Research article

The roles of ACE I/D and *ACTN3* R577X gene variants in heat acclimation

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A B S T R A C T

Roles of genes in heat acclimation (HA, repeated exercise-heat exposures) had not been explored. ACE I/D and ACTN3 R577X genetic polymorphisms are closely associated with outstanding exercise performances. This study investigated whether the two polymorphisms influenced the response to HA. Fifty young Han nationality male subjects were selected and conducted HA for 2 weeks. Exercise indicators (5-km run, push-up and 100-m run) were tested and rest aural thermometry (RTau) was measured before and after HA. ACE gene was grouped by I homozygote and D carrier, and ACTN3 gene was grouped by R homozygote and X carrier. Results showed that there were no differences between groups in age, body mass index, exercise indicators and RTau before HA. After HA, RTau of ACE I homozygote was lower than that of D carrier [$F_{(1, 48)} = 9.12$, $p = 0.004$, $\eta = 0.40$]. Compared with RTau before HA, that of I homozygote decreased after HA ($\Delta = -0.26\,^\circ\text{C}$, 95 % CI -0.34–0.18, $p < 0.001$), while that of D carrier did not change. There was a ACE gene × HA interaction in RTau [$F_{(1, 48)} = 14.26$, $p < 0.001$, $\eta = 0.48$]. No effect of ACTN3 gene on RTau was observed. For exercise indicators, there were no differences between groups after HA, and no gene × HA interactions were observed. There may be a strong interaction of ACE gene and HA in the change of rest core temperature. I homozygote may have an advantage on improving heat tolerance.

1. Introduction

Environmental temperature raise caused by global warming is a hot issue that attracts worldwide attention. Global warming makes it more common for human beings to face high-temperature, and also makes original hot environment worse, which may bring more severe physical challenges to individuals in it. In some special occupations and situations (such as military operations and sports competitions), it is particularly important to overcome the adverse effects of high-temperature on human body. Over the last two decades, many combat operations were carried out in harsh environments such as high-temperature. Some high-level sports events (such as the Summer Olympics, the FIFA world cup and 2020 Tokyo Olympics) are often held in summer or hot spots. When performing military operations and sports competitions in high-temperature environment, individuals not only have to face the high-temperature of external environment, but also deal with the high heat caused by high-load exercise in their bodies (physical work in occupation and sport settings could increase metabolic heat production by > 10-fold [1]). In military operations, individuals often have to wear combat gear and carry a lot of equipments, which further aggravates the thermal load of the body. The synthetic influences of the

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internal and external heat loads not only could significantly impair physical performance, but also bring serious health problems (such as lethal exertional heat stroke). Exercise (especially endurance) performance is very sensitive to the increase of temperature. Environment temperatures >25 °C will decrease running performance at distances ≥800-m [2], with greater decrements when individuals are dehydrated [3]. Military personnel or athletes need to exert the best physical level to ensure excellent completions of military or competition tasks. In those cases, it is very important to reduce the influence of high-temperature, even if the reduction is small, for optimizing physical performance.

Heat acclimation (HA, here it refers to repeated exercise-heat exposures in artificial or natural environment) is one of the most important and effective strategies to reduce heat stress, improve heat tolerance and optimize physical performance in hot environment [4–6]. HA can cause a series of thermal adaptability changes in human body, such as lowering rest core body temperature [7,8], increasing plasma volume [7], increasing skin blood flow and sweating rate, and reducing exercise heart rate [7,9]. Those changes can reduce or stabilize the core temperature during exercise in hot environment, thus effectively reduce heat damage and significantly improve exercise ability [10–12]. However, the researches on soldiers, workers, athletes, experimental volunteers and other different types of individuals show that there is a great inter-individual variability in the responses to HA [13–15]. For example, physiologically, the commonly observed decrease of core temperature following HA occurs in some individuals, but not all [16,17]. Exercise performances in hot environment are also highly individual after HA [4,18]. The HA durations of different individuals to produce significant effect also vary greatly, ranging from one week to one month [5]. Identifying the potential factors associated with those differences is of great practical significance for improving HA effect accurately, efficiently and scientifically. The great inter-individual variability in HA responses implies genetic factors may influence HA effect.

So far, understanding for the genetic mechanism of heat adaptation in humans is very limited. A recent study [19] demonstrated an association between heat shock protein A1B gene and heat tolerance (an important indicator assessing heat adaptation capacity or HA effects). To the best of the author' knowledge, no study on the roles of genes in HA had been reported. In human exercise performance, there are two gene variants that attract very great attentions: angiotensin-converting enzyme (ACE) I/D (rs4340, https://www.ncbi.nlm.nih.gov/gene/1636) and α-actinin-3 (ACTN3) R577X (rs1815739, https://www.ncbi.nlm.nih.gov/snp/rs1815739) polymorphisms. The two variants are both common in general population of different ethnicities. For I/D polymorphism, a large amount of evidence shows that it has extensive effects on human body, and is associated with a series of diseases, including hypertension, cardiovascular disease, kidney disease, cancer, type 2 diabetes, obesity and coronavirus disease 2019 (COVID-19). In exercise, ACE I homozygote is closely related to outstanding endurance performance, and is associated with elite performances in a range of endurance
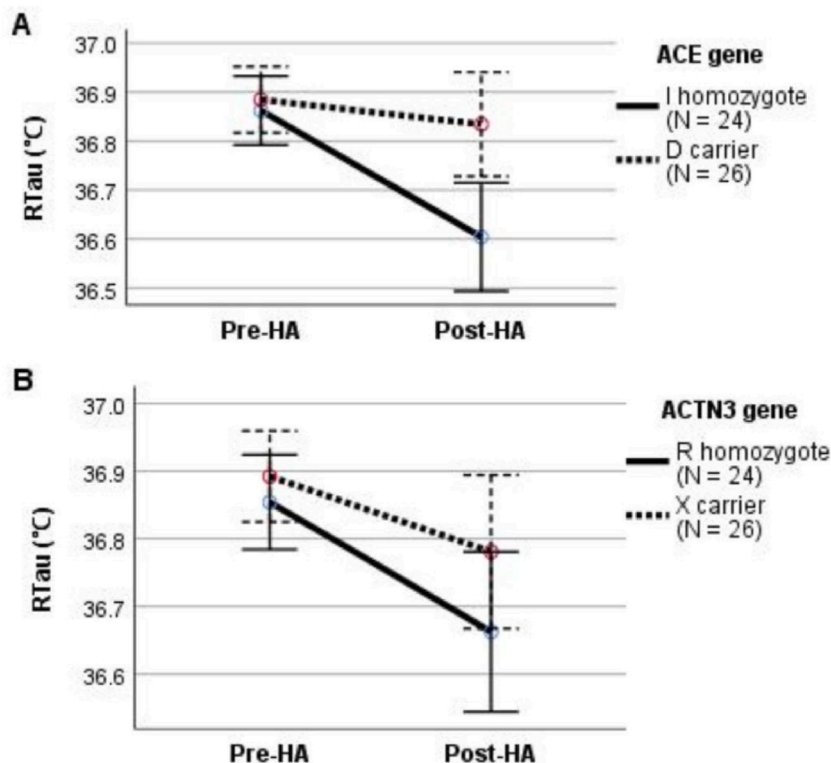
Fig. 1. For ACE gene (A), compared with this of pre-HA, post-HA RTau of I homozygote decreased ($\Delta = -0.26$ °C, 95 % CI -0.34–0.18, $p < 0.001$), but the change of D carrier was not significant ($p = 0.20$). The ACE gene × HA interaction was significant [$F_{(1, 48)} = 14.26$, $p < 0.001$, $\eta = 0.48$]. For ACTN3 gene (B), the genotype within-group changes of RTau were both significant ($p < 0.001$ for R homozygote and $p = 0.01$ for X carrier), but no significance ($p = 0.20$) was observed in gene × HA interaction. RTau, rest aural thermometry. HA, heat acclimation.

sports [20–23]. ACTN3 gene is called "a gene for speed". Strong association of ACTN3 R homozygote with outstanding speed performance has been widely agreed [22,24–26]. Furthermore, R577X polymorphism may have a potentially wide-ranging influence on muscle function, and may be related to post-exercise recovery and sports injuries [24]. So far, ACE and ACTN3 genes are the least controversial in terms of "being considered as candidate genes for sports", and are considered to have the strongest associations with exercise capacity. Therefore, the two genes have been studied mostly when investigating the genetic mechanism of exercise ability. HA, as repeated exercise-heat exposures, is actually a process of exercise training in hot environment, which suggests that it may be influenced by genetic factors related to exercise performances. To the best of the author' knowledge, no study had reported the relationships between ACE and ACTN3 genes and heat tolerance, exercise ability in hot environment or HA response.

The aim of this study was to investigate the roles of ACE I/D and ACTN3 R577X genetic polymorphisms in the response to HA using a young Chinese Han nationality male cohort.

2. Subjects and methods

2.1. Subjects

50 subjects from a certain unit stationed in the tropics were selected. All individuals in the unit had a strict unified daily schedule in activities, rests, and meals. The inclusion criteria were: young Han nationality males; these who were the same batch of newcomers to the unit; before joining in the unit, these had not settled in tropical or cold region, had no experiences in regular high-temperature operations, had no experiences similar to HA the year before joining, and had no history of regular exercise training (except the physical preparation training, which was uniformly organized and performed at a non-tropics before entering the tropics); physical and mental indicators were normal (all individuals of the unit had undergone strict health examinations before and after joining in the unit). Those who had suffered from overheating-related diseases (such as heat shock and thermal spasm) in the past were excluded. Before the subjects were selected, it was especially emphasized that each participant must participate in this study voluntarily. Informed consent from all subjects was obtained prior to conducting this study. The study conformed to the Declaration of Helsinki for Human Research of 1974 (last modified in 2013) and was approved by the Scientific Research and Academic Department of Chinese People's Liberation Army Special Warfare School.

Age was self-report. Height and weight were measured in unified light clothing and without shoes, and body mass index (BMI) was calculated as weight divided by height squared (kg/m^2).

2.2. HA

Although HA can be carried out in artificial thermal environment, outdoor (natural thermal environment) HA is more beneficial to practical application because it is closer to the real environment [4,18]. HA was done outdoors in the present study. In order to widen the temperature gap between HA and tests (see "2.3. Data collections"), and also in consideration of the requirement of HA for the ambient temperature [27], the environment temperature during HA was controlled between 28.5–30.0 °C. All subjects wore unified T-shirt, shorts and exercise shoes during HA.

HA lasted for 2 weeks. The HA duration was referred to the previous studies involved in HA duration [5,7]. The subjects were trained 3 times (Monday, Wednesday, and Friday) a week. Training items of each time were: 100-m run (a trip), push-up (a set), and 3-km run (a trip). 100-m run and 3-km run were both completed in the shortest time as hard as possible for each trip, and push-up was conducted, according to the action standard [28], to achieve the maximum repetitions in 2 min for each set. Before HA, the subjects were randomly divided into 5 batches with 10 subjects each batch (see "2.3.1. Random batches"). Training arrangement each time sees "2.3.3. Exercise indicators tests", but the differences were: 5-km run was replaced by 3-km run, the interval between items was ~5 min for each batch, and no measurement was performed. The organizers and supervisors (from the unit) were both the same each time. All persons (also including all subjects) were blind to genotypes. Even if this study was not done, all subjects were faced with the problem of improving their exercise ability to meet the demands of their work, which better ensured that all subjects tried their best to train. The unit where the subjects were selected had a strict unified daily schedule in rests, activities, and meals, which better ensured the consistency of subjects' sleep, daily activities and nutrient supplies during HA. In order to ensure the consistency of heat exposures of all subjects, it was emphasized that outdoor activities (except at night) were only carried out under a unified organization (the daily activities of the unit were uniformly arranged, which better ensured that). After 2 weeks HA, all subjects reported that they were physically and mentally normal during HA.

2.3. Data collections

Before (pre-) and after (post-) HA, rest aural thermometry (RTau) measurements and exercise indicators (100-m run, push-up and 5-km run) tests were carried out. In order to make the subjects in a good state, the post-HA data collection was conducted 3 days after HA. The environment temperatures at the beginnings of two data collections for exercise indicators tests were both 38.0 °C (both at pm), relative humidity was slightly different (89 % before HA and 88 % after HA), and there both were no winds sensed. All subjects wore unified long sleeve, long trousers and exercise shoes in two data collections (different from HA). The organizers, supervisors and data collectors of two data collections were all the same, and they were familiar with their work procedures (also including measurement instruments) one day in advance. Measurement personnel (belong to data collectors) had rich measurement experiences. The organizers and supervisors (except the author) were different from those during HA. Data collectors were different from data analyzer.

All persons (also including all subjects) were blind to genotypes.

2.3.1. Random batches

Before data collections, the subjects were randomly divided into 10 batches with 5 subjects each batch (the batches were unchanged in two data collections). A blinded assistant was responsible for random allocation using SPSS version 26.0. The order of each batch was the same in all indicators during data collection.

2.3.2. RTau measurement

RTau was measured (unified left ear) one by one in each batch using an infrared ear thermometer (Braun IRT6520) following the manufacturer's instructions strictly after sitting quietly for 5 min. No intervals were arranged between batches in order to maximize time efficiency to reduce the influence of biological rhythm. RTau measurement was arranged at 8: 00 (am).

2.3.3. Exercise indicators tests

For each batch, the test order was: 100-m run, then push-up, and then 5-km run. The interval between exercise indicators was ~10 min for each batch, and slow walk for recovery was conducted during the interval. In 5-km run, supervisors recorded the lap number of each participant to ensure that the 5-km was completed and all participants were required to be in the same runway to the greatest extent after pulling away. Each subject was required to complete the test of each exercise indicator with all efforts. 100-m run, push-up, and 5-km run test methods were same to those (trip and set) of HA training (see "2.2. HA" and 5-km run referred to 3-km run). 100-m run and 5-km run were timed using a stopwatch, respectively, and the repetitions of push-up were recorded.

2.4. Genotyping

Buccal epithelium was collected using buccal swab. DNA isolation was conducted using the Genomic DNA Extraction Kit (Nano-MagBio, Wuhan, China) according to the manufacturer's instructions. All Primers were synthesized with Primer Premier 5 by BGI (Beijing, China). Primers for ACE were F: 5′- CTGGAGACCACTCCCATCCTTTCT-3′ and R: 5′-GATGTGGCCATCACATTCGTCAGAT-3′. Primers for ACTN3 were F: 5′-CTGCTGCCCTTTCTGTTG-3′ and R: 5′-CATATCCCACTTGGTGTTGAT-3′. PCR (conventional) amplification [96 °C for 5min → (96 °C for 20s → 62 °C for 30s → 72 °C for 30s) × 10 cycles (−1 °C/cycle) → (96 °C for 20s → 52 °C for 30s → 72 °C for 30s) × 35 cycles → 72 °C for 10min] was carried out with 2 × Super PCR Mix (with dye, and also was used in ACTN3 sequencing) (BGI, Beijing, China) using ABI 9700 (Thermo Fisher Scientific, Inc.). After 1 % agarose gel electrophoresis, PCR products were visualized under UV light to identify ACE genotypes. When homozygotes were observed for D allele, a second round of amplification was performed to avoid mistyping produced by D allele that prevents the existence of non-amplified allele I, with primers: F: 5′-TGGGACAGCGCCCGCCACTAC-3′ and R: 5′-TCGCCAGCCCTCCCATGCCCATAA-3′. The purification of PCR products was performed using MacroView PCR Kit (Ensure Biologicals, Shanghai, China) following the standard protocol. Sequencing [primer F: 5′-CGGGCTGAGGGTGATGTAGG-3′, 95 °C for 15s → (95 °C for 15s → 50 °C for 5s → 60 °C for 90s) × 35 cycles] was carried out with ABI 3730XL DNA analyzer (Thermo Fisher Scientific, Inc.) to identify ACTN3 genotypes. The personnel detecting genotypes were blind to the experiment, and data analyzer was blind to genotypes.

2.5. Statistical analyses

Two weeks exercise training was involved in the present study. Thus, the training process was difficult to be controlled if sample size was large. In addition, the exercise tests in this study were conducted outdoors, while the tests had high a requirement for environmental temperature, which demanded that the test duration should be as short as possible, so it was not suitable to use a large sample size. As no studies on ACE and ACTN3 genes in HA or heat tolerance were reported, sample size was evaluated by referring to two previous studies on exercise training response (selected experiment indicators and training duration in those studies were somewhat similar to the present study). One study was about explosive power (12 days training), the minimum sample sizes in the ACTN3 and ACE genotype groups were 11 and 10 respectively, but significances were detected [29]. The other was about the influence of ACE gene on running endurance, the sample size of I homozygote group was 12, but significance was detected [30]. Nevertheless, the training duration of the previous study was 6 weeks.

Hardy-Weinberg equilibrium for each genetic polymorphism was tested by χ^2 tests. In the present study, ACE gene was divided into I homozygote (II) group and D carrier (ID + DD) group, and ACTN3 gene was divided into R homozygote (RR) group and X carrier (RX + XX) group. Grouping was based on the following considerations: firstly, genotype II and RR both have outstanding effect (compared with other genotypes, respectively) (see "1. Introduction"); secondly, the sample sizes of DD and XX genotypes were both small in the present study; thirdly, the grouping methods were also used in many previous studies. Considering the difference of the roles of ACE and ACTN3 genes (see "1. Introduction") and the sample size, the ACE × ACTN3 gene interaction was not analyzed in this study. Using skewness and box diagram, the normality of the whole subjects and each group was analyzed. In the present study, all skewness was < or ≈ |1|, while the distribution characteristic was acceptable as T tests and ANOVA, used in this study, are both robust to some deviation from normal distribution. In the whole subjects, the difference between post- and pre- HA was analyzed using paired samples T tests. One-way ANOVA and ANCOVA were performed for comparisons between groups before and after HA. Repeated-measures ANOVA and ANCOVA were used for within-group changes and gene × HA interaction (the difference between groups in within-group changes). Covariate inclusion followed the principles of necessity and frugality. Baseline before HA, age and BMI were considered as covariates in comparisons between groups after HA. Age and BMI were selected as covariates in within-group changes

and gene × HA interactions analyses. Because of an association between core temperature and exercise endurance [31], 5-km run was included in covariates in RTau analysis. Effect size (η) was interpreted using Cohen's (1988) cautious rule of thumb: | 0.10 |, | 0.24 |, | 0.37 |, and | 0.45 |+ are for small, medium, large, and very large, respectively. Statistical significances for all analyses were accepted at $p < 0.05$. Statistical analyses were conducted with SPSS version 26.0.

3. Results

3.1. Basic characteristics and differences between post- and pre-HA in the whole subjects

In the whole subjects, the characteristics of age and BMI are shown in Table 1. The genotype distributions of ACE ($\chi^2 < 0.01$, $df = 2$, $p = 1.00$) and ACTN3 ($\chi^2 = 2.94$, $df = 2$, $p = 0.23$) both conformed to the Hardy-Weinberg equilibrium (Table 1), in which ACE was highly conformed. Compared with these before HA, after HA, neither 5-km run nor push-up was improved ($p > 0.05$) (Table 1), 100-m run was improved ($\Delta = -1.4$ s, 95 % CI -2.0–0.9, $p < 0.001$) (Table 1), and RTau decreased ($\Delta = -0.2$ °C, 95 % CI -0.2–0.1, $p < 0.001$) (Table 1).

3.2. Differences between genotype groups before HA

Before HA, there were no differences ($p > 0.05$) between ACE or ACTN3 genotype groups in age, BMI, 5-km run, push-up, 100-m run and RTau (Table 2).

3.3. Differences between genotype groups after HA

After HA, there were no differences ($p > 0.05$) between ACE or ACTN3 genotype groups in 5-km run, push-up and 100-m run (Table 3). For ACE gene, RTau of I homozygote was lower than that of D carrier [$F_{(1, 48)} = 9.12$, $p = 0.004$, $\eta = 0.40$], and the differences were still significant (both $p = 0.001$) after controlling covariates (Table 3). When covariates were baseline (pre-HA RTau) and 5-km run, the difference was still significant ($p < 0.001$). There were no differences ($p > 0.05$) of RTau between ACTN3 genotype groups (Table 3), and there was still no difference ($p = 0.17$) when covariates were baseline and 5-km run.

3.4. Genotype within-group changes and gene × HA interactions

There were no significances ($p > 0.05$) in within-group changes of ACE or ACTN3 genotypes for 5-km run and push-up (Table 4), and the changes were all significant ($p < 0.01$) in 100-m run (Table 4). For 5-km run, push-up and 100-m run, no gene (ACE or ACTN3) × HA interaction was observed ($p > 0.05$) (Table 4).

For RTau, the genotype within-group changes and gene × HA interactions for ACE (Fig. 1. A) and ACTN3 (Fig. 1. B) are shown in Fig. 1. before controlling covariates. After controlling covariates, the with-group change of ACE I homozygote was still significant ($p < 0.001$ adjusted for age and BMI, $p < 0.001$ adjusted for pre-HA 5-km run, and $p < 0.001$ adjusted for age, BMI and pre-HA 5-km run), while the change of D carrier was still not significant ($p = 0.22$, $p = 0.22$, and $p = 0.23$ adjusted for the above-mentioned covariates, respectively). ACE gene × HA interaction was still significant after controlling covariates ($p = 0.001$ adjusted for age and BMI, $p < 0.001$ adjusted for pre-HA 5-km run, and $p < 0.001$ adjusted for age, BMI and pre-HA 5-km run). There was still not significance after controlling the above-mentioned covariates ($p = 0.24$, $p = 0.15$, and $p = 0.18$, respectively) in ACTN3 gene × HA interaction.

4. Discussion

To the best of the author' knowledge, it is the first study to investigate the roles of genes in HA. The relationships between ACE and ACTN3 genes and exercise performance in hot environment were also explicitly reported for the first time. The present results showed a strong ACE gene × HA interaction in RTau, and the RTau of I homozygote significantly decreased after HA.

In hot environment, core temperature will rise significantly if no effective regulation, which is one key factor leading to individual

Table 1
Basic characteristics and differences between post- and pre-HA in the whole subjects (N = 50).

	Mean (SD), or N (%), or minimum ~ maximum value			p
Age (years)	23 (2)	19–28		
BMI (kg/m^2)	22.4 (1.9)	19.6–26.7		
ACE genotype	24 (48 %) for II	21 (42 %) for ID	5 (10 %) for DD	1.00[a]
ACTN3 genotype	24 (48 %) for RR	15 (30 %) for RX	11 (22 %) for XX	0.23[a]
5-km run (s)	1427 (145)[b]	1419 (150)[c]		0.21
Push-up (n)	71 (20)[b]	73 (21)[c]		0.21
100-m run (s)	13.4 (1.7)[b]	11.9 (0.4)[c]		<0.001
RTau (°C)	36.9 (0.2)[b]	36.7 (0.3)[c]		<0.001

Note. [a] p for Hardy-Weinberg equilibrium test. [b] pre-HA. [c] post-HA. HA, heat acclimation. N, subject number. BMI, body mass index. n, repetitions of push-up. RTau, rest aural thermometry.

Table 2

Differences between ACE or ACTN3 genotype groups before HA.

	ACE genotype		ACTN3 genotype		p for ACE	p for ACTN3
	I homozygote (N = 24)	D carrier (N = 26)	R homozygote (N = 24)	X carrier (N = 26)		
Age (years)	23 (2)	22 (2)	23 (2)	23 (2)	0.35	0.76
BMI (kg/m^2)	22.2 (1.7)	22.6 (2.2)	22.8 (2.1)	22.1 (1.8)	0.40	0.20
5-km run (s)	1417 (141)	1435 (152)	1410 (139)	1442 (152)	0.67	0.44
Push-up (n)	73 (23)	69 (18)	72 (22)	71 (19)	0.51	0.84
100-m run (s)	13.5 (1.7)	13.3 (1.8)	13.2 (1.8)	13.6 (1.7)	0.74	0.47
RTau (°C)	36.9 (0.2)	36.9 (0.2)	36.9 (0.2)	36.6 (0.2)	0.65	0.43

Note. Data are presented as mean (SD). HA, heat acclimation. N, subject number. BMI, body mass index. n, repetitions of push-up. RTau, rest aural thermometry.

Table 3

Differences between ACE or ACTN3 genotype groups after HA.

	ACE genotype		ACTN3 genotype		p for ACE	p for ACTN3
	I homozygote (N = 24)	D carrier (N = 26)	R homozygote (N = 24)	X carrier (N = 26)		
5-km run (s)	1408 (141)	1430 (160)	1405 (131)	1433 (168)	0.60, 0.68, 0.85	0.52, 0.72, 0.78
Push-up (n)	75 (22)	71 (20)	73 (23)	73 (20)	0.52, 0.93, 0.89	0.96, 0.54, 0.35
100-m run (s)	12.0 (0.5)	11.9 (0.4)	11.9 (0.4)	11.9 (0.4)	0.74, 0.71, 0.76	0.99, 0.91, 0.94
RTau (°C)	36.6 (0.3)	36.8 (0.2)	36.7 (0.3)	36.8 (0.3)	0.004, 0.001, 0.001	0.15, 0.23, 0.31

Note. Data are presented as mean (SD). *p* values presented are no adjustment for covariates, adjustment for baseline, and adjustment for baseline, age and body mass index, respectively. HA, heat acclimation. N, subject number. n, repetitions of push-up. RTau, rest aural thermometry.

Table 4

ACE or ACTN3 genotype within-group changes and gene × HA interactions in exercise indicators.

	ACE gene			ACTN3 gene		
	I homozygote (N = 24)	D carrier (N = 26)	ACE × HA	R homozygote (N = 24)	X carrier (N = 26)	ACTN3 × HA
5-km run	0.25, 0.33	0.53, 0.44	0.68, 0.86	0.55, 0.53	0.25, 0.27	0.70, 0.75
Push-up	0.37, 0.35	0.38, 0.40	0.98, 0.92	0.68, 0.86	0.18, 0.12	0.53, 0.34
100-m run	<0.001, <0.001	<0.001, 0.001	0.81, 0.71	0.002, 0.002	<0.001, <0.001	0.50, 0.58

Note. Analyses results are presented as *p* values. *p* values presented are no adjustment for covariates and adjustment for age and body mass index, respectively. HA, heat acclimation. N, subject number.

heat intolerance. Previous studies have strongly established a conclusion: after HA, rest core temperature drops [32,33]. The rest core temperature decrease caused by HA is one of the most important adaptation mechanisms for improving heat tolerance [7,8]. A lower rest core temperature increases the heat-sink capacity of the body, which enables individuals to deal with greater temperature rise before cooling mechanism is up-regulated [12,34,35]. Individuals with lower rest core temperature have greater plasma volume causing enhanced core-to-shell heat conduction via increased cutaneous blood flow [36]. Measuring Tau is a common method for knowing about the core temperature. Especially, at rest, Tau can better accurately reflect the core temperature [37]. Therefore, the observation results on RTau in the present study suggest that ACE gene may have an effect on the response to HA, and I homozygote may have a better HA response in improving the heat tolerance.

Much evidence shows elevation of exercise endurance is very beneficial to improve heat tolerance [31]. ACE I homozygote is closely related to outstanding endurance performance [20–23]. Excellent exercise endurance requires years of training, which means that I homozygote may have a better response to endurance training. On the one hand, the response may be reflected in the improvement of endurance performance. On the other hand, it may be reflected in the most important physiological changes related to the endurance improvement, such as a decrease of core temperature (or heat tolerance improvement). In exercise-heat combination stress, the effect of I homozygote on core temperature may be more significant, therefore we could observe a significant decrease in core temperature during short-term HA. For ACTN3 gene, its association with outstanding speed performance has been strongly confirmed [22,24–26], but very few data show its association with exercise endurance [38], and it is generally recognized that the gene has nothing to do with exercise endurance. An important difference between speed exercise and endurance exercise is the duration of the former is much shorter, which has a lower requirement for temperature regulation ability in body. It may be related to the role of ACTN3 gene in exercise that no significances for ACTN3 were observed in the present study.

An important role of ACE is to transform angiotensin I into angiotensin II, and the latter is a strong vasoconstrictor [39]. ACE I/D polymorphism is closely related to plasma ACE level. I homozygote has the lowest ACE level compared with other genotypes, and the ACE levels of ID heterozygote and D homozygote are both obviously higher than that of I homozygote [40]. Lower ACE level is very beneficial to vasodilation, which is obviously important to reduce body heat in hot environment. Therefore, individuals with I homozygote, as the lower level of ACE, may show lower core temperature. The results on RTau in the present study suggest that the lower

core temperature may be better unfolded through HA.

No significances were observed in exercise indicators whether before or after HA, or in gene × training interactions in the present study. It may be related to the following reasons. Firstly, although previous studies strongly support the close associations between ACE and ACTN3 genes and exercise performance (see "1. Introduction"), most of these studies were based on athletes undergoing long-term professional training. Generally, HA effect could be achieved through 2 weeks HA [5,7]. Based on this view, the training duration of the present study was designated as 2 weeks. Therefore, the negative results on exercise indicators may be related to the short duration. Secondly, different from Tau measurement, exercise test is easily influenced by subjective factors. Thirdly, the present study was about the hot environment, which had not been studied. Perhaps the roles of ACE and ACTN3 genes in exercise performance or training response in hot environment are different from those in general environment. In addition, a study on recruits who originally had lived in hot areas showed that many of the recruits could not stand larger load exercise in hot environment [41], which may implies that the improvement of heat tolerance, which was observed in the present study, does not necessarily significantly improve exercise performance in hot environment.

In the present study, the analyses on the whole subjects showed that after HA, only 100-m run was improved, while 5 km-m run and push-up did not significantly change. Previous studies have shown that $\geq 30\,^{\circ}\text{C}$ provide sufficient stimulation in temperature to induce HA [27]. For athletes, the duration for each exercise-heat exposure during HA should be more than 60 min [42], which means that less trained individuals may need to take longer for each exposure to obtain HA effect. Generally, HA effect could be achieved through 2 weeks HA [5,7]. However, it may take about one month to achieve an ideal effect, if the training interval is increased (for example, an exposure every 2–3 days) [5]. The present experiment was carried out outdoors and had relative more subjects participating in training and exercise test. Thus, it was difficult to fully meet the above-mentioned requirements because of the need of experiment condition control and the restriction of practical situation. Therefore, no significant changes observed in 5-km run and push-up may be related to the ambient temperature, duration each time, and exposure frequency during the HA. Thermal environment has the greatest influence on endurance exercise [43], while the influence is small in speed exercise, and it may even be improved [44]. In the present study, both 5-km run and push-up belong to endurance, while 100-m run belongs to speed, which should be an important reason for the different changes of those indicators.

In the significant results on RTau in the present study, η were all above 0.40, and were all above 0.45 after controlling covariates. Those effect sizes are large or very large. On the one hand, it means that statistically significant differences could be detected by using a small sample size; on the other hand, it also implies that the present results may have a practical significance. During the whole experiment, subjects homogeneity should be better ensured and the experimental conditions should be better strictly controlled, which increases the persuasiveness of the results. In this study, the subjects were young Han nationality males, thus, it is not known whether the results could be generalized to other ethnicities, female, and childhood and elderly individuals. As the limitation of sample size, the heterozygote and homozygote of mutant allele were not analyzed separately in this study, thus, the quantitative effect of mutant allele is not known.

5. Conclusion

The present results suggest that ACE gene may have an effect on the response to HA. There may be a strong interaction of ACE gene and HA in the change of rest core temperature. ACE I homozygote may have a advantage on improving heat tolerance. The association of ACE gene with HA response implies the gene may play a role in human heat adaptation, which may provide an important hint for understanding the genetic mechanism of heat adaptation. The role of ACE I/D polymorphism in improving exercise performance may be limited during short-term HA. However, a decrease of core temperature is obviously beneficial to physical work in hot environment, especially in these cases of having an extreme requirement for physical levels. For ACTN3 gene, there may be no difference between R homozygote and X carrier in the response to HA.

Ethics statement

This work was approved by the Scientific Research and Academic Department of Chinese People's Liberation Army Special Warfare School.

Consent

Informed consent from all subjects participating in the study was obtained prior to conducting the study.

Funding

Data availability statement

Data associated with the study has not been deposited into a publicly available repository. The data that been used is confidential.

CRediT authorship contribution statement

Tao Liu: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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