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Angiotensin-converting gene and hypoxic exercise tolerance: a randomized crossover trial

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ABSTRACT

Hypoxic training enhances endurance sports tolerance. However, individual responses vary due to physiological differences. This study investigated the relationship between genetic factors and exercise tolerance in hypoxic conditions. This randomized crossover study included 22 male university students (age 20.8 ± 1.3 years, peak oxygen uptake 54.5 ± 6.5 mL/min/kg). Incremental load tests were conducted to assess the symptomatic limit on separate days under normoxic and hypoxic conditions (oxygen concentration 15.4 ± 0.8 %) using an ergometer. The initial test environment was randomized. The peak oxygen uptake and blood lactate were monitored every minute, and Δ peak oxygen uptake (peak oxygen uptake under hypoxia – peak oxygen uptake under normoxia) was calculated. Sixteen genotypes linked to exercise tolerance (such as angiotensin-converting enzyme [ACE]) were examined. Peak oxygen uptake significantly decreased under hypoxia ($p < 0.01$). Δ peak oxygen uptake varied among individuals (minimum: 0.7 and maximum: − 18.9). Among analyzed genetic polymorphisms, ACE-II genotypes showed significantly greater Δ peak oxygen uptake than ACE-ID/ACE-DD genotypes ($p = 0.02$). ACE-II genotypes exhibited lower blood lactate elevation at peak exercise in normoxic ($p = 0.01$) and hypoxic ($p = 0.03$) conditions. Participants with the ACE-II genotype had lower lactate concentrations and greater reductions in peak oxygen uptake under hypoxic conditions. Optimizing hypoxic training requires individualized programs incorporating genetic analysis.

Introduction

Reduced oxygen availability during hypoxia has various effects on the human body, particularly on exercise tolerance [1]. Notably, peak VO_2 decreases during incremental load tests under acute hypoxic conditions (HCs) [2, 3]. This decrease is primarily due to a reduction in blood oxygen saturation, which limits oxygen supply to the muscles [3, 4]. Moreover, individual responses to exercise in HCs vary [5]. For instance, endurance athletes with exercise-induced hypoxia experience reduced peak VO_2 under these conditions [1, 6], which may be attributed to the skeletal muscle's capacity to utilize oxygen for energy [1, 6]. Consequently, the response to acute hypoxic exposure may depend on the properties

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of skeletal muscle developed through training. These muscle characteristics may also be influenced by genetic factors [7].

Various genetic polymorphisms, such as those of angiotensin-converting enzyme (ACE, rs4646994) [8], alpha-actin 3 (ACTN, rs18157393) [9], and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A, also known as PGC1-alpha, rs8192678) [10] have been reported to influence skeletal muscle characteristics and exercise characteristics (such as endurance or power in athletes). The skeletal muscle fiber type is determined by ACTN3 and ACE, which shows no change in distribution [7]. In addition, among the genetic polymorphisms related to exercise training, *ACE* and *ACTN3* have been reported to contribute to the skeletal muscle fiber type [8, 9, 11]. ACE is a crucial component of the renin-angiotensin system (RAS), which regulates blood pressure and fluid balance [8, 11]. The RAS optimizes oxygen supply to skeletal muscle by regulating blood flow and pressure, thereby influencing both endurance and explosive power. Athletes with *ACE I*allele have a high percentage of type-I fibers and are associated with high levels of endurance performance [8, 11]. In contrast, those with the *ACE D* allele have a high percentage of type-II fibers and are predominantly at the high level, as power athletes [9, 11]. *ACTN3* is a protein that constitutes the type-II fiber and different skeletal muscle types have been reported based on the R and X alleles in *R577X.* Athletes with RR and RX types are often sprint and power athletes [9, 11].

Beyond these correlations, the relationship between these genetic polymorphisms and changes in endurance capacity during hypoxic exercise conditions is not well established. Therefore, we hypothesized that specific genetic polymorphisms responsible for skeletal muscle characteristics influence the reduction in peak oxygen uptake (VO_2) during acute hypoxic exposure. This study examined the association between genetic factors and their effects on exercise tolerance under HCs in adults who routinely engage in relatively intense exercise training.

Materials and Methods

Participants

This prospective randomized crossover trial used bicycle loading to assess exercise tolerance. Inclusion criteria comprised: college students with 1) exercise habits and 2) familiarity with cycling exercise. Exclusion criteria included: participants with 1) hypertension, 2) diabetes mellitus, and 3) active lung disease. Based on these criteria, 22 male university students were recruited. The study protocol was approved by the Institutional Review Board (permission number: 20221016) and was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent. The trial was conducted between September 2022 and July 2023, and was registered in the Clinical Trials Registry.

Experimental procedure

Participants underwent the incremental exercise test under HC (fraction of inspired oxygen [FiO_2], 15.4 ± 0.8 % equivalent to a simulated altitude of 2,500 m) or normoxic condition (NC; FiO_2, 20.9 %). To create a hypoxic environment, the Hypoxico Altitude Tent System was used (Everest Summit II, HYPOXICO Inc., Gardiner, NY, USA). During exercise, the expired gas flow was monitored using a breath-by-breath automated system (Aeromonitor®, MINATO Medical Science CO., LTD., Osaka, Japan). Heart rate (HR) was monitored using Polar (Polar H10, Polar Electro Japan Co., Ltd., Tokyo, Japan) and blood lactate levels were measured using a standard enzymatic method on a lactate analyzer (Lactate Pro2®, ARKRAY Inc., Kyoto, Japan). Skeletal muscle oxygenation in the right thigh was measured using a near-infrared spectrometer (NIRO Monitor NIRO-200NX; Hamamatsu Photonics K.K., Hamamatsu, Japan). Peripheral arterial oxygen saturation (SpO_2) was measured using a pulse oximeter (HPO-100, OMRON Corporation, Kyoto, Japan). To eliminate the influence of training on the second exercise test, a randomized crossover method was used. During the first test, we randomly assigned the participant to an exercise stress test under HC or NC. The interval between NC and HC tests was less than one month (minimum: 1 day, average: 10.2 ± 8.8 days; ▶ **Fig. 1**). Before the first test, the lower limb muscle mass was measured using a body composition meter (Inbody 470, Inbody Japan Inc., Tokyo, Japan) while the knee joint extension torque was measured using a handheld dynamometer (mobie, SAKAI Medical Co., Ltd., Tokyo, Japan).

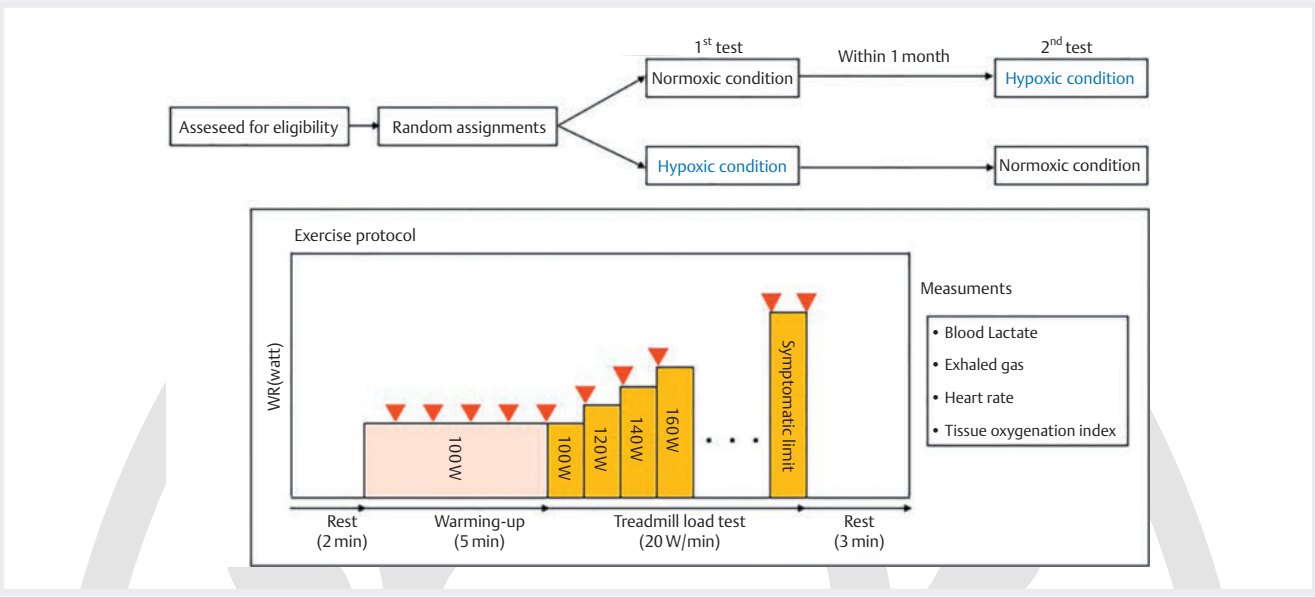
Exercise-testing protocol

On the day of the exercise test, the participants avoided heavy physical activities before the test. In addition, caffeine and food intake were restricted for three hours before the text to avoid potential influence on the exercise results due to their stimulant and nutritional effects. The bicycle was attached to a smart trainer (Minoura KAGURA LSF9300, Minoura Co., Ltd., Gifu, Japan), and incremental load tests were performed using a load control software, Zwift® (Zwift Japan Co., Japan). After measuring the resting data for 2 min, a preparatory exercise was performed for 5 min at a load of 100 W, as a warm-up. The starting load was 100 W, similar to the warm-up load, and the load was increased by 20 W every 60 s (▶ **Fig. 1**). Participants were instructed to pedal at 70 rpm. The incremental load test ended when the participant could no longer pedal because of fatigue or could no longer maintain a speed of 70 rpm for 5 s. The participants were supervised by two exercise physiologists, who are experts in conducting incremental load tests.

Genetic analysis

Two milliliters of saliva were collected using the Saliva DNA Sample Collection Kit (MD-ZSV-001, Zeesan Biotech Co., Ltd., Xiamen, China), extracted using the MGIEasy Nucleic Acid Extraction Kit (MGI Tech Co., Ltd., Shenzhen, China), stored at room temperature, and DNA was extracted immediately according to the manufacturer's instructions. For genotype identification, we used the Infinium Asian Screening Array-24 v1.0 BeadChip (Illumina, Inc., San Diego, CA, USA), according to the manufacturer's instructions. The extracted DNA was amplified and incubated for 24 h at 37 °C. After hybridization and staining, the chip was scanned.

The genotype involved in exercise endurance included [11, 12]: ACE (rs4646994), ACTN3 (rs1815739), PGC1-α (rs8192678), adrenoceptor beta2 (ADRB2: rs1042713), angiotensin II captor type 2 (AGTR2: rs11091046), adenosine monophosphate deaminase (AMPD1: rs17602729), creatine kinase M-type (CKM: rs17602729), collagen type V alpha 1 chain (COL5A1: rs12722),



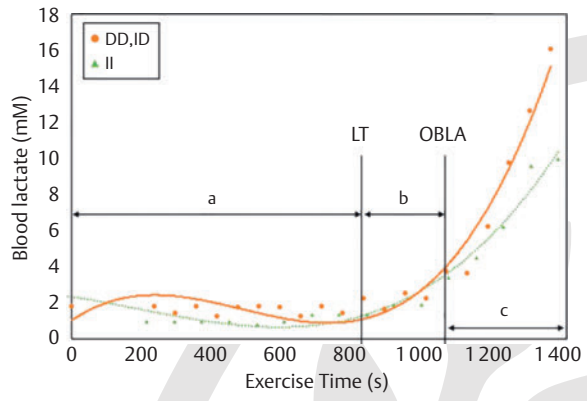
▶ **Fig. 1** Flowchart of study protocol. The exercise protocol consisted of 2 min of rest, followed by a 5-min warm-up at 100 W. Treadmill loading was initiated at 100 W, and was performed up to the symptomatic limit, increasing by 20 W per minute. The exercise was completed after 3 min of rest. The same exercise protocol was followed under both hypoxic and normoxic conditions. Red triangle: timing of blood lactate measurement; WR: work ratio.

GA blinding protein transcription factor subunit beta1 (GABPB1: rs7181866), glutathione S-transferase P1 (GSTP1: rs1695), homeostatic iron regular (HFE: rs1799945), hypoxia inducible factor 1 subunit alpha (HIF1α: rs11549465), monocarboxylate transporter (MCT1: rs1049434), nitric oxide synthase3 (NOS3: rs2070744), peroxisome proliferator-activated receptor alpha (PPARα: rs4253778), transcription factor A, mitochondrial (TFAM: rs7144481), uncoupling protein 2 (UCP2: rs660339), uncoupling protein 3 (UCP3: rs1800849), vascular endothelial growth factor receptor 2 (VEGFR2: rs1870377), guanine nucleotide binding protein (GNB3: rs5443), and nuclear receptor subfamily 1 group H member 3 (NR1H3: rs7120118). These genetic polymorphisms were divided into two groups according to different genotypes (such as ACE: DD + ID vs. II or DD vs. ID + II). Five of the 21 genetic polymorphisms could not be analyzed because sufficient statistical power would not have been achieved after separation of the participants into the two groups. Specifically, the polymorphisms HFE GG, AMPD1 TT, HIF1α TT, NOS3 TC, and GNB3 TT each had only one participant, rendering statistical analysis infeasible due to insufficient power. Therefore, 16 genetic polymorphisms were included in the final analysis.

Measurement data

During the exercise test, respiratory gas exchange, including expiratory ventilation (VE), VO_2, carbon dioxide production (VCO_2), and respiratory rate, were monitored continuously and measured using a 30-s average. Three calibration processes were performed on the system: flow volume sensor, gas analyzer, and delay time calibrations. When exercising under hypoxia, gas calibration was performed using a cylinder for hypoxia (O_2: 8.08 %, CO_2: 4.95 %). Peak VO_2 was calculated as the average oxygen consumption during the last 30 s of the exercise. The ventilation threshold (VT) was determined using a procedure described previously by Gaskill et al. using the ventilatory equivalent, excess carbon dioxide, and modified V-slope methods [13]. VT was evaluated as the point at which VE/VCO_2 was unchanged or decreased, VE/VO_2 increased, end-tidal fractional carbon dioxide concentration ($FETCO_2$) unchanged or decreased, and end-tidal fractional oxygen concentration ($FETO_2$) increased. A plot of the production of CO_2 over VO_2 use showed an increase in the gradient of the slope.

The blood sample for blood lactate measurement was obtained by auricular pricking and gentle squeezing of the ear lobe measured using a blood lactate analyzer. Blood lactate levels were measured every minute during warm-up and incremental load tests. The blood lactate threshold (bLT) was determined using graphical plots [14]. The number of seconds before the bLT was reached was determined from the blood lactate curve. The onset of blood lactate accumulation (OBLA) was defined as the point during exercise when the blood lactate concentration reached 4 mM [14]. The exercise period, from start to end, was divided into three sections: 1) the period from the exercise start to LT "a" in ▶ **Fig. 2**); 2) from the LT to OBLA ("b" in ▶ **Fig. 2**); and 3) from the OBLA to exercise end ("c" in ▶ **Fig. 2**). To determine the speed of blood lactate increase at each interval, changes in blood lactate level (Δ blood lactate) were divided by exercise time as follows: increased blood lactate level = Δ blood lactate level/exercise time. In this study, tissue hemoglobin oxygen saturation (tissue oxygenation index, [TOI]) was calculated using the spatially resolved spectroscopy method [15]. The TOI obtained from the NIRO was assessed. The monitor consisted of light-sending and light-receiving probes. Near-infrared spectroscopy provides non-invasive, continuous measurement of the TOI by determining the ratio of oxidized to reduced hemoglobin in skeletal muscles during exercise [16]. A pair of probes was attached 4 cm apart on the skin over the vastus lateralis muscle in the distal third



▶ **Fig. 2** Representative graphs of blood lactate levels during exercise After OBLA, the speed of blood lactate increase was greater in patients with DD/ID allele (orange) than in those with II allele (green). (**a**) period from the start of exercise to LT; (**b**) period from LT to OBLA; (**c**) period from OBLA to the end of exercise. LT: lactate threshold; OBLA: onset blood lactate accumulation.

▶ **Table 1** Characteristics of participants (mean ± standard deviation).

Characteristics		
Height (cm)		172.8 ± 4.5
Weight (kg)		65.7 ± 7.1
Age (years)		20.8 ± 1.3
Athlete career (years)		4.6 ± 3.6
Skeletal muscle mass (kg)		52.1 ± 4.6
Lower extremity muscle mass (kg)		8.9 ± 0.8
Body fat percentage (%)		15.3 ± 6,3
Peak VO_2 (mL/min/kg)	NC	54.5 ± 6.5
	HC	48.1 ± 8.1
	NC-HC (Δ peak VO_2)	− 6.4 ± 5.1

NC: normoxic condition, HC: hypoxic condition, VO_2: oxygen uptake. Δ peak VO_2 = (peak VO_2 in hypoxia) – (peak VO_2 in normoxia).

of the thigh [17], covered and secured with tape (SELF ADHESIVE TAPE, Lindo Sports Co., Ltd., Moriguchi, Japan) [18].

Statistical analysis

All data are reported using mean and standard deviation or number (percentage). A paired t-test was used to compare the differences between NC and HC. An unpaired t-test was used to compare differences between the randomized groups. The difference in peak VO_2 between HC and NC groups was defined as Δ peak VO_2 (= [peak VO_2 in HC] – [peak VO_2 in NC]). Differences in Δ peak VO_2 corresponding to the genetic polymorphisms involved in exercise tolerance were tested using unpaired t-test. Pearson's correlation analysis was used to determine the relationship between speed of blood lactate increase in the third interval and Δ peak VO_2. Statistical significance was set at $p < 0.05$. The analyses were performed using SPSS software (version 28, IBM Japan Ltd., Tokyo, Japan). Additionally, a post-hoc power analysis was performed using G*Power software (version 3.1.9.7, Heinrich-Heine-Universität Düsseldorf, Germany) to determine the power of the study, using the Δ peak VO_2 variable, which showed significant differences in relation to the genetic polymorphisms involved in exercise tolerance, for the power calculation.

Results

▶ **Table 1** presents the physical characteristics of the participants. The mean height, weight, age, skeletal muscle mass, lower extremity muscle mass, body fat percentage, and peak VO_2 of the participants were 172.8 ± 4.5 cm; 65.7 ± 7.1 kg; 20.8 ± 1.3 years; 52.1 ± 4.6 kg; 8.9 ± 0.8 kg, 15.3 ± 6.3 %, and 54.5 ± 6.5 mL/min/kg, respectively. All participants completed the incremental stress test under both conditions. No significant differences were observed in the first test between participants who exercised under NC and HC (**Supplementary Table 1**). Exercise under NC and HC occurred under similar temperatures (26.4 ± 1.1 vs. 27.0 ± 1.4 °C) and humidity levels (49.5 ± 5.2 % vs. 50.8 ± 4.3 %).

▶ **Table 2** lists the differences in respiratory gas exchange during exercise between both conditions. No significant differences were observed in the respiratory gas exchange at rest. Respiratory rate (mean difference, 1.5 times, 95 % confidence interval [95 % CI], 0.2–2.8, d = 0.5, $p < 0.01$) and HR (mean difference, 41.7 bpm, 95 % CI, 36.6–46.7, d = 3.8, $p < 0.01$) were significantly higher in the HC, during warm-up. VO_2 (mean difference, − 4.3 mL/min/kg, 95 % CI, − 6.5 to − 2.0, d = 0.8, $p < 0.01$) and VCO_2 at VT (mean difference, − 310.2 mL, 95 % CI, − 480.8 to − 139.6, d = 0.8, $p < 0.01$) were significantly lower in HCs. Peak VO_2 (mean difference, − 6.4 mL/min/kg, 95 % CI, − 8.6 to − 4.1, d = 1.2, $p < 0.01$) and VCO_2 (mean difference, − 491.3 mL, 95 % CI, − 731.2 to − 231.4, d = 0.9, $p < 0.01$) were significantly lower at the exercise end point in HCs. However, decreased peak VO_2 varied widely (Δ peak VO_2: − 6.4 ± 5.1 mL/min/kg [minimum: 0.7, maximum: − 18.9]).

Among the 16 analyzed genetic polymorphisms, participants who are homozygous carriers of the insertion genotype in the ACE (ACE-II) had significantly lower Δ peak VO_2 compared to those with ACE-DD/ID genotypes (mean difference = − 4.8 mL/min/kg, 95 % CI, − 9.56 to − 0.09, d = 1.0, p = 0.02; ▶ **Fig. 3**). None of the other genetic polymorphisms showed significant differences in Δ peak VO_2 (Supplementary material; ▶ **Fig. 1** and ▶ **Table 2**).

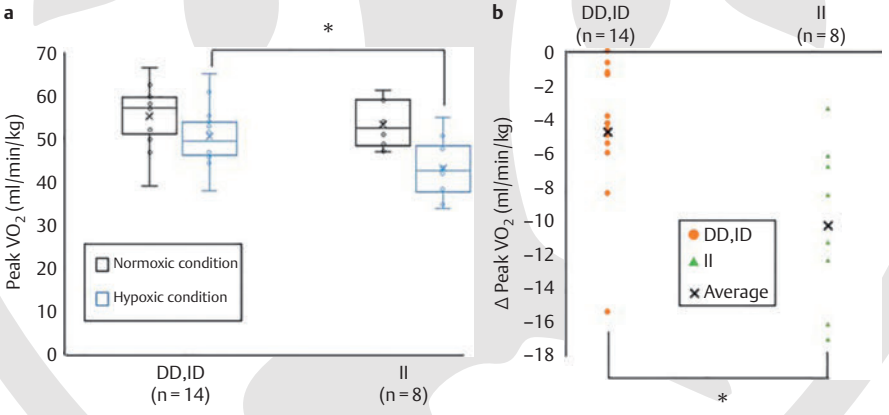
In HCs, athletes with ACE-II genotypes had a lower peak VO_2 (mean difference = − 6.5 mL min/kg, 95 % CI, − 13.67 to − 0.61, d = 0.8, p = 0.04), lower blood lactate level (NC: mean difference = − 3.6, 95 % CI = − 6.19 to − 1.01, d = 1.3, p = 0.01; HC: mean difference = − 3.49, 95 % CI = − 6.27 to − 0.71, d = 1.2, p = 0.02), and lower change in blood lactate levels between rest and peak exercise (NC: mean difference = − 3.7, 95 % CI, − 6.38 to − 0.92, d = 1.3, p = 0.01; HC: mean difference = − 3.1, 95 % CI, − 5.93 to − 0.34, d = 0.9, p = 0.03) compared to those with ACE-DD/ID genotypes. No significant differences were observed in muscle oxygenation during exercise and knee joint extension torque (▶ **Table 3**).

The amount of increase in lactate after OBLA was lower in athletes with *ACE*-II genotypes (NC: mean difference = − 3.6, 95 % CI, − 6.09 to − 1.11, d = 1.3, $p < 0.01$; HC: mean difference = − 3.5, 95 % CI, − 6.17 to − 0.83, d = 1.1, p = 0.02). The speed of blood lactate increase after OBLA was lower in athletes with *ACE*-II genotypes (NC: mean difference = − 1.0, 95 % CI, − 2.16 to − 0.14, d = 0.8, p = 0.04;

▶ **Table 2** Respiratory gas exchange data and physiological indices of participants by normoxic and hypoxic conditions.

	Rest		WU		VT		Peak	
	NC	**HC**	**NC**	**HC**	**NC**	**HC**	**NC**	**HC**
VO_2 (mL/min/kg)								
Mean	5.7	6.0	23.7	24.1	38.5	**34.3***	54.5	**48.1***
SD	1.0	1.1	3.0	2.9	6.1	5.7	6.5	8.1
VCO_2 (mL/min)								
Mean	370.5	387.4	1547.8	1574.4	2538.7	**2220.3***	3618.2	**3134.2***
SD	63.3	60.1	168.4	137.1	435.5	316.3	409.4	458.8
VE (l/min)								
Mean	12.8	13.9	40.7	44.0	63.9	62.7	154.2	153.3
SD	1.6	1.9	4.7	7.9	13.0	9.7	29.9	25.6
RQ								
Mean	0.90	0.90	0.90	0.91	0.93	0.92	1.22	1.21
SD	0.09	0.09	0.07	0.07	0.05	0.06	0.11	0.13
RR (time)								
Mean	17.5	18.6	27.4	**29.0***	31.6	31.7	63.1	65.1
SD	4.2	2.9	5.2	4.3	6.5	5.3	8.7	12.2
HR (bpm)								
Mean	79.5	83.3	115.1	**125.2***	150.9	149.0	192.1	189.9
SD	16.9	17.1	17.2	15.6	9.0	13.9	9.5	6.8
TOI (%)								
Mean	69.5	67.8	66.9	**62.2***	60.3	58.6	56.6	**55.4***
SD	5.3	7.0	4.6	9.1	6.7	8.6	8.0	8.9
SpO_2 (%)								
Mean	97.8	**94.3***	95.0	**89.6***	93.8	**86.2***	87.0	**82.1***
SD	1.4	1.8	3.6	3.3	2.7	4.1	14.2	4.9

*$p < 0.05$, compared with normoxic conditions. WU: 5-min warm-up; VT: ventilator threshold; NC: normoxic condition; HC: hypoxic condition; VO_2: Oxygen Uptake; VCO_2: carbon dioxide production; VE: expiratory ventilation; RQ: respiratory quotient; RR: respiratory rate; HR: heart rate; TOI: tissue oxygen index; SpO_2; oxygen saturation of peripheral artery.



▶ **Fig. 3** Difference in peak VO_2 between participants with ACE-DD/-ID and ACE-II. (**a**) Peak VO_2 under normoxia and hypoxia in participants with ACE-DD/-ID and ACE-II. (**b**) A decrease in peak VO_2 in hypoxic exercise (Δ peak VO_2) in participants with ACE-DD/-ID and ACE-II. *: $p < 0.05$. VO_2: oxygen uptake; Δ peak VO_2 = (peak VO_2 in hypoxia) – (peak VO_2 in normoxia).

▶ **Table 3** Physiological data during exercise according to the ACE genotype (mean ± standard deviation).

		ACE-DD/ID (n = 14)	ACE-II (n = 8)	p-value
VO_2 at VT (ml/min/kg)	NC	38.8 ± 6.3	37.0 ± 4.5	0.45
	HC	36.1 ± 5.8	34.5 ± 4.1	0.49
	HC-NC	− 2.7 ± 3.2	− 2.5 ± 2.6	0.82
Exercise time (sec) at VT (sec)	NC	763.1 ± 106.7	715.6 ± 95.3	0.38
	HC	698.2 ± 101.2	674.5 ± 93.5	0.64
	HC-NC	− 64.9 ± 45.8	− 41.1 ± 32.7	0.28
WR at VT (watt)	NC	188.6 ± 27.5	178.1 ± 24.2	0.33
	HC	160.4 ± 25.3	151.3 ± 23.1	0.42
	HC-NC	− 28.2 ± 12.7	− 26.8 ± 11.3	0.75
Peak VO_2 (ml/min/kg)	NC	55 .1 ± 7.0	53.4 ± 5.8	0.55
	HC	50.6 ± 7.0	43.2 ± 7.5	**0.04***
	HC-NC	− 4.6 ± 4.0	− 10.2 ± 4.8	**0.02***
Endurance time (sec)	NC	1162.7 ± 168.3	1164.3 ± 165.1	0.98
	HC	1088.5 ± 134.6	1095.1 ± 89.0	0.89
	HC-NC	− 74.2 ± 54.4	− 69.1 ± 90.2	0.89
Peak WR (watt)	NC	334.3 ± 56.3	335.0 ± 38.2	0.97
	HC	315.7 ± 47.2	317.5 ± 22.5	0.91
	HC-NC	− 20.0 ± 15.7	− 15.0 ± 20.7	0.28
ΔTOI (%)	NC	− 18.6 ± 10.9	− 15.8 ± 9.2	0.97
	HC	− 21.8 ± 9	− 20.7 ± 7.9	0.91
	HC-NC	− 3.2 ± 10.3	− 4.9 ± 10.0	0.28
ΔSpO_2 (%)	NC	− 7.3 ± 3.9	− 7.5 ± 4.6	0.95
	HC	− 13.3 ± 4.9	− 14.5 ± 5.9	0.73
	HC-NC	− 6.0 ± 3.4	− 7.0 ± 4.0	0.67
ΔHR (bpm)	NC	112.6 ± 15.9	115.4 ± 13.0	0.67
	HC	103.1 ± 13.5	112.0 ± 11.9	0.13
	HC-NC	− 9.6 ± 16.6	− 3.4 ± 6.1	0.22
Lactate at the baseline (mM)	NC	1.7 ± 0.3	1.8 ± 0.5	0.78
	HC	2.1 ± 0.5	1.8 ± 0.3	0.06
Peak lactate (mM)	NC	11.9 ± 3.0	8.3 ± 2.6	**0.01***
	HC	13.1 ± 3.7	9.6 ± 2.3	**0.02***
Δ lactate (mM)	NC	10.2 ± 2.8	6.5 ± 2.8	**0.01***
	HC	11.0 ± 3.8	7.8 ± 2.2	**0.03***
	HC-NC	0.8 ± 2.7	1.3 ± 2.7	0.66
Lower extremity muscle (kg)		9.0 ± 1.0	8.9 ± 0.5	0.93
Torque (N*lower extremity length/kg)		254.7 ± 48.6	226.4 ± 24.1	0.09

*$p < 0.05$; NC: normoxic condition; HC: hypoxic condition; VO_2: oxygen uptake; WR: work ratio; TOI: tissue oxygen index; SpO_2: oxygen saturation of peripheral artery; HR: heart rate. Δ TOI = (peak TOI) – (baseline TOI); ΔSpO_2 = (peak SpO_2) – (baseline SpO_2); ΔHR = (peak HR) – (baseline HR); Δ lactate = (peak lactate level) – (baseline lactate level).

HC: mean difference = − 1.7, 95 % CI, − 3.12 to − 0.18, d = 0.9, p = 0.03) (▶ **Table 4** and ▶ **Fig. 2**). In addition, athletes with *ACE*-II genotypes showed a trend towards spending longer time cycling above OBLA intensity in HCs (mean difference = 71.0 s, 95 % CI, − 4.73 to 146.73, d = 0.9, p = 0.06). No significant differences were observed in the rate of blood lactate increase from the start to bLT or from bLT to OBLA. A moderate correlation was observed between speed of blood lactate increase after OBLA and Δ peak VO_2 (r = 0.48, p = 0.03), and nearly all participants with ACE-DD/ID genotypes were included in the plotted category with the low Δ peak VO_2 and high magnitude of lactate accumulation during hypoxia (▶ **Fig. 4**, orange dotted circle).

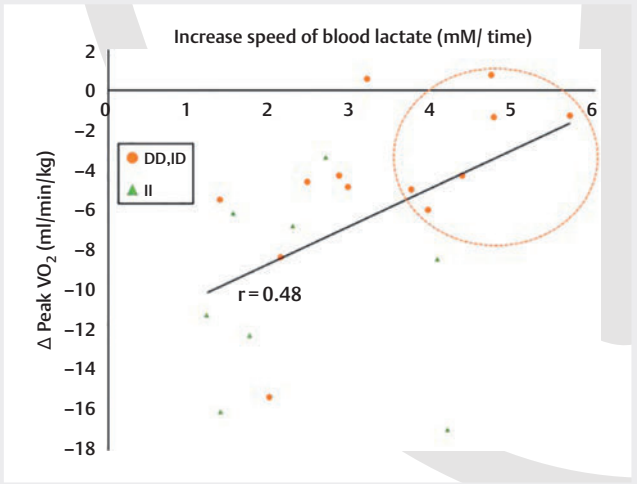
Discussion

In the present study, a progressive exercise stress test was performed until the symptomatic limit was reached, and the results showed normal physiological responses under HCs, such as a decrease in peak VO_2 and work ratio [19, 20]. This prospective study

▶ **Table 4** Physiological data during each exercise interval according to the ACE genotype (mean ± standard deviation).

		ACE-DD/ ID (n = 14)	ACE-II (n = 8)	p-value
The first interval shown in ▶ **Fig. 2a**: the period from the exercise start to LT				
Interval time in seconds	NC	836.3 ± 128.2	856.3 ± 143.9	0,75
	HC	741.9 ± 105.2	758.8 ± 81.2	0.69
	HC-NC	−94.4 ± 76.4	−95.6 ± 108.5	0.98
Increase in lactate (Δ Lactate) in mM	NC	1.0 ± 0.5	0.8 ± 0.5	0.35
	HC	0.9 ± 0.8	1.1 ± 0.5	0.39
	HC-NC	−1.0 ± 0.9	0.7 ± 2.0	0.06
Speed of blood lactate increase in mM/sec	NC	0.1 ± 0.1	0.1 ± 0.1	0.19
	HC	0.1 ± 0.1	0.1 ± 0.1	0.21
	HC-NC	−0.2 ± 0.3	0.1 ± 0.7	0.25
The second interval shown in ▶ **Fig. 2b**: the period from LT to OBLA				
Interval time in seconds	NC	62.0 ± 78.5	125.4 ± 63.3	0.07
	HC	92.7 ± 101.4	90.0 ± 89.6	0.95
	HC-NC	30.9 ± 90.5	−35.4 ± 93.2	0.12
Increase in lactate (Δ Lactate) in mM	NC	1.3 ± 0.5	1.4 ± 0.5	0.43
	HC	1.0 ± 0.6	1.1 ± 0.6	0.74
	HC-NC	0.1 ± 2.5	−0.7 ± 1.3	0.34
Speed of blood lactate increase in mM/sec	NC	2.1 ± 1.7	1.3 ± 0.5	0.13
	HC	1.2 ± 0.8	1.3 ± 1.2	0.72
	HC-NC	0.07 ± 0.8	0.5 ± 1.3	0.32
The third interval shown in ▶ **Fig. 2c**: the period from OBLA to exercise end				
Interval time in seconds	NC	251.0 ± 106,7	182.6 ± 65.7	0.09
	HC	243.8 ± 86.5	246.3 ± 54.2	0.93
	HC-NC	−7.2 ± 73.2	63.8 ± 86.5	0.06
Increase in lactate (Δ Lactate) in mM	NC	7.9 ± 3.0	4.3 ± 2.5	**< 0.01***
	HC	9.1 ± 3.7	5.6 ± 2.3	**0.02***
	HC-NC	0.4 ± 3.0	0.9 ± 2.3	0.63
Speed of blood lactate increase in mM/sec	NC	3.4 ± 1.3	2.4 ± 1.2	**0.04***
	HC	4.0 ± 2.3	2.3 ± 0.8	**0.03***
	HC-NC	0.2 ± 1.3	0.2 ± 0.8	0.97

*p < 0.05; NC: normoxic condition; HC: hypoxic condition; OBLA: onset of blood lactate; Δ lactate: increase in lactate in each of the sections.



▶ **Fig. 4** Scatter plot of decreased peak VO_2 in hypoxic exercise condition (Δ peak VO_2) and speed of blood lactate increase after OBLA. VO_2: oxygen uptake; OBLA: onset blood lactate accumulation.

suggests that among the various genetic polymorphisms contributing to exercise tolerance, the ACE gene may significantly influence the reduction in peak VO_2 in hypoxic environments. Athletes who are homozygous carriers of the insertion genotype in the gene for ACE (ACE-II) genotypes had significantly lower lactate concentration during an incremental exercise test and a large decrease in peak VO_2 under hypoxia compared to those with ACE-ID/ACE-DD genotypes. These findings suggest that individuals with ACE-II genotypes primarily generate energy through oxidative phosphorylation in the mitochondria and are highly sensitive to exercise under hypoxia. They experienced a significant reduction in peak VO_2, although this did not correspond to significant changes in peak work ratio. ACE-II genotypes showed poorer increases in lactate towards the end of exercise after OBLA and a slower rate of lactate increase in both NC and HC compared to ACE-ID/-DD genotypes.

Additionally, ACE-II genotypes showed a trend towards longer exercise times after OBLA in HC compared to NC and to ACE-ID/-DD genotypes. These results suggest that 'wastage' and 'utiliza-

tion' of lactate are higher in ACE-II genotypes, particularly after OBLA. Furthermore, this anaerobic compensation mechanism was enhanced in HCs, preventing a reduction in peak work ratio.

ACE is an important enzyme in the control of blood flow and blood glucose availability to the muscles [21–23]. ACE is an important enzyme in RAS and is widely distributed in various tissues, including skeletal muscle, where it degrades bradykinin and converts from angiotensin I to angiotensin II [21–23]. Angiotensin II has vasoconstrictive capabilities and may significantly influence muscle perfusion during contraction. ACE-I/D genotypes are associated with endurance performance via the modulation of ACE activity [23, 24]. The ACE-I/D genotypes are distinguished by the insertion (I-allele) or deletion (D-allele) of a silencer sequence within intron 16 of the ACE gene. The function attributed to the I allele is to attenuate ACE activity in serum and tissues through a decline in ACE protein expression [24], resulting in a reduction of angiotensin II production capacity. Vasoconstriction induced by angiotensin II may be inhibited, leading to augmented muscle perfusion during muscle contraction and enhanced provision of oxygen and substrates. Blood glucose uptake is heightened by the ACE I allele and correlates with a lower incidence of diabetes [25, 26]. Abundant substrate and oxygen supply leads to characteristics similar to those occurring in slow-twitch muscle fibers, such as increased capillary formation, denser mitochondrial volume, and increased intracellular lipids [27]. In this study, oxygen status was assessed using near-infrared spectroscopy and SpO_2 monitoring. The results demonstrated no significant differences in TOI and SpO_2 reduction under NC or HC, irrespective of the ACE genotype. Although the ACE II genotypes were associated with reduced peak VO_2 under acute HCs, no variations in peripheral and muscle oxygenation were observed among genetic variants. Thus, ACE II genotypes did not produce a negative response in skeletal muscle oxygenation, even when peak VO_2 was reduced under hypoxia.

Furthermore, they showed a relatively modest increase in blood lactate levels compared to those with the ACE-ID/-DD genotypes. These findings suggest that energy production depends on oxidative phosphorylation in the mitochondria during exercise and is characteristic of the slow-twitch muscles. Stress in a hypoxic environment can be an important trigger of mechanical, metabolic, and biochemical changes in the skeletal muscle [28]. Training under hypoxia is expected to enhance the hypoxic responsiveness of the skeletal muscle and enhance its ability to adapt to HCs during exercise. In other words, the ACE II genotypes were expected to benefit from hypoxic training.

Conversely, athletes with the D allele showed limited or no decrease in peak VO_2 even under HCs, in addition to a drastic increase in blood lactate concentration after OBLA. Previous studies reported that athletes with the ACE-DD genotypes tend to have increased blood lactate concentrations during exercise [29–31]. A possible explanation is that individuals with the D allele show increased angiotensin II activity, resulting in decreased peripheral blood flow and oxygen supply during exercise [30, 31]. Consequently, in athletes with the D allele, skeletal muscle becomes more regularly prone to exercise-induced hypoxia, and increasing the relative contribution of anaerobic metabolism in energy production is essential, rather than relying on mitochondrial metabolism. Therefore, athletes with the D allele are unlikely to exhibit additive adaptive responses in the skeletal muscle when exposed to HCs, suggesting the limited effectiveness of hypoxic training.

Hypoxic training is currently receiving increasing attention and widespread acceptance in endurance sports. Despite performance at sea level being better with hypoxic training, high altitude training has negative health effects such as the risk of altitude sickness and problems related to the cost of travel [32]. Therefore, alternatives to high-altitude training, such as fitness centers and comparable facilities that provide substantial isobaric hypoxia are expanding in urban areas [32]. However, hypoxic training requires careful consideration because of its potentially limited effectiveness, differences in effectiveness based on physical characteristics and types of sports, and adaptation to hypoxic environments. To promote the effective use of hypoxic training, original programs tailored to each athlete, including genetic analysis, should be developed. This may contribute to finding other genetic polymorphisms that might have an effect on hypoxic training such as ACE.

Limitations

This study has certain limitations. First, a post hoc power analysis using G*Power indicated that the power of the study was 0.82, exceeding the threshold of 0.80. The sample size required to achieve 80 % power was 26 individuals, implying that the sample size in this study was smaller than that required. A smaller sample size increases the likelihood of type-II errors and significance of the results could be due to false positives or type-I errors. Based on the exploratory findings of this study, further investigation with a substantial number of participants is warranted. Second, the study was limited to college students, resulting in a concentration of participants with similar levels of competition. Future studies should include a broader range of athletic levels to facilitate a more comprehensive analysis. Third, our results were limited to male participants. Sex differences should also be considered in future studies. Fourth, although the temporal assessment of arterial oxygen saturation was a crucial element, it was not assessed due to the invasive nature of frequent blood gas sampling during exercise.

Practical applications

The present study, to our knowledge, is the first validation of an attempt to compare genetic responses in the incremental load test conducted under HCs. However, the exact involvement of genetic polymorphisms in endurance-related training effects is unclear from the response to hypoxia observed in the present study alone. Future investigations are therefore essential to examine the possible additive effects of hypoxia training due to differences in ACE genotypes.

Conclusion

Athletes with ACE-II had significantly lower lactate concentration during an incremental exercise test and a large decrease in peak VO_2 under hypoxia compared to those with ACE-ID/-DD genotypes. To promote the effective use of hypoxic training, original programs tailored to each participant, including genetic analysis, should be developed.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Supplementary material

Online Supplemental Table1: Comparison of exercise capacity of participants first tested under normoxia vs. hypoxia

	Normoxia first (n=11)	Hypoxia first (n=11)	Mean difference	Cohen's d	p-value
Peak VO_2/W (ml/min/kg)					
NC	53.8±7.1	55.1±6.1	-1.3	-0.20	0.65
HC	46.6±8.0	48.5±8.6	-1.9	-0.21	0.60
VO_2/W at VT ml/min/kg)					
NC	41.2±7.9	41.0±5.5	-0.8	-0.11	0.79
HC	37.3±5.9	37.2±4.8	1.1	0.21	0.96

Normoxia first: The first group underwent the incremental load test in normoxia; hypoxia first: The first group underwent the incremental load test in hypoxia.

Peak VO_2/W: peak oxygen uptake / weight; NC: normoxic condition; HC: hypoxic condition; VT: ventilation threshold

Supplementary material

Online supplemental Table2: Comparison of all endurance related-genes to Δ Peak VO_2

Gene (n)	Ave	SD	Mean difference	Cohen's d	p-value
ACE					
DD, ID (14)	-4.6	4.0	-5.6	1.25	**0.02***
ACE II (8)	-10.2	4.8			
DD (4)	-3.6	2.8	-3.7	0.72	0.08
ID, II (18)	-7.3	5.2			
ACTN					
XX (6)	-4.2	3.6	-3.9	0.65	0.14
XR, RR (16)	-8.1	5.7			
PPATGC1					
AA (5)	-9.9	3.6	-4.3	0.89	0.10
AG, GG (17)	-5.6	5.1			
AA, AG (16)	-7.6	5.6	-3.6	0.73	0.14
GG (6)	-4.0	1.5			
GABPB1					
AA (12)	-7.7	4.9	-2.3	0.44	0.30
AG, GG (10)	-5.4	5.2			
AGTR					
CC (12)	-5.5	5.1	-3.0	0.63	0.20
AA (7)	-8.5	4.0			
NRF					
AA (15)	-6.7	5.0	-1.0	0.18	0.71
GG (6)	-5.7	5.6			
TMPRSS6					
TT (12)	-6.9	5.3	-0.7	0.14	0.75
TC, CC (10)	-6.2	5.0			
ADRB2					
AA (8)	-5.4	5.6	-1.7	0.32	0.50

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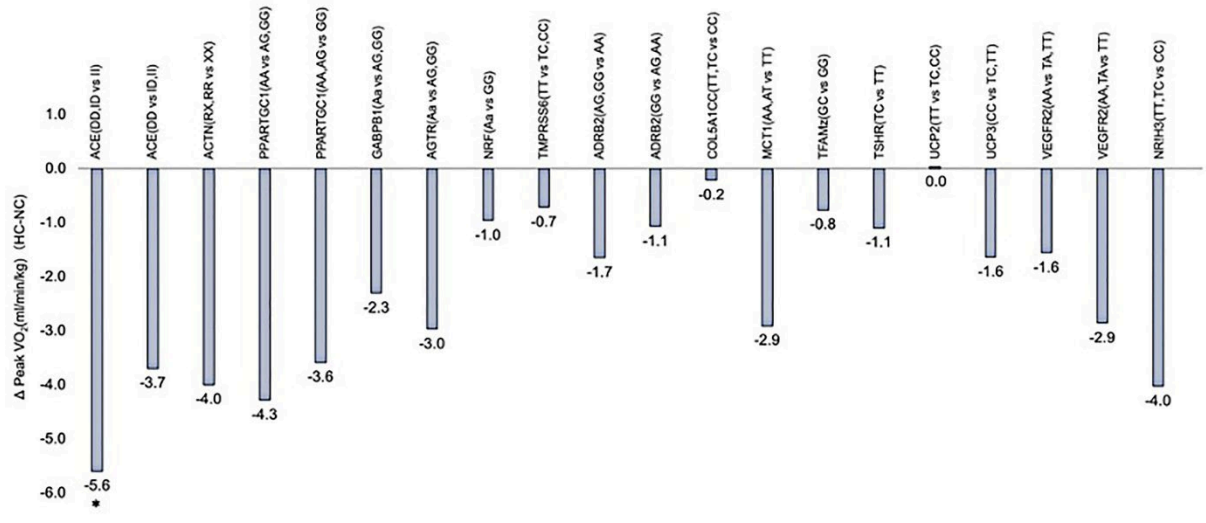
AG, GG (13)	-7.0	4.8			
AA, AG (13)	-6.0	4.7	-1.1	0.21	0.67
GG (8)	-7.1	5.8			
COL5A1					
CC (13)	-5.6	4.4	-0.2	0.04	0.94
TC, TT (4)	-5.8	5.2			
MCT1					
AA (11)	-5.0	3.4	-2.9	0.56	0.21
AT, TT (10)	-7.9	6.3			
TFAMz					
GG (17)	-6.4	4.8	-0.8	0.15	0.82
GC (5)	-7.2	6.6			
TSHR					
TT (17)	-6.2	4.8	-1.1	0.21	0.78
TC (4)	-7.3	7.0			
UCP2					
CC, TC (16)	-6.6	4.8	0.0	0.01	0.99
TT (6)	-6.6	6.2			
UCP3					
CC (8)	-7.7	4.8	-1.6	0.32	0.47
TC, TT (14)	-6.0	5.3			
VEGFR2					
AA (7)	-7.7	5.0	-1.6	0.30	0.26
TA, TT (15)	-6.1	5.2			
AA, TA (17)	-7.3	5.4	-2.9	0.57	0.28
TT (5)	-4.4	2.9			
NR1H3					
TT, TC (14)	-7.7	5.4	-4.0	0.84	0.09
CC (7)	-3.7	3.2			

Supplementary material

* $p < 0.05$

Supplemental Figure 1



supplemental Figure.1 Mean difference in Δ Peak VO2 between pairs of endurance-related genes

Un-paired t-test used for compared all genes could compare in both groups. The result, significant differences were found only for compared between ACE DD, ID and II.

* $p < 0.05$

Δ Peak VO2/kg: Amount of change in VO2 by hypoxia, HC: Hypoxic condition, NC: Normoxic condition, Angiotensin-converting; ACE, actinin alpha 3;ACTN3, peroxisome proliferator-activated receptor gamma coactivator 1-alpha;PGC1-a, adrenoceptor beta2;ADRB2, angiotensin II receptor type 2;AGTR2, adenosine monophosphate deaminase;AMPD1, creatine kinase M-type;CKM, collagen type V alpha 1 chain;COL5A1, GA binding protein transcription factor subunit beta1;GABPB1, glutathione S-transferase P1;GSTP1, homeostatic iron regulator, hypoxia-inducible factor 1 subunit alpha;HIF1a, monocarboxylate transporter;MCT1, nitric oxide synthase 3;NOS3, peroxisome proliferator-activated receptor alpha; Para, transcription factor A, mitochondrial;TFAM, uncoupling protein 2;UCP2, uncoupling protein 3;UCP3, vascular endothelial growth factor receptor 2;VEGFR2, guanine nucleotide-binding protein;GNB3, and nuclear receptor subfamily 1 group H member 3;NR1H3.