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Original Article

Acute effect of different normobaric hypoxic conditions on shuttle repeated sprint performance in futsal players

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Abstract:
This study aimed to investigate the acute effects of different environmental oxygen conditions on shuttle repeated sprint performance in futsal players. Using a counterbalanced design, 16 male university-level futsal players performed 3 sets of shuttle repeated sprints (6 × 10 s) over a 5m distance with 20 s active rest between reps and 5 min active rest between sets. Participants completed 4 trials in a random order over 4 weeks with different inspired oxygen fractions for each trial (FIO2 = 20.9%, 14.5%, 13.5% and 12.5%). Electromyography (RMSEMG) data was recorded from the vastus lateralis muscle during sprinting. The number of completed shuttles in each 10 s period, blood lactate concentration, rating of perceived exertion, oxygen saturation (SpO2), and cardiorespiratory variables were recorded after each set. We found that after set3, blood lactate concentration was substantially higher ($p < 0.05$) in the 12.5% FIO2 (10.4 ± 2.1 mmol/L) compared to normoxia, 14.5% or 13.5% condition (5.0 ± 1.6, 7.8 ± 1.5, and 9.4 ± 1.9 mmol/L, respectively). Compared to normoxia, the RMSEMG signal was 34.1% lower in the 12.5% condition, 14.1% and 18.6% lower in the 14.5% and 13.5% oxygen condition respectively. Moreover, SpO2 after set3 was substantially lower ($p < 0.05$) in 12.5% (73.7 ± 3.4%) compared to normoxia, 14.5% or 13.5% condition (95.1 ± 1.8, 81.1 ± 2.1, and 79.1 ± 2.4%, respectively). Overall the lower FIO2 conditions produced lower in number of sprints, and highest heart rate, ventilation and rating of perceived exertion levels. In conclusion, FIO2 13.5% is an appropriate choice for shuttle repeated sprint training in hypoxia. Although, FIO2 13.5% was lower in benefitcial responses than FIO2 12.5% but FIO2 12.5% might produce unnecessary and unwanted fatigue from insufficient oxygen to athletes.
Key words: hypoxic training, altitude training, fatigue, shuttle running, team sport, muscle oxygenation.

Introduction

Futsal is a team-sport which requires high physical demands including endurance, speed, strength and the ability to change direction quickly and regularly (Makaje et al., 2012). It is estimated that 26% of a futsal match is completed at high intensity with changes in direction occurring every 3 s (Doğramacı & Watsford, 2006). The demands of the game with regular possession turn-overs and a substantially smaller pitch size (Vaeyens et al., 2007), results in a high number of sprints (a sprint occurs every 79 s), covering a relatively short distance (10.5 m) with little recovery between sprints (< 40 s) (Castagna et al., 2009). As a result of the high anaerobic demand, blood lactate concentrations during futsal games reach relatively high levels (5.3-5.5 mmol/L) (Castagna et al., 2009; Makaje et al., 2012). Elite futsal players also need high aerobic fitness with players usually covering over 5 km during a match (Makaje et al., 2012). Because futsal is a multiple sprint sport requiring more high-intensity phases than soccer and other intermittent sports (Barbero-Alvarez et al., 2008) repeated sprint ability is an important component of fitness.

As the aerobic system is heavily involved in regenerating ATP during recovery from repeated sprints (Spencer et al., 2005), it is thought that strategies used to improve aerobic metabolism may also help to improve repeat sprint ability. A lower rate of oxygen delivery to the muscle during hypoxic training has been suggested to increase the stress on the anaerobic metabolic pathways thereby resulting in upregulation of anaerobic metabolism (Faiss, Leger, et al., 2013), and ultimately improving repeated sprint performance. In addition, high-intensity sprinting in hypoxia itself, may contribute to changes in the muscle to counteract the reduced oxygen concentration during exercise (Hoppeler & Vogt, 2001) and result in enhanced repeat sprint ability. Such adaptations may include compensatory vasodilation, greater microvascular oxygen delivery to the fast twitch fibers, specific HIF1-alpha related molecular changes, and increased removal of waste metabolites (Brocherie et al., 2017). A large amount of evidence suggests that combining repeated sprint training with hypoxia improves anaerobic power output and sprint capacity (Billaut, Gore, & Aughey, 2012; Brocherie et al., 2017; Faiss, Leger, et al., 2013). In an attempt to find the optimal level of hypoxic exposure for repeated sprint training, Bowtell et

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al. (2014) had well trained multi-sport athletes perform one set of 10 reps of 6 s all-out running sprints interspersed with 30 s recovery on a non-motorized treadmill at different oxygen concentrations (F_IO_2: 12%, 13%, 14%, 15%, 21%). The result suggested that physiological responses to the exercise were incrementally greater as F_IO_2 decreased to 13%, yet fatigue development was significantly exacerbated relative to normoxia (F_IO_2: 21%) only at the 12% F_IO_2. Similarly, Goods et al. (2014) in an attempt to uncover the best hypoxic dose for repeated sprinting, had Australian Rules Football athletes complete 3 sets of 9 x 4 s all-out sprint on a non-motorized treadmill at sea-level and at 2000, 3000 and 4000 m. In agreement with previous studies, Goods et al. (2014) recommended repeated sprints should be completed at altitudes of 2000-3000 m (~16.5-14.5% F_IO_2) due to the potential positive adaptations at such altitudes without the unwanted effects of fatigued-induced decreased training quality found at higher altitudes (Bowtell et al., 2014; Goods et al., 2014).

Because futsal is a game that requires repeated sprinting, but with multiple changes in direction over very small distances, we wondered whether the results of previous hypoxic training studies using linear sprints without changes in direction on non-motorized treadmills would equally apply to futsal athletes. In an attempt to investigate the effect of repeated sprinting under hypoxic conditions in a more ecologically valid way, we had futsal players undergo a series of over-ground repeated sprints with repeated directional changes in a variety of hypoxic conditions to try and identify the most appropriate hypoxic dose for such players.

Material & methods

Participants

Sixteen university futsal players (age 20.4 ± 0.9, height 171.2 ± 5.5 cm, weight 63.4 ± 7.5 kg, BMI 21.6 ± 2.2, mean ± *SD*) participated in this study. All the players were born and lived at or near sea level. Participants were recruited and gave their written informed consent to participate in this study, which was approved by the Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University (098.1/59) and conformed to the current Declaration of Helsinki guidelines.

Procedure

To investigate the acute effects of shuttle repeated sprint performance in various normobaric oxygen conditions, a randomly selected, counterbalanced, and repeated measures design was employed during this study which consisted of 4 trials. The shuttle repeated sprints were held in a hypoxic chamber (ATS-5KHP 750 SYSTEM, Altitude Training Systems, NSW, Australia) at the same time of day, on the same day of the week for each participant over 4 continuous weeks. Each trial was identical and was performed in the normoxic condition (F_IO_2: 20.9%) or a normobaric hypoxic condition (F_IO_2: 14.5%, 13.5%, and 12.5%). A temperature of 25^o C was maintained in the chamber throughout the experiments, and to reduce CO_2 build-up a CO_2 absorbent (Drägersorb 800 plus, Dräger, Lübeck, Germany) was placed in the chamber. The weekly microcycle of training before a trial was kept consistent, and participants did not train in the morning before a trial. Moreover, participants were instructed to consume the same meal before each trial and to not eat in the 2 hours before each trial.

Electromyography

The overlying skin was carefully prepared before placing the electrodes. The hair was shaved, and the skin was gently abraded to remove the outer layer of epidermal cells and thoroughly cleansed with alcohol to reduce the skin electrode interface impedance. After skin preparation, an active bipolar bar electrode (99.9% Ag, 10 mm length, 1 mm width, 10 mm pole spacing, CMRR > 80 dB, model DE2.1, DelSys Inc, Boston, MA) was placed over the belly of vastus lateralis muscle of the right leg distal to the motor endplate region and oriented parallel to the muscle fibres, according to recommendations from the SENIAM (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles) project. The ground electrode was placed over the patella of the right leg. The EMG signal was pre-amplified (×1000) and band-pass filtered between 20 and 450 Hz at the source (Bagnoli-8, DelSys Inc, Boston, MA), and further high-pass filtered at 10 Hz when transferred to a computer. EMG of vastus lateralis recording was activated at the start of the set and data collection stopped at the end of the set by a digital signal. During post-processing, all EMG data were full-wave rectified and quantified using the root mean square (RMS). After removing the signal for each of the 20-s recovery periods between sprints, the RMS was calculated for each of the 10-s sprints. As well as the RMSEMG of individual sprints, we averaged all sprints over the 3 sets to get an overall change in RMSEMG between hypoxic conditions. RMSEMG is reported as raw data (μV) and as a percent difference from the normoxia condition.

Heart Rate, Cardiorespiratory Parameters, Oxygen Saturation and Rating of Perceived Exertion

A portable metabolic system (MetaMax 3B, Cortex, Leipzig, Germany) was used to measure the cardiorespiratory parameters including oxygen consumption (VO_2), carbon dioxide production (VCO_2), minute ventilation (V_E), respiratory exchange rate (RER), and the heart rate (HR). The MetaMax 3B system consisted of a face mask (attached to a volume transducer), a Polar HR chest strap, a transmitter (with expiratory gas analyzing system), and a receiving unit. The equipment, which weights approximately 0.7 kg, was carried on the back of the subject in an adjustable harness. Data was collected breath-by-breath and separated into sprint and recovery periods. Data for the 20-s recovery periods were removed and the cardiorespiratory parameters for each of the six 10-s sprints were averaged to give an overall mean change per exercise set. At the end of each set, a finger pulse oximeter (Nonin GO2 Achieve, Nonin Medical Inc, MN, USA) was immediately used to measure blood oxygen saturation (SpO_2) and a rating of perceived exertion (RPE via the Borg 6–20 scale) was recorded.

Blood Lactate Concentration

Blood lactate concentration was measured by taking capillary blood samples (30–50 μL) from the fingertip using standard lancets and capillary tubes. Samples were taken at rest, and after set 1, 2, and 3. Blood samples were immediately analyzed to determine the whole blood lactate concentration by a calibrated blood lactate analyzer (Analox LM5, Analox Instruments Ltd, London, UK).

Shuttle Repeated Sprint Exercise

After equipment familiarization, participants were randomly allocated to a treatment order. Subsequently, participants entered the environmental chamber and were seated for 10 minutes to acclimatize and record blood lactate concentration, rating of perceived exertion, SpO_2 and cardiorespiratory parameters. After rest, a warm-up comprising of 10 minutes light jogging followed by 5 minutes stretching and another 5 minutes jog at 60-65% of HRmax was completed. Following the warm-up, participants completed the repeated sprint exercise which comprised of 3 sets of maximal sprints (6 × 10-s). In an attempt to replicate the type of sprinting that occurs in futsal, we had participants complete the repeated sprints over a 5 m distance. The distance was also constrained by the dimensions of the chamber which was 5.6 x 4.2 m. When the participants started they would continue to sprint between cones spaced 5 m apart until the 10-s rep was completed. The number of completed sprints per rep was calculated by the number of completed 5-m sprints each participant was able to cover in the 10-s period. Recovery time between reps was 20-s of active rest (walking) which made each set 160 s in duration. After each set, participants were asked to complete 5 minutes of active rest (walking or jogging) to maintain HR at 60-65% of HRmax. Total session duration from commencement of set 1 to the conclusion of set 3 was 18 minutes. After finishing the 3 sets, participants spent 10 minutes cooling down which calculates to 58 minutes in total within the chamber (warmup, sprint exercise and cooldown). During this time, participants were permitted to drink ad libitum from a water bottle.

Statistical Analysis

All data are presented as mean ± *SD*. Statistical analyses were performed using Statistical software (SPSS version 24.0, SPSS Inc., Chicago, IL, USA). The data set for each variable was analyzed by a within-subject, repeated measures ANOVA with a Bonferroni confidence interval adjustment when comparing main effects. Alpha intervals for all testing were set at $p < 0.05$.

Results

In general, shuttle repeated sprint performance decreased over the 3 sets of sprints (Table 1), which was exacerbated particularly in set 2 and 3 by hypoxia. Only in F_IO_2 of 12.5%, and only during the last 2-3 reps, was the total number of sprints completed in 10 s substantially reduced compared to the normoxic condition (F_IO_2 = 20.9%; $p < 0.05$). The overall mean number of sprints completed during the 3 sets was substantially lower ($p < 0.05$) in the 12.5% oxygen condition (5.7 ± 0.3) compared to normoxia (6.0 ± 0.3), and the less severe hypoxic conditions (5.9 ± 0.4, 5.8 ± 0.3 in F_IO2 of 14.5% and 13.5% respectively).

Table 1. Number of sprints completed in 10 seconds under normoxic and hypoxic conditions.

Set	Rep	F_IO_2			
		20.9%	14.5%	13.5%	12.5%
1	1	6.2 ± 0.4	6.1 ± 0.3	6.0 ± 0.4	5.9 ± 0.3
	2	6.1 ± 0.5	5.9 ± 0.3	6.0 ± 0.4	5.9 ± 0.3
	3	6.1 ± 0.3	6.1 ± 0.5	5.9 ± 0.4	5.9 ± 0.3
	4	6.1 ± 0.3	6.0 ± 0.4	5.9 ± 0.3	5.8 ± 0.4
	5	6.1 ± 0.3	5.9 ± 0.3	5.9 ± 0.3	5.7 ± 0.5
	6	5.9 ± 0.3	5.9 ± 0.3	5.9 ± 0.3	5.6 ± 0.5
2	1	6.3 ± 0.5	6.1 ± 0.4	6.1 ± 0.4	5.9 ± 0.3
	2	6.1 ± 0.3	5.9 ± 0.3	5.9 ± 0.3	5.7 ± 0.5
	3	6.1 ± 0.3	5.9 ± 0.3	5.9 ± 0.3	5.8 ± 0.4
	4	6.1 ± 0.3	5.9 ± 0.3	5.8 ± 0.4	5.6 ± 0.5 [a]
	5	5.9 ± 0.3	5.9 ± 0.3	5.8 ± 0.4	5.4 ± 0.5 [a, b]
	6	5.9 ± 0.3	5.9 ± 0.4	5.8 ± 0.5	5.5 ± 0.5
3	1	5.9 ± 0.3	5.9 ± 0.3	5.8 ± 0.4	5.8 ± 0.5
	2	6.0 ± 0.0	5.9 ± 0.3	5.9 ± 0.3	5.6 ± 0.5
	3	5.9 ± 0.3	5.8 ± 0.4	5.7 ± 0.5	5.6 ± 0.5
	4	5.9 ± 0.3	5.8 ± 0.4	5.8 ± 0.5	5.5 ± 0.5
	5	5.9 ± 0.3	5.8 ± 0.5	5.7 ± 0.5	5.4 ± 0.5 [a]
	6	5.9 ± 0.3	5.7 ± 0.5	5.6 ± 0.5	5.4 ± 0.5 [a]
Overall mean		6.0 ± 0.3	5.9 ± 0.4 [a]	5.8 ± 0.3 [a]	5.7 ± 0.3 [a, b, c]
% Difference			-1.8%	-2.8%	-5.6%

Data are mean ± *SD*. Overall mean is the average of all sprints; % Difference is the percentage difference in overall mean sprints compared to normoxic condition; [a] Significantly different from 20.9%; [b] Significantly different from 14.5%; [c] Significantly different from 13.5%.

Compared to normoxic conditions, average oxygen saturations at rest and at the end of each set were substantially lower in all hypoxic conditions (p <0.05) (Table 2). As the level of hypoxia increased the oxygen saturation levels decreased with the greatest desaturation occurring in the F_IO_2 of 12.5% condition reaching 73.7 ± 3.4% by the end of the last sprint in the last set (p <0.05). Sprinting under hypoxic conditions resulted in higher post-sprint blood lactate concentrations, with an F_IO_2 of 12.5% producing the highest blood lactate concentration (10.4 ± 2.1 mmol/L) after set 3 (p <0.05). Similarly, hypoxia increased the post-sprint rating of perceived exertion compared to normoxia, with the highest levels reported again at the end of the 12.5% F_IO_2 condition (p <0.05).

Table 2. Physiological and psychological measures taken at rest and at the end of each exercise set performed at different inspired oxygen concentrations.

Variable	Period	F_IO_2			
		20.9%	14.5%	13.5%	12.5%
Blood lactate (mmol/L)	Rest	2.2 ± .7	3.4 ± 1 [a]	3.9 ± 1[a]	4.5 ± 1[a,b]
	Set1	4.3 ± 1.8	7.5 ± 1.8 [a]	8.7 ± 2 [a]	9.3 ± 2 [a, b]
	Set2	5.1 ± 1.9	8.1 ± 1.6 [a]	9.2 ± 1.8 [a]	10.2 ± 1.8 [a, b]
	Set3	5.0 ± 1.6	7.8 ± 1.5 [a]	9.4 ± 1.9 [a, b]	10.4 ± 2.1 [a, b]
Rating of perceived exertion	Rest	6.1 ± 0.3	6.2 ± 0.4	6.4 ± 0.8	6.8 ± 0.7 [a, b]
	Set1	13.8 ± 0.8	14.7 ± 0.5 [a]	14.8 ± 0.9 [a]	15.4 ± 1.1[a]
	Set2	13.2 ± 1.1	14.6 ± 1[a]	15.3 ± 0.9 [a]	15.7 ± 1 [a, b]
	Set3	14.1 ± 2	16 ± 1.2 [a]	16.3 ± 1.5 [a]	17.1 ± 0.7 [a, b]
SpO_2 (%)	Rest	97.4 ± .9	91.8 ± 2.1[a]	89.4 ± 2.3 [a]	85.5 ± 2.7 [a, b, c]
	Set1	95.0 ± 2.3	84.4 ± 1.9 [a]	81.3 ± 1.4 [a, b]	75.6 ± 2.8 [a, b, c]
	Set2	95.2 ± 1.9	82.3 ± 2.7 [a]	79.8 ± 2.3 [a, b]	74.2 ± 2.4 [a, b, c]
	Set3	95.1 ± 1.8	81.1 ± 2.1[a]	79.1 ± 2.4 [a, b]	73.7 ± 3.4 [a, b, c]

Data are mean ± *SD*. [a] Significantly different from 20.9%; [b] Significantly different from 14.5%; [c] Significantly different from 13.5%.

Heart rate during the sprints increased across sets and across hypoxic conditions with the highest heart rates found during the 3rd set and in the most severe hypoxic conditions (F_IO_2 = 12.5%; p <0.05) (Table 3). Similarly minute ventilation increased across the 3 sets of sprints and across the different hypoxic conditions. Minute ventilation was highest in the lowest oxygen concentration condition (F_IO_2 = 12.5%; p <0.05). Average oxygen consumption and carbon dioxide production increased as subjects completed the sets of sprints (i.e. highest in set 3). In addition, the more hypoxic the conditions were the more oxygen consumed and carbon dioxide produced during the sprints (p <0.05) (Table 3).

Table 3. Cardiorespiratory responses during the repeated sprint exercise performed at different inspired oxygen concentrations.

Variable	Period	F_IO_2			
		20.9%	14.5%	13.5%	12.5%
Heart Rate (bpm)	Rest	75.6 ± 11.5	84.9 ± 8.8 [a]	88.3 ± 9.2 [a]	89.4 ± 11.9 [a]
	Set1	134.7 ± 8.5	137.4 ± 7.5	142.9 ± 7.8 [a, b]	147.0 ± 9.5 [a, b]
	Set2	141.7 ± 9.3	146.2 ± 6.1	148.5 ± 6.3 [a]	153.3 ± 8.5 [a, b]
	Set3	143.6 ± 9.1	148.4 ± 7.2	151.7 ± 6.1 [a]	155.2 ± 7.2 [a, b]
VO_2 (L/min)	Rest	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2
	Set1	1.6 ± 0.3	1.9 ± 0.2 [a]	1.9 ± 0.3 [a]	2.2 ± 0.4 [a, b, c]
	Set2	1.8 ± 0.3	2.0 ± 0.3 [a]	2.1 ± 0.4 [a]	2.3 ± 0.4 [a, b, c]
	Set3	1.9 ± 0.3	2.1 ± 0.3	2.1 ± 0.3 [a]	2.4 ± 0.4 [a, b, c]
VCO_2 (L/min)	Rest	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.2
	Set1	1.5 ± 0.3	1.8 ± 0.3 [a]	1.9 ± 0.3 [a, b]	2.2 ± 0.5 [a, b, c]
	Set2	1.6 ± 0.3	1.8 ± 0.3 [a]	2.0 ± 0.3 [a, b]	2.2 ± 0.4 [a, b, c]
	Set3	1.7 ± 0.3	1.9 ± 0.3 [a]	2.0 ± 0.3 [a]	2.2 ± 0.4 [a, b, c]
V_E (L/min)	Rest	13.4 ± 3.9	16.6 ± 5.4	16.9 ± 5.7	16.7 ± 6.9
	Set1	52.0 ± 9.0	57.1 ± 9.7 [a]	65.3 ± 10.5 [a, b]	73.3 ± 13.2 [a, b, c]
	Set2	58.5 ± 10.6	62.9 ± 10.7	70.4 ± 9.2 [a, b]	76.9 ± 13.3 [a, b]
	Set3	63.4 ± 12.6	66.6 ± 10.5	73.5 ± 11.9 [a]	79.8 ± 14.5 [a, b]
RER	Rest	0.87 ± 0.07	0.88 ± 0.09	0.89 ± 0.05	0.86 ± 0.08
	Set1	0.93 ± 0.05	0.94 ± 0.08	0.97 ± 0.06	0.98 ± 0.09
	Set2	0.91 ± 0.05	0.91 ± 0.06	0.94 ± 0.04	0.95 ± 0.06
	Set3	0.90 ± 0.05	0.92 ± 0.05	0.93 ± 0.05	0.93 ± 0.06

Data are mean ± *SD*. [a] Significantly different from 20.9%; [b] Significantly different from 14.5%; [c] Significantly different from 13.5%.

RMSEMG of the vastus lateralis muscle decreased across reps and sets of sprints. Compared to normoxia, completing repeated sprints in hypoxic conditions resulted in substantially lower RMSEMG (p <0.05), with the magnitude of change in the RMSEMG signal being proportional to the level of hypoxia (i.e. greatest RMSEMG decrease in lowest oxygen environment). Compared to the normoxic condition, the overall of the RMSEMG signal was 34.1% lower in the 12.5% condition which was substantially lower than the 14.5% (-14.1%) and 13.5% (-18.6%) conditions (Table 4).

Table 4. Changes in surface electromyogram (EMG) amplitude (root mean square RMS) during each 10-s sprint performed at different inspired oxygen concentrations.

Set	Rep	F_IO_2			
		20.9%	14.5%	13.5%	12.5%
1	1	369.1 ± 81.3	320.3 ± 59.6 [a]	318.8 ± 73.8 [a]	267.2 ± 77.9 [a, b, c]
	2	367.2 ± 78.5	312.8 ± 76.5 [a]	300.3 ± 78.2 [a]	249.7 ± 81.6 [a, b, c]
	3	349.4 ± 75.2	310.0 ± 76.9 [a]	291.6 ± 76.9 [a]	230.9 ± 78.7 [a, b, c]
	4	353.8 ± 75.7	291.3 ± 72.6 [a]	272.8 ± 77.4 [a]	218.8 ± 71.1 [a, b, c]
	5	325.3 ± 75.1	277.2 ± 66.7 [a]	259.4 ± 71.9 [a]	213.4 ± 66.7 [a, b, c]
	6	314.7 ± 57.6	269.7 ± 73.7 [a]	249.7 ± 74.9 [a]	198.4 ± 61.8 [a, b, c]
2	1	365.0 ± 67.7	316.9 ± 67.7 [a]	318.4 ± 70.5 [a]	265.3 ± 65.7 [a, c]
	2	358.4 ± 70.5	306.3 ± 67.7 [a]	304.1 ± 77.0 [a]	252.2 ± 69.0 [a, c]
	3	348.8 ± 72.0	300.9 ± 73.0 [a]	287.5 ± 73.5 [a]	236.9 ± 69.9 [a, b, c]
	4	340.3 ± 71.0	289.1 ± 68.9 [a]	270.6 ± 71.6 [a]	219.7 ± 64.6 [a, b, c]
	5	322.5 ± 74.6	271.9 ± 69.5 [a]	250.0 ± 75.2 [a]	208.4 ± 68.9 [a, b, c]
	6	304.7 ± 65.9	262.8 ± 65.3 [a]	237.5 ± 70.4 [a]	191.9 ± 57.2 [a, b, c]
3	1	354.7 ± 60.8	313.1 ± 70.7 [a]	307.2 ± 73.7 [a]	248.1 ± 66.0 [a, b, c]
	2	351.9 ± 63.7	307.8 ± 67.4 [a]	292.8 ± 64.0 [a]	230.6 ± 64.3 [a, b, c]
	3	342.5 ± 69.8	298.8 ± 65.8 [a]	285.0 ± 80.9 [a]	222.8 ± 66.2 [a, b, c]
	4	330.0 ± 68.2	283.8 ± 68.9 [a]	264.1 ± 71.6 [a]	200.3 ± 63.6 [a, b, c]
	5	310.6 ± 72.6	263.1 ± 69.5 [a]	241.6 ± 74.7 [a]	186.6 ± 55.5 [a, b, c]
	6	301.3 ± 70.1	251.3 ± 67.9 [a]	223.1 ± 58.0 [a]	187.5 ± 43.1 [a, b, c]
Overall mean		339.4 ± 71.9	291.5 ± 70.5 [a]	276.4 ± 76.3 [a, b]	223.8 ± 69.4 [a, b, c]
% Difference			-14.1%	-18.6%	-34.1%

Data are mean ± *SD* of the RMS EMG signal in microvolts; Overall mean, average of all sprints; % Difference, percentage difference in overall mean sprints compared to normoxic condition; [a] Significantly different from 20.9%; [b] Significantly different from 14.5%; [c] Significantly different from 13.5%.

Discussion

We have found that completing shuttle repeated sprint training under hypoxic conditions, using more ecologically valid sprint training protocols for futsal players (e.g. very short over-ground sprints with many changes in direction), elicits physiological changes that are closely related to the severity of hypoxia (i.e. the more hypoxic the environment the greater the physiological change). Repeated sprint training using 13.5% or 14.5% (equivalent to 3000-3500 m altitude) oxygen stimulated physiological changes that are necessary for subsequent training adaptation (e.g. decreases S_PO_2, increases blood lactate and heart rate), without a significant decrement in repeated sprint performance (e.g. no substantial difference in number of sprints completed during each rep) We found shuttle repeated sprint performance decrement was similar at 13.5% and 14.5% oxygen (approximately 2-3%) but decreased substantially more under the 12.5% oxygen conditions (approximately 6%). As the environment became more hypoxic the futsal players attempted to bring in more oxygen for the exercising muscles. Minute ventilation was significantly higher in the 12.5% and 13.5% oxygen conditions, with oxygen consumption being significantly higher in the 12.5% condition compared to all other conditions. Despite this increase in oxygen consumption, the amount of oxygen available was obviously not sufficient resulting in significantly higher heart rates and an increased reliance on the anaerobic metabolic systems (i.e. increased blood lactate concentration). Interestingly, Bowtell et al. (2014) found that oxygen consumption during one set of 10 x 6-s sprints was reduced in hypoxic conditions (F_IO_2 of 15-12%). Contrastingly, we found oxygen consumption increased during sprinting as the environmental oxygen concentration was reduced. We suggest the slightly longer on-feet repeated sprints were completed at a lower intensity by our futsal players resulting in a greater reliance on the aerobic energy pathway resulting in higher VO_2 levels. Moreover, the increase in VO_2 to maintain O_2 delivery to the tissues may be related to the increase in minute ventilation likely driven by the lower partial pressure of oxygen and lactate acidosis (Faiss, Pialoux, et al., 2013; Wagner et al., 1986). However, the heart rates recorded in our subjects during sprinting would indicate a work-intensity that was not maximal. Furthermore, the blood lactate levels in our subjects (average over 3 sets in the F_IO_2 12.5% condition of 9.9 mmol/L) were not as high as the levels witnessed by Bowtell et al. (2014) (e.g. in the F_IO_2 12% condition 11.2 mmol/L).

Despite working at a lower intensity during shuttle sprinting our futsal players still became fatigued, particularly in the 12.5% F_IO_2 condition and particularly towards the final few sprints. The fatigue witnessed is probably a result of a number of physiological changes that occur when exercising in hypoxic environments. The significantly lower partial pressure of oxygen results in less oxygen attaching to the hemoglobin molecule and a lower SpO_2. What oxygen is available will be used quickly to reconstitute the creatine phosphate metabolized during the sprinting exercise, but with repeat sprinting ultimately oxygen will become scarce and the anaerobic systems will be required to contribute to creatine phosphate and ATP reconstitution. The substantially higher

blood lactate concentrations found in our players along with presumably higher levels of hydrogen ion accumulation that occurs with anaerobic metabolism, probably contributed to the subsequent fatigue witnessed in our players (Girard, Mendez-Villanueva, & Bishop, 2011). In addition it is well known that high-intensity sprinting uses predominantly creatine phosphate (Bogdanis et al., 1996) resulting in high levels of inorganic phosphate which may also contribute to fatigue particularly in fast-twitch muscle fibers (Westerblad, Allen, & Lannergren, 2002). Indeed a lack of oxygen has been shown to progressively increase muscle deoxyhemoglobin levels in the subjects during a 10 x 6 s sprint test (Bowtell et al., 2014). This lack of oxygen and the subsequent increased reliance on anaerobic energy pathways probably resulted in increased metabolic by-products which resulted in decreased performance in our futsal players particularly in the later sprints.

Hypoxic environments can also result in altered central drive resulting in heightened fatigue. Similar to previous research (Billaut et al., 2013; Bowtell et al., 2014; Smith & Billaut, 2010), participants showed a progressive decrease in RMSEMG during the shuttle repeated sprint exercise which was exacerbated with hypoxia. The decrease in RMSEMG was associated with the degree of hypoxia with the greatest decrease (-34.1%) found in the lowest oxygen condition (F_IO_2 = 12.5%). This reduction in RMSEMG is not thought to be due to peripheral changes but are probably central in origin (Billaut et al., 2013; Smith & Billaut, 2012). Shuttle repeated sprinting under hypoxic conditions (F_IO_2 = 13.3%) was associated with greater cerebral deoxygenation which resulted in a reduction in muscle fiber recruitment (Smith & Billaut, 2010). It is possible that hypoxia at the muscles stimulates the type III/IV sensory nerve fibers leading to inhibition of the motor neurons (Amann & Calbet, 2008). Reduced cerebral blood flow as a result of hypoxia-induced hyperventilation leading to hypocapnia and subsequently cerebral vasoconstriction may also play a role (Ainslie & Ogoh, 2010), if hypoxia is severe enough.

We found a very high correlation between changes in SpO_2 and rating of perceived exertion during the shuttle repeated sprinting exercise in participants (r = 0.85). This relationship has been observed previously with sprinting in hypoxia (Billaut & Smith, 2010) and suggest hypoxia has a strong association with the sense of effort. Monroe et al. (2016) suggest that deoxygenation of some parts of the brain (dorsolateral prefrontal cortex) may be associated with perceived feelings of exertion and fatigue (Monroe et al., 2016). It is also possible that such feelings are associated with the increased sensory feedback from the hypoxic muscles during shuttle repeated sprint exercise in hypoxia (Amann & Calbet, 2008).

Conclusions

In conclusion, we have found that shuttle repeated sprinting (which does not have to be at maximal intensity) under hypoxic conditions (particularly at an F_IO_2 of 12.5%) results in an increased reliance on anaerobic glycolytic processes. Excessive accumulation of the by-products from the maximal stimulation of these anaerobic processes is probably a major factor in the subsequent development of fatigue when completing exercise in hypoxia but other mechanisms may also be involved including, altered central motor drive and increased feelings of exertion and fatigue. Under the conditions in this experiment, F_IO_2 13.5% is suitable condition for futsal players who wished to be trained in hypoxia with an on-feet shuttle repeated sprint protocol. Although, at F_IO_2 12.5% % was higher in benefitcial responses than F_IO_2 13.5 but F_IO_2 12.5% might produce unnecessary and unwanted fatigue from insufficient oxygen to athletes. Thus, F_IO_2 13.5% was an appropriate choice to use for training.

Conflicts of interest

The authors declare that they have no conflict of interest.

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