

Research article

## A Combined Hot and Hypoxic Environment during Maximal Cycling Sprints Reduced Muscle Oxygen Saturation: A Pilot Study

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### Abstract

The present study investigated the effects of a combined hot and hypoxic environment on muscle oxygenation during repeated 15-s maximal cycling sprints. In a single-blind, cross-over study, nine trained sprinters performed three 15-s maximal cycling sprints interspersed with 7-min passive recovery in normoxic (NOR; 23°C, 50%, FiO<sub>2</sub> 20.9%), normobaric hypoxic (HYP; 23°C, FiO<sub>2</sub> 14.5%), and hot normobaric hypoxic (HH; 35°C, FiO<sub>2</sub> 14.5%) environments. Relative humidity was set to 50% in all trials. The *vastus lateralis* muscle oxygenation was evaluated during exercise using near-infrared spectroscopy. The oxygen uptake (VO<sub>2</sub>) and arterial oxygen saturation (SpO<sub>2</sub>) were also monitored. There was no significant difference in peak or mean power output among the three conditions. The reduction in tissue saturation index was significantly greater in the HH (-17.0 ± 2.7%) than in the HYP (-10.4 ± 2.8%) condition during the second sprint ( $p < 0.05$ ). The average VO<sub>2</sub> and SpO<sub>2</sub> were significantly lower in the HYP (VO<sub>2</sub> = 980 ± 52 mL/min, SpO<sub>2</sub> = 82.9 ± 0.8%) and HH (VO<sub>2</sub> = 965 ± 42 mL/min, SpO<sub>2</sub> = 83.2 ± 1.2%) than in the NOR (VO<sub>2</sub> = 1149 ± 40 mL/min, SpO<sub>2</sub> = 90.6 ± 1.4%;  $p < 0.05$ ) condition. In conclusion, muscle oxygen saturation was reduced to a greater extent in the HH than in the HYP condition during the second bout of three 15-s maximal cycling sprints, despite the equivalent hypoxic stress between HH and HYP.

**Key words:** Heat stress, normobaric hypoxia, environmental stressor, muscle oxygenation.

### Introduction

Repeated-sprint training in hypoxia (RSH) is known as an efficient procedure to improve sprint performance (Brocherie et al., 2017; Millet et al., 2019). One of the key physiological factors of greater training adaptations following RSH would be larger muscle deoxygenation/reoxygenation during each training session (Faiss et al., 2013). In fact, a hypoxic environment promoted muscle deoxygenation during a single session of repeated-sprint exercise compared with a normoxic environment (Billaut and Buchheit, 2013; Girard et al., 2017; Willis et al., 2017). In addition to hypoxia, a hot environment (40°C) also promoted muscle deoxygenation during prolonged moderate-intensity cycling compared with a thermoneutral environment (18°C), although the same trend was not evident during sprint exercises (Periard et al., 2013). Taken together, hot and hypoxic environments enhance muscle deoxygenation through different proposed mechanisms, (increased muscle oxygen [O<sub>2</sub>] extraction in hot vs. restricted O<sub>2</sub> availability in hypoxic environments)(Girard et al., 2017; Periard et al., 2013), perhaps to a greater extent in a

combination of two environments than hot or hypoxic alone. A few studies examined this hypothesis, but no consensus was obtained for the effect of combined hot and hypoxic environments on muscle oxygenation during exercise. Yatsutani et al. (2020) reported that the tissue saturation index (TSI) during 60-min moderate-intensity cycling tended to be lower in hot hypoxic than thermoneutral hypoxic environments. On the other hand, Yamaguchi et al. (2021) failed to observe larger deoxygenation during repeated cycling sprints in a hot hypoxic environment compared with a thermoneutral hypoxic environment. The magnitude of exercise-induced muscle deoxygenation is related to exercise intensity and duration (Racinais et al., 2014; Shibuya et al., 2004), therefore the effect of combined hot and hypoxia on muscle oxygenation may also depend on exercise intensity and regimen utilized. Although muscle oxygenation during repeated-sprint exercise ( $\leq 10$  s) in hypoxia-only (Billaut and Buchheit, 2013; Willis et al., 2017) and combined hot and hypoxia (Yamaguchi et al., 2021) have been previously examined, muscle oxygenation response during longer sprint exercise ( $> 10$  s) is still unknown. Track and field sprinters typically incorporate sprint interval exercise, in particular a relatively longer duration of sprints (15-30 s) with complete recovery, into their training routines. If the combination of hypoxia and heat stress promotes muscle deoxygenation during the sprint exercise, it would be beneficial information for athletes because the transient reduction in O<sub>2</sub> partial pressure in the muscles during training sessions plays a key role in muscular adaptations (Hoppeler et al., 2008; Hoppeler et al., 2003).

Therefore, this study examined the effects of a combined hot and hypoxic environment on muscle oxygenation during three 15-s maximal cycling sprints. We hypothesized that this environment would enhance muscle deoxygenation compared with thermoneutral normoxic and hypoxic environments.

### Methods

#### Subjects

Nine trained sprinters (100–200 m) were recruited (age = 19.3 ± 0.4 years, height = 172.1 ± 1.8 cm, weight = 63.8 ± 2.2 kg). The subjects were informed about the experiment and provided informed consent. They were asked to avoid intense exercise, caffeine, alcohol, and supplements for 24 h before each session. This study was approved by the Ethics Committee of Ritsumeikan University, Japan.

## Experimental protocol

During the study, the subjects visited the laboratory four times, with the visits being at least 1 week apart (familiarization session followed by three experimental trials). The trials were conducted in normoxic (NOR; 23°C, relative humidity [RH] = 50%,  $FiO_2$  = 20.9% [sea level]), normobaric hypoxic (HYP; 23°C, RH = 50%,  $FiO_2$  = 14.5% [simulated altitude of 3,000 m]) and hot normobaric hypoxic (HH; 35°C, RH = 50%,  $FiO_2$  = 14.5%) conditions in a single-blind, cross-over study. Moderate hypoxia ( $FiO_2$  = 14.5%) and heat stress (35°C) were selected based on previous studies combining the hypoxic and hot environments during exercise (Girard and Racinais, 2014; Yamaguchi et al., 2020). The order of the trials was randomized and counterbalanced. The trials were conducted in the afternoon (16:00~19:00; same time of the day among trials within each subject). All subjects consumed an identical lunch at least 2 h before arriving at the laboratory. Following baseline measurements, the subjects entered an environmental chamber (FCC-5000S; Fuji Medical Science, Chiba, Japan). After a 30-min exposure period, they performed a warm-up exercise (5-min cycling [60 rpm, 60 W] followed by  $2 \times 6$ -s maximal sprints). Subsequently, three 15-s maximal sprints interspersed with 7-min passive rest periods were performed on an electromagnetically braked cycle ergometer (Power Max VIII; Konami, Tokyo, Japan). The pedaling load was fixed at 7.5% of body weight. This exercise protocol mimicked one of the typical training regimens in track and field sprinters. The 7-min long rest period was inserted to prevent power output reduction, unlike repeated-sprint exercise.

## Measurements

Near-infrared spectroscopy (NIRS) was used to measure the TSI. The NIRS probe (Hb14; ASTEM, Kanagawa, Japan) was attached to the skin surface above the muscle belly of the right *vastus lateralis* (middle of thigh) and covered by an elastic band. The inter-optode distance was 30 mm (near-infrared light was transmitted 15 mm below the skin surface), and the sampling frequency was set to 10 Hz. The TSI was averaged for each sprint and expressed as the change ( $\Delta$ ) from the baseline value, which was recorded while sitting on a chair for 60 s before entering the chamber. Moderate to high day-to-day reliability (ICC: 0.70-0.87, CV: 1.5-2.6%) was reported for TSI during rest and several intensities of submaximal exercise (Lucero et al., 2018).

Pulmonary oxygen uptake ( $VO_2$ ) was measured breath-by-breath during each sprint using an automatic gas analyzer (AE-300S; Minato Medical Science, Tokyo, Japan). Heart rate (HR) and arterial oxygen saturation ( $SpO_2$ ) were recorded at 1 Hz throughout the experiment using a wireless HR monitor (RCX5; Polar Electro Oy, Kempele, Finland) and a finger pulse oximeter (PULSOX-Me300; Teijin Pharma Ltd., Tokyo, Japan), respectively. The peak HR and the lowest  $SpO_2$  during each sprint were obtained.

Muscle temperature was monitored noninvasively with the zero-heat-flow method (Fox et al., 1973; Matsukawa et al., 1996; Muravchick, 1983; Togwa et al.,

1976; Yamakage et al., 2002; Yamakage and Namiki, 2003) using a surface thermometer (CM-210; Terumo, Tokyo, Japan) attached to the belly of the left *vastus lateralis* muscle (Ito et al., 2020; Yamaguchi et al., 2020); the thermometer detected muscle temperature at a depth of approximately 10 mm. The temperature measured using this procedure was strongly correlated with the temperature at a depth of 18 mm measured using a needle thermocouple (Matsukawa et al., 1996). Before the measurement, the subcutaneous fat thickness of all participants was confirmed to be less than 7 mm using ultrasound (Prosound SSD-3500; Aloka, Tokyo Japan). Skin temperature (left side of the chest, arm, thigh, and calf) was monitored using wired probes (ITP082-24; Nikkiso-Therm, Tokyo, Japan), and the mean skin temperature was calculated (Ramanathan, 1964). Muscle and skin temperatures were recorded from the end of the 30-min acclimation period until completion of the exercise.

The blood lactate concentration was measured from capillary blood samples using a lactate analyzer (Lactate Pro 2; Arkray, Kyoto, Japan), before and 5 min after completion of the exercise.

The rating of perceived exertion (RPE; 10-point scale) and thermal sensation (TS; 9-point scale) were assessed immediately after completion of the exercise.

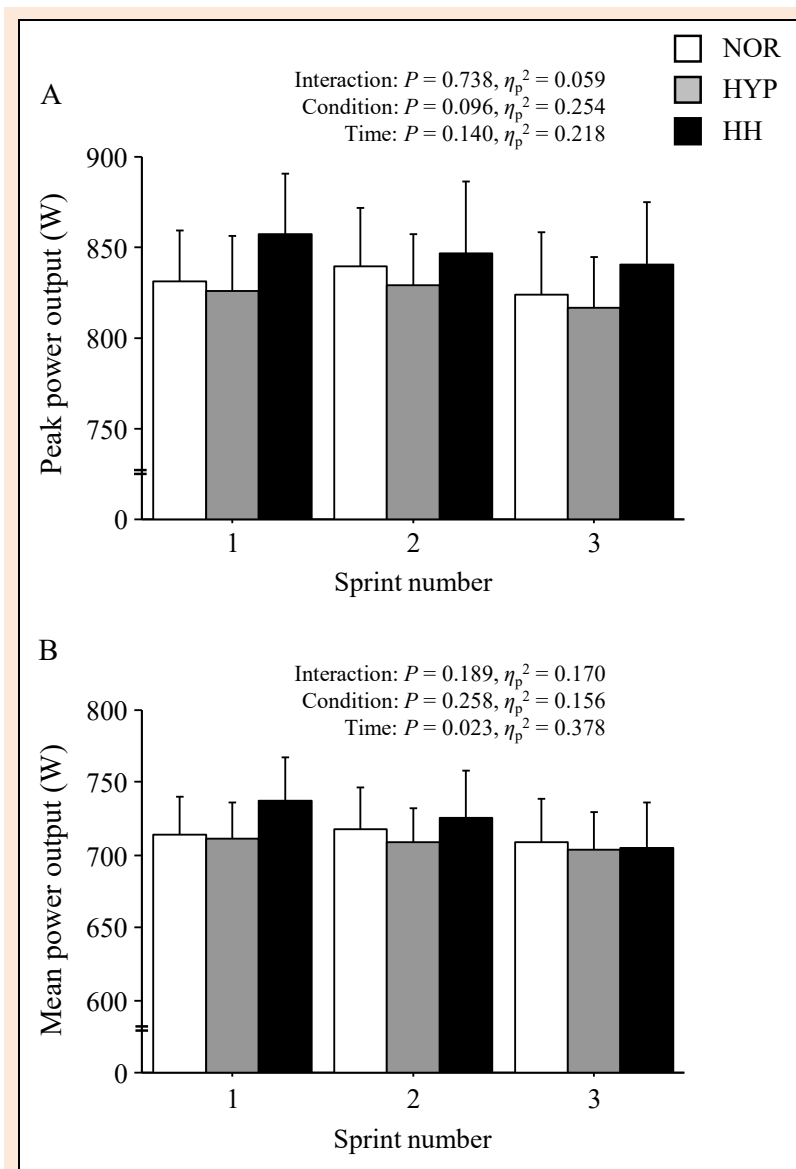
## Statistical analyses

All data are presented as the mean  $\pm$  standard error of the mean. Two-way repeated-measures analysis of variance (ANOVA) was performed using SPSS software (ver. 27.0; IBM, Armonk, NY, USA). Effect size is evaluated using the partial eta squared ( $\eta_p^2$ ). Values of 0.01, 0.06, and  $> 0.14$  were considered as small, medium, and large, respectively (Cohen, 1988). When ANOVA revealed a significant main effect (condition or time) or interaction (condition  $\times$  time), the Tukey-Kramer *post-hoc* test was performed. Statistical significance was set at  $P < 0.05$ .

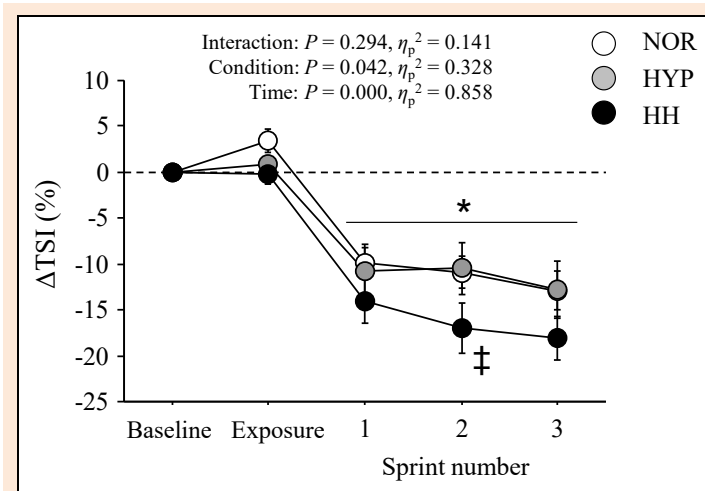
## Results

There was no significant difference in peak or mean power output among the three conditions (Figure 1).  $\Delta$ TSI was significantly lower in the HH than in the HYP condition during the second sprint ( $P < 0.05$ , Figure 2). The averaged  $VO_2$  and  $SpO_2$  during the sprints were significantly lower in the HYP and HH than in the NOR ( $P < 0.05$ , Table 1) condition. By contrast, in the HH condition, the HR and muscle and skin temperatures were significantly higher than in the NOR and HYP conditions throughout the three sprints ( $P < 0.05$ , Table 1).

The blood lactate concentration was significantly elevated after exercise, with no difference in post-exercise concentration among the three conditions (NOR,  $16.8 \pm 1.3$  mmol/L; HYP,  $17.0 \pm 1.2$  mmol/L; HH,  $17.0 \pm 1.2$  mmol/L). The RPE at the completion of all exercises did not differ significantly among the conditions (NOR,  $7.3 \pm 0.4$ ; HYP,  $7.6 \pm 0.5$ ; HH,  $8.3 \pm 0.5$ ), while the TS was significantly higher in the HH ( $8.7 \pm 0.4$ ) than in the NOR ( $6.3 \pm 0.6$ ) or HYP ( $6.1 \pm 0.4$ ) condition.



**Figure 1.** Peak (A) and mean power outputs (B) during each sprint. Values are means  $\pm$  standard error of the mean.



**Figure 2.** Changes in ATSI during each sprint. Values were expressed as absolute change ( $\Delta$ ) from the baseline. Values are means  $\pm$  standard error of the mean. \*:  $p < 0.05$  vs. Baseline. ‡:  $p < 0.05$  vs. HYP.

**Table 1.** Cardiorespiratory variables and body temperatures during each sprint and average of all sprints.

		Sprint number				ANOVA ( $\eta_p^2$ )		
		1	2	3	Average	Interaction	Condition	Time
Peak HR (bpm)	CON	155 ± 4	162 ± 5 *	166 ± 4 *	161 ± 4			
	HYP	155 ± 4	161 ± 4 *	164 ± 4 *	160 ± 4	0.648	< 0.001	< 0.001
	HH	166 ± 5 †‡	172 ± 4 *†‡	174 ± 4 *†‡	171 ± 4 †‡	(0.082)	(0.852)	(0.938)
Lowest SpO <sub>2</sub> (%)	CON	90.7 ± 1.4	89.1 ± 2.2	92.0 ± 1.1	90.6 ± 1.4			
	HYP	81.6 ± 1.1 †	83.3 ± 1.1 †	83.7 ± 0.7 †	82.9 ± 0.8 †	0.094	< 0.001	0.703
	HH	83.9 ± 1.4 †	83.8 ± 1.2 †	82.0 ± 1.7 †	83.2 ± 1.2 †	(0.240)	(0.735)	(0.049)
VO <sub>2</sub> (mL/min)	CON	940 ± 84	1253 ± 45 *	1253 ± 50 *	1149 ± 40			
	HYP	831 ± 71	1042 ± 81 *†	1067 ± 50 *	980 ± 52 †	0.888	0.018	0.012
	HH	815 ± 105	1034 ± 34 †	1045 ± 72	965 ± 42 †	(0.039)	(0.435)	(0.612)
Muscle temperature (°C)	CON	36.1 ± 0.7	36.5 ± 0.6 *	36.8 ± 0.5 *	36.5 ± 0.2			
	HYP	36.0 ± 0.6	36.5 ± 0.5 *	36.8 ± 0.4 *	36.4 ± 0.2	0.089	< 0.001	< 0.001
	HH	37.1 ± 0.3 †‡	37.3 ± 0.3 *†‡	37.5 ± 0.3 *†‡	37.3 ± 0.1 †‡	(0.266)	(0.663)	(0.927)
Skin temperature (°C)	CON	31.9 ± 0.2	32.2 ± 0.2 *	32.4 ± 0.2 *	32.1 ± 0.2			
	HYP	31.7 ± 0.2	32.0 ± 0.2 *	32.3 ± 0.2 *	32.0 ± 0.2	< 0.001	< 0.001	0.001
	HH	35.6 ± 0.1 †‡	35.1 ± 0.1 *†‡	35.0 ± 0.1 *†‡	35.2 ± 0.1 †‡	(0.739)	(0.963)	(0.573)

Values are means ± standard error of the mean. \*:  $P < 0.05$  vs. the first sprint, †:  $P < 0.05$  vs. CON, ‡:  $P < 0.05$  vs. HYP.

## Discussion

This study compared changes in muscle oxygenation variables during maximal cycling sprints among NOR, HYP, and HH conditions. The main finding was that  $\Delta$ TSI during the second 15-s maximal cycling sprint was significantly lower in the HH than in the HYP condition, despite  $F_iO_2$  being equal between those two conditions. Notably, the lower TSI during the second sprint in the HH condition occurred despite the power output being comparable among the three conditions.

During exercise, the TSI generally reflects the balance between O<sub>2</sub> supply and use in muscles (Ferrari et al., 2011). In the present study, the lower  $\Delta$ TSI in the HH condition suggested enhanced muscle deoxygenation. This is in line with a study demonstrating that a combined hot and hypoxic environment while cycling at moderate intensity for 60 min tended to produce a lower TSI than thermoneutral normoxic or hypoxic environments (Yatsutani et al., 2020). Hot and hypoxic environments were reported to independently enhance muscle deoxygenation during exercise (Periard et al., 2013; Yamaguchi et al., 2019). In a hypoxic environment, the limited O<sub>2</sub> availability (i.e., lower SpO<sub>2</sub>) and decreased O<sub>2</sub> supply to muscles would facilitate muscle deoxygenation (Hoppeler et al., 2003). Furthermore, fractional O<sub>2</sub> extraction is increased when O<sub>2</sub> tension is lowered (e.g., hypoxic condition) in fast-twitch fibers (Faiss et al., 2013; McDonough et al., 2005). By contrast, elevated body temperature in a hot environment causes a rightward shift in the O<sub>2</sub>-hemoglobin dissociation curve (Barcroft and King, 1909), thus promoting muscle O<sub>2</sub> extraction (muscle deoxygenation). Since the hypoxic stimulus (i.e. reduction in SpO<sub>2</sub>) was comparable between the HYP and HH conditions, adding heat exposure to hypoxia further promoted muscle deoxygenation by combining the above mechanisms in HH.

While  $\Delta$ TSI was lower in HH during 15-s maximal sprints, a previous study reported that reduction of TSI during repeated-cycling sprints did not differ between hypoxic-only and combined hot and hypoxic environments (Yamaguchi et al., 2021). The differences in exercise

regimen (three 15-s sprints in the present study vs. three sets of 5 × 6-s sprints in the previous study), pre-exercise exposure duration (30-min in the present study vs. 5-min in the previous study), and subjective characteristics (track and field sprinters in the present study vs. active males in the previous study) would be potential reasons for inconsistent results. The significantly greater muscle deoxygenation was found only during the second sprint, but not during the first and the third sprints. Although  $\Delta$ TSI level was consistently lower in the HH versus NOR and HYP through three sprints, it did not reach statistical difference during the first and the third sprints, which may be due to the small sample size and interindividual differences.

The 15-s sprint performance was not different among conditions. Combining hypoxia and hot condition decreased moderate-intensity cycling time to exhaustion (Girard and Racinais, 2014) and 90-min simulated soccer performance (Aldous et al., 2015) compared with hypoxia or hot alone, probably due to higher blood lactate concentration and greater reduction of plasma volume related to the combination of impaired O<sub>2</sub> availability and increased cardiovascular strain (Aldous et al., 2015; Girard and Racinais, 2014). In contrast to prolonged exercise, repeated short maximal sprint performance was not negatively affected by combined hot and hypoxia (Dennis et al., 2021; Yamaguchi et al., 2020). Therefore, the performance outcome in the present study was supported by the previous studies.

In practical terms, maximal cycling sprints in a hot and hypoxic environment reduced muscle O<sub>2</sub> saturation compared with a hypoxic environment, without negatively affecting the power output. Therefore, performing maximal sprints in a combined hot and hypoxic condition would increase hypoxia-related stress in muscles while maintaining a mechanical load.

## Conclusion

The muscle oxygen saturation was reduced to a greater extent in the HH than in the HYP condition during the second bout of three 15-s maximal cycling sprints, although the

hypoxic stresses (FiO<sub>2</sub> and SpO<sub>2</sub>) and power output did not differ between these conditions. This suggests that a combined hot and hypoxic environment would partially promote local hypoxia in the working muscles during maximal sprint exercise compared with a hypoxic environment.

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**Key points**

- The muscle oxygen saturation was reduced to a greater extent in the combined hot and hypoxia than in hypoxia alone during the second bout of three 15-s maximal cycling sprints, despite similar arterial oxygen saturation.
- There was no significant difference among conditions for peak and mean power outputs during three 15-s maximal sprints.
- These results suggest that acute exposure to a combined hot and hypoxia would partially promote local hypoxia in the working muscles without a negative effect on sprint performance.

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