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Research article

Cardiorespiratory Effects of One-Legged High-Intensity Interval Training in Normoxia and Hypoxia: A Pilot Study

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Abstract
A higher-than-average maximal oxygen consumption (VO_{2max}), is closely associated with decreased morbidity and mortality and improved quality of life and acts as a marker of cardiorespiratory fitness. Although there is no consensus about an optimal training method to enhance VO_{2max}, nevertheless training of small muscle groups and repeated exposure to hypoxia seem to be promising approaches. Therefore, this study was aimed at gaining innovative insights into the effects of small muscle group training in normoxia and hypoxia. Thirteen healthy participants were randomly assigned to the hypoxic (HG, n = 7) or normoxic (NG, n = 6) training group. Both groups completed nine high-intensity interval training sessions in 3 wks. The NG performed the training in normoxia (F_iO_2: 0.21; ~ 600 m) and the HG in hypoxia (F_iO_2: 0.126; ~ 4500 m). Each session consisted of 4 x 4 min one-legged cycling at 90% of maximal heart rate separated by 4 min recovery periods. Before and after the intervention period, VO_{2max} and peak power output (W_{max}) and responses to submaximal cycling (100 and 150 watts) were assessed in a laboratory cycling test. Peak power output significantly improved within both groups (9.6 ± 4.8% and 12.6 ± 8.9% for HG and NG, respectively) with no significant interaction ($p = 0.277$). However, VO_{2max} only significantly increased after training in hypoxia from 45.4 ± 10.1 to 50.0 ± 9.8 ml/min/kg (10.8 ± 6.0%; $p = 0.002$) with no significant interaction ($p = 0.146$). The maximal O_2-pulse improved within the HG and demonstrated a significant interaction ($p = 0.040$). One-legged cycling training significantly improved VO_{2max} and peak power output. Training under hypoxic conditions may generate greater effects on VO_{2max} than a similar training in normoxia and is considered as a promising training method for improving cardiorespiratory fitness.

Key words: Local muscle training, altitude training, exercise physiology.

Introduction

The most important limiting factor for the maximum oxygen uptake (VO_{2max}) in healthy persons is the maximal achieved cardiac output (Murias et al., 2010). For this reason most training interventions employ large muscle groups (e.g. running, cycling), resulting in sufficient stimuli for the cardiorespiratory system (Burtscher, 2013; Murias et al., 2010). In untrained and/or chronically ill persons (e.g. respiratory and cardiovascular disease) the use of a large muscle mass provokes pronounced stress responses of the cardiorespiratory system. This often causes patients to stop e xercising at an early stage without the training stimulus being intense enough to provoke peripheral adaptations.

Training of smaller muscle mass (quadriceps) was found to cause local muscular adaptations and to improve the exercise tolerance for subsequent whole-body training (Esposito et al., 2011). Several studies reported an increase in cardiorespiratory fitness following small muscle group training, particularly in diseased participants e.g. in patients suffering from heart disease (Esposito et al., 2011; Jankowska et al., 2008; Tyni-Lenné et al., 2001). Peripheral adaptations in these patients, such as increases in O_2-delivery, muscle capillarization, mitochondrial density and oxygen extraction were shown after isolated quadriceps training (Esposito et al., 2011). Specific one-legged training proved to be more effective than traditional two-legged aerobic training in improving peak oxygen uptake (Bjørgen et al., 2009; Dolmage and Goldstein, 2008).

Furthermore, passive intermittent hypoxic intervention is widely used among healthy athletes (Katayama et al., 2003) but also among chronically ill persons (Burtscher et al., 2004; 2009) aiming to improve aerobic capacity. Investigations demonstrated increased exercise tolerance and improved resistance to ischaemic stress in people with and without cardiovascular diseases due to intermittent hypoxic interventions (Katayama et al., 2003; Wang et al., 2014). Moreover, there are also investigations on healthy individuals that proved the beneficial effect of active intermittent hypoxic training on exercise tolerance at sea level (Czuba et al., 2011; Hoppeler and Vogt, 2001; Zoll et al., 2006), whereas the increase in exercise tolerance due to normoxic local muscle group training could be demonstrated for both diseased (chronic heart failure) (Jankowska et al., 2008; Magnusson et al., 1996; 1997) and healthy participants (Cesar et al., 2009; Miyachi et al., 2001). However, only a small number of studies have investigated the effects of local muscle group training in hypoxia in healthy people, which elicited contrasting results (Bakkman et al., 2007; Melissa et al., 1997).

Therefore, the aim of the present study was to investigate the exercise responses and effects of local muscle group training in hypoxia and normoxia in healthy participants, as a potential basis for further investigations with chronically ill patients. We hypothesized that high-intensity training (HIT) performed in hypoxia may improve exercise tolerance even in healthy participants to a greater extent than HIT performed under normoxic conditions.

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Methods

Participants

A total of 13 (eight females and five males) healthy young participants (mean age 26 ± 3 years) were recruited for participation in this study. The physical activity status of the participants was recreationally active. Group and gender-specific age and physical characteristics of the participants are presented in Table 1. The study was carried out according to the Declaration of Helsinki and was approved by the Institutional Review Board of the Department of Sport Science (University of Innsbruck). All participants gave written informed consent to participation in the study.

Table 1. Baseline age and physical characteristics of the hypoxic, normoxic and entire group. Data are presented as means (±SD).

	Height (m)	BMI	VO_{2max} ($ml \cdot min^{-1} \cdot kg^{-1}$)
HG (n = 7)	1.70 (.10)	22 (2)	45.4 (10.1)
F (n = 5)	1.66 (.07)	22 (2)	39.9 (4.9)
M (n = 2)	1.81 (.01)	21 (2)	59.0 (.7)
NG (n = 6)	1.71 (.05)	23 (3)	48.1 (12.4)
F (n = 3)	1.68 (.04)	21 (1)	42.2 (9.9)
M (n = 3)	1.74 (.05)	24 (3)	53.8 (13.8)
Total (n = 13)	1.70 (.08)	22 (3)	46.7 (10.3)

F, females; HG, hypoxic training group; M, males; NG, normoxic training group; N, quantity; BMI, Body Mass Index

Study protocol

The study was designed as a randomized training study including hypoxic and normoxic training groups (HG and NG, respectively) and two measurement times (pre- vs. post-training). Pre- and post-tests included an incremental cycle test (ergospirometry). After the pretest all participants were randomly assigned, stratified by VO_{2max} and gender, either to the NG (F = 3; M = 3) or HG group (F = 5; M = 2). There was no significant difference between the two groups for VO_{2max} at baseline. After a break of one week, the HG and NG started the 3-wk one-legged HIT programme. Post-testing measurements were the same as for baseline condition and were conducted 2–3 days after the last HIT session.

Pre-and post-test measurements

All measurements were performed in the laboratories of the Department of Sport Science of the University Innsbruck (Austria) at an altitude of 590 m. Participants were instructed to refrain from intense exercise 24 h before the pre- and post-training measurements and to appear fully hydrated on the test day. Participants were also advised to maintain their normal eating and drinking habits throughout the study period. Alcohol was restricted 24 h before the tests. As part of the pretest, participants were informed in detail about the content and the aim of the study. The pre-and post-tests consisted of the same measurements. Participants initially performed a two-legged incremental cycling test to volitional fatigue on an electronically braked cycle ergometer (Ergoline 900, Schiller, Dietikon, Switzerland) to determine VO_{2max} and peak power output (W_{max}, achieved at the highest stage in the test). After a 10-min cycling warm-up at a self-chosen resistance, workload was increased by 25 watts each minute, which initially started at zero watts, until volitional exhaustion. Gas analysis was performed using an open spirometric system (Oxycon Mobile, Care Fusion, Würzburg, Germany), which was calibrated before each measurement, according to the manufacturer's guidelines. Ventilatory parameters [i.e. ventilation (VE), oxygen pulse (O_2-pulse), oxygen uptake (VO_2), carbon dioxide output (VCO_2)] were recorded breath by breath during the ergospirometry. Heart rate (HR) was determined by the use of a chest belt sensor (Wear Link, Polar, Kempele, Finland) and signals were transmitted to the spirometric device. The blood sample for the lactate (La) concentration was obtained from the earlobe after stopping the test, while the participant was still sitting on the ergometer. Mean VO_{2max} was determined as the highest value averaged over 30 seconds and maximal heart rate (HR_{max}) as the highest 5 s average during the last 2 min of the test. Submaximal values were obtained at 100 and 150 watts (after 4 and 6 min).

Before the participants started the exercise test, hyperaemized capillary blood was taken from a fingertip to analyse haemoglobin concentration (Hb) (g/dl) by the use of a miniphotometer (Miniphotometer Plus LP 20, Dr. Lange, Germany).

Intervention

All participants started the training protocol one week following the pretesting, which consisted of nine one-legged HIT sessions over 3 wks (training every 2–3 days). Each training session consisted of 4 x 4 min one-legged high-intensity cycling (on a standard cycling ergometer) bouts at an HR that corresponded to the 90% of the HR_{max} achieved during the incremental cycling test. Participants from the HG and NG groups trained at the same relative intensity, as it has become apparent that training at the same relative intensity is more adequate in investigating the effect of hypoxic training than training at the same absolute intensity (Bakkman et al., 2007). At each session, at least one study supervisor was present for guiding and observing the training as well as to register the stress reactions (HR, oxygen saturation, perceived exertion). The participants had to report on the rated perceived exertion scale directly after each 4-min interval bout. Each training session started with a 10-min two-legged warm-up on the ergometer and was followed by 4 x 4 min single-legged intervals. Heart rate was measured and controlled with a heart rate monitor (Polar, Kempele, Finland). Every other session was performed with the opposite leg, whiles the other, inactive leg, was placed on the ergometer. Participants performed active recovery (cycling with low resistance with both legs) between the intervals (work/rest ratio 1:1). The HG trained in the altitude chamber of the Institute for Sport Science in Innsbruck (4500 m; F_iO_2: 0.126). The training sessions of the NG took place in the gyms of the Institute under normoxic conditions (Innsbruck 600 m; F_iO_2: 0.21).

On the remaining days, participants continued their individual training in the usual manner. For the additional training we recommended low-intensity, regenerative training.

Statistical analysis

Statistical analyses were conducted by PASW Statistics 19 (IBM, Vienna, Austria).

Normal distribution of data was tested by the Kolmogorov–Smirnov test. ANOVA with repeated measurement design was used to determine changes due to the training intervention (main effect: training) and to determine different changes between the hypoxia and normoxia training group (interaction: training × group). ANOVA was also applied to evaluate a possible gender effect (group × training × gender). In addition, paired student's *t* tests were carried out to evaluate within-group effects. The relationships between variables were assessed by correlation analyses (Pearson). *p* values <0.05 (two-tailed) were considered to indicate statistical significance. Values are presented as mean ± SD. For the analysis of the maximal values, the full data set was available, but for submaximal analyses, due to malfunctions of the heart rate monitor and gas analyser, some submaximal values for two participants of the NG were missing. Additionally, in HG, one female participant had already finished the pretest before 150 watts were reached, so her data set could not be included for submaximal evaluation at 150 watts, resulting in a sample size of 6 in HG and 4 in NG for the evaluation at 150 watts, and 7 in HG and 5 in NG for the evaluation at 100 watts.

Results

No gender effect was identified with regard to the parameters of interest, allowing us to pool the main variables of both sexes.

Measurements recorded during the training program

Heart rate, SpO_2 and ratings of perceived exertion (RPE) have been recorded during the HIT intervention. Mean training HR for the HG ranged between 88.5 ± 5.5% and 90.3 ± 6.6% of HR_{max}, for NG, mean training HR ranged between 87.8 ± 5.3% and 90.3 ± 3.6%. Training HR over all nine HIT session did not differ between the two groups (p = 0.953). Mean RPE during all nine HIT sessions ranged between 16.4 ± 1.3 and 17.3 ± 1.6 for the HG and between 15.9 ± 1.5 and 17.3 ± 1.2 for the NG (with no significant between-group difference, p = 0.832). Mean SpO_2 at each HIT session significantly differed between HG and NG (p = 0.001). In HG, mean SpO_2 ranged between 80.7 ± 7.2% and 84.4 ±5.7%, and between 94.8 ±1.1% and 96.6 ±1.6% for the NG.

The entire group

Assessing the main effect (n = 13), W_{max} and VO_{2max} (two-legged cycling) significantly improved (p < 0.001, p = 0.003, respectively). In the post-test, peak power output (W_{max}) increased by an average of 24.2 ± 14.4 watts (+11.0%). VO_{2max} improved by 8.5% from 46.7 ± 10.3 ml/min/kg to 50.1 ± 9.1 ml/min/kg.

No significant main effects were found for submaximal values in the entire group.

The hypoxic- and normoxic group

Maximal values: Outcomes of the performance testing for HG and NG are shown in Table 2. HG and NG significantly improved W_{max} by 9.6% and 12.6%, respectively, with no significant interaction. VO_{2max} significantly increased in HG (+10.8%; p = 0.002) from 45.4 ± 10.1 to 50.0 ± 9.8 ml·min^{-1}·kg^{-1}. In the NG, VO_{2max} improved by 6.6% from 48.1 ± 12.4 to 50.1 ± 9.3 ml·min^{-1}·kg^{-1}, but without reaching significance (p = 0.269). No significant interaction was seen for VO_{2max} (p = 0.146). There was a significant interaction for maximal O_2-pulse (ml/beat)

Table 2. Changes of physiological and performance parameters from pre- to post-training of the hypoxic and normoxic training group. Data are presented as means (±SD).

	HG (n = 7)		NG (n = 6)		ANOVA	
	pre	post	pre	post	main effect (training)	interaction (training x group)
VO_{2max} (ml·min^{-1}·kg^{-1})	45.4 (10.1)	50.0 (9.8) *	48.1 (12.4)	50.1 (9.3)	**.003**	.146
VO_{2max} (ml·min^{-1})	2905 (925)	3183 (919) *	3234 (1143)	3380 (943)	**.004**	.284
Maximal O_2-pulse (ml/beat)	15.4 (4.7)	17.0 (4.3) *	18.9 (6.6)	18.3 (5.0)	.256	.040
§O_2-pulse$_{sub\text{-}max\ 150}$ (ml/beat)	13.1 (2.7)	14.4 (2.2) *	15.3 (3.3)	14.7 (2.8)	.471	.078
§§O_2-pulse$_{sub\text{-}max\ 100}$ (ml/beat)	11.3 (2.8)	11.5 (2.3)	12.6 (3.9)	13.3 (2.1)	.407	.538
§$EqO_{2sub\text{-}max\ 150}$	26 (4)	25 (4)	23 (1)	25 (2)	.543	.008
§§$EqO_{2sub\text{-}max\ 100}$	24 (3)	24 (3)	22 (2)	23 (2)	.292	.348
W_{max} (Watt)	231 (74)	251 (75) **	248 (68)	277 (68) *	**≤.001**	.277
W_{max}/BW (Watt/kg)	3.6 (.8)	3.9 (.9) **	3.8 (.9)	4.1 (.7) *	**≤.001**	.946
HR_{max} (bpm)	190 (12)	189 (12)	180 (9)	185 (7)	.234	.081
§$HR_{sub\text{-}max\ 150}$ (bpm)	160 (22)	154 (18)	144 (10)	147 (21)	.679	.332
§§$HR_{sub\text{-}max\ 100}$ (bpm)	143 (27)	141 (19)	130 (20)	124 (11)	.679	.332
§$BF_{sub\text{-}max\ 150}$ (ml·min^{-1})	27 (8)	27 (6)	27 (4)	30 (4)	.142	.142
§§$BF_{sub\text{-}max\ 100}$ (ml·min^{-1})	25 (8)	24 (8)	25 (5)	25 (2)	.897	.897
Weight (kg)	62.9 (7.5)	62.6 (7.5)	66.9 (12.7)	67.4 (12.3)	.694	.135
Hb (g/dl)	13.9 (.9)	13.0 (1.8)	12.6 (2.3)	13.1 (1.2)	.633	.168
La_{max} (mmol/l)	8.9 (2.2)	9.1 (3.2)	9.7 (1.9)	11.5 (1.3)	.118	.211
RER_{max}	1.18 (.06)	1.19 (.06)	1.18 (.08)	1.20 (.06)	.470	.630

BF, breathing frequency; EqO_2, ventilatory equivalent for oxygen; Hb, hemoglobin concentration; La_{max}, maximal lactate; RER, respiratory exchange ratio; sub-max 100, submaximal responses to 100 watts; sub-max 150, submaximal responses to 150 watts. *significant within-group changes from pre-to post-training ($p \leq 0.05$). ** significant within-group changes from pre- to post-training ($p \leq 0.001$). §sample size: NG= 4; HG= 6. §§sample size: NG=5; HG=7

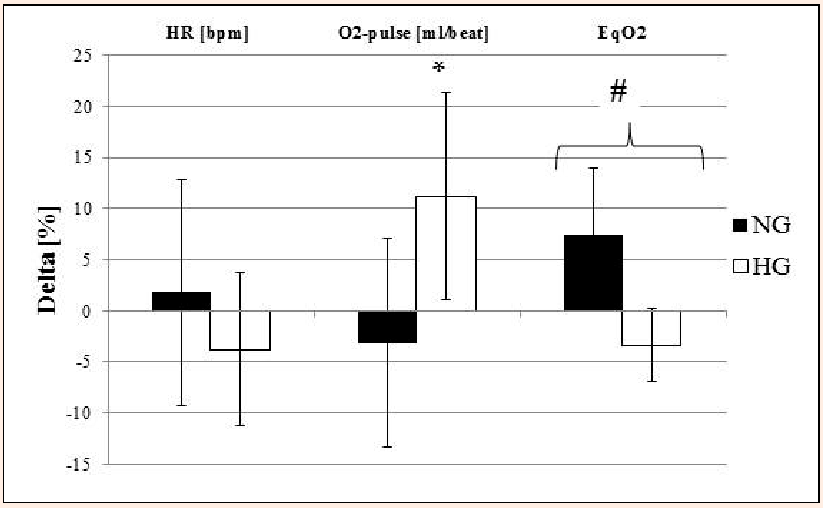

Figure 1. Mean changes of HR, O_2-pulse and EqO_2 at the submaximal workload of 150 W for the hypoxic and normoxic training group. Data are presented as percentage (%) change. Sample size : NG= 4; HG= 6. HG, hypoxic training group; HR, heart rate; NG, normoxic training group; EqO_2, ventilatory equivalent for oxygen. *significant within-group changes ($p \leq 0.05$). #significant between-group changes ($p \leq 0.05$)

(p = 0.040). Maximal O_2-pulse significantly increased in the HG from 15.4 ± 4.7 to 17.0 ± 4.3 ml/beat (+12.1%; p = 0.003) but remained unchanged in the NG.

Submaximal values: Responses to submaximal cycling were obtained at 100 watts (27–82% of W_{max}; mean: 45%) and 150 watts (40–82% of W_{max}; mean: 62%). The ventilatory equivalent for oxygen (EqO_2) at submaximal load (150 W) decreased within the HG by 3.3 ± 3.3%, whereas in NG EqO_2 increased by 7.5 ± 6.5% and showed a significant interaction (p = 0.008). O_2-pulse at 150 W significantly increased in HG by 11.2 ± 10.2% (p = 0.041) and decreased by 3.1 ± 10.2% in the HG. No significant interaction was found for submaximal HR at 150 watts (see Figure 1). However, no significant interaction was found for O_2-pulse at 150 W (p = 0.078). No significant interactions or main effects were found for submaximal values evaluated at 100 watts.

Discussion

One main finding of this project was that a 3-wk one-legged cycling training leads to an improved maximal performance (W_{max}) and improved VO_{2max} regardless of hypoxic or normoxic conditions. Additionally, VO_{2max} and maximal O_2-pulse significantly increased after training by over 10% and 12% respectively, only under hypoxic conditions. However, despite a within-group change of VO_{2max} in the HG, no interaction (group × time) was seen for VO_{2max}, indicating no significant effect of training in hypoxia. The findings from the present investigation do not support our hypothesis that one-legged HIT performed in hypoxia may improve exercise tolerance to a greater extent than one-legged HIT performed under normoxic conditions.

The observed improved maximal exercise performance due to one-legged cycling has already been demonstrated in several studies (Bakkman et al., 2007; Miyachi et al., 2001). The associated adaptations, as well as in normoxia and hypoxia, seem to be more due to a local rather than a systemic nature. An enlargement of the femoral artery diameter increases blood flow and thereby increase the capillarization of working muscles, the oxidative capacity of mitochondria, and reduces the sympathetic tone during exercise. These seem to be the primary mechanisms involved in improvement of exercise performance due to one-legged cycling exercise (Bakkman et al., 2007; Geiser et al., 2001; Miyachi et al., 2001; Ray, 1999). Another finding was that responses to submaximal exercise (150 watts) improved after training in a hypoxic environment. The results showed a tendency towards a decreased HR at submaximal loads after training in hypoxia, which might be related to a decrease in sympathetic tone (Figure 1). The slight reduction in submaximal HR due to training in hypoxia may indicate an improvement in cardiovascular fitness (Holliss et al., 2014). Submaximal O_2-pulse rose after one-legged training in hypoxia compared to training in normoxia, which might be a consequence of reduced HR. The results of the present study indicated that cardiorespiratory fitness at submaximal loads may be beneficially influenced by one-legged training in hypoxic conditions due to attenuated submaximal HR in HG. This may be the result of attenuated muscle metaboreflex sensitivity due to high-intensity hypoxic exercise. This reflex downregulation may result from the effect of repeated exposures and the resulting adaptation to the aggregated metabolites. Furthermore, one-legged hypoxic HIT induced a decrease in EqO_2 whereas no alterations were found for the NG at 150 watts, indicating that ventilatory efficiency had improved (Miyachi and Katayama, 1999) due to hypoxic endurance training. Moreover, we found that VO_{2max} significantly improved after one-legged hypoxic training, which is controversially discussed in the literature. Furthermore, Bakkman et al. (2007) demonstrated that training in hypoxia may inhibit the development of oxidative capacity. The authors demonstrated significantly increased citrate synthase (CS) activity after one-legged training in normoxia whereas in hypoxic training conditions CS remained unaffected. Contrarily, Geiser et al. (2001) reported HIT in combination with hypoxic conditions were the most effective stimulus for improved muscle oxidative capacity, when compared to low-intensity training in hypoxia, as well as to low- and high-intensity training in normoxia. Moreover, intermittent hypoxic whole-body training was found to generate an improved oxidative capacity, increased capillarization as well as elevated muscle buffering capacity and anaerobic capacity (Czuba et al., 2011).

Taken together, high-intensity exercise training performed with small muscle groups in hypoxia might cause more favourable effects on whole-body VO_{2max} than a similar training in normoxia.

Limitations

There are at least some limiting factors that need to be mentioned. First, findings were obtained from a relatively small sample size. However, at least for the investigation of the main research question, the sample size is appropriate but for subgroup analyses (150 watts) it may be too low. A second limitation may arise from the fact that the investigation was not blinded. As males and females were investigated, the question may arise whether the menstrual cycle may have had an effect on the measured values. As the performance tests were conducted in normoxia under ambient temperature, it is unlikely that the menstrual cycle may have influenced the parameters, which is supported by Janse de Jonge et al. (2012), who couldn´t find differences in exercise performance between menstrual cycle phases in temperate conditions. Furthermore, 4–5 training sessions per leg may be an insufficient training load to have significant training effects and differences between normoxia and hypoxia. However, because participants were not accustomed to this type of training, significant training effects – as at least partly demonstrated by the findings of the present investigation – were expected. The training period may have been too short to detect more clear differences between HG and NG.

Conclusion

Training of small muscle groups seems to be a still overlooked but promising method for improving the physical fitness and exercise tolerance of healthy persons, which could probably be even more important for patients suffering from chronic diseases. One-legged training significantly improved VO_{2max} and peak power output. The results of the present study showed that local muscle group training performed in hypoxia did not improve exercise tolerance to a greater extent than normoxic training. Future research should focus on performance effects after a more prolonged one-legged training period in hypoxia.

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Key points

- Nine sessions of one-legged high-intensity interval training significantly improved physical fitness.
- One-legged hypoxic training significantly improved W_{max}, VO_{2max} and submaximal performance.
- One-legged training in normoxia only improved W_{max} but did not significantly improve VO_{2max} and submaximal performance.

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