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ORIGINAL ARTICLE

Effect of 3-week high-intensity interval training on VO_{2max}, total haemoglobin mass, plasma and blood volume in well-trained athletes

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Abstract
Purpose This study examined the haematological adaptations to high-intensity interval training (HIT), i.e. total haemoglobin mass (tHb-mass), blood volume (BV), and plasma volume (PV), and its effects on VO_{2max} in well-trained athletes.
Methods Twenty-seven male and eight female well-trained (VO_{2max} 63.7 ± 7.7 ml/min/kg) athletes were randomly assigned to the HIT (HITG, $N = 19$) or the control group (CG, $N = 16$). Over a 3-week period, the HITG performed 11 HIT sessions, consisting of four 4-min interval bouts at an exercise intensity of 90–95 % of the individual maximal heart rate (HR_{max}), separated by 4-min active recovery periods. Before and 5 ± 2 days after the intervention, tHb-mass, BV and PV were determined by the CO-rebreathing method. VO_{2max} was assessed in a laboratory treadmill test.
Results tHb-mass (from 753 ± 124 to 760 ± 121 g), BV (from 5.6 ± 0.8 to 5.6 ± 0.9 l) and PV (from 3.2 ± 0.5 to 3.2 ± 0.5 l) remained unchanged after HIT and did not show an interaction (group × time). Within the HITG, VO_{2max} improved from baseline by +3.5 % ($p = 0.011$), but remained unchanged in the CG. No interaction (group × time) was seen for VO_{2max}. The HITG showed a significant reduction in HR_{max} compared to the baseline measurement (−2.3 %, $p \leq 0.001$), but HR_{max} remained unchanged in the CG. There was a significant interaction (group × time) for HR_{max} ($p = 0.006$). Also, oxygen pulse significantly increased only in HITG from 22.9 ± 4.4 to 23.9 ± 4.2 ml/beat, with no interaction ($p = 0.150$).
Conclusions Eleven HIT sessions added to usual training did neither improve VO_{2max} nor haematological parameters compared to the CG.

Keywords High-intensity interval training · Maximal oxygen uptake · Blood volume · Total haemoglobin mass · Plasma volume · Well-trained individuals

Abbreviations

ANOVA	Analysis of variance
$avDO_2$	Arterial–venous O_2 content difference
BV	Blood volume
CG	Control group
CO	Carbon monoxide
Hb	Haemoglobin
Hct	Haematocrit
HIT	High-intensity interval training
HITG	High-intensity interval training group
HR	Heart rate
HR_{max}	Maximal heart rate
O_2-pulse	Oxygen pulse
PV	Plasma volume
Q	Cardiac output
Q_{max}	Maximal cardiac output
SV	Stroke volume
tHb-mass	Total haemoglobin mass
VO_{2max}	Maximal oxygen uptake

Introduction

High-intensity interval training (HIT) consisting of short bouts of vigorous intensity exercise, interspersed with

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Table 1 Baseline age and physical characteristics of the training and the control group (gender specific and for the total group)

	HIT group			Control group		
	Female ($n = 5$)	Male ($n = 14$)	Total ($n = 19$)	Female ($n = 3$)	Male ($n = 13$)	Total ($n = 16$)
Age (years)	25 ± 1	28 ± 3	27 ± 3	23 ± 1	25 ± 1	24 ± 2
Height (cm)	169 ± 7	172 ± 4	173 ± 7	169 ± 5	181 ± 6	176 ± 8
BMI	21 ± 2	22 ± 2	22 ± 2	20 ± 1	22 ± 1	21 ± 1
VO_{2max} (ml/min/kg)	55.0 ± 4.9	66.7 ± 5.5	63.6 ± 7.5	52.6 ± 3.0	66.3 ± 6.7	63.7 ± 8.2

Data are presented as mean ± SD

low-intensity recovery periods, seems to be the most effective method to improve exercise performance not only in well-trained athletes but also in sedentary and diseased individuals (Astorino et al. 2012; Breil et al. 2010a; Helgerud et al. 2007; Iaia et al. 2009; Jacobs et al. 2013; Laursen et al. 2002). However, as the favourable effects of HIT on VO_{2max} are well established, the underlying mechanism explaining these improvements, especially in well-trained athletes, remains partly elusive. It has been shown that in endurance-trained athletes, VO_{2max} is predominantly determined by the oxygen supply (Steiner and Wehrlin 2011). Both blood volume (BV) and haemoglobin (Hb) particularly contribute to the amount of oxygen delivery by increasing both cardiac output (Q) and oxygen content of arterial blood (Pottgiesser and Schumacher 2013; Warburton et al. 2000). In contrast to their sedentary or not endurance-trained counterparts, endurance-trained athletes possess a considerably higher body weight-related haemoglobin mass (Hb-mass) and a higher BV (Krip et al. 1997; Schmidt et al. 2002; Steiner and Wehrlin 2011; Warburton et al. 2004). However, whether the Hb-mass and BV of already well-trained athletes can be further increased by high-intensity training and therefore contribute to enhanced VO_{2max}, remains unclear. Several investigations demonstrated unchanged Hb-mass in trained athletes, independently of intensification of the training (Glass et al. 1969; Gore et al. 1997). In contrast, Breil et al. (2010b) have shown significantly increased Hb-mass (+9.9 %) and BV (+10.0 %) after 15 HIT sessions (4 × 4 min at 90–95 % of maximal heart rate (HR_{max})) in elite junior skiers with concomitant increase of VO_{2max}, suggesting Hb-mass and BV are responsible for improved VO_{2max} after HIT. However, Helgerud et al. (2007) failed to demonstrate training-induced changes in BV after 8 weeks of HIT (4 × 4 min at 90–95 % HR_{max}) despite significantly improved VO_{2max} in moderately trained subjects. Investigations showed exercise intensity as the main stimulus for training-induced hypervolaemia (Convertino 1991; Nagashima et al. 1999). This is manifested by significant increases in BV after short-term HIT in trained athletes (Green et al. 1984; Richardson et al. 1996). Richardson et al. (1996) reported increased PV (+4.4 %) even after 2 days of HIT in trained (VO_{2max} 60.7 ± 6.4 ml/min/kg) men. In untrained males, 12 weeks of interval training resulted in significant increased VO_{2max} and BV. The training-induced hypervolaemia accounted for 47 % of the VO_{2max} changes (Warburton et al. 2004). Nevertheless, in a recent investigation, Jacobs et al. (2013) failed to demonstrate increases in total haemoglobin mass (tHb-mass), BV, PV, and maximal cardiac output (Q_{max}) after six HIT sessions in untrained individuals, although VO_{2max} and endurance performance improved.

Due to these contradictory findings, as well the paucity of data on haematological adaptations to HIT in well-trained athletes, the present study aimed to examine the impact of HIT on changes in tHb-mass, BV, and PV, explaining VO_{2max} in already well-trained athletes. We hypothesized that the increase in VO_{2max} caused by HIT would be directly related to differences in training-induced hypervolaemia and increased tHb-mass.

Materials and methods

The present paper pools the main outcome variables (tHb-mass, BV, PV, VO_{2max} and maximal oxygen pulse (O_2-pulse)) of two HIT investigations. Both sought to investigate haematological adaptations to 3-week HIT in well-trained individuals with the same training intervention and main outcome variables but different treadmill protocols.

Participants

Overall, eight female and twenty-seven male well-trained sport students participated in the study. All study participants underwent a routine pre-participation screening prior to the baseline testing. Age and physical characteristics of the participants are presented in Table 1. Normal exercise load of the subjects was 9.5 ± 5.0 h/week. Possible exclusion criteria were all types of acute and chronic diseases, smoking or pregnancy.

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

Innsbruck). All participants gave written informed consent to participate in the study.

Study protocol

The study was designed as a randomized controlled training study including HIT and control group (HITG and CG, respectively) and two measurement times (baseline vs. post-training). Baseline measurements included the determination of haematological parameters and a laboratory treadmill test. After baseline measurements, the participants were randomly assigned, stratified by gender and VO_{2max} (determined in the laboratory treadmill test) either to the HITG or the CG. After a break of at least 4 days, the HITG started the 3-week HIT program whereas CG maintained their usual training during this period. Post-training measurements were the same as for baseline condition and were conducted 5 ± 2 days after the last HIT session. Haematological parameters were measured by a blinded investigator.

Baseline and post-training 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 baseline and post-training measurements and to appear fully hydrated on the test day.

Haematological parameters

The carbon monoxide (CO) rebreathing method, according to Schmidt and Prommer (2005), was used to determine tHb-mass and BV. After the participants had spent 5 min in a sitting position, hyperaemized capillary blood was taken from a fingertip to analyse Hb (g/dl), Hct (%), and HbCO (Auto Counter AC 920, Swelab Instruments, Stockholm, Sweden). Subsequently, participants inhaled a bolus of carbon monoxide (CO) corresponding to 1 mL × kg^{-1} body mass. Then, they had to stop breathing for 10 s before they started rebreathing the gas mixture for another 1.50 min in a closed circuit (glass spirometer, Blood Tec GbR, Bayreuth, Germany). Subsequently, capillary blood samples were analysed for HbCO 6 and 8 min after the inhalation of the CO. The end-tidal CO concentration was measured with a CO gas-tester (CO-220, Fluke, Canada).

According to Schmidt and Prommer (2005, 2008), this method is valid for the estimation of tHb-mass with a typical error of 1.7 %. After familiarization with this method, a typical error between 1.5 and 1.7 % was observed in our lab derived from duplicate and triplicate determinations.

Laboratory treadmill testing

Tests were carried out on the electrically driven treadmill (h/p/cosmos pulsar, h/p/cosmos Sports and Medical, Nussdorf-Traunstein, Germany). As mentioned above, two different treadmill protocols were used to assess VO_{2max}, with no differences in achieving VO_{2max}. The following treadmill protocol, as described in detail by Burtscher et al. (2008), was used to assess VO_{2max} in the first investigation. Exercise started at 5 km/h and 5 % inclination, after 2 min inclination was set at 10 % for 2 min. Subsequently, running speed was increased to 6 km/h and inclination was augmented by 2 % every minute until 20 %. Finally, inclination was kept constant at 20 % and running speed was increased by 1 km/h per minute (Burtscher et al. 2008). The test was completed when the athlete was subjectively exhausted despite verbal encouragement. In investigation 2, the treadmill protocol according to Burtscher et al. (2010) was used. Participants started at 10 km/h and 1 % inclination for 1 min followed by 1 min at 11 km/h and 1 % inclination. Afterwards, running speed was set to 12, 14 and 16 km/h with the same inclination, each speed level was performed for 4 min. Afterwards, speed was increased by 1 km/h every minute. When 20 km/h were reached, speed was kept constant and inclination increased by 2 % every minute (Burtscher et al. 2010).

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. VE, O_2-pulse, VO_2, VCO_2) were recorded breath by breath during the ergospirometry. Heart rate (HR) was determined by chest belt (Wear Link, Polar, Kempele, Finland) and transmitted to the spirometric device. VAT was determined at the point at which VE/VO_2 reaches the minimum value and begins to rise without a concomitant rise in VE/VCO_2 (Wassermann et al. 1973). A test was considered maximal when three of the following criteria were fulfilled: (1) VO_2 plateau at peak exercise (2) respiratory exchange ratio ≥1.10 (3) peak HR ≥90 % of the theoretic maximal HR (220-age), and (4) indication of maximal exhaustion by the athlete (Cunha et al. 2010). VO_{2max} was defined as the highest 30 s average in oxygen uptake and maximal heart rate (HR_{max}) as the highest 5 s average during the test.

HIT program

The HITG performed 11 HIT sessions during the 3-week HIT period. Each HIT session consisted of four 4-min interval bouts at an exercise intensity of 90–95 % of the $HR_{max,}$ separated by 4-min active recovery periods (work/rest ratio = 1:1). In the first week, athletes completed three

HIT sessions and in the following 2 weeks four HIT sessions each week. On the remaining days, the HITG continued their individual training. For the additional training of the HITG, we recommended low-intensity, regenerative training.

All HIT sessions took place on an athletic 400 m track and were supervised by two investigators. Training intensity was controlled by continuous HR monitoring (Polar, Kempele, Finland) and the rating of perceived exertion (RPE) was determined according to the Borg scale (6–20; Borg 1982). HR after 2 min and HR at the end of the interval bout, as well as RPE, were directly listed after each interval bout. The HIT sessions were performed in training groups of 4–6 athletes and started with a standardized 15-min warm-up at 70–75 % HR_{max} (determined at the maximal treadmill test), five running drills and three submaximal progressive sprints over about 100 m. The athletes of the CG maintained their usual endurance and strength training during the 3-week intervention period. In particular, they were advised not to include additional high-intensity training. The training data (i.e. includes all endurance training performed during the 3-week training period) for the HITG and the CG were recorded in a training log book and the total endurance training loads were determined according to Foster et al. (2001) as perceived exertion × endurance training session time.

Statistical analysis

Statistical analyses were conducted by PASW Statistics 19 (IBM, Vienna, Austria). Statistical power for the chosen sample size of 35 (19 in the HITG and 16 in the CG) amounted to >90 %; alpha = 0.05. Power calculation (G*Power 3.1.7) was based on the findings of Warburton et al. (2004) considering expected changes in VO_{2max}, PV and BV due to HIT. Normal distribution of data was tested by the Kolmogorov–Smirnov test. A two-way analysis (group × time) of variance (ANOVA) with repeated measurements was used to verify between-group changes. ANOVA was also applied to evaluate a possible gender effect (group × time × 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.

Results

No gender effect was identified with regard to the parameters of interest (tHb-mass, BV, PV, VO_{2max}, HR_{max} and maximal O_2-pulse), allowing us to pool the main outcome variables of both sexes.

Table 2 Changes of physiological, haematological and performance parameters from pre- to post-training of the HITG and CG

	HIT group ($n = 19$)		Control group ($n = 16$)		ANOVA (interaction)
	Pre	Post	Pre	Post	Time × group
tHb-mass (g)	753 ± 124	760 ± 121	767 ± 134	768 ± 127	0.370
tHb-mass (g/kg)	10.9 ± 1.4	11.0 ± 1.5	11.0 ± 1.5	11.0 ± 1.4	0.357
BV (l)	5.6 ± 0.8	5.6 ± 0.9	5.6 ± 0.7	5.7 ± 0.7	0.867
BV (ml/kg)	80 ± 10	81 ± 11	81 ± 11	82 ± 12	0.864
PV (l)	3.2 ± 0.5	3.2 ± 0.5	3.2 ± 0.6	3.1 ± 0.4	0.446
PV (ml/kg)	46 ± 6	46 ± 7	46 ± 8	45 ± 7	0.433
Hb (g/dl)	14.9 ± 1.2	14.9 ± 1.4	15.0 ± 1.0	15.0 ± 0.9	0.719
Hct (%)	42 ± 3	43 ± 4	43 ± 4	44 ± 2	0.406
VO_{2max} (ml/min/kg)	63.6 ± 7.5	65.8 ± 7.1*	63.7 ± 8.2	64.7 ± 7.1	0.243
VO_{2max} (ml/min)	4405 ± 823	4546 ± 815*	4513 ± 890	4566 ± 818	0.208
HR_{max} (bpm)	196 ± 6	191 ± 5§	197 ± 11	196 ± 10	0.006
Maximal O_2-pulse (ml/beat)	22.9 ± 4.4	23.9 ± 4.2*	23.1 ± 4.7	23.5 ± 4.6	0.150
RER_{max}	1.18 ± 0.07	1.15 ± 0.06	1.15 ± 0.06	1.15 ± 0.07	0.384
Weight (kg)	69.2 ± 7.8	69.2 ± 7.7	69.8 ± 9.1	69.9 ± 9.3	0.848

Data are presented as mean ± SD

BV blood volume, *PV* plasma volume, *tHb-mass* total haemoglobin mass

* Significant within-group changes from pre- to post-training ($p \leq 0.05$)

§ Significant within-group changes from pre- to post-training ($p \leq 0.001$)

Table 3 Training data (all endurance training performed during the 3-week training period) for HITG and CG

	HITG			CG		
	Endurance training time (min)	Perceived exertion (BORG)	TRIMP	Endurance training time (min)	Perceived exertion (BORG)	TRIMP
Week 1	250 ± 154	13.4 ± 1.9	3120 ± 1817	314 ± 160	13.3 ± 0.9	4160 ± 2224
Week 2	303 ± 208	14.0 ± 1.9	3759 ± 2310	273 ± 176	13.2 ± 1.1	3578 ± 2503
Week 3	284 ± 184	14.5 ± 1.8	3895 ± 2676	355 ± 173	13.3 ± 0.8	4574 ± 2243
Total/overall	1086 ± 1024	14.0 ± 1.4	11,087 ± 5193	943 ± 441	13.3 ± 0.7	12,576 ± 5766

Data are presented as mean ± SD

TRIMP training impulse (perceived exertion × endurance training session time)

Haematological parameters

No interaction (group × time) was found for tHb-mass, BV, PV and Hct (Table 2). Within the HITG, tHb-mass (from 753 ± 124 to 760 ± 121 g), BV (from 5. 6 ± 0.8 to 5.6 ± 0.9 l) and PV (from 3.2 ± 0.5 to 3.2 ± 0.5 l) remained unchanged after HIT. Mean values for tHb-mass, BV and PV are shown in Table 2.

Laboratory treadmill testing

Outcomes of the performance testing are shown in Table 2. The participants were all sport students and appeared motivated to take the tests. Of all the participants, 88.6 % reached a plateau of VO_2 during the baseline test and 85.7 % during the post-test. For the baseline test, 16 out of 35 participants and for the post-test, 10 out of 35 persons fulfilled all four criteria for a maximal test according to Cunha et al. (2010). All participants reached a RER ≥1.05. For the baseline and post-test, 80 and 71.4 %, respectively, of all participants reached a RER ≥1.1. There was no difference in attaining VO_{2max} between both treadmill protocols.

VO_{2max} (ml/min/kg) significantly increased in the HITG (+3.5 %, $p = 0.011$) from 63.6 ± 7.5 to 65.8 ± 7.1 ml/min/kg, but remained unchanged in the CG. There was no interaction (group × time) for VO_{2max}. The HITG showed a significant reduction in HR_{max} compared to the baseline measurement from 196 ± 6 to 191 ± 5 bpm (−2.3 %, $p \leq 0.001$), but HR_{max} remained unchanged in the CG. There was a significant interaction (group × time) for HR_{max} ($p = 0.006$). Maximal O_2-pulse significantly increased in the HITG (+4.7 %, $p = 0.012$) from 22.9 ± 4.4 to 23.9 ± 4.2 ml/beat but remained unchanged in the CG. However, there was no interaction (group × time) for maximal O_2-pulse. No significant correlation between changes in HR_{max} and changes in VO_{2max} ($r = -0.079$; $p = 0.748$) was seen for the HITG.

Training data

All participants completed the study with no adverse events. Except for one person, who performed only 10 HIT sessions, all other athletes of the HITG completed 11 HIT sessions. Twelve out of 19 training log books were filled in completely and used for the evaluation of the entire training load. The remaining seven training report forms could not be included in the evaluation due to incomplete information (missing BORG and/or training session time). Data of endurance training loads for each week are presented in Table 3. The total training load did not differ between groups during the 3-week intervention period (HITG: 11,087 ± 5193; CG: 12,576 ± 5766; $p = 0.489$) nor did any other of the training data. Mean heart rate responses and mean ratings of perceived exertion over all HIT sessions are presented in Table 4. In all but the last HIT session, participants reached their target heart rate of 90–95 % of HR_{max} within the first 2 min and maintained it to the end of the HIT bout. Mean ratings of perceived exertion (BORG) during all eleven HIT sessions were ranged between 16.7 ± 1.4 and 17.3 ± 1.5. Two participants did not entirely reach their target heart rate in all exercise sessions (average training heart rate 88.3 and 87.3 % of HR_{max}, respectively).

Discussion

The main results of the present study are that after a 3-week HIT program, VO_{2max} only increased within the HITG with concomitant decreases of HR_{max} and increases in maximal O_2-pulse but without any changes in tHb-mass, BV and PV. However, despite a within-group change of VO_{2max}, no interaction (group × time) was seen for VO_{2max}, tHb-mass, BV, PV and maximal O_2-pulse, indicating no significant effect of HIT. The findings from the present investigation do not support our hypothesis that VO_{2max} changes after 3 weeks of HIT are directly related to differences in training-induced

Table 4 Mean heart rate responses (%HR_{max}) and mean ratings of perceived exertion (BORG) over all HIT sessions

	%HR_{max} at 2 min	%HR_{max} at 4 min	BORG
HIT 1	92.2 ± 3.2	93.7 ± 1.5	16.9 ± 1.7
HIT 2	92.7 ± 2.7	93.6 ± 1.7	17.3 ± 1.5
HIT 3	91.1 ± 3.0	93.4 ± 2.9	17.1 ± 1.5
HIT 4	92.2 ± 2.8	93.4 ± 2.5	17.1 ± 1.0
HIT 5	91.6 ± 3.5	93.4 ± 3.3	17.0 ± 1.4
HIT 6	90.4 ± 4.0	93.4 ± 3.3	17.1 ± 1.9
HIT 7	90.3 ± 3.1	93.0 ± 2.8	16.7 ± 1.4
HIT 8	91.5 ± 2.9	93.7 ± 3.4	17.1 ± 1.3
HIT 9	90.5 ± 4.2	92.2 ± 4.8	17.1 ± 1.2
HIT 10	90.7 ± 3.4	92.3 ± 3.1	16.8 ± 1.4
HIT 11	87.8 ± 4.7	89.8 ± 3.5	17.0 ± 1.3

%HR_{max} 2 min heart rate response at minute 2, *%HR_{max} end* heart rate response at the end of the interval bout

hypervolaemia and/or increases in tHb-mass. There are several studies, demonstrating no significant relationship between VO_{2max} and tHb-mass/vascular volume after a HIT intervention in trained subjects (Glass et al. 1969; Gore et al. 1997; Laursen et al. 2005; Helgerud et al. 2007). In their study, Laursen et al. (2005) investigated the influence of three different HIT regimes on PV, anaerobic capacity and ventilatory thresholds in well-trained cyclists. The authors demonstrated increased 40 km time-trial performance, improved VO_{2peak}, ventilatory thresholds, and anaerobic capacity while PV did not change significantly. They suspected that peripheral adaptations and not central adaptation are more likely to be responsible for performance improvements. However, previous investigations, particularly with untrained subjects, reported improved VO_{2max} and increased vascular volume after HIT (Esfandiari et al. 2014; Warburton et al. 2004). Moreover, several investigations exist, reporting increases in tHb-mass and vascular volume after HIT in well-trained individuals (Breil et al. 2010b; Richardson et al. 1996). The considerable discrepancy is likely due to different training protocols and primarily to different participant characteristics. In endurance-trained athletes, body weight-related tHb-mass and BV levels are described as considerably higher than in untrained subjects (Krip et al. 1997; Schmidt et al. 2002; Steiner and Wehrlin 2011; Warburton et al. 2004), suggesting endurance-trained athletes may already be well adapted and seem to be at their optimal BV (Warburton et al. 1999, 2000). Thus, in well-trained athletes higher HIT doses might be necessary to generate changes in vascular volume and tHb-mass when compared to untrained individuals. In untrained individuals, tHb-mass and BV were reported to be 11.5 g/kg and 75 ml/kg, whereas endurance-trained athletes exhibit values of 15.7 g/kg and 103.4 ml/kg, respectively (Schmidt et al. 2002), showing a training-dependent increase in both tHb-mass and BV. On the other hand, in their recent investigation, Jacobs et al. (2013) reported similar findings to our present study, but in untrained individuals. After six HIT sessions within 2 weeks (8–12 × 60 s cycling intervals at 100 % peak power output), VO_{2max} improved by 8 % while tHb-mass, BV, PV and Q remained unaltered. Although several investigations reported high-intensity training-induced PV expansion (Esfandiari et al. 2014; Richardson et al. 1996), it can therefore be assumed to be a transient effect (Jacobs et al. 2013).

We found significantly improved maximal O_2-pulse only in HITG. Considering the facts that BV and Q_{max} are closely related (Bonne et al. 2014; Krip et al. 1997) and BV did not change significantly after the 3-week HIT, it is suggested that Q remained essentially unchanged. Thus, increased SV should have compensated for the reduced HR_{max} partly explaining the elevated maximal O_2-pulse. If true, it will be mainly the maximal arterial–venous O_2 content difference ($avDO_2$) that explains the increased VO_{2max}. HIT adaptations may result from peripheral adaptation as alterations in maximal $avDO_2$, based on increased capillarization and mitochondrial density (Daussin et al. 2008; Jensen et al. 2004; Wahl et al. 2014).

The presented data demonstrate a significant improved VO_{2max} of +3.5 % after 3 weeks of HIT in already well-trained individuals. Helgerud et al. (2007), using the same four × 4-min intervals, achieved an improvement of 7.2 % after 4 weeks of HIT. However, in contrast to the present study, baseline VO_{2max} values were markedly lower in that study compared to our participants (55.5 ± 7.4 vs. 63.6 ± 7.5 ml/min/kg). The effect that subjects with lower baseline values benefited more than subjects with higher VO_{2max} is in line with previous studies showing that VO_{2max} at baseline and changes in VO_{2max} are significantly correlated (McMillan et al. 2005). However, when considering the average increase of VO_{2max} per training session, the present study showed an increase in VO_{2max} of 0.32 % per session, while VO_{2max} increased by about 0.3 % per session in the study of Helgerud et al. (2007).

Another interesting finding of the present study was that HR_{max} decreased due to HIT. However, changes in HR_{max} and changes in VO_{2max} were unrelated, suggesting that HR_{max} reduction does not contribute to VO_{2max} improvements after 3 weeks of HIT. The suppressed HR_{max} was already observed in other HIT investigations (Breil et al. 2010a; Etxebarria et al. 2014) and is most likely due to increased cardiac vagal activity (Kiviniemi et al. 2014) and cardiovagal baroreflex function (Zavorsky 2000).

Study limitations

Some limitations have to be mentioned. We combined the outcomes of two independent investigations to obtain a high number of participants, necessary for investigating the underlying mechanism. Therefore, two different laboratory treadmill tests were performed. Nonetheless, the intervention

and the main outcome variables remained the same in both investigations (VO_{2max}, tHb-mass, BV and PV). In addition, we did not determine HIT-related changes on the level of working muscles but this was not a study goal as we aimed at investigating possible changes of tHb-mass, BV and PV, explaining VO_{2max}. Despite the high calculated power for the chosen sample size, we were unable to detect between-group changes of VO_{2max}. However, power calculation was based on not well-trained subjects and therefore we cannot exclude that our study may have been underpowered particularly with regard to BV and PV. Regarding VO_{2max}, other HIT studies (Breil et al. 2010a; Helgerud et al. 2007) with the same HIT protocol (4 × 4 min at 90–95 % of HR_{max}) and with well-trained subjects had only 10 and 13 subjects, respectively (in the HITG), and showed significant changes. This indicates that 19 participants in the HITG should have been sufficient do detect VO_{2max} changes.

Conclusion

Eleven HIT sessions added to usual training did neither improve VO_{2max} nor haematological parameters when compared to the CG. Probably, 11 HIT sessions did not provide a sufficient overload to improve VO_{2max} and stimulate haematological changes in well-trained athletes. Future research should focus on potential performance and blood effects after more intensified HIT.

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Conflict of interest The authors declare that they have no conflict of interest.

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