

Hypoxia affects tissue oxygenation differently in the thigh and calf muscles during incremental running

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Abstract

Purpose The present study was performed to determine the impact of hypoxia on working muscle oxygenation during incremental running, and to compare tissue oxygenation between the thigh and calf muscles.

Methods Nine distance runners and triathletes performed incremental running tests to exhaustion under normoxic and hypoxic conditions (fraction of inspired oxygen = 0.15). Peak pulmonary oxygen uptake ($\dot{V}O_{2\text{peak}}$) and tissue oxygen saturation (StO_2) were measured simultaneously in both the vastus lateralis and medial gastrocnemius.

Results Hypoxia significantly decreased peak running speed and $\dot{V}O_{2\text{peak}}$ ($p < 0.01$). During incremental running, StO_2 in the vastus lateralis decreased almost linearly, and the rate of decrease from warm-up (180 m min^{-1}) to $\dot{V}O_{2\text{peak}}$ was significantly greater than in the medial gastrocnemius under both normoxic and hypoxic conditions ($p < 0.01$). StO_2 in both muscles was significantly decreased under hypoxic compared with normoxic conditions at all running speeds ($p < 0.01$). The rate at which StO_2 was decreased by hypoxia was greater in the vastus lateralis as the running speed increased, whereas it changed little in the medial gastrocnemius.

Conclusions These results suggest that the thigh is more deoxygenated than the calf under hypoxic conditions, and that the effects of hypoxia on tissue oxygenation differ between these two muscles during incremental running.

Keywords Running · Hypoxic condition · Near infrared spectroscopy · Muscle deoxygenation · Heterogeneity

Abbreviations

F_{IO_2}	Fraction of inspired oxygen fraction
Hb	Hemoglobin
HHb	Deoxygenated hemoglobin and myoglobin
NIRS	Near infrared spectroscopy
O_2Hb	Oxygenated hemoglobin and myoglobin
SD	Standard division
SpO_2	Arterial oxygen saturation
StO_2	Tissue oxygen saturation
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2\text{peak}}$	Peak oxygen uptake

Introduction

Previous studies have shown that hypoxia influences exercise performance and cardiopulmonary responses during whole-body exercises such as running (Ogawa et al. 2007), cycling (Osawa et al. 2011; Subudhi et al. 2007), swimming (Ogita and Tabata 1992), and rowing (Peltonen et al. 1995). Under hypoxic conditions, pulmonary oxygen uptake ($\dot{V}O_2$) at a given exercise intensity does not change, while peak $\dot{V}O_2$ ($\dot{V}O_{2\text{peak}}$) at maximal exercise decreases. In addition to the influence on cardiopulmonary responses, hypoxia affects regional muscle circulation and metabolism. Near infrared spectroscopy (NIRS) is a useful method to evaluate tissue oxygen metabolism and can be used to continuously and non-invasively

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monitor tissue oxygenation from various parts of the body during dynamic exercise (Bhambhani 2012; Hamaoka et al. 2007). Previous studies have indicated that muscle oxygenation under hypoxic conditions does not change at rest and during single-knee extension (DeLorey et al. 2004), but decreases during intermittent sprint (Bowtell et al. 2014), constant-load (Spencer et al. 2012a, b), and incremental cycling (Osawa et al. 2011; Subudhi et al. 2007). However, few studies have investigated the effects of hypoxia on working muscles during running, which is a major whole-body exercise mode.

During incremental cycling, systemic $\dot{V}O_2$ increases almost linearly until $\dot{V}O_{2peak}$ (Day et al. 2003), but working muscle deoxygenation monitored by NIRS changes non-linearly and is attenuated before $\dot{V}O_{2peak}$, despite increases in $\dot{V}O_2$ (Boone et al. 2015; Osawa et al. 2011; Subudhi et al. 2007). However, tissue oxygenation in the thigh muscles differs during running compared with cycling. Quaresima et al. (2002) showed that the difference in tissue oxygenation between walking and running was larger in the vastus lateralis muscle than in the medial gastrocnemius muscle. Another study showed that during walking and running at incremental speeds, tissue oxygenation decreased in the vastus lateralis until exhaustion, but this did not occur in the lateral gastrocnemius (Hiroyuki et al. 2002).

The decrease in arterial oxygen content between muscles induced by systemic hypoxia would not be different, while the microvascular responses and recruitment pattern of muscle fibers are different (Heinonen et al. 2010). During high-speed running there was a higher contribution to workload by the thigh as compared with the calf (Simpson and Bates 1990), and tissue deoxygenation of the working muscle was exacerbated by systemic hypoxia (Osawa et al. 2011; Subudhi et al. 2007). The purpose of this study was thus to compare the impact of hypoxia on tissue oxygenation between the thigh and calf muscles during incremental running. We hypothesized that hypoxia-induced tissue deoxygenation would be greater in the thigh than in the calf during high-speed running.

Methods

Subjects

Nine male distance runners and triathletes (age = 24 ± 3 years, height = 1.69 ± 0.05 m, body mass = 57.1 ± 3.6 kg, body mass index = 20.1 ± 1.3 kg·m⁻² [means \pm standard deviation (SD)]) volunteered to participate in this study. The health status of each subject was assessed based on medical history and physical examination. All procedures and any potential risks were explained to the participants before starting the study, and all provided written informed consent. This study was approved by the local

ethics committee, and all experiments were performed in accordance with the Declaration of Helsinki.

Experimental procedure and protocol

After familiarization with the experimental procedures and the treadmill machine, the participants performed two incremental running tests on the treadmill [inclination = 1% (Jones and Doust 1996)] in an environmentally controlled, simulated room under normoxic or normobaric hypoxic (fraction of inspired oxygen (F_{IO_2}) = 0.15, balanced with nitrogen) conditions in random order. Ambient conditions were controlled automatically (temperature = 23.0°, relative humidity = 40%). The participants performed the two sessions 72 h apart and the tests were at the same time each day. The participants were instructed to refrain from caffeine consumption for 12 h and stressful exercise for 24 h before each test. After breathing in each gas mixture at rest for at least 30 min to equilibrate body gas stores of oxygen and carbon dioxide, the participants performed incremental running tests under either normoxic or hypoxic conditions. The protocol included at least 5 min of standing rest, 3 min of running at 180 m·min⁻¹ (warm-up), and then a 10 m·min⁻¹ increase in pace every minute until exhaustion. The actual speeds on the treadmill were measured every minute with a handheld tachometer (DT-105 N; Nidec-Shimpo Corporation, Kyoto, Japan), as the actual treadmill speeds varied slightly compared with the set speeds during running.

Cardiopulmonary responses

Breath-by-breath measurements of pulmonary gas exchange and minute ventilation were obtained using a computerized metabolic cart (AE300; Minato Medical Science, Osaka, Japan). $\dot{V}O_2$ was expressed in standard temperature, pressure, and dry, and minute ventilation was expressed in body temperature, ambient pressure, and saturated with water vapor. Heart rate was determined using a heart rate monitor (Polar RS800CX; Polar Electro, Kempele, Finland). Arterial oxygen saturation (SpO₂) was monitored non-invasively using a pulse oximeter (OLV-3100; Nihon Kohden, Tokyo, Japan) on the index finger. To ensure the accuracy of the data, pulse data were acquired simultaneously and confirmed to match the heart rate. We were not able to collect complete data from two participants, so the results of SpO₂ were averaged in the other seven participants. Respiratory responses, heart rates, and SpO₂ were measured throughout the exercise bouts. Blood was acquired from fingertip sticks and blood lactate concentration were measured using a blood lactate test meter (Lactate-Pro; Arkray, Kyoto, Japan) before exercise and at the 1st and 3rd minutes after exercise; the highest value was recorded as the blood lactate at $\dot{V}O_{2peak}$. The participants were allowed to stop running when they fell

more than one meter behind on the treadmill despite verbal encouragement.

Tissue oxygenation

Changes in oxygenated (O_2Hb) and deoxygenated (HHb) hemoglobin and myoglobin concentrations were calculated using modified Beer–Lambert methods (Hamaoka et al. 2007), in which the concentration of absorber was calculated by the optical density and pathlength. Tissue oxygen saturation (StO_2) was assessed with spatially resolved spectroscopy and measured using a multi-distance spatially resolved tissue oximeter (NIRO-200NX; Hamamatsu Photonics, Shizuoka, Japan). The baseline values of O_2Hb and HHb were obtained while the participants stood on the treadmill for at least 5 min before the test. Changes in total hemoglobin (Hb) were calculated by adding the changes in O_2Hb and HHb, and these were considered to reflect changes in blood volume of the measurement site. Optodes were placed on the lower third of the vastus lateralis and the middle of the medial gastrocnemius using double-sided and surgical tape and housed in plastic holders. The thigh and calf were wrapped with elastic bandages. The light source consisted of three laser diodes of different wavelengths (775, 810, and 850 nm). The intensity of the transmitted light was measured at 5 Hz. The distance between the emitter and receiver was 3 cm. The thickness of the fat layer in the regions of interest

was <5 mm (mean \pm SD = 2.7 ± 0.7 mm), ensuring that the NIRS signals would be minimally influenced by the fat layer (Niwayama et al. 2007).

Statistical analyses

All data were averaged in the last 30 s of exercise at each speed and all values were calculated as mean \pm SD. Statistical analyses were performed using statistical software (IBM SPSS Statistics 19; IBM Cooperation, Chicago, IL). Exercise-induced changes were compared by two-way analysis of variance, and the variables included the muscles, F_{IO_2} conditions, and running speeds. When significant effects were observed, the Bonferroni post hoc test was performed. In all analyses, $p < 0.05$ was taken to indicate statistical significance.

Results

Running speeds and cardiopulmonary responses at rest and at $\dot{V}O_{2peak}$ under normoxic and hypoxic conditions are shown in Table 1. Compared with normoxic conditions, running speed and $\dot{V}O_2$ at $\dot{V}O_{2peak}$ under hypoxic conditions were significantly reduced by $8.9 \pm 2.4\%$ and $12.8 \pm 7.0\%$, respectively. SpO_2 was lower in hypoxic than in normoxic conditions by 8.8 ± 4.2 percentage points at rest and 20.0 ± 5.2 percentage points at $\dot{V}O_{2peak}$. Heart

Table 1 Running speed, cardiopulmonary responses, and blood lactate at rest and at $\dot{V}O_{2peak}$ under normoxic and hypoxic conditions

Variables	Trials	Rest	$\dot{V}O_{2peak}$
Running speed ($m \cdot min^{-1}$)	Normoxia	0 ± 0	326 ± 11
	Hypoxia	0 ± 0	$297 \pm 12^{**}$
$\dot{V}O_2$ ($mL \cdot min^{-1}$)	Normoxia	372 ± 82	3618 ± 640
	Hypoxia	421 ± 90	$3126 \pm 357^{**}$
$\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Normoxia	6.6 ± 1.7	63.4 ± 10.8
	Hypoxia	7.4 ± 1.8	$54.8 \pm 6.3^{**}$
Minute ventilation ($L \cdot min^{-1}$)	Normoxia	12 ± 2	134 ± 19
	Hypoxia	13 ± 3	139 ± 16
Respiratory exchange ratio (A.U.)	Normoxia	0.86 ± 0.11	1.08 ± 0.04
	Hypoxia	0.84 ± 0.12	1.08 ± 0.05
Partial pressure of end-tidal oxygen (mmHg)	Normoxia	104 ± 16	108 ± 15
	Hypoxia	$71 \pm 14^{**}$	$81 \pm 9^{**}$
Partial pressure of end-tidal carbon dioxide (mmHg)	Normoxia	36 ± 3	37 ± 4
	Hypoxia	35 ± 4	$31 \pm 3^{**}$
Heart rate (bpm)	Normoxia	73 ± 13	196 ± 9
	Hypoxia	80 ± 13	191 ± 9
SpO_2 (%) ($N = 7$)	Normoxia	99.4 ± 0.5	91.3 ± 2.4
	Hypoxia	$90.6 \pm 4.5^{**}$	$71.3 \pm 5.0^{**}$
Blood lactate (mM)	Normoxia	1.3 ± 0.4	10.3 ± 2.1
	Hypoxia	1.2 ± 0.6	11.3 ± 2.2

Values are mean \pm SD. $N = 9$. Significantly different from normoxic conditions (** $p < 0.01$)

rates at $\dot{V}O_{2\text{peak}}$ were significantly different between the two conditions, but there were no significant difference in minute ventilation and blood lactate concentration.

StO₂ in the vastus lateralis and medial gastrocnemius at rest did not differ significantly between normoxic and hypoxic conditions. However, the StO₂ was significantly decreased by both changes in F_{IO_2} and running speed, and there was a significant interaction between these changes (Fig. 1). The decreases in StO₂ from warm-up (180 m·min⁻¹) to $\dot{V}O_{2\text{peak}}$ in the vastus lateralis (19.5 ± 6.5% points and 18.7 ± 4.8% points under normoxic and hypoxic conditions, respectively) were significantly larger than those in the medial gastrocnemius (6.6 ± 8.9% points and 8.2 ± 6.6% points under normoxic and hypoxic conditions, respectively), but no significant differences were found between normoxic and hypoxic conditions. At $\dot{V}O_{2\text{peak}}$, StO₂ was lower in the vastus lateralis than in the medial gastrocnemius, and the vastus lateralis, but not the medial gastrocnemius, was more deoxygenated in hypoxic than in normoxic conditions.

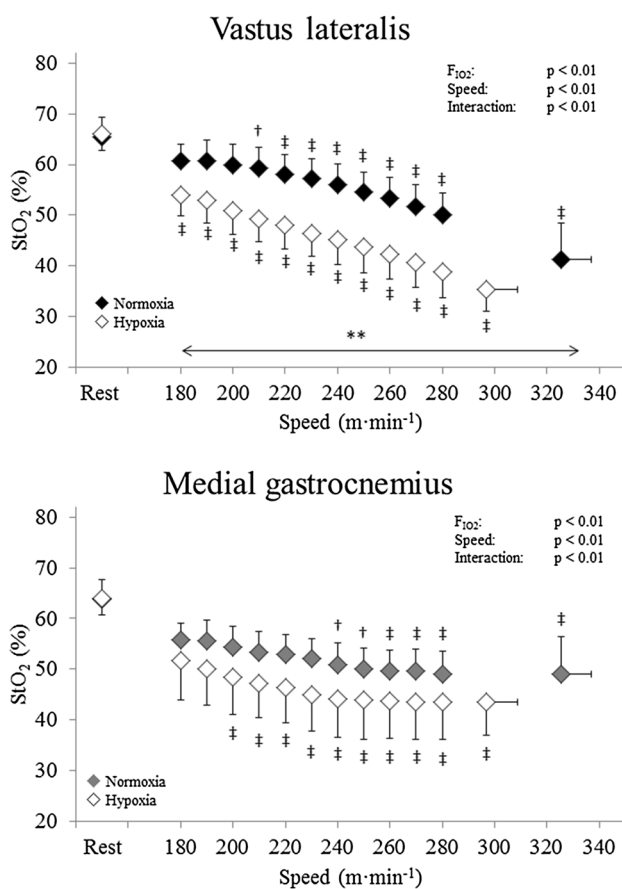


Fig. 1 StO₂ in the vastus lateralis (upper) and the medial gastrocnemius (lower) under normoxic (shaded squares) and hypoxic (clear squares) conditions. Values are mean ± SD. Significantly different from rest under each condition ([†] $p < 0.05$, [‡] $p < 0.01$). Significantly different between normoxic and hypoxic conditions (** $p < 0.01$)

HHb in the vastus lateralis was significantly increased both by hypoxic conditions (compared with normoxic conditions) and increasing running speeds, and there was an interaction between these changes. Similarly, HHb in the medial gastrocnemius interacted significantly with the changes in F_{IO_2} and running speed (Fig. 2).

Figure 3 shows the average responses of total Hb in the vastus lateralis and medial gastrocnemius with progression from rest to $\dot{V}O_{2\text{peak}}$. The change in the vastus lateralis was similar between normoxic and hypoxic conditions, but that of the medial gastrocnemius interacted significantly with changes in F_{IO_2} and speeds. At $\dot{V}O_{2\text{peak}}$, total Hb values were not significantly different in either the vastus lateralis or the medial gastrocnemius in normoxic compared with hypoxic conditions.

At rest, the rate at which StO₂ was decreased from normoxic to hypoxic conditions did not differ between the vastus lateralis and medial gastrocnemius. However, there was a significant interaction in the decrease in StO₂ under hypoxic

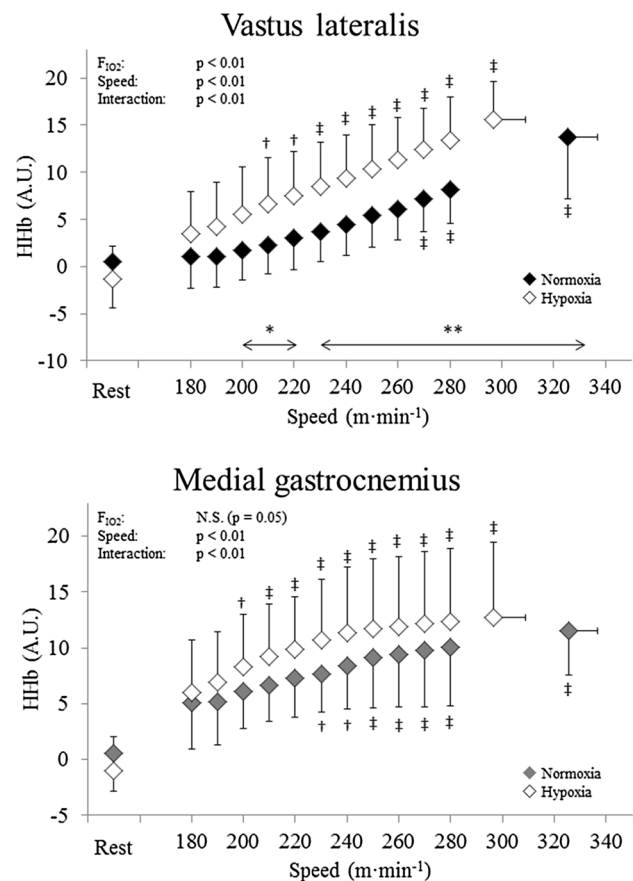


Fig. 2 HHb in the vastus lateralis (upper) and the medial gastrocnemius (lower) under normoxic (shaded squares) and hypoxic (clear squares) conditions. Values are mean ± SD. Significantly different from rest under each condition ([†] $p < 0.05$, [‡] $p < 0.01$). Significantly different between normoxic and hypoxic conditions (* $p < 0.05$, ** $p < 0.01$)

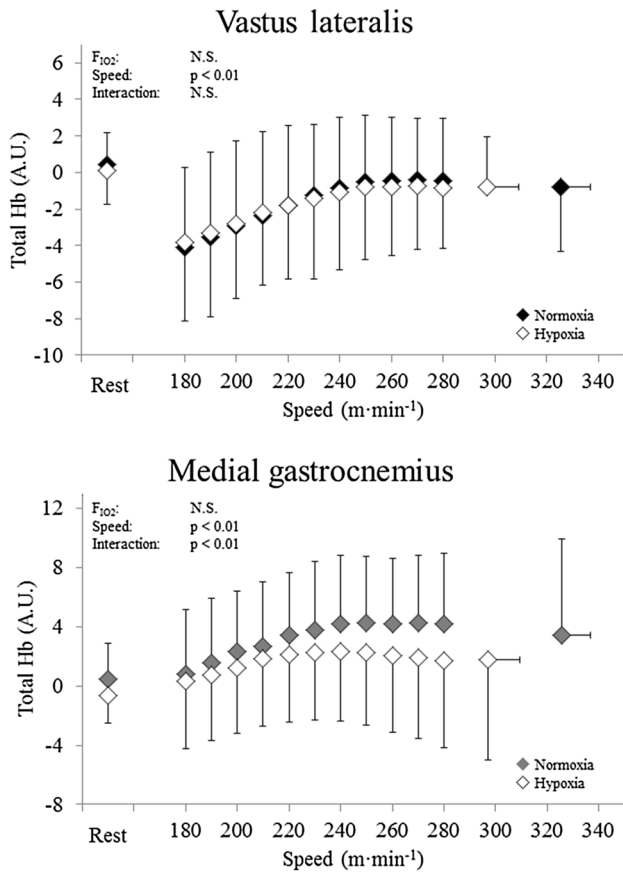


Fig. 3 Total Hb in the vastus lateralis (upper) and the medial gastrocnemius (lower) under normoxic (shaded squares) and hypoxic (clear squares) conditions. Values are mean \pm SD

compared normoxic conditions between the muscles and speeds (Fig. 4 upper). Similarly, the increase in HHb from normoxic to hypoxic conditions was significantly greater in the vastus lateralis during incremental running compared with at rest, whereas that in the medial gastrocnemius it was not (Fig. 4 lower).

Discussion

We examined the difference in the effects of hypoxia on tissue oxygenation between the thigh and calf muscles during incremental running. The main findings of this study were that hypoxia decreased the tissue oxygenation of both the vastus lateralis and medial gastrocnemius while running, and that the decreases were more pronounced in the vastus lateralis than in the medial gastrocnemius. These results suggest that the effect of hypoxia on tissue oxygenation in the two working muscles was influenced by the running speed, and that the decreases in tissue oxygenation induced by hypoxia were greater in the

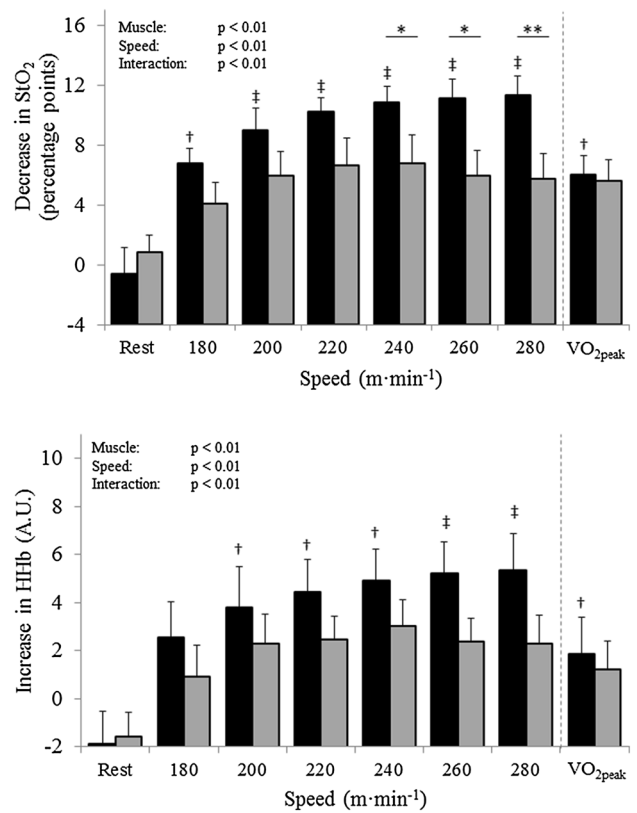


Fig. 4 Decrease in StO₂ (upper) and increase in HHb (lower) in the vastus lateralis (black bars) and medial gastrocnemius (gray bars) at increasing absolute running speeds. Values are mean \pm SD. Significantly different from rest under each condition ($\dagger p < 0.05$, $\ddagger p < 0.01$). Significantly different between normoxic and hypoxic conditions ($*p < 0.05$, $**p < 0.01$)

vastus lateralis than in the medial gastrocnemius, especially during high-speed running. The simulated altitude in this study (calculated from inspired oxygen pressure) was ~2700 m, and this is commonly used as high-altitude training by elite athletes. The data from our study suggest that hypoxic training has disparate effects on different muscles.

Previous studies using positron emission tomography have shown that carbohydrate metabolism is greater in the calf than in other muscles while running (Fujimoto et al. 2000). Likewise, glycogen depletion rates after ~2.5 h of intense running have been shown to be almost 1.7-fold greater in the calf than in the thigh muscles (Krssak et al. 2000). It is possible that carbohydrate metabolism in the medial gastrocnemius—a muscle that is less oxidative than the vastus lateralis (Johnson et al. 1973)—should increase during high-speed running since whole-body carbohydrate usage increases when running speed increases (Zavorsky et al. 1998). Thus, it has been thought that more energy is consumed by the calf than the thigh muscles while running, and thus, the medial gastrocnemius is

more deoxygenated than the vastus lateralis during high-speed running. Moreover, the effects of hypoxia on tissue oxygenation are thought to be more pronounced in the medial gastrocnemius than in the vastus lateralis because hypoxia-induced tissue deoxygenation in working muscles is accelerated by increased exercise intensity (Osawa et al. 2011; Subudhi et al. 2007). However, we showed that tissue deoxygenation increased more in the vastus lateralis than in the medial gastrocnemius during incremental running under both normoxic and hypoxic conditions. These results could be partly attributable to changes in the relative contribution rates of the muscles to propulsive force generation (Novacheck 1998). Previous studies have shown that the movement parameters of the hip, knee, and ankle joints increase as running speed increases, but that the contribution rates of various muscles at these joints differ (Simpson and Bates 1990). For example, the relative contribution of the calf muscles to propulsive forces decreased gradually as running speed increased, while the contribution in the thigh muscles increased. This is partly supported by previous studies showing that the knee velocity increased as the running speed and stride length increased (Collins et al. 2000). The vastus lateralis became deoxygenated nearly linearly until $\dot{V}O_{2peak}$, whereas the medial gastrocnemius became deoxygenated at a slower rate.

NIRS responses

Tissue oxygenation (as monitored by NIRS) is thought to reflect the balance between oxygen supply and consumption at the measured sites (Grassi and Quaresima 2016). The decrease in tissue oxygenation during exercise is attributed not only to increased oxygen consumption, but also to decreased oxygen supply. In other words, there is a relative decrease in the oxygen supply/consumption ratio during exercise. A decrease in StO_2 of the vastus lateralis during incremental running may be primarily caused by increased muscle oxygen consumption, but it could also be affected by increases or decreases in oxygen supply. Decreases in oxygen supply can be caused by a decrease in arterial oxygen content resulting from exercise-induced hypoxemia and a decrease in F_{IO_2} (Miyachi and Tabata 1992). In addition, tissue blood flow can be decreased by vasoconstriction due to increases in sympathetic nerve activity (Hansen et al. 2000). Increases in oxygen supply can be caused by increases in cardiac output and leg blood flow as well as vasodilation induced by endothelium-derived nitric oxide (Rådegran and Hellsten 2000). Tissue oxygenation is thus regulated both by control systems affecting oxygen supply to the muscles and by changes in muscle oxygen consumption.

Tissue oxygenation in hypoxia

As seen in cycling (Osawa et al. 2011; Subudhi et al. 2007), working muscles became deoxygenated during incremental running under hypoxic conditions, and this was not completely attributed to decreases in SpO_2 . At rest, StO_2 was similar between the two conditions despite a significant decrease in SpO_2 in hypoxia. This was likely because the decrease in SpO_2 was modulated by increases in microvascular blood flow. Indeed, muscle oxygenation was unchanged at low $\dot{V}O_2$, such as at rest or during low-intensity exercise, and in exercises utilizing a small muscle mass (DeLorey et al. 2004). Therefore, decreases in StO_2 in the vastus lateralis and medial gastrocnemius while running in hypoxic conditions are likely related to an inability to compensate for the decrease in SpO_2 .

The balance between tissue oxygen delivery and consumption determines tissue oxygenation, which mainly reflects the microcirculation (Hamaoka et al. 2007). It has traditionally been thought that the effects of hypoxia on muscle oxygen supply are not different between muscles and that the decrease in StO_2 in the vastus lateralis and medial gastrocnemius are similar. However, our results indicate that the effects of hypoxia on tissue oxygenation do indeed differ between these muscles. In addition to physiological responses affected by hypoxia, including sympathetic nerve (Saito et al. 1988) and microcirculatory responses (Bourdillon et al. 2009; Heinonen et al. 2010), intramuscular metabolites likely accumulate more in hypoxic than in normoxic conditions (Wadley et al. 2006), which stimulates the recruitment of fast-twitch muscle fibers (Moritani et al. 1992). The muscle fiber compositions in both the vastus lateralis and medial gastrocnemius are mixed, although the vastus lateralis is slightly more oxidative than the medial gastrocnemius (Johnson et al. 1973). The relative contribution of the thigh to propulsive forces increases more than the calf during incremental running (Simpson and Bates 1990), so the recruitment of fast-twitch muscle fibers, which are thought to influence oxygen delivery and consumption (McDonough et al. 2005), may be greater in the vastus lateralis.

Tissue oxygenation during running

During incremental running, StO_2 in the vastus lateralis decreased almost linearly. The deoxygenation dynamics differed from those seen in cycling, in which tissue deoxygenation in working muscles becomes attenuated at high intensity as $\dot{V}O_{2peak}$ is approached (Boone et al. 2015, 2016 (review); Fontana et al. 2015; Keir et al. 2015; Osawa et al. 2011; Spencer et al. 2012a, b; Subudhi et al. 2007). The difference in tissue deoxygenation dynamics between running and cycling could be related to various factors, including the range of knee joint motion, the types of muscle

contraction, and the duration of muscle contraction per step (running) and per pedal (cycling). However, oxygenation of the medial gastrocnemius formed an inverted arc compared with the vastus lateralis, and the change in amplitude from warm-up to $\dot{V}O_{2\text{peak}}$ was smaller than that of the vastus lateralis. This is similar to what has been observed in previous reports (Hiroyuki et al. 2002). The small change in tissue oxygenation observed in the medial gastrocnemius during incremental running was likely not mediated by unchanged or decreased muscle oxygen consumption, but instead by concurrent increases in both muscle oxygen supply and consumption. This could explain the near tissue oxygen balance during running. Electromyographic activity (Bijker et al. 2002) and muscle oxygen consumption in the calf both increase during incremental running, although electromyographic activity and muscle deoxygenation do not always correlate (Osawa et al. 2011). Moreover, as tissue oxygenation is partially related to the Bohr Effect (Grassi et al. 1999; Wang et al. 2006), it is possible that intramuscular metabolites and pH are different between the vastus lateralis and medial gastrocnemius.

Previous reports have shown that tissue circulation and metabolism during exercise (Cannon et al. 2013; Fujimoto et al. 2000; Heinonen et al. 2010) differed between muscles, as did muscle oxygenation (Chin et al. 2011) and electromyographic activities (Hug et al. 2004). Moreover, heterogeneous oxygenation within a single muscle has also been reported (Kime et al. 2010; Vogiatzis et al. 2015). However, the present study assessed only the vastus lateralis and medial gastrocnemius as being representative of the thigh and calf muscles, respectively. Therefore, the possibility that tissue oxygenation in other muscles differs from that in the vastus lateralis and medial gastrocnemius cannot be excluded. Further studies are required to elucidate the heterogeneity among working muscles.

Conclusions

We showed that the effects of hypoxia on tissue oxygenation varied between working muscles during incremental running, and that hypoxia-induced tissue deoxygenation was exacerbated by faster running speeds in the vastus lateralis but not in the medial gastrocnemius. It is likely that the disparate contributions to running propulsive forces play a role in the differences in tissue oxygenation between the thigh and calf during incremental running. These results help to understand the microcirculatory and metabolic alterations to different muscles that are induced by hypoxia during whole-body exercise.

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Compliance with ethical standards

Conflict of interest This study was performed without any conflict of interest, financial or otherwise.

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