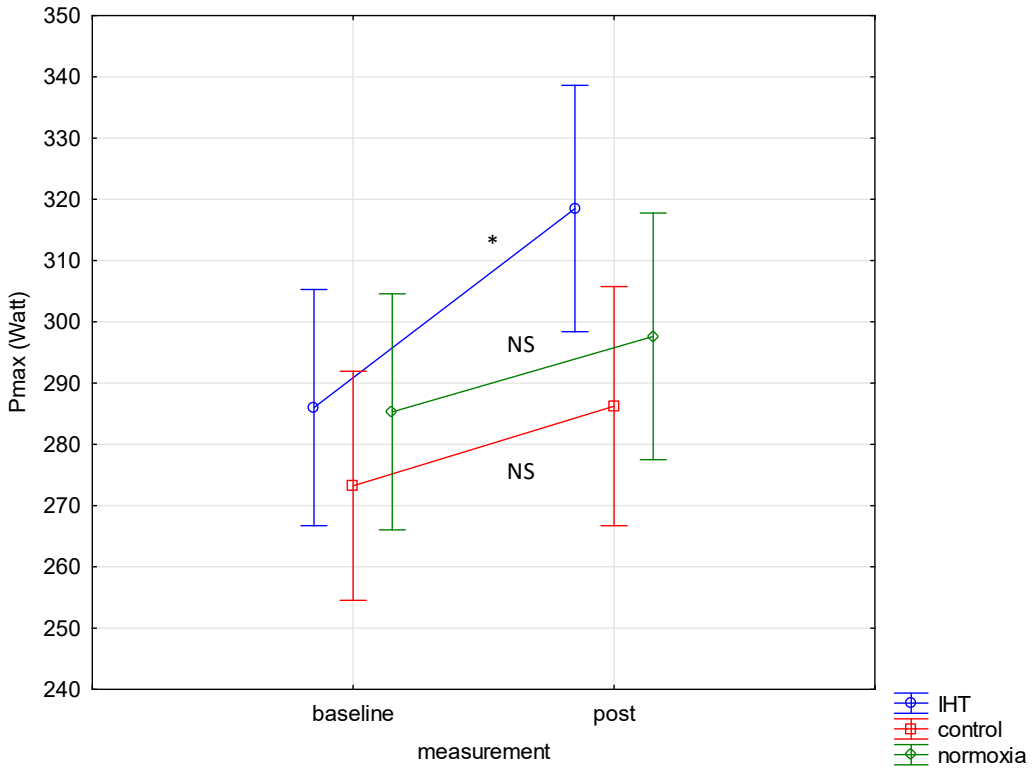

Figure 2. Effect of interval training in normoxia (NT) and in hypoxia (IHT) on maximal power output (Pmax) (*: $p < 0.05$; NS: non-significant; vertical bars indicate 0.95 confidence interval).

Table 3. Effects of interval training performed in normoxia and hypoxia on parameters noted at the second ventilatory threshold (data are presented as mean ± SD).

Variable	Group	Pre	Post	Effect: Group F *p* ηp^2	Effect: Time F *p* ηp^2	Interaction F *p* ηp^2	Post Hoc Pre vs. Post *p*	Pre vs. Post ES
P_{VT2} (W)	ctrl	166.2 ± 20.2	163.9 ± 37.4	8.02 0.001 0.26	6.44 0.01 0.12	3.23 0.04 0.13	0.99	0.08
	NT	162.5 ± 21.1	173.4 ± 26.5				0.72	0.46
	IHT	183.8 ± 29.2	209.2 ± 36.1				0.02	0.78
rel_P_{VT2} (W/kg)	ctrl	2.23 ± 0.39	2.20 ± 0.54	2.89 0.06 0.11	6.00 0.02 0.12	3.19 0.05 0.12	0.99	0.06
	NT	2.14 ± 0.37	2.27 ± 0.37				0.76	0.35
	IHT	2.34 ± 0.46	2.65 ± 0.44				0.02	0.69
%Pmax	ctrl	61.4 ± 7.92	57.9 ± 12.8	4.11 0.02 0.15	0.10 0.74 0.002	1.24 0.29 0.05	0.67	-
	NT	57.4 ± 8.66	58.5 ± 7.92				0.99	-
	IHT	64.5 ± 6.87	65.5 ± 6.55				0.99	-
HR_{VT2} (bpm)	ctrl	153 ± 17	144 ± 18	0.95 0.39 0.04	0.48 0.49 0.01	6.51 0.003 0.22	0.02	-
	NT	148 ± 13	148 ± 16				0.49	-
	IHT	151 ± 7	157 ± 8				0.45	-
%HRmax	ctrl	83.5 ± 6.3	79.2 ± 8.2	2.24 0.11 0.09	1.53 0.22 0.03	4.31 0.01 0.16	0.03	-
	NT	80.5 ± 6.1	80.4 ± 6.0				0.99	-
	IHT	83.5 ± 3.8	54.9 ± 3.9				0.92	-
VO_{2_VT2} (L/min)	ctrl	2.12 ± 0.22	2.13 ± 0.35	8.03 0.001 0.25	11.73 0.001 0.20	2.96 0.06 0.11	0.99	0.04
	NT	2.15 ± 0.28	2.34 ± 0.55				0.27	0.46
	IHT	2.38 ± 0.35	2.68 ± 0.43				0.01	0.77
%VO_2max	ctrl	64.1 ± 8.1	63.6 ± 11.1	3.09 0.05 0.11	1.35 0.25 0.03	0.83 0.44 0.03	-	-
	NT	61.2 ± 9.9	62.7 ± 8.4				-	-
	IHT	66.5 ± 5.9	69.8 ± 4.4				-	-

P: power output, VT2: second ventilatory threshold; HR: heart rate; VO_2: oxygen uptake; ctrl: control group; NT: normoxia training; IHT: intermittent hypoxia training.

In the control group, there was a significant decrease in HR as well as work intensity at VT2 (%HRmax) after observation. None of the trainings significantly affected the intensity at VT2 (%HRmax, %VO_2max) (Table 3).

Maximal heart rate and RER were similar in all groups in both the first and second measurements (Table 2)

4. Discussion

In this study, we hypothesized that interval training in normobaric hypoxia (IHT) may be a more effective method of training to improve endurance performance than training only in normoxia. Our results only partially confirmed our hypothesis. Absolute maximal oxygen uptake significantly increased in both the NT and IHT groups, and the effect size was comparable in both groups. Absolute VO_2max significantly increased in both training groups, indicating that the conditions under which training took place (normoxia or hypoxia) did not significantly affect VO_2max. The post-training changes in relative VO_2max are due not to the effect of hypoxia, but to small changes in body mass after training: in the IHT group, body mass decreased by an average of 0.4 kg and in the NT group, body mass increased by 0.2 kg, with the consequence that the post-training change in relative VO_2max was significant in the IHT group and was insignificant in the NT group. This indicates that IHT did not strengthen the effect of interval training on VO_2max, and when applied in this study, IHT proved to be an ineffective method of improving VO_2max. At the same time, we observed other beneficial effects of IHT that did not occur after

training in normoxia–maximal power output and power output at VT2 and VO_2 at VT2 were improved after training.

In this study, IHT was ineffective in improving VO_2max, which is consistent with what has been reported in previous studies [28,29] in which a non-significant increase in VO_2max was observed, and in contrast to data presented by Roels et al. [30], who showed significant improvement in VO_2max after brief IHT. The lack of improvement in maximal oxygen uptake indicates that the hypoxic stimulation used in this study ('hypoxic dose'), may have been too weak to improve hematological parameters and the efficiency of oxygen delivery to working muscles. The IHT protocol also may be insufficient to accelerate erythropoiesis [28]. Despite years of research, the characteristics of the optimal hypoxia 'dose' (magnitude, duration, frequency) are not clear [28]. This dose may be highly individual [28]. Undoubtedly, the results of previous studies may be influenced by the selection of participants to take part in the study because this type of study typically involves well-trained athletes in whom there may already be limited possibilities for improving VO_2max due to already high physical performance. Also, the variety of hypoxic and training protocols used or genetic factors [19] may have a significant impact on the effectiveness of IHT.

Although the hypoxia applied in our study was found to be ineffective in improving VO_2max, our results indicate that IHT affected other aspects affecting endurance performance, which is consistent with what has been previously reported [31]. The IHT used was effective in improving maximal power output, power output and absolute VO_2 at VT2. These results confirm other previously published data, where improvement in maximal power output was observed in a graded test, while reporting an unclear effect of IHT on the anaerobic threshold and VO_2max [32]. In another similar study, Ventura et al. [33] reported no beneficial effect of IHT on Pmax and VO_2max. Improvements in maximal and/or threshold power output can be attributed to non-hematological effects of IHT, while beneficial changes in skeletal muscle—e.g., mitochondrial density, capillary-to-fiber ratio and fiber cross-sectional area [14,34]—have been reported in untrained participants after hypoxia training. Improved non-hematological factors may also be the cause of improved anaerobic power, which has also been reported after IHT [35]. Improved anaerobic metabolism and sprint performance after IHT may possibly be through enhanced glycolysis [36]. Enhanced glycolysis after IHT may also affect the anaerobic threshold (second ventilatory threshold), as observed in this study.

To maximize the effects of applied training, we used interval training in this study in which loads were determined based on metabolic (ventilation) thresholds, and further strengthened by normobaric hypoxia stimulation. The interval loads used in this study allowed us to control the exercise metabolism of this training, and exercise bout was performed with power output at VT2, i.e., the highest intensity at which there is still a balance between lactate production and lactate removal [25]. In contrast, active recovery was performed with power output at VT1, i.e., the intensity corresponding to the highest intensity of aerobic metabolism [25]. By applying the workloads in this way, it was possible to adapt to high concentrations of lactate, and at the same time perform effective recovery based on aerobic metabolism. In this study, interval training was relatively short (4 weeks)—shorter than the usual 6–12 weeks used to improve VO_2max [20]—and proved to be an effective training method. Our training (both in normoxia and normobaric hypoxia) proved to be effective in improving VO_2max, which is an important practical application of the results of our study, especially in sports where the pre-season is short and coaches have little time for effective physical conditioning in athletes

Limitation

In this study, we focused on the effects of applied intermittent hypoxic interval training on endurance determinants. We did not investigate either hematologic or non-hematologic mechanisms that may underlie the observed effects, which should be a direction for further research on IHT.

5. Conclusions

The effect of interval training in normoxia and hypoxia on absolute maximal oxygen uptake was similar: both trainings were effective and significantly improved the absolute VO_2max. However, the applied IHT was effective in improving maximal power and power at VT2, which was not observed after training in normoxia. The effects of IHT reported in this study have great practical application for coaches. Short IHT can be useful if the goal of training is to increase maximal power output or power output at the second ventilatory threshold.

Author Contributions: Conceptualization, M.M. and T.P.; methodology, M.M., T.P., M.B. and M.W.; validation, M.M. and T.P.; formal analysis, M.M.; investigation, M.W., J.K., J.S., M.B., S.M. and Z.S.; resources, M.M. and T.P.; data curation, M.M.; writing—original draft preparation, M.M.; writing—review and editing, T.P., M.B. and M.W.; visualization, M.M.; supervision, M.M. and T.P.; project administration, M.M.; funding acquisition, M.M. and T.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ministry of Education and Science (Poland), grant number MEIN 2021/DPI/229.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by Bioethical Commission of the Regional Medical Chamber in Krakow, Poland (opinion No. 47/KB/OIL/2022; date 11 April 2022).

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

Data Availability Statement: The datasets analyzed during the study are available from the corresponding author (S.M.) upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Coyle, E.F. Integration of the physiological factors determining endurance performance ability. *Exerc. Sport Sci. Rev.* **1995**, *23*, 25–64. [CrossRef] [PubMed]
2. Mahood, N.V.; Kenefick, R.W.; Kertzer, R.; Quinn, T.J. Physiological determinants of cross-country ski racing performance. *Med. Sci. Sports Exerc.* **2001**, *33*, 1379–1384. [CrossRef] [PubMed]
3. Wasserman, K.; Beaver, W.L.; Whipp, B.J. Gas exchange theory and the lactic acidosis (anaerobic) threshold. *Circulation* **1990**, *81* (Suppl. S1), II14-30.
4. Maciejczyk, M.; Szymura, J.; Cempla, J.; Gradek, J.; Więcek, M.; Bawelski, M. Respiratory compensation point during incremental test in overweight and normoweight boys: Is it useful in assessing aerobic performance? A longitudinal study. *Clin. Physiol. Funct. Imaging* **2014**, *34*, 56–63. [CrossRef] [PubMed]
5. Maciejczyk, M.; Wiecek, M.; Szymura, J.; Cempla, J.; Wiecha, S.; Szygula, Z.; Brown, L.E. Effect of body composition on respiratory compensation point during an incremental test. *J. Strength Cond. Res.* **2014**, *28*, 2071–2077. [CrossRef] [PubMed]
6. Meyer, T.; Faude, O.; Scharhag, J.; Urhausen, A.; Kindermann, W. Is lactic acidosis a cause of exercise induced hyperventilation at the respiratory compensation point? *Br. J. Sports Med.* **2004**, *38*, 622–625. [CrossRef] [PubMed]
7. Álvarez-Herms, J.; Julià-Sánchez, S.; Hamlin, M.J.; Corbi, F.; Pagès, T.; Viscor, G. Popularity of hypoxic training methods for endurance-based professional and amateur athletes. *Physiol. Behav.* **2015**, *143*, 35–38. [CrossRef]
8. Millet, G.P.; Faiss, R.; Brocherie, F.; Girard, O. Hypoxic training and team sports: A challenge to traditional methods? *Br. J. Sports Med.* **2013**, *47* (Suppl. S1), i6–i7. [CrossRef]
9. Sinex, J.A.; Chapman, R.F. Hypoxic training methods for improving endurance exercise performance. *J. Sport Health Sci.* **2015**, *4*, 325–332. [CrossRef]
10. Girard, O.; Brocherie, F.; Goods, P.S.; Millet, G.P. An updated panorama of "living low-training high" altitude/hypoxic methods. *Front. Sports Act. Living* **2020**, *2*, 26. [CrossRef]
11. Faiss, R.; Girard, O.; Millet, G.P. Advancing hypoxic training in team sports: From intermittent hypoxic training to repeated sprint training in hypoxia. *Br. J. Sports Med.* **2013**, *47* (Suppl. S1), i45–i50. [CrossRef]
12. McLean, B.D.; Gore, C.J.; Kemp, J. Application of 'live low-train high'for enhancing normoxic exercise performance in team sport athletes. *Sports Med.* **2014**, *44*, 1275–1287. [CrossRef]
13. Millet, G.P.; Brocherie, F. Hypoxic training is beneficial in elite athletes. *Med. Sci. Sports Exerc.* **2020**, *52*, 515–518. [CrossRef]
14. Wilber, R.L. Application of altitude/hypoxic training by elite athletes. *Med. Sci. Sports Exerc.* **2007**, *39*, 1610–1624. [CrossRef]

15. Ambroży, T.; Maciejczyk, M.; Klimek, A.T.; Wiecha, S.; Stanula, A.; Snopkowski, P.; Palka, T.; Jaworski, J.; Ambrozy, D.; Rydzik, L.; et al. The effects of intermittent hypoxic training on anaerobic and aerobic power in boxers. *Int. J. Environ. Res. Public Health* **2020**, *17*, 9361. [CrossRef]
16. Czuba, M.; Waskiewicz, Z.; Zajac, A.; Poprzecki, S.; Cholewa, J.; Roczniok, R. The effects of intermittent hypoxic training on aerobic capacity and endurance performance in cyclists. *J. Sports Sci. Med.* **2011**, *10*, 175–183.
17. Morton, J.P.; Cable, N.T. The effects of intermittent hypoxic training on aerobic and anaerobic performance. *Ergonomics* **2005**, *48*, 1535–1546. [CrossRef]
18. Levine, B.D. Intermittent hypoxic training: Fact and fancy. *High Alt. Med. Biol.* **2002**, *3*, 177–193. [CrossRef]
19. Treff, G.; Sareban, M.; Schmidt, W. Hypoxic training in natural and artificial altitude. *Dtsch. Z. Sportmed* **2022**, *73*, 112–117. [CrossRef]
20. MacInnis, M.J.; Gibala, M.J. Physiological adaptations to interval training and the role of exercise intensity. *J. Phys.* **2017**, *595*, 2915–2930. [CrossRef]
21. Blair, S.N.; Haskell, W.L.; Ho, P.; Paffenbarger, R.S., Jr.; Vranizan, K.M.; Farquhar, J.W.; Wood, P.D. Assessment of habitual physical activity by a seven-day recall in a community survey and controlled experiments. *Am. J. Epidemiol.* **1985**, *122*, 794–804. [CrossRef]
22. Sarkin, J.; Campbell, J.; Gross, L.; Roby, J.; Bazzo, S.; Sallis, J.; Calfas, K. Seven-day physical activity recall. *Med. Sci. Sports Exerc.* **1997**, *29*, 89–103.
23. Mistura, L.; Comendador Azcarraga, F.J.; D'Addezio, L.; Martone, D.; Turrini, A. An Italian case study for assessing nutrient intake through nutrition-related mobile apps. *Nutrients* **2021**, *13*, 3073. [CrossRef]
24. Bhambhani, Y.; Singh, M. Ventilatory thresholds during a graded exercise test. *Respiration* **1985**, *47*, 120–128. [CrossRef] [PubMed]
25. Binder, R.K.; Wonisch, M.; Corra, U.; Cohen-Solal, A.; Vanhees, L.; Saner, H.; Schmid, J. Methodological approach to the firstand second lactate threshold in incremental cardiopulmonary exercise testing. *Eur. J. Cardiovasc. Prev. Rehabil.* **2008**, *15*, 726–734. [CrossRef]
26. Howley, E.T.; Basset, D.R.; Welch, H.G. Criteria for maximal oxygen uptake: Review and commentary. *Med. Sci. Sports Exerc.* **1995**, *27*, 1292–1301. [CrossRef] [PubMed]
27. Cohen, J. *Statistical Power Analysis for the Behavioral Sciences*; Routledge Academic: New York, NY, USA, 1988; ISBN 9780203771587.
28. Julian, C.G.; Gore, C.J.; Wilber, R.L.; Daniels, J.T.; Fredericson, M.; Stray-Gundersen, J.; Hahn, A.G.; Parisotto, R.; Levine, B.D. Intermittent normobaric hypoxia does not alter performance or erythropoietic markers in highly trained distance runners. *J. Appl. Physiol.* **2004**, *96*, 1800–1807. [CrossRef]
29. Rodriguez, F.A.; Truijens, M.J.; Townsend, N.E.; Martini, E.R.; Stray-Gundersen, J.; Gore, C.J.; Levine, B.D. Effects of four weeks of intermittent hypobaric hypoxia on sea level running and swimming performance. *Med. Sci. Sports Exerc.* **2004**, *36* (Suppl. S5), S338.
30. Roels, B.; Millet, G.P.; Marcoux, C.J.; Coste, O.; Bentley, D.J.; Candau, R.B. Effects of hypoxic interval training on cycling performance. *Med. Sci. Sports Exerc.* **2005**, *37*, 138–146. [CrossRef] [PubMed]
31. Katayama, K.; Matsuo, H.; Ishida, K.; Mori, S.; Miyamura, M. Intermittent hypoxia improves endurance performance and submaximal exercise efficiency. *High Alt. Med. Biol.* **2003**, *4*, 291–304. [CrossRef] [PubMed]
32. Bonetti, D.L.; Hopkins, W.G.; Kilding, A.E. High-intensity kayak performance after adaptation to intermittent hypoxia. *Int. J. Sports Physiol. Perform.* **2006**, *1*, 246–260. [CrossRef] [PubMed]
33. Ventura, N.; Hoppeler, H.; Seiler, R.; Binggeli, A.; Mullis, P.; Vogt, M. The response of trained athletes to six weeks of endurance training in hypoxia or normoxia. *Int J. Sports Med.* **2003**, *24*, 166–172. [CrossRef]
34. Vogt, M.; Puntschart, A.; Geiser, J.; Zuleger, C.; Billeter, R.; Hoppeler, H. Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. *J. Appl. Phys.* **2001**, *91*, 173–182. [CrossRef] [PubMed]
35. Hendriksen, I.J.; Meeuwsen, T. The effect of intermittent training in hypobaric hypoxia on sea-level exercise: A cross-over study in humans. *Eur. J. Appl. Phys.* **2003**, *88*, 396–403. [CrossRef] [PubMed]
36. Hamlin, M.J.; Marshall, H.C.; Hellemans, J.; Ainslie, P.N.; Anglem, N. Effect of intermittent hypoxic training on 20 km time trial and 30 s anaerobic performance. *Scand. J. Med. Sci. Sports* **2010**, *20*, 651–661. [CrossRef] [PubMed]