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Abstract

Background. The purpose of this study was to investigate the use of hypoxic sprint interval training (SIT) for the improvement of aerobic capacity.

Method. 27 subjects (mean ± SD), age 21 ± 1 yrs, body mass 72.4 ± 9.7 kg and height 175 ± 7 cm, completed an $\dot{V}O_{2peak}$ incremental exercise test and a time to exhaustion (TTE) trial (80% $\dot{V}O_{2peak}$) pre and post SIT. Subjects were randomly assigned to one of three groups, control (CONT), normoxic (NORM) and hypoxic (FiO_2: 0.15) (HYP). The SIT involved 30s sprints interspersed with 4min rest. The number of sprints performed progressed from four to seven sprints over six sessions separated by 1-2 days rest. Two-way mixed design ANOVA was performed to determine changes in baseline measures between conditions.

Results. $\dot{V}O_{2peak}$ improved ($p < 0.05$) from pre to post SIT in NORM (11.2 ± 10.8 %) and HYP (10.9 ± 6.2 %), but not CONT (0.7 ± 14.3 %). TTE post SIT was significantly improved from pre SIT in NORM and HYP but not CONT (CONT = 1 ± 6, NORM = 56 ± 25, HYP = 34 ± 25%, $p < 0.05$). Peak and recovery heart rate was significantly lower in NORM than HYP as SIT sessions progressed. SpO_2 (%) was lower in HYP (86.1 ±4.3%) compared to NORM (97.1 ±0.7%), decreasing within all HYP sessions, and increasing with SIT.

Conclusions. Both hypoxic and normoxic SIT using 30s sprints, progressing in number over 2 weeks, caused improvement in $\dot{V}O_{2peak}$ and TTE compared to a control. Hypoxic SIT did not cause further improvements of this magnitude, indicating that hypoxia based SIT offers no additional benefit for improvement of endurance performance.

Keywords:

Altitude, Anaerobic, Cycling, Sprint Training, High Intensity.

Introduction

Sprint interval training (SIT) is a time-efficient method to improve skeletal muscle oxidative capacity and exercise performance characterised by repeated sprints at supramaximal workloads, interspersed by short recovery bouts [1,2]. During repeated sprints oxygen uptake ($\dot{V}O_2$) is elevated during recovery to facilitate replenishment of myoglobin, resynthesis of creatine phosphate (PCr), and to metabolise lactate and remove intracellular inorganic

phosphates. Oxygen (O_2) availability is directly associated with accumulation of anaerobic metabolites during sprint training [3].

High intensity training predominantly augments aerobic adaptations, possibly due to the increased requirement of aerobic metabolism during training, with high intensity training resulting in improved $\dot{V}O_2$ and O_2 transport capacity [4,5].

High intensity intermittent training is a successful method for eliciting improvements in aerobic metabolism pathways and endurance performance [6,7]. McConnell *et al* [8] implemented a time efficient 2 week training study that elicited significant VO_{2peak} improvement using continuous endurance training and intermittent interval training. Literature suggests that improvements in aerobic performance are a result of metabolic changes to the muscle, notably increased skeletal muscle capillarisation, oxidative and glycolytic enzyme activity [9], increased glycogen availability [10,11], muscle buffering capacity through mitochondrial density [12]; and as a result of neural adaptations (motor unit recruitment and synchronisation) [13]. It is widely acknowledged that high maximal oxygen uptake has a strong correlation with endurance performance. It is believed that when short bouts of exercise are repeated, phosphocreatine stores deplete [14]; and since resynthesis is dependent on availability of O_2, assumptions can be made that a greater VO_{2max} and O_2 delivery to muscles will aid rephosphorylation [12].

Recent literature has identified that SIT in hypoxia can augment adaptation in both six [2] and four [15] week training periods with reduced inspired O_2 during SIT limiting aerobic contribution to recovery accelerating cardiovascular adaptation. Adaptations were observed that six weeks of hypoxic SIT increased phosphofructokinase (PFK) and power output to a greater extent than normoxia, with both groups demonstrating improvements in VO_{2max} compared to a controls [2]. Four weeks of SIT in hypoxia is known to increase repeated sprint performance with trends towards improve O_2 uptake and attenuation of cerebral deoxygenation [15].

SIT over a two week period is known to improve aerobic capacity [1,16,17], augmenting mitochondrial [18] and vascular [19] adaptation, reducing the presence of inflammatory markers [20], improving insulin sensitivity [21,22] and exercise performance [1,18,22] in both diseased and healthy populations [7]. It is unknown whether the addition of hypoxia to SIT can augment greater adaptation in comparison to normoxia over a two week training period.

Although, not currently investigating individuals with compromised health, this is the first study to consider enhancing SIT through environmental modification during a short training intervention, and could indicate future directions for developing SIT to optimise rapid changes in endurance capacity in clinical, healthy and athletic populations.

It is hypothesised that SIT in hypoxia will inhibit the aerobic contribution during recovery, consequently inducing additional cardiovascular performance gains, further optimising the efficiency of SIT as a means for improving endurance performance.

Methods

Subjects

Twenty-seven healthy individuals (15 males, 12 females) volunteered to take part in this experiment. Subjects were informed of the procedures to be employed in the study and associated risks, which had the approval of the University of Brighton Research Ethics Committee. All subjects provided written, informed consent. The subjects were non-smokers and had not spent time above 2000m in the 2 months prior to the study. Subjects were advised to refrain from alcohol and caffeine for 24 hours prior to testing.

Experimental design

The 27 subjects were randomly assigned and equally split for number (n = 9) and gender (3 females) to one of the three intervention groups; a normoxic (NORM) (FiO_2: 0.2093) environment, a moderate hypoxic (HYP) (FiO_2: 0.15) environment and a control (CONT) normoxic non training group (Table 1). All testing was carried out in the hypoxic chamber to blind subjects and control temperature (19°C) and humidity (40%).

Familiarisation of the Wingate anaerobic test (WaNT) and time to exhaustion (TTE) was performed before any experimental testing began. Pre and post intervention blood was taken to measure haematocrit (Hct) and haemoglobin (Hb). Baseline testing included completing a $\dot{V}O_{2peak}$ incremental test and a time to exhaustion cycle test (TTE) 48 hours apart and 16 hours prior to the start of the SIT. SIT using the WaNT was spread over a two week period with 24 – 48 hours between each session (see Figure 1). Each training session consisted of between four and seven 30s "all out" efforts on a cycle ergometer interspersed with 4min warm up/recovery (Figure 1). Heart rate (HR), peripheral arterial oxygen saturation (SpO_2)

and rating of perceived exertion (RPE) were measured immediately after each WaNT and every minute thereafter during recovery. The number of WaNTs increased over the two week period and 48 hours after the final SIT session subjects repeated the $\dot{V}O_{2peak}$ and TTE (figure 1).

Preliminary and Post SIT Testing

Subjects performed an incremental test to volitional exhaustion on a cycle ergometer (Monark, model 864, Sweden) to determine $\dot{V}O_{2peak}$ using indirect calorimetry. Starting at 100w, the power was increased by 25w per minute. Expired gas was collected in the last 45s of each stage using Douglas bags (Harvard, Cranlea UK). Heart rate ($bts \cdot min^{-1}$) and RPE (Borg Scale 6 -20) were taken at every stage. Exercise continued until volitional exhaustion.

HR was monitored by short range telemetry (Polar Electro Oyo, Temple, Finland). VO_{2peak} was determined using Douglas bags (Harvard, Cranlea UK) to collect expired air, which was analysed with a gas analyser (Servomex 144, Servomex Group Ltd, England).

A TTE was performed 48 hours later whereby subjects cycled on the ergometer (Monark, model 864, Sweden) at a calculated 80% of $\dot{V}O_{2peak}$, as used by others (1). The test was terminated at volitional exhaustion when the subjects' cadence fell below $40revs \cdot min^{-1}$. Exercise duration was then determined.

Pre and post each $\dot{V}O_{2peak}$ test blood was collected using a finger prick pen (Accucheck Softclix Pro, Roche, England) after the finger had been cleaned using an alcohol wipe. Using heparinised capillary tubes (Hawksley & Sons Ltd, England) and clay at each end, the blood was spun in a centrifuge (Hematospin 1300, Hawksley & Sons Ltd, England) at 1000rpm for 1.5min to calculate the haematocrit. To measure haemoglobin, blood was placed on a Hemocue slide (B-Hemoglobin Photometer, Hemocue, Sweden) and using the Hemocue, haemoglobin device (B-Hemoglobin Microvettes, Hemocue, Sweden).

Sprint Interval Training

Subjects gave a 30s "all out" effort on a cycle ergometer (Monark, model 864, Sweden) against a resistance of $0.075kg.kg^{-1}$ body mass, from a rolling start of $70revs \cdot min^{-1}$. The subjects were verbally encouraged throughout. The WaNT's were interspersed with a 4min warm up/active recovery period of cycling at 60W. Power measures were recorded using Monark Anaerobic Test software (Monark, Sweden) continuously throughout the sprints.

The normobaric hypoxic environment was achieved using a purposed built nitrogen-enriched chamber (Altitude Centre, London). Peripheral arterial oxygen saturation (SpO_2) and heart rate was monitored using a finger pulse oximeter (Nonin 2500, Nonin Medical Inc., USA) every minute during recovery.

Statistical analysis

Data were tested for normality, skewness and kurtosis. Data were normally distributed unless otherwise stated. A Two Way Mixed Design ANOVA was performed separately on each of the independent variables; VO_{2peak}, TTE, Hb and Hct, to calculate whether there was a significant change between the three conditions. When significant, post hoc analysis was performed using the Bonferroni corrected t-test and Tukey's HSD. All data were reported as Mean ± Standard Deviation. All statistical tests followed a significance level of $p<0.05$. The statistical package used was SPSS (SPSS Inc. Chicago, USA, version 20.0).

Results

Exercise Performance

$\dot{V}O_{2peak}$ ($L.min^{-1}$) increased from pre to post test ($f = 13.659$, $p = 0.001$) overall, and different for the pre-post*group interaction ($f = 3.684$, $p = 0.040$). Post hoc analysis observed increases for HYP ($p = 0.003$; +10.9%; 3.13 ± 0.95 to 3.49 ± 1.14 $L.min^{-1}$) and NORM ($p = 0.004$; +11.2%; 2.91 ± 0.42 to 3.26 ± 0.71 $L.min^{-1}$), but not CONT ($p = 0.935$; +0.7%; 3.02 ± 0.93 to 3.01 ± 0.94 $L.min^{-1}$) (Figure 2).

TTE (min) increased from pre to post test ($f = 39.109$, $p < 0.001$) overall, and was different for the pre-post*group interaction ($f = 10.310$, $p = 0.001$). Post hoc analysis observed increases as occurring pre-post HYP ($p < 0.001$; +34.8%; 8.0 ± 3.1 to 10.5 ± 4.2 min) and NORM ($p < 0.001$; +56.3% 6.56 ± 2.1 to 10.0 ± 3.0 min) but not CONT ($p = 0.962$; 1.6%; 9.0 ± 3.6 to 9.0 ± 3.2 min) (Figure 3).

The mean PPO ($W.kg^{-1}$) of the first four sprints was different from pre to post test ($f = 15.948$, $p = 0.001$) overall, but not for the pre-post*group interaction ($f = 3.552$, $p = 0.001$). Post hoc analysis observed increases from pre-post in NORM ($p = 0.001$; +16.2%; 8.4 ± 2.2 to 9.4 ± 1.9 $W.kg^{-1}$) but not HYP ($p = 0.155$; +5.1%; 8.9 ± 1.7 to 9.2 ± 1.7 $W.kg^{-1}$) see Figure 5.

Blood markers

Hb ($g.dL^{-1}$) was not different from pre to post test ($f = 2.247$, $p = 0.147$) overall, or for the pre-post*group interaction ($f = 3.044$, $p = 0.066$) for HYP (14.9 ± 1.7 to 15.4 ± 1.6 $g.dL^{-1}$), NORM (14.6 ± 1.4 to 14.7 ± 1.2 $g.dL^{-1}$), and CONT 14.7 ± 1.3 to 14.5 ± 1.4 $g.dL^{-1}$).

Hct (%) was not different from pre to post test ($f = 0.803$, $p = 0.379$) overall, or for the pre-post*group interaction ($f = 0.102$, $p = 0.903$) for HYP (45.4 ± 3.5 to 45.9 ± 3.8 %), NORM (44.2 ± 2.4 to 44.3 ± 3.3 %), and CONT 43.5 ± 3.2 to 43.7 ± 2.9 %).

Physiological markers

Peak HR ($b.min^{-1}$) was different overall between HYP (175.5 ± 2.7 $b.min^{-1}$) compared to NORM (172.2 ± 3.1 $b.min^{-1}$) ($f = 31.523$; $p = 0.000$), significant differences were also observed between SIT sessions ($f = 5.461$; $p = 0.000$) and for the group*SIT interaction ($f = 2.918$; $p = 0.021$). Post-hoc analysis observed HYP to be significantly higher than NORM during SIT 1 ($p = 0.039$; HYP 176.2 ± 4.4, NORM 172.6 ± 1.4 $b.min^{-1}$), SIT3 ($p = 0.049$; HYP 176.8 ± 2.7, NORM 173.4 ± 2.0 $b.min^{-1}$), SIT4 ($p = 0.000$; HYP 174.9 ± 2.5, NORM 169.3 ± 2.0 $b.min^{-1}$), and SIT5 ($p = 0.000$; HYP 176.3 ± 2.4, NORM 170.1 ± 1.1 $b.min^{-1}$), but not SIT2 ($p = 0.336$; HYP 172.8 ± 1.8, NORM 171.3 ± 2.3 $b.min^{-1}$) or SIT6 ($p = 0.867$; HYP 176.0 ± 1.6, NORM 175.8 ± 1.1 $b.min^{-1}$). No differences were observed between SIT sessions within each group.

Recovery HR ($b.min^{-1}$) was significantly different overall between groups, HYP (140.7 ± 5.0 $b.min^{-1}$) compared to NORM (135.6 ± 4.9 $b.min^{-1}$) ($f = 21.568$; $p = 0.000$), significant differences were also observed between SIT sessions ($f = 2.890$; $p = 0.022$) but not the group*SIT interaction ($f = 2.918$; $p = 0.052$). Post-hoc analysis observed HYP to be significantly higher than NORM during SIT 1 ($p = 0.001$; HYP 146.7 ± 4.6 NORM 135.5 ± 4.8 $b.min^{-1}$), SIT4 ($p = 0.031$; HYP 139.0 ± 3.8, NORM 133.3 ± 4.4 $b.min^{-1}$), SIT5 ($p = 0.003$; HYP 140.3 ± 3.5, NORM 132.4 ± 6.0 $b.min^{-1}$) and SIT6 ($p = 0.025$; HYP 142.5 ± 4.0, NORM 137.1 ± 3.0 $b.min^{-1}$), but not SIT2 ($p = 0.496$; HYP 137.1 ± 6.0, NORM 135.1 ± 4.6 $b.min^{-1}$) or SIT3 ($p = 0.621$; HYP 139.5 ± 5.2, NORM 140.9 ± 2.7 $b.min^{-1}$). The only difference between SIT sessions within each group was SIT1 as different to SIT2 ($p = 0.028$) within HYP.

SpO_2 (%) was significantly different overall between groups, HYP (86.1 ± 4.3 %) compared to NORM (97.1 ± 0.7 %) ($f = 2677.786$; $p = 0.001$), significant differences were also observed between SIT sessions ($f = 4.710$; $p = 0.001$) and for the group*SIT interaction ($f =$

4.423; p = 0.001). Post-hoc analysis observed HYP to be significantly lower than NORM in all SIT sessions (p = 0.001). No difference existed between any SIT sessions in NORM. Significant differences were observed within sessions with 1.1 (88.2 ± 3.0 %) greater than 1.3 (82.9 ± 4.6 %; p = 0.009) and 1.4 (81.1 ± 5.0; p = 0.001), 2.1 (88.4 ± 3.4 %) from 2.4 (82.0 ± 4.8 %; p = 0.001 %) and 2.5 (81.7 ± 4.5; p = 0.001), 3.1 (88.9 ± 3.8 %) from 3.5 (83.3 ± 3.9 %; p = 0.004), 4.1 (89.4 ± 2.7) from 4.5 (83.2 ± 4.0 %; p = 0.001 %) and 4.6 (82.7 ± 3.7 %; p = 0.001), 5.1 (90.6 ± 2.6) from 5.6 (84.1 ± 3.5 %; p = 0.001 %) and 6.1 (91.6 ± 1.7 %) from 6.5 (86.1 ± 2.6 %; p = 0.006), 6.6 (85.2 ± 3.2 %; p = 0.001) and 6.7(84.2 ± 3.1 %; p = 0.001). No difference was observed between the first sprint of any session (p = 1.000) although a trend existed whereby SpO_2 increased daily (Figure 4).

Discussion

The main purpose of this study was to examine the influence on 2 weeks of SIT in normoxia and hypoxia on endurance capacity. To this end, the training protocol was designed to enhance aerobic performance measures [1,23] with the inclusion of a hypoxic condition, designed to stress the aerobic metabolic contribution to SIT.

There were no significant changes in TTE or $\dot{V}O_{2peak}$ pre and post intervention for the control group. $\dot{V}O_{2peak}$ and TTE increased in HYP and NORM after SIT however no difference was observed between HYP and NORM. These data contradict our hypothesis that additional physiological strain of hypoxia during SIT, increases aerobic contribution during recovery, consequently inducing additional cardiovascular performance gains. Six sessions of SIT may not have provided enough additional training stimuli for our HYP group to benefit from the additional physiological strain, and a longer training period may have yielded statistically significant differences between NORM and HYP [2,15].

Longer duration studies [24] have also shown a significant increase in $\dot{V}O_{2peak}$ from 51.06 to 54.5 $ml \cdot kg^{-1} \cdot min^{-1}$ over 7 weeks of SIT ($p<0.05$). Burgomaster et al [25] demonstrated that $\dot{V}O_{2peak}$ improved following 6week of SIT. Studies that implemented SIT for 2 weeks [15,18] found significant improvement in time trial performance in agreement with our data. Burgomaster et al [1] also found 2 weeks SIT to improve TTE. In the present study TTE did not improve to the extent of that seen by Burgomaster's group[1]. Improvement in TTE ranged from 81 to 169% [1], where as the current study reports smaller improvement range for NORM (20 – 104%) and HYP (3 – 62%). Differences may be a consequence of the poorer training

status of those in the current study, finding the considerable training intensity too severe in latter sprints.

Burgomaster et al [1] implied that the training-induced increases in mitochondrial potential, which was measured by citrate synthase activity. Macdougall et al [24] also found significant improvement in aerobic performance measures with SIT, attributing this to improvements in oxidative and glycolytic enzyme activity. The precise mechanisms behind endurance performance are complex and results from other studies suggest that SIT can stimulate a range of adaptations that facilitate performance: Increases of resting glycogen [4], changes in enzymatic activity [24,27,28], sarcoplasmic reticulum function and COX activity [17]. As expected, blood parameters were not altered by the hypoxic stimulus, as such short durations and a relatively low altitude dosage [30] was not likely to induce erythropoiesis, although erythropoietin increase would be possible it was not measured [31].

Oxygen availability has a significant influence on the rate of $\dot{V}O_2$ at the onset of high intensity exercise [23], and specifically to this study, hypoxic conditions result in the slowing of $\dot{V}O_2$ kinetics. This increases the magnitude of the O_2 deficit incurred during each sprint and places more demand on anaerobic sources to maintain ATP production. This increased rate of fatigue under hypoxic conditions may be the result of inorganic phosphate (Pi) accumulation during each sprint and the reduced rate of removal during recovery [(4)]. Having not tested $\dot{V}O_2$ throughout the SIT, the metabolic differences between HYP and NORM are difficult to decipher, however HR would give an indication of physiological effort during SIT. Mean HR was significantly greater in HYP than NORM, demonstrating greater autonomic requirement to recover from a hypoxic sprint. After each 4min recovery HR during HYP progressively increased over each SIT, recovery was not achieved and O_2 deficit accumulated, this provides insight into the mechanism for differences in PPO between HYP and NORM during SIT session 6, where insufficient recovery was made in HYP [34] and therefore PPO was maintained, not improved.

SpO_2 reduced in HYP, meaning a reduction in O_2 availability at the cellular level for the recovery processes as further evidenced by a greater HR during recovery. The trend of this response demonstrates an acute acclimatisation to the hypoxic training over the six sessions, whereby SpO_2 was significantly greater at respective time points in the latter sessions. This may be well documented in longer simulated acclimatisation training. Yet this response may be of use to those considering short term, high intensity hypoxic training for improvement in

altitude tolerance. This high intensity, simulated altitude acclimatisation training warrants further research.

The explanation for non-significant results for the HYP group could be attributed to the strength of hypoxia. HR and RPE were high throughout the 2 weeks and recovery periods were not sufficient in comparison to NORM, potentially resulting in a reduced anaerobic power and training load across the sprints. While it is possible that the hypoxic stimulus was too severe, it could also be suggested that the recovery phases were too short. Yet altitude training classically uses moderate hypoxia, approximately ~2,500m [35] (FiO_2: 0.15) allowing for a sufficient training intensity. It is not thought that the normobaric nature of the exposure is different to hypobaric hypoxia due to the short durations of exposure [36]. Future research may wish to consider such hypoxic SIT at higher inspired oxygen fractions to allow greater aerobic recovery for consecutive sprints.

Morton & Cable [37] studied the use of moderate to high intensity 30min cycle training in normobaric hypoxia (2750m) over 4weeks. $\dot{V}O_{2peak}$, OBLA, mean power and peak power increased with both normoxic and hypoxic training, yet no differences were seen between normoxic and hypoxic training conditions. These findings are similar to that of the current study whereby improvements in endurance were seen, yet hypoxia offered no additional benefit than to that of high intensity normoxic training. Roels et al [38] used intermittent hypoxic (FiO_2: 0.14) training and found a similar cardiovascular improvement with no additional benefit of hypoxic training. Additionally, Roels et al [38] conclude that this use of training has greater implications for short term acclimatisation to altitude for altitude performance, as supported by the SpO_2 data presented within this study.

The recognition of high intensity exercise is growing, not only for performance training but for weight management and various diseased populations. The intensity of this training presents some significant feasibility and safety implications for these populations [7]. Hypoxic SIT only exacerbates the difficulty of this training modality and therefore should not be considered by most.

The use of hypoxia or altitude as a training stimulus can be difficult to evaluate due to the individual responses to a hypoxic or altitude environment. As a result, the current study is limited by the relatively small number of recreationally active participants. Further, measurement of power outputs for training loads throughout the SIT sessions would have been a useful tool for evaluating training intensities managed for each environment. For

greater interpretation of mechanisms future work may investigate cellular or inflammatory responses to hypoxic SIT. Future research evaluating sprint or recovery durations for hypoxic SIT may well discover beneficial training methods.

Conclusion

Both normoxic and hypoxic sprint interval training (SIT) using 30s sprints interspersed with 4min rest progressing in number over 2 weeks, caused improvement in TTE and VO_2peak. This study indicates that hypoxia based SIT offers no additional benefit for improvement of endurance performance.

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Figure Captions

Figure 1 Schematic of the study protocol

Figure 2 Absolute $\dot{V}O_{2peak}$ changes shown across all groups and between conditions. Values are ± SD. *denotes significant difference within group. ($p < 0.05$). HYP = hypoxic training, (FiO_2: 0.15) NORM = normoxic training, (FiO_2: 0.2093), CONT = no training.

Figure 3 Time to Exhaustion changes shown across all groups and between conditions. Values are ± SD. *denotes significant difference within trial ($p<0.05$). HYP = hypoxic training, (FiO_2: 0.15) NORM = normoxic training, (FiO_2: 0.2093), CONT = no training**.**

Figure 4 SpO_2 (%) during sprint interval training (SIT). * denotes significant difference ($p < 0.05$) from first sprint within day. Values are ± SD. # denotes significant difference ($p < 0.05$) between conditions. HYP = hypoxic training, (FiO_2: 0.15) NORM = normoxic training, (FiO_2: 0.2093)

Table Captions

Table 1: Subject characteristics. Values are means ± SD