DOI: https://doi.org/10.5114/biolsport.2019.79975

The influence of angiotensin I-converting enzyme (ACE) I/D gene polymorphism on cardiovascular and muscular adaptations following 8 weeks of isometric handgrip training (IHG) in untrained normotensive males

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ABSTRACT: We examined the association between the angiotensin I-converting enzyme (ACE) I/D gene polymorphism and isometric handgrip (IHG) training on cardiovascular and muscular responses among normotensive males. Thirty (II = 10, ID = 10, and DD = 10) normotensive untrained males underwent IHG training at 30% of their maximal voluntary contraction 3 days per week for 8 weeks. Cardiovascular and muscular variables were measured before IHG, after a session of IHG and after 8 weeks of IHG. No significant interaction effect was found between ACE I/D genotype and IHG training session on all dependent variables (all $p > 0.05$). There was a significant main effect of IHG training session on systolic blood pressure (SBP) ($p = 0.002$), mean arterial pressure (MAP) ($p = 0.015$) and handgrip strength (HGS) ($p = 0.001$) scores, while no difference in diastolic blood pressure (DBP), pulse pressure, or heart rate scores was found. A greater improvement in cardiovascular parameters following 8 weeks of IHG training was observed in participants with the D allele than the I allele (SBP reduction: ID+DD genotype group (-5.53 ± 6.2 mmHg) vs. II genotype group (-1.52 ± 5.3 mmHg)); MAP reduction: ID + DD genotype group (-2.80 ± 4.5 mmHg) vs. II genotype group (-1.45 ± 3.5 mmHg). Eight weeks of IHG training improved cardiovascular and muscular performances of normotensive men. Reduction in SBP and MAP scores in D allele carriers compared to I allele carriers indicates that the ACE I/D polymorphism may have an influence on IHG training adaptation in a normotensive population.

CITATION: Ahmad Yusof H, Aziz AR, Che Muhamed AM et al. The influence of angiotensin I-converting enzyme (ACE) I/D gene polymorphism on cardiovascular and muscular adaptations following 8 weeks of isometric handgrip training (IHG) in untrained normotensive males. Biol Sport. 2019;36(1):81–94.

Received: 2017-07-27; Reviewed: 2017-11-05; Re-submitted: 2018-10-12; Accepted: 2018-10-13; Published: 2018-11-05.

Key words:
ACE I/D gene polymorphism
IHG training
Normotensive
Blood pressure management

INTRODUCTION

Resistance exercise training, which has not been previously recommended for blood pressure (BP) management in hypertensive patients [1], has been shown to lower resting BP in normotensive and hypertensive individuals [2-5]. Reductions of 3 to 4 mmHg in resting systolic and diastolic BP were observed following four weeks of resistance exercise training [5]. Meanwhile, in another meta-analysis study conducted by Cornelissen and Smart [6], the largest reductions in resting BP were reported following the isometric resistance exercise training (systolic: -10.9 ± 2.86 mmHg), diastolic: -6.2 ± 3.34 mmHg) compared to after endurance (systolic: -3.5 ± 6.01 mmHg, diastolic: -3.7 ± 3.92 mmHg) and dynamic resistance exercise training (systolic: -1.8 ± 4.85 mmHg, diastolic: -2.5 ± 3.29 mmHg). It has been suggested that an isometric exercise training protocol consisting of four sets of 2-minute handgrip [7, 8] or leg contractions [9] at 30–50% of maximal voluntary contraction (MVC) [2, 10] with 1–4 minutes of passive rest between each contraction [2, 7] performed 3–5 times per week for 4–10 weeks [11, 12] is more effective at lowering resting BP than endurance and dynamic resistance exercise training.

Although the benefit of isometric exercise training for the management of hypertension has been well documented [13], it has remained unclear how factors [14] that can influence BP, such as sex and genetics, may influence the efficiency of this isometric exercise programme. Indeed, several studies using twins as subjects reported that BP is controlled by genetic factors [15, 16]. Given the fact that BP has a genetic basis, research efforts have been directed towards identifying the candidate genes involved in BP regulation [17, 18]. Among the proposed candidate genes, the angiotensin I-converting enzyme (*ACE*) gene has attracted much attention due to its role in the renin-angiotensin system (RAS), which is the body's primary physiological system that regulates BP [19-21].

Within the *ACE* gene, the *ACE I/D* gene polymorphism showed a strong link with the level of ACE [22] in the RAS and accounted for 47% of the total phenotypic variance of ACE [23]. Rigat et al. [23]

observed that ACE levels were higher among individuals with the *DD* genotype, followed by those with the *ID* and *II* genotypes of the *ACE I/D* gene polymorphism. Wong et al. (2012) reported that individuals with the *II* genotype had lower resting BP than those with the *ID* and *DD* genotypes. It has been suggested that individuals with two copies of the *I* allele might have lower resting BP compared to those with two copies of the *D* allele as the lower level of ACE decreased the production of ANG II, a potent vasodepressor and aldosterone stimulating peptide, besides activating bradykinin, a potent vasodilator that leads to a drop in BP [24]. Hence, given that the *ACE I/D* gene polymorphism has an important role in BP regulation, the BP response to exercise training may vary among individuals with different genotypes of *ACE I/D* gene polymorphism.

The influences of the *ACE I/D* gene polymorphism on BP in response to exercise training have been investigated previously, but the results have been inconsistent [25-31]. It has been reported that the

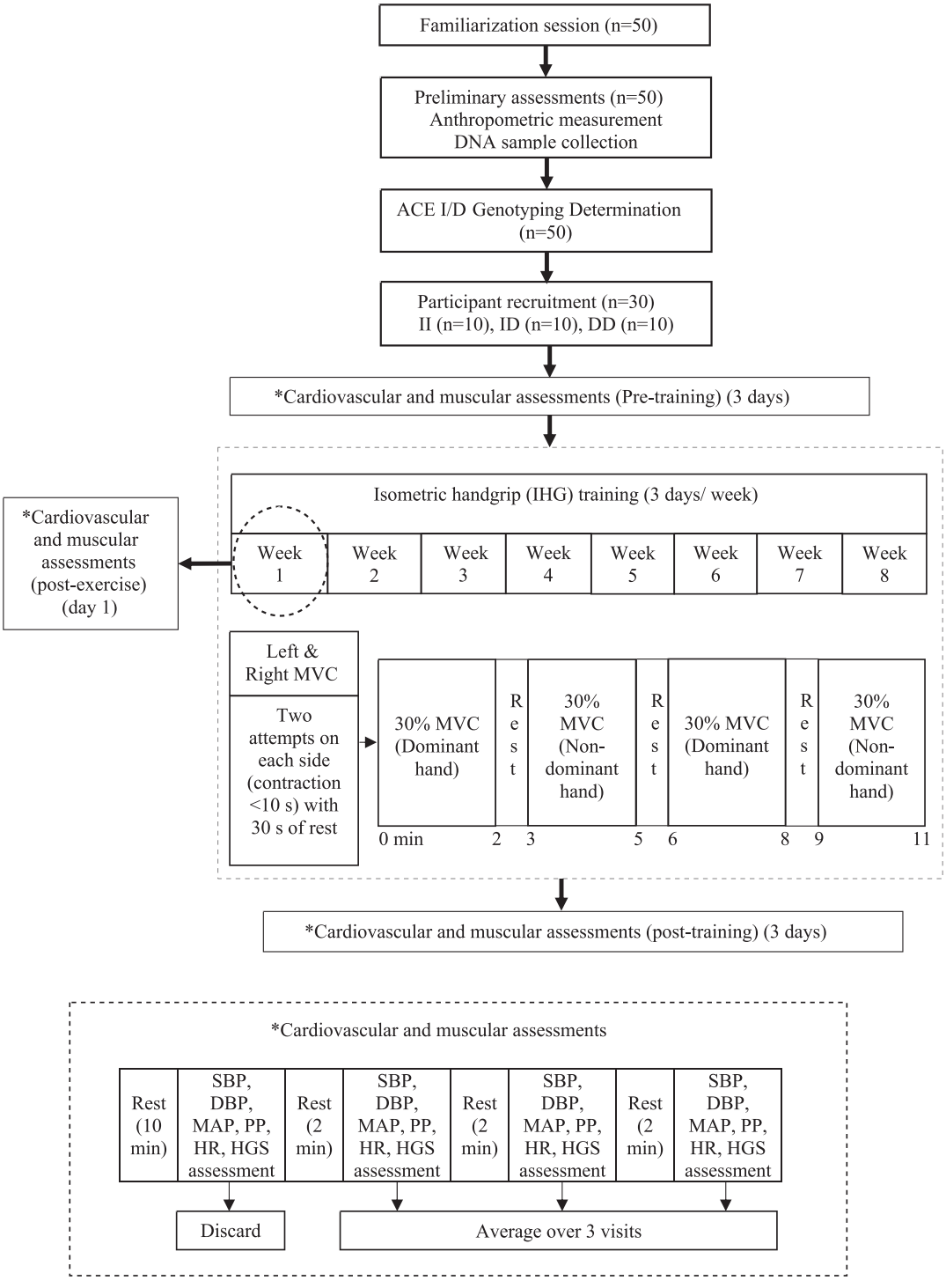

FIG. 1. Flow chart of study design.

ACE I/D gene polymorphism did not influence BP response to endurance [25, 27, 29] and dynamic resistance [31] exercise training. On the other hand, Hagberg et al. [26] reported a greater drop in resting BP among hypertensive men with the *II* and *ID* genotypes compared to those with the *DD* genotype after 9 months of endurance exercise training at 75–85% of maximal oxygen consumption. Zhang et al. [28] reported similar results for the impact of the *ACE I/D* gene polymorphism on BP response to 10 weeks of exercise therapy on a bicycle ergometer among 64 Japanese participants (16 males, 48 females) with mild to moderate essential hypertension. In contrast to these results, Kim [30] observed that adult women with the *DD* genotype had greater reduction in BP (diastolic) than those with the *II* and *ID* genotypes following 12 weeks of combined aerobic and resistance exercise training. The reasons for these inconsistent results are unclear, but differences in sample sizes, training protocols and ethnic background of the participants for eliciting substantial changes in resting BP may be involved. There is also a possibility that the effects of *ACE I/D* gene polymorphism on BP in response to exercise training may vary depending on the ethnic origin, which could explain the different findings in Korean and US populations as investigated by Kim [30] and Hagberg et al. [26], respectively. Ethnic variation has been demonstrated to exist in the distribution of *ACE I/D* gene polymorphism, with the highest frequency of the *I* allele being reported in the Black (Australian Aboriginal) population (0.97) [32], while the *D* allele was reported to be highest among the Caucasian population (0.77) [22]. Based on the previous reports that the distribution of *ACE I/D* gene polymorphism varies across ethnic groups and the disparity in findings between different ethnic groups, population-specific/ethnic-specific research is suggested to control this potential bias.

Based on the above-mentioned findings, there is a possibility that the *ACE I/D* gene polymorphism might also influence the BP response to isometric exercise training. More specifically, it raises the question of which if any *ACE* genotype group would be likely to benefit from this exercise programme. To our knowledge, no study has investigated the effect of isometric exercise training on BP response among individuals with different genotypes of the *ACE I/D* gene polymorphism. Thus, this investigation is important for determining whether *ACE I/D* gene polymorphism influences BP in response to exercise training. The results of this investigation would help to identify individuals who could reap potential clinical benefits from this type of exercise training programme.

MATERIALS AND METHODS

Study Design

A single-blind, repeated measures study design was used in the present study. All participants underwent identical cardiovascular and muscular assessments before training (pre-training), after the initial acute exercise session (post-exercise), and after 8 weeks of training (post-training). They performed isometric handgrip (IHG) exercise 3 days per week for 8 weeks. All assessments and IHG training were performed under the supervision of the researchers and were conducted in a quiet, temperature-controlled room (20–25ºC). The study protocol was approved by the Human Research Ethics Committee in Universiti Sains Malaysia in accordance with the ethical standards of the Helsinki Declaration. A flowchart of the study design is presented in Figure 1.

Participants

ifty healthy, normotensive, untrained males aged 30.3 ± 5.1 years, reportedly of Malay ancestry within the last three generations from Malaysia, were initially recruited to the study. After obtaining written consent from the participants, they were asked to complete a participant's information detail form and the Physical Activity Readiness Questionnaire (PAR-Q) to record their health and physical activity status. The details obtained from the PAR-Q were used to determine whether the participant was sedentary (determined as 2 or fewer days a week of recreational exercise for < 30 minutes a day for the preceding 3 months [33]). These sedentary individuals were then screened for *ACE I/D* gene polymorphism (rs4646994). Thirty of these initial participants (10 each with the *II*, *ID*, and *DD* genotypes of the *ACE I/D* gene polymorphism) were then selected for IHG training.

The number of participants for this study was based on the results obtained by Hagberg et al. [26] and the sample size was calculated using the Power and Sample Size Calculation version 3.1.2 software [34] [Calculated sample size = 27 participants;Research sample size = 27 participants + (27*10% (expected drop out)) = 30 participants]. The statistical power of the study was set at 0.80 with a 95% confidence interval and an effect size of 0.25. The participants were briefed about the testing protocol and familiarized with the instruments and procedures involved in the isometric handgrip test. After they had signed and completed the consent form, they were interviewed to obtain personal information, including sex, age, ethnicity, and health status. Body height was measured using a portable stadiometer (Seca 213, Seca Corporation, Chino, California, USA). Body mass, body mass index, and body fat were measured using an Omron KARADA Scan Body Composition & Scale (HBF-362, Omron Corporation, Kyoto, Japan).

ACE I/D Genotyping

A deoxyribonucleic acid (DNA) sample from each participant was collected using a buccal swab with a sterile swab applicator (Classic Swabs by Copan Flock Technologies, Brescia, Italy). Genomic DNA was isolated from the swab samples using the GeneAll Exgene Cell SV kit following the manufacturer's protocol (GeneAll Biotechnology Co. Ltd., Seoul, South Korea). Polymerase chain reaction (PCR) was carried out in a final volume of 25 μl consisting of 2.5 μl of 10X standard reaction buffer (25 mm Mg^{2+}, 50 mm Tris-HCl, 50 mm KCl, 0.1 mm EDTA, 1 mm DTT, 0.5 mm PMSF, and 50% glycerol (GeneAll Biotechnology Co. Ltd., Seoul, South Korea), 2.0 μl of dNTP mix (200 μm from each dNTP (dATP, dCTP, dGTP, and dTTP)), 0.8 μm of

each primer (forward primer: 5′-CTGGAGACCACTCCCATCCTTTCT-3′: reverse primer: 5′-CTGGAGACCACTCCCATCCTTTCT-3′), 0.5 units of Taq DNA polymerase, 2.5 μl of dimethylsulfoxide, 10.8 μl of sterilize distilled water, and 5 μl of genomic DNA (2–8 ng/μl). The target fragment bearing the *ACE I/D* gene polymorphism was amplified under the following conditions: 7 minutes at 95 °C followed by 25 cycles of 30 seconds at 95 °C, 30 seconds at 62 °C, and 1 minute at 72 °C, with a final step of 7 minutes at 72 °C. The amplified products were electrophoresed on a 1.5% agarose gel that was pre-stained with ethidium bromide at 70 volts for 1 hour. The presence of 490 base pair (bp) and 190 bp bands indicated the *ACE* insertion (*I*) and deletion (*D*) alleles, respectively. The PCR products for *ACE I/D* gene polymorphism were confirmed by sequencing (First BASE Laboratories Sdn Bhd, Selangor, Malaysia). The genotyping protocol used in the present study was adapted and modified from the established protocol by Mayne et al. [35].

Isometric Handgrip Training

Before every training session, the left and the right hand MVC values of the participants were assessed to reassess daily MVC of the participants with two attempts on each side (contraction duration < 10 seconds), separated by 30 seconds of passive rest between the attempts. If the variance from the two recordings for left or right hand MVC was < 5%, the highest value was taken as the participant's MVC for that side. If the recordings for left or right hand MVC differed by > 5%, further attempts were made at 1-minute intervals until a stable maximal value was obtained. All participants were trained using unilateral (one hand), alternate IHG exercise 3 days per week for 8 weeks. During each session, participants performed four trials of 2-minute IHG exercise at 30% of their MVC. They performed the IHG exercise using alternate hands, starting with the dominant hand, with a 1-minute rest period between each trial while sitting with the working arm extended towards the front.

Cardiovascular and Muscular Assessments

Prior to measurement of the study variables, the participants were asked to refrain from performing vigorous exercise and consuming caffeinated beverages within 24 h before the assessments. A handgrip dynamometer (Takei A5401, Takei Scientific Instruments Co. Ltd., Tokyo, Japan) was used to measure the muscular variable, whereas the cardiovascular variables were assessed using a non-invasive automated brachial oscillometer (Omron HEM907XL, Omron Healthcare, Inc., Lake Forest, Illinois, USA). Overall, the cardiovascular and muscular assessments took approximately 30 minutes.

Cardiovascular systolic BP (SBP), diastolic BP (DBP), mean arterial pressure (MAP), pulse pressure (PP), and heart rate (HR) and muscular handgrip strength (HGS) variables were measured on 3 consecutive days at the same time (± 2 h) of the day, immediately prior to commencing training. During each visit, after 10 minutes of seated rest, all variables were measured on the dominant arm hand (self-reported by the participant) in the sitting position four successive times with 2-minute rest intervals. The first of the four measurements of all variables in each visit was discarded (due to the white coat effect), whereas the remaining three measurements were averaged over the three visits to represent the pre-training value [8].

One hour after the initial training session (post-exercise), the cardiovascular and muscular variables were assessed using the procedure described above to examine the acute effects of IHG exercise [36]. Considering the white coat effect following one hour of exercise, the first and the second measurements were discarded, whereas the last two measurements were averaged to represent the post-exercise value [36].

For 3 consecutive days after 8 weeks of training (post-training), the cardiovascular and muscular variables were again assessed using the procedure described above. The measurements of cardiovascular and muscular variables were averaged in the same way as described for the pre-training value to represent the post-training value [8].

Statistical Analysis

The descriptive data are presented as mean ± standard deviation (SD). Differences in pre- and post-exercise/post-training values were calculated as the final (post-exercise/post-training) minus the initial (pre-training) value. Positive and negative results indicated an increase and a decrease with IHG training, respectively. The mean values of all variables at pre-training, post-exercise, and post-training were compared for *II*, *ID*, and *DD* genotype groups via one-way analysis

TABLE 1. Physical characteristics of participants according to *ACE I/D* genotype.

Variables	II (n=10)	ID (n=10)	DD (n=10)	F value	p value
Age (years)	27.8 ± 6.2	32.9 ± 3.1	30.0 ± 4.5	2.834	0.071
Height (cm)	169.0 ± 6.2	170.4 ± 9.3	169.0 ± 5.4	0.133	0.876
Body Weight (kg)	72.6 ± 10.0	74.7 ± 24.0	72.4 ± 7.4	0.066	0.961
Body Mass Index (kg/m^2)	25.4 ± 3.1	27.1 ± 4.4	25.6 ± 3.2	0.672	0.519
Body Fat (%)	23.8 ± 3.5	26.0 ± 4.7	24.1 ± 3.7	0.937	0.404

Data shown as mean ± SD.

of variance (ANOVA) followed by Bonferroni's post-hoc test when appropriate. A two-way repeated measures ANOVA test was used to examine whether there was an interaction effect between the *ACE I/D* genotype and IHG training on the cardiovascular and muscular variables (interaction: genotype x training;training effect (within subject);genotype effect (between subjects)). The main effects of training and genotype are presented as estimated marginal mean ± standard error of mean (SEM). All statistical evaluations were performed using IBM SPSS Statistics version 20.0 (Armonk, New York, USA), with the level of significance set at $p < 0.050$.

RESULTS

Characteristics of Participants

Table 1 shows the physical characteristics of the participants according to *ACE I/D* genotype. All *ACE I/D* genotype groups were similar in age, height, body weight, body mass index, and body fat.

Cardiovascular and Muscular Responses in the Whole Samples

Table 2 shows the cardiovascular and muscular responses in the whole samples (regardless of their *ACE I/D* genotype) following a single session of IHG exercise (post-exercise) and 8 weeks of IHG training. SBP ($t(29) = 2.456$, $p = 0.020$) and MAP ($t(29) = 2.506$, $p = 0.018$), but not DBP, PP, and HR, were significantly lower following the initial session of IHG exercise than at pre-training. HGS did not increase significantly after the first session of IHG exercise. SBP ($t(29) = 3.753$, $p = 0.001$), MAP ($t(29) = 3.008$, $p = 0.004$), PP ($t(29) = 2.401$, $p = 0.023$), and HR ($t(29) = 2.398$, $p = 0.023$), but not DBP, were significantly lower following 8 weeks of IHG training than at pre-training. HGS ($t(29) = -3.175$, $p = 0.004$) significantly increased after IHG training.

ACE I/D Genotype and Cardiovascular and Muscular Responses

Cardiovascular and muscular variables among *ACE* genotype groups (*II*, *ID*, *DD*) at pre-training, post-exercise and post-training are shown in Table 3. Two-way repeated measures ANOVA revealed no significant interaction effect between *ACE I/D* gene polymorphism and IHG training session on all dependent variables. However, a significant main effect of IHG intervention (pre-training, post-exercise and post-training) on SBP ($p = 0.002$), MAP ($p = 0.015$) and HGS ($p = 0.001$) scores was found, while no significant differences were found in DBP, PP, and HR scores across the IHG training sessions.

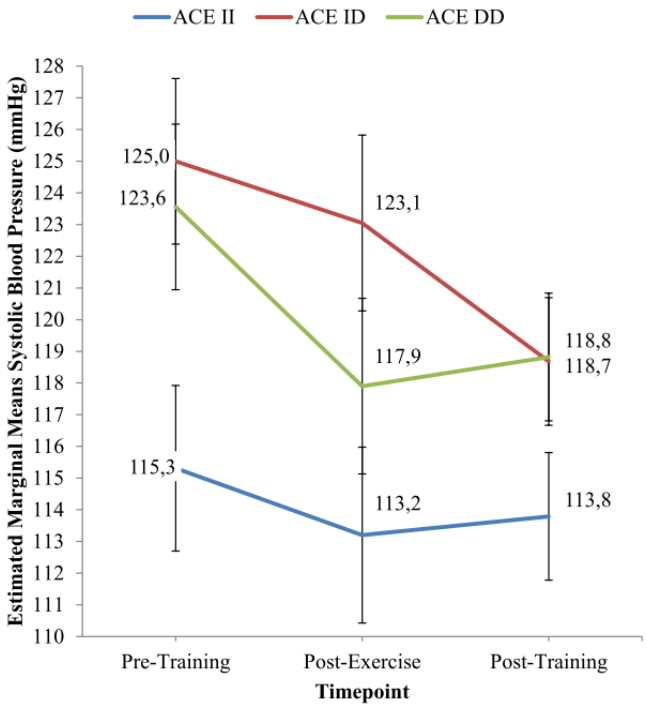

FIG. 2. Estimated marginal means of Systolic Blood Pressure (SBP) in *ACE II*, *ACE ID* and *ACE DD* genotype groups at Pre-Training, Post-Exercise and Post-Training.

TABLE 2. Cardiovascular and muscular responses to a session of IHG exercise and 8 weeks of IHG training.

Variables	Pre-training	Mid-training	Post-training	Change (Δ) with IHG exercise	Change (Δ) with IHG training	t value[a]	p value[a]	t value[b]	p value[b]
SBP (mmHg)	121.3 ± 9.1	118.1 ± 9.4*	117.1 ± 6.6*	-3.2 ± 7.2	-4.2 ± 6.1	2.456	0.020	3.753	0.001
DBP (mmHg)	76.2 ± 7.2	73.8 ± 8.5	74.7 ± 7.9	-2.4 ± 6.5	-1.4 ± 4.5	2.024	0.052	1.737	0.093
MAP (mmHg)	91.2 ± 6.9	88.5 ± 8.0*	88.9 ± 6.8*	-2.7 ± 5.8	-2.4 ± 4.2	2.506	0.018	3.008	0.004
PP (mmHg)	45.1 ± 8.2	44.3 ± 8.0	42.4 ± 6.8*	-0.9 ± 7.1	-2.8 ± 6.3	0.655	0.518	2.401	0.023
HR (bpm)	79.4 ± 7.7	78.5 ± 9.1	76.8 ± 8.9*	-0.9 ± 7.6	-2.6 ± 5.9	0.608	0.548	2.398	0.023
HGS (kg)	43.5 ± 6.7	43.2 ± 6.9	46.0 ± 7.5*	-0.3 ± 2.3	2.6 ± 4.4	0.800	0.430	-3.175	0.004

Data shown as mean ± SD; *Significantly different compared to pre-training value ($p < 0.050$); [a]p value for change (Δ) with IHG exercise; [b]p value for change (Δ) with IHG training.

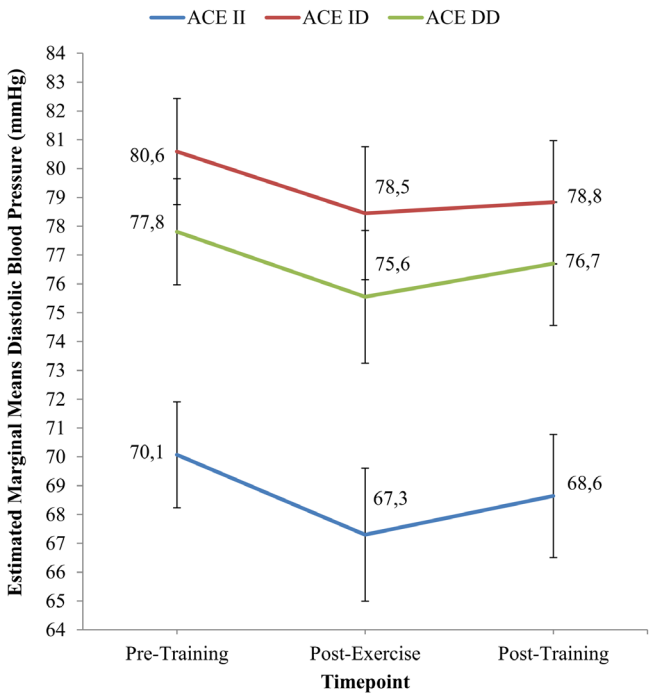

FIG. 3. Estimated marginal means of Diastolic Blood Pressure (DBP) in *ACE II*, *ACE ID* and *ACE DD* genotype groups at Pre-Training, Post-Exercise and Post-Training.

Post hoc tests using the Bonferroni correction revealed that IHG training elicited a significant reduction in SBP score from pre-training to after 8 weeks of IHG training ($p = 0.02$). However, a session of IHG exercise (post-exercise) elicited a slight reduction in SBP score from pre-training, which was not statistically significant ($p = 0.064$). There was also a significant main effect of *ACE I/D* gene polymorphism on SBP score ($p = 0.038$). Figure 2 shows that the SBP scores (estimated marginal means) after 8 weeks of IHG training for the *II* genotype group (113.8 ± 2.0 mmHg) are lower than those for the *ID* (118.7 ± 2.0 mmHg) and *DD* (118.8 ± 2.0 mmHg) genotype groups. However, as shown in Table 4, the SBP reduction was significantly greater in the *ID* genotype group (-6.32 ± 7.3 mmHg) than the *II* (-1.52 ± 5.3 mmHg) and *DD* (-4.74 ± 5.2 mmHg) genotype groups.

There was also a significant main effect of *ACE I/D* gene polymorphism on DBP score ($p = 0.001$). The DBP scores (estimated marginal means) after 8 week of IHG training for the *II* genotype group (68.6 ± 2.1 mmHg) are lower than those for the *ID* (78.8 ± 2.1 mmHg) and *DD* (76.7 ± 2.1 mmHg) genotype groups (Figure 3). However, Table 4 shows that the DBP reduction was greater in the *ID* genotype group (-1.76 ± 5.7 mmHg) than *II* (-1.43 ± 3.7 mmHg) and *DD* (-1.11 ± 4.4 mmHg) genotype groups, though it was not significantly difference from pre-training values.

TABLE 3. Repeated-Measures 2-way ANOVA on Cardiovascular and muscular responses to a session of IHG exercise and 8 weeks of IHG training according to *ACE I/D* genotype.

Variables	Genotype	n	Pre-training	Mid-training	Post-training	Repeated-Measures 2-way ANOVA		
						Interaction (genotype x training session)	Training effect (within subject)	Genotype effect (between subjects)
SBP (mmHg)	*II*	10	115.3 ± 7.2	113.2 ± 7.5	113.8 ± 6.3	0.198	0.002*	0.038*
	ID	10	125.0 ± 9.3	123.1 ± 8.9	118.7 ± 4.9			
	DD	10	123.6 ± 8.2	117.9 ± 9.8	118.8 ± 7.6			
DBP (mmHg)	*II*	10	70.1 ± 4.6	67.3 ± 7.0	68.6 ± 6.7	0.996	0.098	0.001*
	ID	10	80.6 ± 5.9	78.5 ± 5.2	78.8 ± 6.7			
	DD	10	77.8 ± 6.7	75.6 ± 9.1	76.7 ± 6.9			
MAP (mmHg)	*II*	10	85.2 ± 4.5	82.6 ± 6.4	83.7 ± 5.3	0.839	0.015*	0.001*
	ID	10	95.4 ± 5.8	93.3 ± 4.5	92.1 ± 5.3			
	DD	10	93.1 ± 6.0	89.7 ± 8.8	90.7 ± 6.7			
PP (mmHg)	*II*	10	45.2 ± 7.3	45.9 ± 7.0	45.2 ± 8.2	0.278	0.067	0.692
	ID	10	44.4 ± 9.2	44.6 ± 10.3	39.9 ± 6.4			
	DD	10	45.8 ± 8.7	42.4 ± 6.6	42.1 ± 5.1			
HR (bpm)	*II*	10	78.6 ± 6.5	77.0 ± 8.7	78.9 ± 8.1	0.140	0.164	0.923
	ID	10	79.8 ± 9.5	77.0 ± 10.2	76.1 ± 9.3			
	DD	10	79.8 ± 7.7	81.7 ± 8.3	75.5 ± 9.8			
HGS (kg)	*II*	10	43.4 ± 4.9	43.3 ± 5.7	47.6 ± 4.6	0.199	0.001*	0.223
	ID	10	46.3 ± 8.0	46.3 ± 7.9	47.1 ± 9.5			
	DD	10	40.8 ± 6.3	39.9 ± 6.0	43.5 ± 7.8			

Data shown as mean ± SD; * Represent the main effect (Significant at $p < 0.050$).

For MAP, IHG exercise elicited a significant reduction in MAP score from pre-training to 8 weeks of IHG training ($p = 0.016$). However, the MAP score was not significantly lower at post-exercise compared to pre-training ($p = 0.066$). There was a significant main effect of *ACE I/D* gene polymorphism on MAP score ($p = 0.001$). The MAP score (estimated marginal means) after 8 weeks of IHG training for the *II* genotype group (83.7 ± 1.8 mmHg) was lower than those for the *ID* (92.1 ± 1.8 mmHg) and *DD* genotype groups (90.7 ± 1.8 mmHg) (Figure 4). However, Table 4 shows that the MAP reduction was greater in the *ID* genotype group (-3.28 ± 5.3 mmHg) than *II* (-1.45 ± 3.5 mmHg) and *DD* (-2.32 ± 3.7 mmHg) genotype groups though it was not significantly different from pre-training values.

Post hoc tests using the Bonferroni correction also revealed that the increase in HGS as a result of IHG training was statistically significant following 8 weeks of IHG training ($p = 0.001$). Conversely, a session of IHG exercise did not lead to any increment in HGS score ($p > 0.095$). Additionally, the gains realized in HGS score were not significantly affected by *ACE I/D* gene polymorphism ($p = 0.223$) (Table 3).

To further analyse the association between the *ACE I/D* gene polymorphism and training adaptation, the dominant model was used in which the data for *ID* and *DD* genotype groups were combined

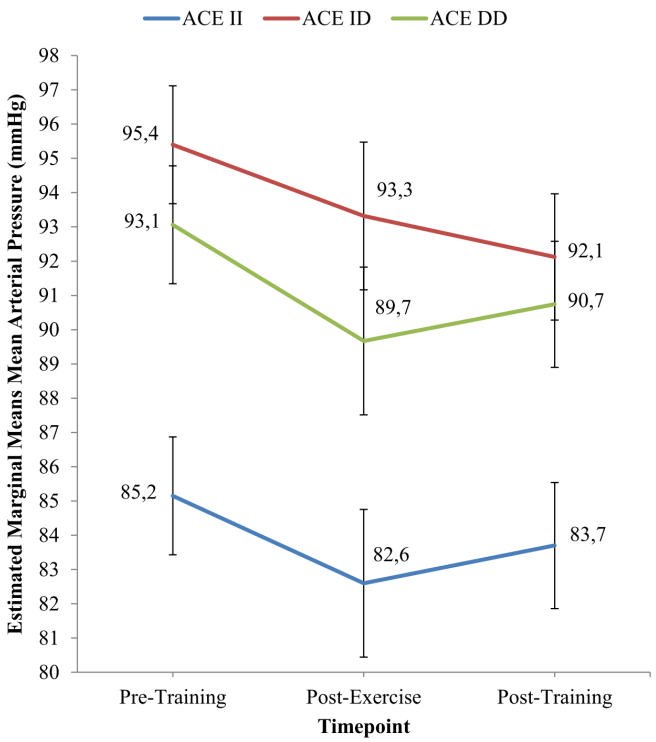

FIG. 4. Estimated marginal means of Mean Arterial Pressure (MAP) in *ACE II*, *ACE ID* and *ACE DD* genotype groups at Pre-Training, Post-Exercise and Post-Training.

TABLE 4. Cardiovascular and muscular changes to a session of IHG exercise and 8 weeks of IHG training according to *ACE I/D* genotype.

Variables	Genotype	n	Pre-training	Mid-training	Post-training	Change (Δ) with IHG exercise	Change (Δ) with IHG training	t value[a]	p value[a]	t value[b]	p value[b]
SBP (mmHg)	*II*	10	115.3 ± 7.2	113.2 ± 7.5	113.8 ± 6.3	-2.11 ± 6.8	-1.52 ± 5.3	-0.986	0.350	-0.909	0.387
	ID	10	125.0 ± 9.3	123.1 ± 8.9	118.7 ± 4.9*	-1.95 ± 8.4	-6.32 ± 7.3	-0.738	0.479	-2.750	0.022
	DD	10	123.6 ± 8.2	117.9 ± 9.8*	118.8 ± 7.6*	-5.66 ± 6.5	-4.74 ± 5.2	-2.734	0.023	-2.905	0.017
DBP (mmHg)	*II*	10	70.1 ± 4.6	67.3 ± 7.0	68.6 ± 6.7	-2.77 ± 5.6	-1.43 ± 3.7	-1.576	0.149	-1.231	0.250
	ID	10	80.6 ± 5.9	78.5 ± 5.2	78.8 ± 6.7	-2.14 ± 3.9	-1.76 ± 5.7	-1.747	0.115	-0.971	0.357
	DD	10	77.8 ± 6.7	75.6 ± 9.1	76.7 ± 6.9	-2.26 ± 9.4	-1.11 ± 4.4	-0.759	0.467	-0.801	0.444
MAP (mmHg)	*II*	10	85.2 ± 4.5	82.6 ± 6.4	83.7 ± 5.3	-2.55 ± 5.0	-1.45 ± 3.5	-1.599	0.144	-1.309	0.223
	ID	10	95.4 ± 5.8	93.3 ± 4.5	92.1 ± 5.3	-2.08 ± 4.9	-3.28 ± 5.3	-1.347	0.211	-1.953	0.083
	DD	10	93.1 ± 6.0	89.7 ± 8.8	90.7 ± 6.7	-3.39 ± 7.7	-2.32 ± 3.7	-1.387	0.199	-1.987	0.078
PP (mmHg)	*II*	10	45.2 ± 7.3	45.9 ± 7.0	45.2 ± 8.2	0.66 ± 6.9	-0.09 ± 5.2	0.303	0.769	-0.054	0.958
	ID	10	44.4 ± 9.2	44.6 ± 10.3	39.9 ± 6.4	0.19 ± 6.5	-4.56 ± 7.2	0.092	0.928	-2.009	0.075
	DD	10	45.8 ± 8.7	42.4 ± 6.6	42.1 ± 5.1	-3.40 ± 7.9	-3.63 ± 6.0	-1.364	0.206	-1.899	0.090
HR (bpm)	*II*	10	78.6 ± 6.5	77.0 ± 8.7	78.9 ± 8.1	-1.55 ± 6.8	0.37 ± 8.1	-0.726	0.486	0.144	0.889
	ID	10	79.8 ± 9.5	77.0 ± 10.2	76.1 ± 9.3*	-2.89 ± 9.1	-3.75 ± 3.5	-1.009	0.339	-3.392	0.008
	DD	10	79.8 ± 7.7	81.7 ± 8.3	75.5 ± 9.8*	1.90 ± 6.8	-4.30 ± 4.2	0.884	0.400	-3.246	0.010
HGS (kg)	*II*	10	43.4 ± 4.9	43.3 ± 5.7	47.6 ± 4.6*	-0.11 ± 2.1	4.18 ± 4.1	-0.149	0.885	3.212	0.011
	ID	10	46.3 ± 8.0	46.3 ± 7.9	47.1 ± 9.5	-0.06 ± 1.4	0.75 ± 2.8	-0.136	0.895	0.851	0.417
	DD	10	40.8 ± 6.3	39.9 ± 6.0	43.5 ± 7.8	-0.84 ± 3.2	2.71 ± 5.6	0.844	0.400	0.420	0.158

Data shown as mean ± SD; *Significantly different compared to pre-training value ($p < 0.050$); [a]p value for change (Δ) with IHG exercise; [b]p value for change (Δ) with IHG training.

and then compared to the data of the *II* genotype group. Table 5 shows the cardiovascular and muscular variables at pre-training, post-exercise and post-training according to the dominant model (*II* vs. *ID + DD* genotypes). A two-way repeated measure ANOVA revealed no significant interaction effect between dominant ACE genotype (*II* vs. *ID+DD* groups) and IHG training session on all dependent variables. However, a significant main effect of IHG intervention (pre-training, post-exercise and post-training) was found in SBP ($p = 0.015$), MAP ($p = 0.029$), and HGS ($p = 0.002$) scores, while no difference was found in DBP, PP, and HR scores across the IHG training session.

There was also a significant main effect of *ACE I/D* gene polymorphism on SBP ($p = 0.013$), DBP ($p = 0.002$) and MAP ($p = 0.002$) according to the dominant model. Figure 5 shows a main effect of IHG training and *ACE* genotype according to the dominant model, and no interaction between these two variables on SBP. The SBP score after 8 weeks of IHG training (post-training) for the *II* genotype group (113.8 ± 2.0 mmHg) was lower than that for the *ID + DD* genotype group (118.8 ± 1.4 mmHg). However, as shown in Table 6, the SBP reduction following the 8 weeks of IHG training was greater in the *ID+DD* genotype group (-5.53 ± 6.2 mmHg) than the *II* genotype group (-1.52 ± 5.3 mmHg).

The DBP score (estimated marginal means) after 8 weeks of IHG training for the *II* genotype group (68.6 ± 2.1 mmHg) was lower than that for the *ID + DD* group (77.8 ± 1.5 mmHg) (Figure 6). However, Table 6 shows that the DBP reduction was slightly greater in the *ID + DD* genotype group (-1.44 ± 5.0 mmHg) than the *II* genotype group (-1.43 ± 3.7 mmHg), though it was not significantly different from pre-training values.

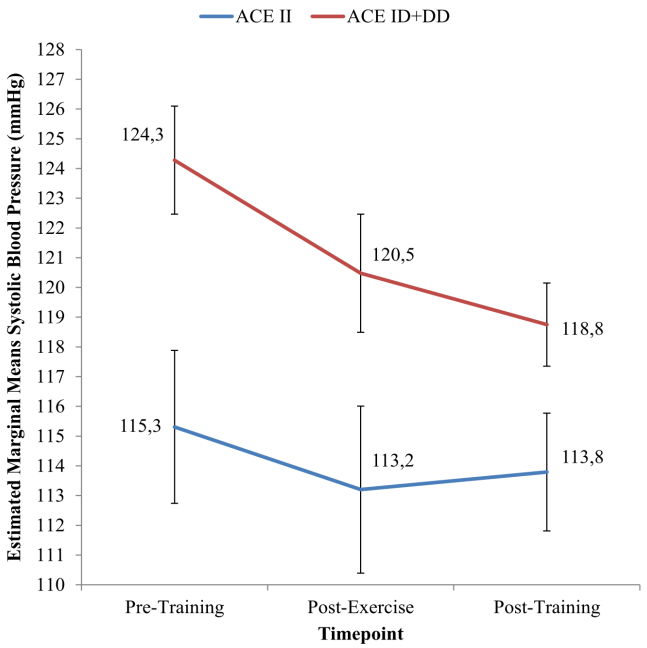

FIG. 5. Estimated marginal means of Systolic Blood Pressure (SBP) in *ACE II* and *ACE ID + DD* genotype groups at Pre-Training, Post-Exercise and Post-Training.

The MAP score (estimated marginal means) after 8 weeks of IHG training for the *II* genotype group (83.7 ± 1.8 mmHg) was lower than for the *ID + DD* genotype group (91.4 ± 1.3 mmHg) (Figure 7). However, Table 6 shows that the MAP reduction was significantly

TABLE 5. Repeated-Measures 2-way ANOVA on Cardiovascular and muscular responses to a session of IHG exercise and 8 weeks of IHG training according to dominant model (*II vs. ID+DD*).

Variables	Genotype	n	Pre-training	Mid-training	Post-training	Repeated-Measures 2-way ANOVA		
						Interaction (dominant genotype x training session)	Training effect (within subject)	Dominant Genotype effect (between subjects)
SBP (mmHg)	*II*	10	115.3 ± 7.2	113.2 ± 7.5	113.8 ± 6.3	0.285	0.015*	0.013*
	ID+DD	20	124.3 ± 8.6	120.5 ± 9.5	118.8 ± 6.2			
DBP (mmHg)	*II*	10	70.1 ± 4.6	67.3 ± 7.0	68.6 ± 6.7	0.959	0.100	0.002*
	ID+DD	20	79.2 ± 6.3	77.0 ± 7.4	77.8 ± 6.7			
MAP (mmHg)	*II*	10	85.2 ± 4.5	82.6 ± 6.4	83.7 ± 5.3	0.774	0.029*	0.002*
	ID+DD	20	94.2 ± 5.8	91.5 ± 7.1	91.4 ± 6.0			
PP (mmHg)	*II*	10	45.2 ± 7.3	45.9 ± 7.0	45.2 ± 8.2	0.292	0.232	0.394
	ID+DD	20	45.1 ± 8.7	43.5 ± 8.5	41.0 ± 5.8			
HR (bpm)	*II*	10	78.6 ± 6.5	77.0 ± 8.7	78.9 ± 8.1	0.135	0.431	0.960
	ID+DD	20	79.8 ± 8.4	79.3 ± 9.4	75.8 ± 9.3			
HGS (kg)	*II*	10	43.4 ± 4.9	43.3 ± 5.7	47.6 ± 4.6	0.196	0.002*	0.776
	ID+DD	20	43.6 ± 7.6	43.1 ± 7.6	45.3 ± 8.7			

Data shown as mean ± SD; *Represent the main effect (Significant at $p < 0.050$).

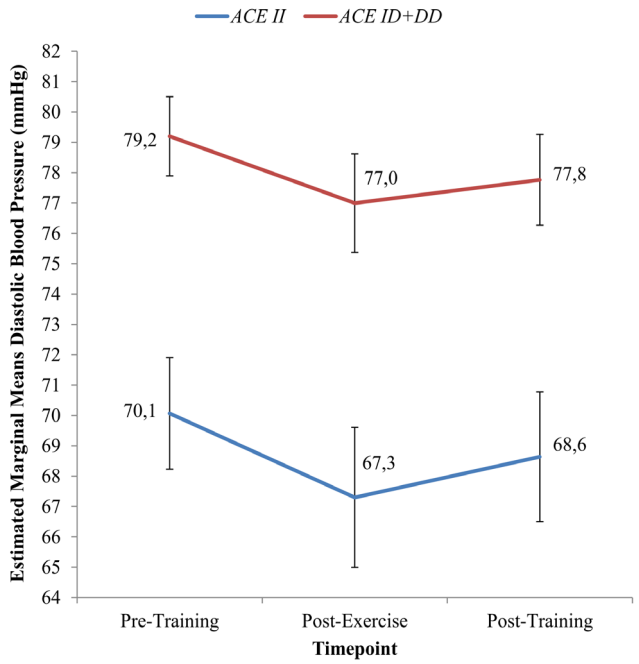

FIG. 6. Estimated marginal means of Diastolic Blood Pressure (DBP) in *ACE II* and *ACE ID + DD* genotype groups at Pre-Training, Post-Exercise and Post-Training.

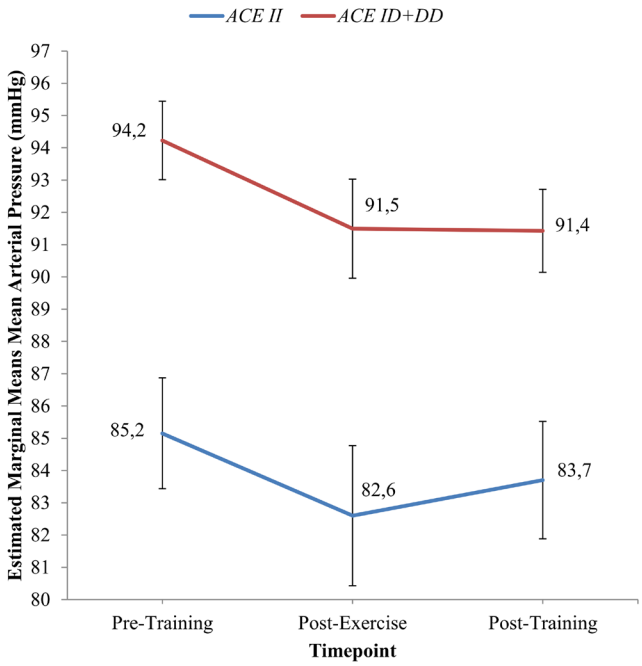

FIG. 7. Estimated marginal means of Mean Arterial Pressure (MAP) in *ACE II* and *ACE ID + DD* genotype groups at Pre-Training, Post-Exercise and Post-Training.

greater in the *ID + DD* genotype group (-2.80 ± 4.5 mmHg) than the *II* genotype group (-1.45 ± 3.5 mmHg).

Figure 8 presents the cardiovascular and muscular changes in all variables following 8 weeks of IHG training among three *ACE* genotype groups (*II*, *ID* and *DD*). Meanwhile, Figure 9 displays the changes in the studied variables following 8 weeks of IHG training according to the dominant model (*II* vs. *ID+DD)*.

TABLE 6. Cardiovascular and muscular changes to a session of IHG exercise and 8 weeks of IHG training according to according to dominant model (*II vs. ID+DD*).

Variables	Genotype	n	Pre-training	Mid-training	Post-training	Change (Δ) with IHG exercise	Change (Δ) with IHG training	t value[a]	p value[a]	t value[b]	p value[b]
SBP	*II*	10	115.3 ± 7.2	113.2 ± 7.5	113.8 ± 6.3	-2.11 ± 6.8	-1.52 ± 5.3	-0.986	0.350	-0.909	0.387
(mmHg)	*ID+DD*	20	125.0 ± 9.3	123.1 ± 8.9*	118.7 ± 4.9*	-3.81 ± 7.6	-5.53 ± 6.2	-2.254	0.036	-3.997	0.001
DBP	*II*	10	70.1 ± 4.6	67.3 ± 7.0	68.6 ± 6.7	-2.77 ± 5.6	-1.43 ± 3.7	-1.576	0.149	-1.231	0.250
(mmHg)	*ID+DD*	20	79.2 ± 6.3	77.0 ± 7.4	77.8 ± 6.7	-2.20 ± 7.0	-1.44 ± 5.0	-1.404	0.213	-1.289	0.176
MAP	*II*	10	85.2 ± 4.5	82.6 ± 6.4	83.7 ± 5.3	-2.55 ± 5.0	-1.45 ± 3.5	-1.599	0.144	-1.309	0.223
(mmHg)	*ID+DD*	20	94.2 ± 5.8	91.5 ± 7.1	91.4 ± 6.0*	-2.74 ± 6.3	-2.80 ± 4.5	-1.933	0.068	-0.704	0.012
PP	*II*	10	45.2 ± 7.3	45.9 ± 7.0	45.2 ± 8.2	0.66 ± 6.9	-0.09 ± 5.2	0.303	0.769	-0.054	0.958
(mmHg)	*ID+DD*	20	45.1 ± 8.7	43.5 ± 8.5	41.0 ± 5.8*	-1.61 ± 7.3	-4.10 ± 6.5	-0.987	0.336	-2.828	0.011
HR	*II*	10	78.6 ± 6.5	77.0 ± 8.7	78.9 ± 8.1	-1.55 ± 6.8	0.37 ± 8.1	-0.726	0.486	0.144	0.889
(bpm)	*ID+DD*	20	79.8 ± 8.4	79.3 ± 9.4	75.8 ± 9.3*	-0.50 ± 8.2	-4.03 ± 3.8	-0.271	0.789	-4.780	0.001
HGS	*II*	10	43.4 ± 4.9	43.3 ± 5.7	47.6 ± 4.6*	-0.11 ± 2.1	4.18 ± 4.1	-0.149	0.885	3.212	0.011
(kg)	*ID+DD*	20	46.6 ± 7.6	43.1 ± 7.6	45.3 ± 7.6	-0.45 ± 2.4	1.73 ± 4.4	-0.838	0.413	1.760	0.095

Data shown as mean ± SD; *Significantly different compared to pre-training value ($p < 0.050$); [a]p value for change (Δ) with IHG exercise; [b]p value for change (Δ) with IHG training.

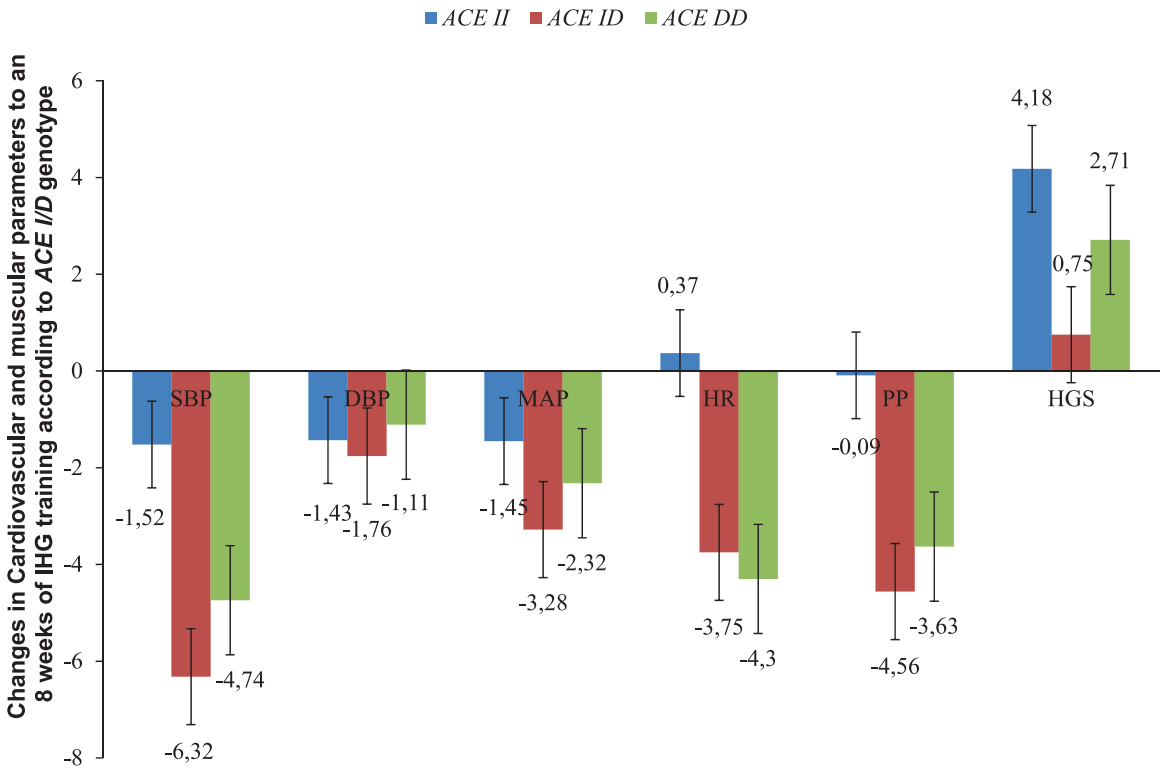

FIG. 8. Cardiovascular and muscular changes to an 8 weeks of IHG training (Post-Training value minus Pre-Training value) according to *ACE I/D* genotype.
SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; MAP=Mean Arterial Pressure; PP=Pulse Pressure; HGS=Handgrip Strengt.

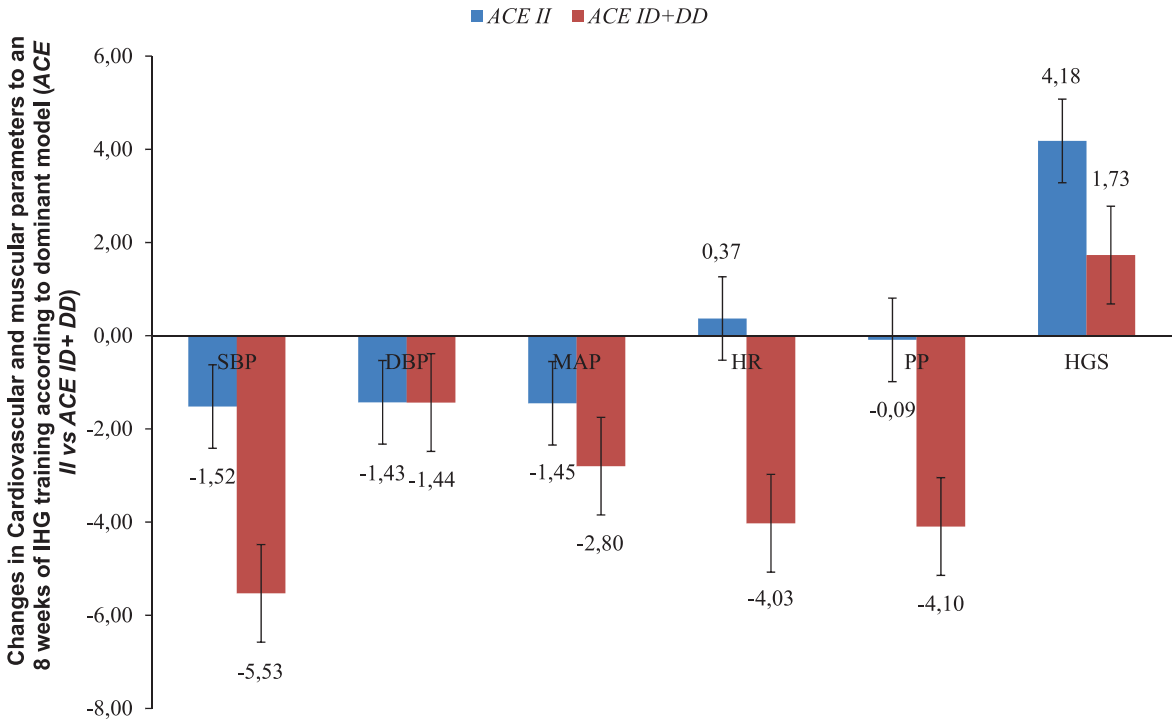

FIG. 9. Cardiovascular and muscular changes to 8 weeks of IHG training (Post-Training value minus Pre-Training value) according to dominant model (*ACE II vs ACE ID+DD*).
SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; MAP=Mean Arterial Pressure; PP=Pulse Pressure; HGS=Handgrip Strength.

DISCUSSION

The present study found significant differences in SBP, MAP and HGS scores between the pre-training and post-training, which showed that an 8-week IHG training programme elicits improvements in cardiovascular and muscular performances of normotensive men. In addition, greater improvements in cardiovascular parameters (SBP, DBP and MAP) were observed in participants with the *D* allele than those with the *I* allele. This finding was indicated by significant reductions in SBP, DBP and MAP scores (parameters for cardiovascular parameters) in the *D* allele group compared to the *I* allele group from the genotyping analysis (Table 4) and additional analysis by using the dominant model (Table 6).

The current study showed that cardiovascular responses to IHG training varied among normotensive individuals with different *ACE I/D* genotypes. Consistent with the results obtained by Kim [30], this study revealed that normotensive men with *DD* and *ID* genotypes tended to have decreased resting SBP and HR with IHG training more than those with *II* genotype (Table 6). Nevertheless, this finding was not comparable to the findings obtained from some previous studies [26, 28] which showed that individuals with the *II* genotype had lower resting BP than other genotypes after exercise. This inconsistency may be due to the differences in the mode of exercise performed, as for instance a study by Kim [30] which involved mixed aerobic and resistance exercise training compared with only aerobic training (endurance training) in a study by Hagberg et al. [26]. Several studies have demonstrated that the physiological adaptation for aerobic training was greater among individuals with the *II* genotype and those with the *DD* genotype responded better to resistance training [37-39]. Thus, the inconsistency between the above-mentioned studies may be due to differences in the intensity of exercise performed.

At present, the mechanism by which isometric exercise training elicits a reduction in BP has remained unclear [40]. Wiles et al. [41] suggested that the rise in BP during isometric exercise will stimulate the baroreceptors, which are sensory afferent nerve endings located in the carotid sinus and the aortic arch. When the BP is elevated, the baroreceptors are stretched and result in a reflex-mediated increase in parasympathetic nerve activity, as well as a decrease in sympathetic nerve activity [41]. Consequently, it causes a decline in the heart rate, while the diameter of blood vessels increases and further leads to a drop in the BP [41]. Moreover, it has been suggested that the reduction in BP after isometric exercise training is related to the repeated power of hydrogen (pH) changes due to muscle fatigue and lactate production that act as a metaboreceptor stimulus [42], augmentation in vasodilator substances, for instance, nitric oxide (NO) [43], and reduction in peripheral vascular adaptations [44].

Data from the *HE*alth, *RI*sk factors, exercise *T*raining *A*nd *G*enetics (HERITAGE) Family Study suggest that reduction in BP after exercise may be influenced by genetic factors [45]. In this regard, the *ACE* gene was initially believed to influence the BP response to exercise [19-21] due to its role in the RAS. Rigat et al. [23] reported that individuals with the *DD* genotype had higher ACE activity compared to those with the *II* genotype. A higher level of ACE in the circulation and skeletal muscle renin-angiotensin system (RAS) would increase the production of angiotensin II (ANG II) [46, 47]. Nevertheless, ANG II has different effects on circulating and skeletal muscle RAS [46]. In circulating RAS, ANG II binds to several receptors that construct the blood vessels to increase BP [47]. However, ANG II in skeletal muscle RAS stimulates the production of angiotensin (ANG) (1-7) peptide, a potent vasodilator that causes a decrease in BP [46]. As this study employed IHG training that particularly involved the contraction of skeletal muscle, the reduction in SBP and HR in individuals with *DD* genotype observed in this study might be interpreted as due to high production of ANG (1-7) during exercise. Nonetheless, the present study did not measure the components of the skeletal muscle RAS. This therefore warrants future studies to confirm this possible mechanism.

The greater reduction in SBP and HR among *DD* genotype carriers could also be due to their higher baseline BP values. This present result was consistent with the finding obtained by Millar et al. [48], who demonstrated that normotensive individuals with higher baseline values of resting SBP and HR had a more pronounced reduction in these parameters after IHG training than those with lower baseline values. This finding was also consistent with Wiley et al. [2], who observed a larger reduction in resting BP in hypertensive patients following isometric exercise training compared with normotensive individuals. Collectively, these findings support the idea of Badrov et al. [12], who had previously suggested that individuals with higher baseline BP values might have greater capacity for reduction in BP following IHG training compared to those with lower baseline BP values. This speculation is supported by the fact that those with higher resting BP, such as hypertensive patients, present greater sympathetic activity at rest [49], which could lead to greater hemodynamic responsiveness to sympathetic activation [50].

The findings of the current study reaffirm previous reports of a reduction in resting BP after 8 weeks of IHG training among normotensive individuals [7, 8, 12]. Similar to previous studies [7, 8, 12], this study found that 8 weeks of IHG training significantly decreased resting SBP, MAP, and PP by 4.2 ± 6.1 mmHg, 2.4 ± 4.2 mmHg, and 2.8 ± 6.3 mmHg, respectively (Table 2). However, no significant difference was observed in resting DBP after the IHG training programme, which was similar to the observations of Badrov et al. [12] in normotensive women. However, the reason for the lack of change in DBP following 8 weeks of IHG training remains unclear. The underlying mechanism could be that DBP has a smaller range of values than SBP, which would limit the maximal change in DBP value [51]. Future studies with larger sample sizes may increase the chance of discovering a significant difference.

Concomitant with the reduction in resting BP, resting HR was also significantly lower after 8 weeks of IHG training in this study (Table 2). This decrease was similar to that reported previously by Singh et al. [52]. In fact, previous studies have suggested that the decrease

in resting BP and HR may be due to a reduction in sympathetic nerve activity during IHG exercise [2, 53]. When BP is elevated during IHG exercise, the baroreceptors are stretched, resulting in a reflex-mediated increase in parasympathetic nerve activity, and a decrease in sympathetic nerve activity [2]. Consequently, it caused the decline in the heart rate, while the diameter of blood vessels increased, leading to a drop in the BP [2].

Besides resting BP and HR, the present study also showed that 8 weeks of IHG training significantly improved muscle strength (Table 2). The increase in muscle strength observed in the present study is consistent with results of previous studies demonstrating that IHG exercise at 30% of MVC improved muscle strength [9, 54]. Considering the significant changes in resting BP and HR as well as muscle strength following IHG training, the training protocol adopted in the present study may be prescribed as part of lifestyle modification for maintaining a desirable BP level.

Comparable with the findings of a cardiovascular response to IHG training, a significant reduction in cardiovascular variables immediately following the first session of IHG exercise was observed (acute effects) (Table 2). Resting SBP and MAP were significantly reduced by 3.2 ± 7.2 mm Hg and 2.7 ± 5.8 mmHg, respectively, in response to acute IHG exercise. These findings indicated that IHG exercise may provide substantial benefits for hypertensive patients by lowering their resting BP and HR for a period of time after acute isometric exercise exposure. Nevertheless, for individuals with uncontrolled hypertension, the IHG exercise can cause a temporary increase in BP due to the increase in muscle tension. Hence, to prevent any potential risk, the IHG exercise should be performed at low intensity (30% MVC) and needs to be closely monitored by the clinician.

Although the present study has yielded some useful findings, there is a limitation that should be taken into consideration. The sample size in each genotype group may have been too small, and further, larger studies are required to confirm these results. The present study did not include a non-exercising control group to reduce the variability of the results, as the study was primarily designed to investigate the effects of *ACE I/D* gene polymorphism on IHG training adaptation by comparison among genotype groups. Despite this limitation, the current results remain valid and applicable, as the participants in each group were relatively homogeneous in terms of sex, physical characteristics, health status, and ethnic background. The training intervention was standardized, and the same trained investigator conducted all the training sessions.

CONCLUSIONS

In conclusion, the present study showed that individuals with the *D* allele exhibited a significantly greater improvement in cardiovascular parameters after IHG training compared to individuals with the *I* allele. Future studies on hypertensive patients are warranted to observe whether the *ACE I/D* gene polymorphism drives similar cardiovascular and muscular changes in hypertensive patients following 8 weeks of IHG training. Moreover, as hypertension is a multifactorial outcome, future studies are needed to address the role of *ACE I/D* gene polymorphism and other genetic variants in the BP response following IHG training.

Acknowledgements: The authors thank the volunteers who made this study possible. This study was funded by the Student Research Fund of the Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia.

Conflict of Interest: The authors declare that they have no conflict of interest.

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