Effects of Genetic Variations in HIF-1α on Physiological Response to Hypoxia
（低酸素に対する生理的応答における HIF-1α 遺伝子多型の影響）

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ABSTRACT

Altitude training in hypoxic conditions, such as living high and training low, is used to improve athlete performance. The benefits of altitude training vary widely among individuals, however, even resulting in reduced performance in some individuals. We hypothesized that hypoxia inducible factor-1α gene (HIF1A) polymorphisms partially affect physiological responses to hypoxia, and may predict adaptation to hypoxia. Changes in erythropoietin concentration, ventilation (V_E), and arterial O_2 saturation (SpO_2) at rest and during exercise in hypoxia were examined in subjects who have T allele of the C1772T SNP or A allele of the G1790A SNP in the HIF-1A. After 12 h of exposure to hypoxia at rest, erythropoietin concentration was higher in individuals with the SNP of G1790A than in those without. The ventilatory response to hypoxia at rest was not represented difference between the two genotypes. In all individuals, V_E increased with declining SpO_2 in response to hypoxia. Changes in V_E and SpO_2 during acute exercise under hypoxic conditions were also similar between the two genotypes. These findings give preliminary evidence to explain whether HIF-1α gene polymorphisms affect individual capacity to respond to hypoxia.

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I. INTRODUCTION

Altitude training is frequently used to improve performance in elite athletes. Many studies have focused on the adaptive mechanisms that underlie changes in aerobic (Gore and Hopkins, 2005; Levine and Stray-Gundersen, 2005) and anaerobic performance (Nummela and Rusko, 2000; Roberts et al., 2003). Although altitude training appears to be statically effective for improved performance, there is an individual difference in the extent of adaptation to hypoxia (Chapman et al., 1998; Julian et al., 2004). Chapman et al. (1998) divided athletes into improved group and declined group from 5000 m race time after altitude training and named responder and nonresponder, respectively. Furthermore, they reported that plasma erythropoietin (Epo) concentration was higher after 30 h at altitude in responder compared with nonresponder. The authors suggested that individual differences in the magnitude of Epo response may influence high altitude acclimation via changes of total red cell volume and volume per time oxygen maximum (VO_2 max). The hypoxic ventilatory response (HVR) also differs between individuals under hypoxic conditions, and correlates with arterial O_2 saturation (Moore et al., 1986). Accordingly, arterial O_2 saturation at altitude is expected to be lower in those individuals with a lower HVR. Lower HVR

values are associated with lower exercise capacity at high altitude (Masuyama et al., 1986; Schoene et al., 1984). Collectively, Epo and HVR may influence physiological acclimation to hypoxic conditions.

Genetic factors may explain individual responses to high altitude. Recently, gene polymorphism of hypoxia inducible factor (HIF) -1α has been focused as a predictive gene of physiological adaptation to hypoxic condition. HIF-1, which is composed of HIF-1α and HIF-1β, plays an important role as a transcriptional factor that regulates the expression of hypoxic-responsive genes. HIF-1α is stabilized under hypoxic conditions, and the conjugated form of HIF1-β induces multiple hypoxic-responsive genes, including Epo and vascular endothelial growth factor (VEGF) (Manalo et al., 2005; Tanimoto et al., 2003). The HIF-1α gene (HIF1A) in humans has many single nucleotide polymorphisms (SNPs), among which C1772T (P582S) and G1790A (A588T) are located in exon 12 of the HIF1α gene, and are well known. These gene polymorphisms are found within the oxygen-dependent degradation domain of the HIF-1A gene. Compared with normal gene which has CC for C1772T and GG for G1790A, these gene polymorphisms stimulate stronger transcriptional activity of HIF-1α under normoxic and hypoxic conditions (Tanimoto et al., 2003). In humans with the C1772T polymorphism, VO_2 max decreases to a greater extent with age, and adaptation to aerobic training is impaired compared with humans with a normal genotype (Prior et al., 2003). On the other hand, recent study reported that significant tendency for greater frequency of C1772T has been found in elite endurance athletes compared with non-athlete controls (Döring et al. 2010).

It is unknown, however, whether these genetic variations modify the function of HIF-1α. On the other hand, Peng et al. (2006) indicated that cardio-respiratory responses to hypoxia exposure might be affected by HIF-1A through O2 sensing in carotid body. Therefore, variations in the HIF-1α gene may influence hypoxia-inducible physiological responses, such as increased Epo in blood, and ventilation to hypoxic conditions.

Present study aimed to examine physiological effect of HIF1-α gene polymorphisms in short term and acute hypoxia exposure. We compared Epo (a target gene of HIF-1) concentration in blood after exposure to hypoxia for 12 h in subjects with the SNPs C1772T and G1790A, and in subjects with a normal genotype. In addition, arterial oxygen saturation and ventilatory responses investigated at rest and during exercise in acute hypoxia exposure in these genotypes group. We hypothesized that physiological responses to short tern or acute hypoxia exposure would differ between subjects with a normal genotype and those with the SNPs C1772T and G1790A.

II. METHODS

Study subjects

Sixty-nine healthy adult Japanese men participated in this study. All subjects lived at sea level. The study protocols and procedures followed the guidelines of the Ethical Committee at Waseda University and the Physiological Society of Japan. Informed consent was obtained from all subjects, and they agreed to gene profiling to detect SNPs on C1772T (P582S, rs11549465) and G1790A (A588T, rs11549467) in the HIF-1α gene. Individuals with a normal genotype were randomly selected to participate in the study.

DNA extraction and SNPs detection

Genomic DNA was extracted from whole venous blood using a whole blood kit (FUJI FILM, Tokyo). SNPs were detected using an automated detection system that employs bacterial magnetic particles (BMPs), and dissociation curve analysis (Maruyama et al., 2004). Fluorescently labeled detection probes for SNP detection were obtained from SIGMA

Genosys (Hokkaido, Japan). The sequences for the primer oligonucleotide of HIF-1α were as follows: forward primer
5'-TACGTTCCTTCGATCAGTTGTC-3'
and reverse primer
5'-TTTGAGGACTTGCGCTTTC-3'.
Allele-specific oligonucleotide detection probes in HIF-1α were as follows: C1772T detection probe, 5'-Cy5-CAGTTGTCATCATTAGAAA-3' and G1790A detection probe,
5'-Cy5-AGTTCCACAAGCCC-3'.

Amplification was performed using a thermal cycler (MJ Research, USA) in a total reaction volume of 100 µl containing 80 ng DNA sample, 2.5 U AmpliTaq DNA polymerase (Applied Biosystems, Canada), 0.2 mM dNTPs, and 0.2 µM primer and 1 × GeneAmp (Applied Biosystems) PCR buffer (10 mM Tris-HCl, pH 8.3 at 25°C, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% w/v gelatin). The thermal profile included pre-running at 95°C for 5 min and 35 cycles of denaturation at 95°C for 30 sec, primer annealing at 55°C for 1 min, and primer extension at 72°C for 1 min, followed by extra-long extension at 72°C for 15 min. After amplification, florescent intensity with BMPs was measured and SNPs were identified in each sample.

Measurement of erythropoietin in blood

To evaluate changes in blood erythropoietin concentration after exposure to hypoxic conditions, subjects remained for 12 h in a hypoxia room set at 15.4% oxygen concentration. This period coincided with the time of sleep between 8 PM and 8 AM. Subjects were monitored for changes in arterial O_2 saturation by pulse oximetry (SpO_2, KONICA MINOLTA, Japan) while in the hypoxia room. The severity of acute mountain sickness (AMS) score was recorded at both 1 and 12 h after entering the hypoxia room to evaluate subject well-being. A venous blood sample (2 mL) was collected immediately after exiting the hypoxia room to determine serum erythropoietin concentration. A control blood sample was also collected on another day at the same time of day. Serum was separated from whole blood by centrifugation, and stored at −80°C until analysis. Erythropoietin concentration was determined using the radioimmunoassay methods reported by Mufson and Gesner (Mufson and Gesner, 1987).

Ventilatory response and O_2 saturation under hypoxia inspiration

HVR at rest was measured using the progressive isocapnic hypoxic test described by Weil et al. (Weil et al., 1970). In the progressive hypoxia test, the subject, holding a mouthpiece in place, breathed room air at first, then started rebreathing in a small bag to a closed by-pass circuit containing CO2 absorber. HVR was measured in all subjects at the same time in the morning to avoid circadian variation. Inspired minute ventilation (V_I), end-tidal partial pressure of O_2 (PETO2), and end-tidal partial pressure of CO_2 were continuously measured using a gas analyzer (AE3000S, MINATO Ikagaku, Japan). Rebreathing system was adjusted PETO2 to reach 40 torr (5.61 % O2 concentration) in 4-5 minutes. During ventilation measurements, SpO_2 was also monitored by pulse oximetry. The ventilatory response to isocapnic progressive hypoxia at rest was evaluated using the slope of the SpO_2–VE response line ($\Delta V_E/\Delta SpO_2$) as the HVR value.

O_2 saturation and ventilatory responses during exercise were measured while exercising on a bicycle ergometer and breathing normoxic air (20.9% O_2 mixture balanced with nitrogen) or hypoxic air (15.4% O_2 mixture balanced with nitrogen). Exercise intensity during the measurement period was gradually increased from 30 to 120 watts by 30 watts every 3 min. Expired minute ventilation (V_E) was measured continuously during exercise under normoxia and hypoxia using a gas analyzer, and calculated as ΔV_E (hypoxic V_E − normoxic V_E). SpO_2 was measured by finger pulse oxymetry during

exercise, and was calculated as ΔSpO_2.

Statistics

The Hardy-Weinberg equation was used to determine whether the proportion of each genotype obtained was in agreement with expected values as calculated from allele frequencies. The X^2-test was used to compare the distribution of genotypes. All data are represented as mean ± SEM. Significant differences among normal genotype and SNPs groups were determined by one-way or two-way ANOVA. If a significant *F* value was observed, the Tukey–Kramer post hoc test was used to locate the difference. Values of $P<0.05$ were considered significant.

III. RESULTS

Screening of HIF-1α polymorphisms

The genotype frequencies of the HIF1A SNPs investigated in 69 subjects are shown in **Tables 1**. In every group, the genotype frequencies were in accordance with the assumption of the Hardy-Weinberg equilibrium. Screening of polymorphisms in HIF-1α gene in 69 subjects revealed 61 subjects with a normal genotype (normal subjects have C1772C and G1790G sequences), four subjects with C1772T (CT), and four subjects with G1790A (GA; including one A1790A subject). Ten subjects were randomly selected from the 61 normal subjects to participate in this study. The physical characteristics of each group are listed in **Table 2**.

Table 1. Appearance two types of allele frequencies of HIF1A gene in 69 subjects

	Nucleotide	Aminoacids	Genotype	Number	%
rs11549465	C1772T	P582S	C/C	65	94.2
			C/T	4	5.8
			T/T	0	0
rs11549467	G1790A	A588T	G/G	65	94.2
			G/A	3	4.35
			A/A	1	1.45

Table 2. Subject characteristics

	Number	Age	Height (cm)	Body weight (kg)
Normal	10	23.0±2.2	174.6±3.4	70.9±7.9
C1772T	4	23.4±2.3	171.6±4.6	64.8±7.2
G1790A (A1790A)	4 (1)	21.3±0.6	170.9±2.6	64.2±4.3

Allele (C1772T and G1790A) frequencies within HIF-1A gene are shown in 69 subjects. 10 subjects who participated in this study were selected at random from 61 normal genotype subjects. Values are means ± SEM.

Response of erythopoietin after hypoxic exposure for 12 h

After remaining in the hypoxia room (15.4% O_2 concentration) for 12 h, we measured the Epo concentration in blood. Compared with pre-exposure values, serum Epo concentration increased in subjects with a normal genotype (126.6 ± 9.4%), subjects with C1772T (133.5 ± 13.8%) and subjects with G1790A (173.6 ± 12.3%) (**Figure 1**). Epo was significantly higher

after hypoxia exposure in subjects with G1790A compared with subjects with a normal genotype, despite the lack of any difference in Epo concentration before hypoxia exposure.

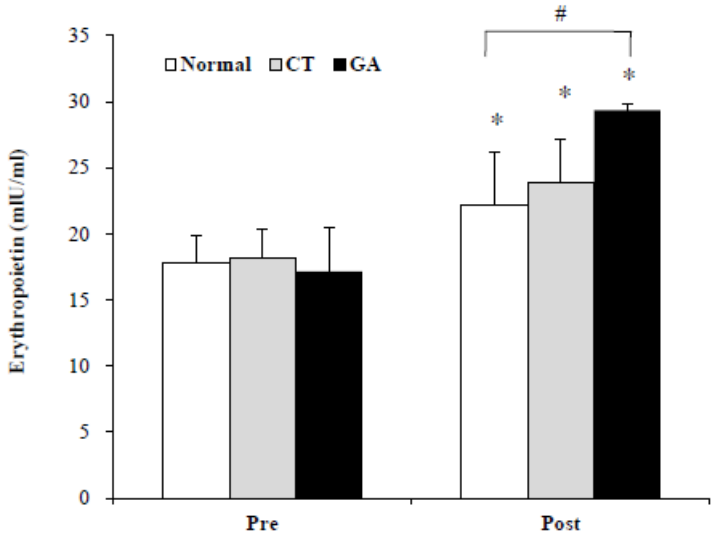

Figure 1. Erythropoietin responses immediately after staying in the hypoxia room for 12 h, in normal (n=10) genotype, C1772T (n=4), and G1790A (n=4) gene groups. Values are means ± SEM. *$P< 0.05$ compared with the 'pre' value; #$P<0.05$ compared with normal genotype. CT, C1772T; GA, G1790A.

Hypoxic ventilatory response under resting condition

With declining O_2 concentration, V_E increased as SpO_2 decreased in all three groups (Figure 2A). HVR at rest condition was calculated using the slope of the SpO_2–V_E response line ($\Delta V_E/\Delta SpO_2$) and mean values were 0.73±0.11 L/min/% in normal, 0.53 ± 0.20 L/min/% in C1772T, and 0.59 ± 0.16 L/min/% in G1790A group, respectively. However, there was variation in the data among individuals in each group (Figure 2B).

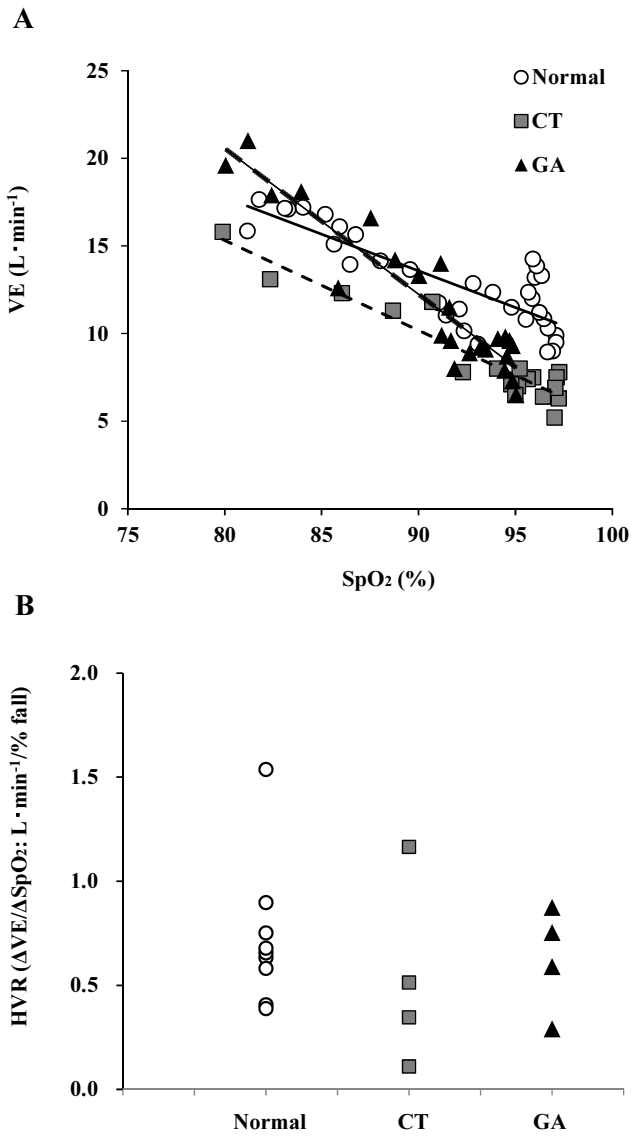

Figure 2. Hypoxic ventilatory response (HVR) at rest in the normal (n=10) genotype, C1772T (n=4), and G1790A (n=4) groups. Representative data for the relationship between SpO_2 and V_E responses to isocapnic progressive hypoxia are shown for each group (A). HVR was evaluated using the slope of the SpO_2–V_E response line ($\Delta V_E/\Delta SpO_2$) and individual data was represented in each genotype (B). SpO_2, saturation of peripheral oxygen; V_E, ventilation; CT, C1772T; GA, G1790A.

Arterial O_2 saturation and ventilatory response during exercise under hypoxia

SpO_2 and V_E during cycle ergometer exercise were measured under normoxia (20.9% O_2 mixture) and hypoxia (15.4% O_2 mixture); changes are shown as delta values (hypoxia – normoxia) (**Figure 3**). SpO_2 during exercise gradually decreased with increasing exercise intensity, and this effect was greater under hypoxic conditions (**Figure 3A**). Conversely, V_E increased with exercise intensity, and was greater under hypoxic conditions (**Figure 3B**). Changes in SpO_2 and VE during exercise were similar among the three groups.

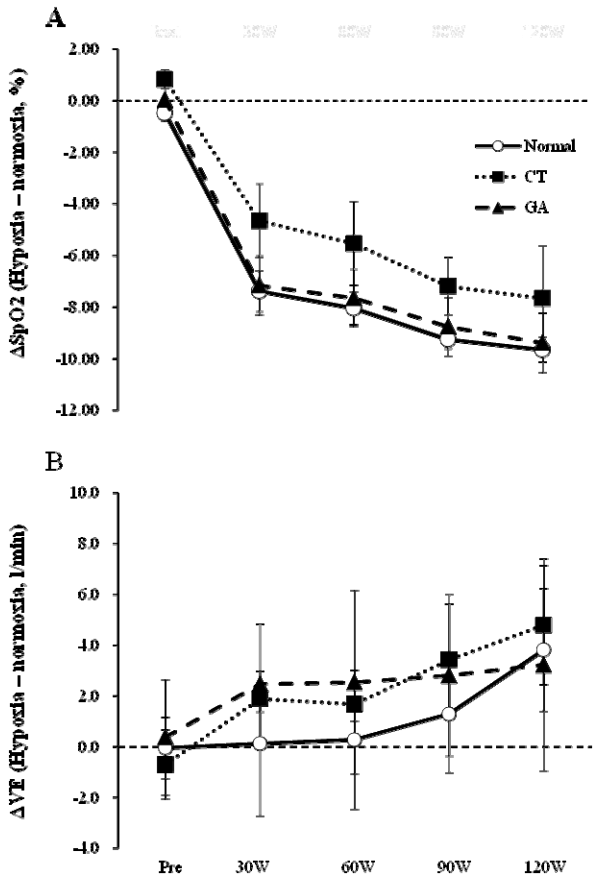

Figure 3. Hypoxia-related changes in SpO_2 and V_E during cycling exercise in the normal (n=10) genotype, C1772T (n=4), and G1790A (n=4) groups. SpO_2 (A) and V_E (B) are shown as delta values (hypoxia – normoxia). Exercise intensity was gradually increased from 30 to 120 watts by 30 watts every 3 min under normoxia (20.9%) or hypoxia (15.4%). Values are means ± SEM. SpO_2, saturation of peripheral oxygen; V_E, ventilation; CT, C1772T; GA, G1790A.

Ⅳ. DISCUSSION

Physical training under hypoxic conditions, such as at high altitude or in a hypoxia room, is used to improve exercise performance at sea level. Performance outcomes in response to altitude training vary widely among individuals, however, and performance even deteriorates in some athletes (Chapman et al., 1998). However, there are currently no predictive factors associated with adaptability to hypoxia. In the present study, we focused on gene polymorphisms in HIF-1α and examined physiological response in response to short term and acute hypoxia. The main findings were as follows: 1) Epo immediately after 12 h in an altitude room was higher in subjects with G1790A compared with subjects with a normal genotype; 2) changes in ventilation and arterial O_2 saturation following acute hypoxia exposure at rest and during exercise were similar among subjects with normal genotype, C1772T, and G1790A.

HIF-1 regulates the transcription of multiple hypoxia-responsive genes, and Epo is one of its downstream targets (Guillemin and Krasnow, 1997; Manalo et al., 2005). Tanimoto et al. (2003) reported that the HIF-1α polymorphisms C1772T and G1790A induce greater transcriptional activity of target genes compared with the normal genotype under both normoxic and hypoxic conditions. In the present study, Epo increased to a greater extent in subjects with G1790A after 12 h exposure to hypoxia compared with subjects with a normal genotype.

It is unclear how the G1790A SNP influences Epo production in vivo. Greater production of Epo under hypoxic conditions might be important for hypoxic adaptation, because Epo is associated with red blood cell production and oxygen delivery (Fandrey, 2004). Performance tends to deteriorate in athletes who have lower Epo production at high altitude (Chapman et al., 1998). Epo responsiveness to hypoxia varies between individuals (Chapman et al., 1998; Ge et al., 2002). Ge et al. (2002) observed that Epo levels simulated by altitude exposure demonstrate marked individual variability, ranging from −41 to 400% after 24 h of exposure to 2,800 m altitude. Genetic polymorphisms, such as G1790A in HIF-1α, may therefore partially contribute to the variability in Epo responses to hypoxia observed in this study. In contrast, we cannot explain why serum Epo concentration did not differ between subjects with the C1772T allele and those with a normal genotype. Further research on longer-term exposure to hypoxia is necessary to understand the effect of SNPs on Epo production and oxygen transport capacity.

The responsiveness of V_E with SpO_2 is also an important determinant of physiological adaptation to hypoxic conditions (Powell and Fu, 2008) and of exercise capacity at high altitude (Masuyama et al., 1986; Schoene et al., 1984). Ventilatory acclimation to chronic hypoxia is involved in an increase in the HVR, and hypocapnia is prevented by compensatory hyperventilation under hypoxic conditions (Powell and Fu, 2008). HVR increases in response to higher O_2-sensitivity in the carotid body (Bisgard, 2000). Carotid body-mediated cardio-respiratory responses to intermittent hypoxia are impaired in heterozygous HIF-1α knockout mice, indicating that HIF-1α contributes to O_2-sensing in the carotid body (Peng et al., 2006). Based on these findings, HIF-1α may partially regulate acclimatization to hypoxic conditions by regulating HVR. In the present study, however, there was no difference for HVR between the three groups. Furthermore, there were no clear effects of HIF-1α genotype on V_E and SpO_2 during exercise in 15.4% oxygen. Therefore, we could not show evidence for a link between HVR and gene polymorphisms of HIF1A gene.

In summary, this study aimed to examine whether SNPs of HIF-1α influence physiological response to hypoxia exposure. Serum Epo concentration was shown the different response in between subjects with the G1790A allele and normal genotype after short-term hypoxia exposure. However, we should not be established physiological effect with HIF-1α gene polymorphism by only finding of serum Epo concentration. In addition, SNPs of C1772T and G1790A were detected in only a small number of subjects. Future research using a larger number of subjects may provide more information concerning the role of gene polymorphisms of HIF-1α in regulating adaptation to hypoxia.

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