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Angiotensin-converting enzyme genotype and physical performance during US Army basic training

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[1]*Thermal and Mountain Medicine Division, and* [3]*Military Performance Division, US Army Research Institute of Environmental Medicine, Natick 01760;* [2]*Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115; and* [4]*US Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, Maryland 21005*

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Sonna, Larry A., Marilyn A. Sharp, Joseph J. Knapik, Michael Cullivan, Karen C. Angel, John F. Patton, and Craig M. Lilly. Angiotensin-converting enzyme genotype and physical performance during US Army basic training. *J Appl Physiol* 91: 1355–1363, 2001.—Prior studies have suggested that angiotensin I-converting enzyme (ACE) genotype correlates with superior physical performance in highly selected populations. This study assessed whether such an association exists in a heterogeneous population. Using polymerase chain reaction techniques, we determined the ACE genotypes (insertion/insertion, deletion/insertion, or deletion/deletion) of 62 male and 85 female US Army recruits. Before and after 8 wk of basic training, we determined peak oxygen uptake and performance on the Army Physical Fitness Test (APFT), which includes standardized measures of muscular endurance (sit-ups, push-ups) and a 2-mile run. Subjects of different ACE genotypes had similar peak oxygen uptakes and APFT scores, both before and after training. Subjects with genotype II had higher APFT scores than others, but the differences were not statistically significant. Furthermore, no ACE genotype group had a performance advantage in analyses that adjusted for baseline fitness. We conclude that ACE genotype does not have a strong effect on aerobic power or muscular endurance in healthy, young American adults drawn from an ethnically and geographically diverse population.

physical training; physical fitness; oxygen uptake; genetics; exercise

ANGIOTENSIN I-CONVERTING ENZYME (ACE) (kininase II, EC 3.4.15.1) is a widely expressed enzyme whose physiological function is to convert angiotensin I to angiotensin II and to inactivate bradykinin and tachykinins (4). There is a 287-bp insertion (I)/deletion (D) polymorphism in *intron 16* of the ACE gene that occurs commonly and that accounts for a substantial portion of the variance in serum ACE levels (26), with the highest mean levels of serum ACE occurring in subjects with genotype DD and the lowest in subjects with genotype II. This polymorphism can be reliably detected by PCR techniques (16). Because of its effect on serum and tissue ACE levels and because ACE is involved in the metabolism of substances that affect vascular remodeling (4), it has been hypothesized that the ACE ID polymorphism might account for some of the differences among individuals in the response to physical training. In support of this, a study in British Army soldiers (21) found a significant association between ACE genotype and the performance gains realized over the course of basic training. Specifically, recruits homozygous for the I polymorphism (ACE genotype II) had 11-fold greater gains in the ability to perform repetitive elbow flexions with a 15-kg barbell compared with those who were homozygous for the D polymorphism (ACE genotype DD). Individuals who were heterozygous (ACE genotype DI) had intermediate levels of performance. It has subsequently been suggested that the II genotype conveys an improved efficiency of skeletal muscle contraction (34). The possibility that ACE genotype plays an important role in physical performance is further supported by epidemiological studies of elite athletes (1, 5, 22), which have found a higher prevalence of individuals with ACE genotype II among elite athletes than in the general population. Finally, in a study of postmenopausal women (7), there was a significant association between ACE genotype and maximal oxygen uptake ($\dot{V}O_{2\,max}$), with the highest values recorded among individuals with ACE genotype II. Together, these findings have been taken to suggest that these variants in the ACE gene may have a strong influence on physical performance, the response to physical training, or both.

One significant limitation of these studies is the fact that the predictive power of genetic variants differs as a function of the genetic background of the population under study. Studies that have found a strong effect of ACE genotype on performance in young athletes have typically been performed in populations of limited ethnic

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or geographic diversity, which may have resulted in an overestimate of the effect of this polymorphism on physical performance. Furthermore, genetic association studies that are performed in relatively homogeneous populations can be confounded by linkage disequilibrium between the gene polymorphism investigated and a nearby locus that actually influences the phenotypic trait under study.

The role of ACE genotype in aerobic performance has recently been examined in detail, in relatively large cohorts of subjects drawn from widely dispersed geographic backgrounds. Two studies found no relationship between ACE genotype and $\dot{V}O_{2\,max}$ (24, 25). Furthermore, a genomewide scan for markers linked with $\dot{V}O_{2\,max}$ found none on *chromosome 17*, the location of the ACE gene (3). Although these studies convincingly ruled out a role for ACE genotype in aerobic performance, they did not examine measures of muscular endurance, which is the performance attribute most convincingly associated with ACE genotype in previous work (21, 34). In summary, the literature to date suggests that the ACE genotype does not generally affect aerobic power but might be a major contributor to muscular endurance in select populations. Whether or not this apparent association holds true in the general population is as of yet unknown.

To help clarify this issue, we studied the effect of the ACE ID variant on the peak oxygen uptake and physical performance response to basic training in an ethnically and geographically diverse population consisting of US Army recruits undergoing basic training. Based on the literature, our hypothesis was that ACE genotype would have a significant effect on measures of muscular endurance but not on measures of aerobic power. We chose to study a heterogeneous population for two reasons: first, to determine whether the apparent association between ACE genotype and muscular endurance previously found in select populations holds true in a cohort drawn from the general population and, second, because doing so would reduce the chance that any detected associations would be attributable to linkage disequilibrium. To test our hypothesis, we examined the effect of ACE genotype on measures of aerobic capacity (peak oxygen uptake and 2-mile run time) and muscular endurance (push-ups and sit-ups). The tool that we used to assess physical performance in our recruits was the Army Physical Fitness Test (APFT) (9, 12). Unlike repetitive elbow flexions, the distribution of APFT scores in US military populations is known, and the comparability of APFT performance scores across gender and age lines has been extensively quantified (12, 13).

MATERIALS AND METHODS

Research volunteers. This double-blind study was one of a number of studies conducted from May through July 1998 at Ft. Jackson, SC, on a cohort of Army recruits undergoing the 8-wk basic training course. Among others, these studies included *1*) an assessment of the fitness of these soldiers compared with historical standards (27); *2*) an evaluation of the prevalence of exercise-induced bronchospasm and its effect on physical performance (28); and *3*) an evaluation of risk factors for injury during basic training (14, 15). Participating in the physical fitness study were 182 men and 168 women from two basic training battalions at Ft. Jackson, SC, from which the subjects in this study were drawn. Of these, 62 men and 85 women also agreed to participate in this study. The study protocol was approved by the appropriate Army institutional review boards, and subjects gave voluntary and informed consent to participate in accordance with Army Regulation 70–25 (8).

Subject characteristics. Gender and ethnic origin were as stated by the subjects (obtained, where necessary, from records in the subject's military unit). US state of enlistment was obtained from the subject's military records. Stature and body mass were measured with an anthropometer and digital scale, respectively, with subjects in shorts, T-shirts, and socks. From these values, body mass index (mass divided by the square of the height, in units of kg/m^2) was calculated. Body composition was measured by dual-energy X-ray absorptiometry (DEXA) (17) on subjects in the supine position with palms down and legs at 45° of external femoral rotation, using a DEXA system (LUNAR, Madison, WI) that had been set up and calibrated at the test site by a manufacturer's representative. Scanning began at the head and progressed in 1-cm slices to the toes with the machine set to the (fast) 10-min scanning speed. LUNAR software (version 3.6) provided estimates of percent body fat.

Army basic training. Subjects participated in 8 wk of physical training four to six mornings per week for 1–1.5 h, beginning around 5:30 AM. Training sessions alternated between aerobic and muscle strength sessions. On average, they performed 2 aerobic days and 2 strength training days per week. On aerobic training days, soldiers ran between 0.5 and 3 miles, sometimes performing sprints. To maximize the chances that a training effect would be achieved in all individuals, soldiers ran in one of four ability groups, established based on quartiles from a 2-mile timed run performed during the first week of basic training. All four ability groups ran for the same duration of time, but those in the higher fitness groups ran at a faster pace and thus covered a greater distance in the time allowed. On muscle strength days, training consisted of push-ups, sit-ups, and calisthenic-type exercises. In addition to morning physical training, trainees participated in many other physical activities, such as road marches (with and without additional loads, such as backpacks, weapons, and combat equipment), fitness obstacle courses, rappelling, as well as physical training involving short sprints, rolling, climbing, pulling, and pushing.

Peak oxygen uptake. Aerobic power was measured at the beginning and end of basic training, with the use of open-circuit spirometry and a continuous uphill treadmill running protocol, similar to that used by Patton et al. (23) and based on standard methods (20, 31). The initial 5-min warm-up was run at 0% grade and 2.68 m/s (6 mph) for men and 2.24 m/s (5 mph) for women. If the heart rate was <150 beats/min by *minute 5* of the warm-up, treadmill speed was increased 0.45 m/s (0.5 mph) for the remainder of the test. After the warm-up, the treadmill grade was increased by 2% every 3 min until voluntary exhaustion. Although the protocol was designed to measure peak oxygen uptake, the subject was considered to have achieved $\dot{V}O_{2\,max}$ if there was an increase of $<2\ ml \cdot kg^{-1} \cdot min^{-1}$ (or 0.15 l/min) with an increase in treadmill grade. Volunteers wore a nose clip and were connected to the oxygen uptake-measuring device via a low-resistance non-rebreathing valve (Hans Rudolf, Kansas City, MO) by a mouthpiece. The on-line oxygen uptake system consisted of an Applied Electrochemistry S-3A oxygen ana-

lyzer (AEI Technologies, Pittsburgh, PA), a Beckman LB-2 carbon dioxide analyzer (Sensormedics, Yorba Linda, CA) and a K. L. Engineering flowmeter turbine (K. L. Engineering Turbine, Northridge, CA), interfaced with a Hewlett-Packard computer (model 9122, Hewlett-Packard, Palo Alto, CA). A single-lead electrocardiogram (model 1511B, Hewlett-Packard) was monitored by trained personnel during the test to determine heart rate and ensure the safety of the volunteer.

APFT. The APFT (9, 10) is a standard assessment of physical fitness administered to all active duty personnel at least twice per year, to recruits at the beginning and end of basic training, and to all members of the reserve components at least once per year. It consists of three events: *1*) a push-up event, where the soldier performs as many Army-standard push-ups as possible in 2 min, *2*) a sit-up event, where the soldier performs as many Army-standard sit-ups as possible in 2 min, and *3*) a timed 2-mile run. For the push-up, the subject is required to start from a position with elbows fully extended, lower his or her body in a generally straight line to a point where the upper arms are parallel to the ground, then return to the starting point. For the sit-up, the subject's knees are maintained at a 90° angle with ankles held in place by a second person to keep the subject's feet firmly on the ground, and hands are behind the head with interlocked fingers. The subject is then required to raise the upper body to a vertical position, such that the base of the neck is anterior to the base of the spine, and then return to the starting position. The accepted techniques for performing Army-standard push-ups and sit-ups are explained and demonstrated to the soldiers just before each event. The precise wordings of the explanations given before each event are standard throughout the US Army (9, 10). A soldier who does not perform push-ups or sit-ups to standard during the first 10 repetitions is stopped, given an explanation of why the performance technique is incorrect, and, after a rest period, retested. Any push-up or sit-up repetition not performed to Army standards is not counted. The 2-mile run is unassisted and performed at a pace determined by the subject; soldiers are not disqualified if they alternate running and walking on this performance test.

Performance on each event is given an age- and gender-adjusted score on a scale of 1–100, based on the number of repetitions performed or the time taken to run 2 miles. A minimum score of 50 in each event is required to graduate from basic training, but soldiers are encouraged to achieve a score of $\geq$60, which is the standard they will subsequently be required to meet. In healthy, trained individuals, the 2-mile run event is known to correlate well with a subject's $\dot{V}o_{2\,max}$ (12, 19); the sit-up and push-up events are generally considered to be measures of muscular endurance (12). The APFT scoring system accounts for individuals who are unable to complete a 2-mile run by assigning them a score of zero for the run event. Under the standards in place at the time of this study, only ~5% of the general military population would achieve a score of 100 on any given event (13). The test scoring system, therefore, reduces the statistical impact of outliers on the overall population without excluding them from the analysis and, importantly, allows grouping of men and women into a single distribution for purposes of statistical analysis. The APFT scoring system has been extensively evaluated (12), and, as a result, the distribution of APFT scores in military populations is well-known, and the comparability of APFT performance scores across gender and age lines has been quantified (13). The APFT has been in use since 1984, although a refinement of the scoring system was introduced shortly after this study was completed in 1998 (10).

The APFT was administered by the soldiers' basic training units according to Army standards by individuals not otherwise directly involved with this study. The age- and gender-adjusted APFT scores for each subject were calculated in accordance with the standards in effect at the time (9).

ACE genotype. A 20-ml sample of blood was drawn from each subject. Leukocytes were separated by red blood cell lysis and Ficoll gradient centrifugation. Leukocyte DNA was extracted by using Qiagen MAXI-prep kits (Qiagen, Carlsbad, CA) and was frozen in distilled water at a concentration of 35–100 ng/μl until ready for analysis.

ACE genotypes were determined by PCR methods using the human angiotensin-converting enzyme (HACE) 3/HACE 5 primers described by others (16). Both the HACE 3 (identifying) and HACE 5 (confirming) PCRs were run in duplicate on each sample. In the event of a discrepancy between the first and second genotype assignment, the PCR runs were repeated, as needed, until a consensus genotype assignment could be made. An investigator who was blinded to the physical performance data assigned the genotypes.

Statistics. Statistical analyses were performed using SPSS for Windows (30). All P values are two-tailed. Differences in the distributions of ACE genotypes by gender and by ethnic origin were examined using Pearson's χ^2. For Hardy-Weinberg equilibrium calculations, a χ^2 statistic (one degree of freedom) was computed from the observed distribution of genotypes and the distribution of genotypes expected from applying the Hardy-Weinberg equilibrium assumption to the observed allele frequencies in the population. The P value corresponding to this statistic was calculated assuming a single degree of freedom.

Parameters measured during exercise treadmill testing and APFT performance scores achieved at the beginning and end of basic training were analyzed by one-way ANOVA after checking for normality by Kolmogorov-Smirnov and for homogeneity of variance by Levene's test. Because one-way ANOVA is robust with respect to deviations from normality and homogeneity of variance, a P value of 0.01 was chosen as the threshold for rejecting the null hypothesis on the Kolmogorov-Smirnov and Levene's tests. When the assumption of normality or homogeneity of variance was violated, testing for statistical significance was performed by Kruskal-Wallis ANOVA on ranks. Post hoc testing for significant differences in the ANOVA was performed by the Tukey's honestly significant difference test.

The gains in physical performance realized during basic training were also analyzed by two-way, repeated-measures ANOVA, using each performance measure as the within-subjects variable and ACE genotype as the between-subjects variable. Peak oxygen uptake data were stratified by gender before analysis. However, because APFT scores are gender and age adjusted, no stratification of these data by gender was performed. The two-way, repeated-measures ANOVAs were performed after checking the data for equality of covariance matrices by Box's test, for sphericity by Mauchly's test, and for equality of error variances by Levene's test. Because there were no violations of the assumption of equality of covariance matrices, no adjustments for sphericity were required. With one exception, there were no violations of the assumption of equal variance. This exception was in the adjusted ANOVA for push-ups in which ethnic origin and baseline performance quartile in the event had been incorporated as covariates; in this analysis, Levene's test revealed $P = 0.026$ for pretraining push-up scores.

To control for baseline level of fitness, we stratified the cohort for each event by quartiles of baseline APFT score. The performance gains realized by subjects in each quartile were analyzed by paired *t*-test, without regard to genotype. The effect of ACE genotype on performance within each quartile stratum was analyzed by one-way ANOVA as described above.

Power analysis was performed using SigmaStat 2.0 for Windows (29). With 30 subjects of each genotype, one-way ANOVA can detect a difference of 12.5 in mean APFT score between the genotype groups with the lowest and highest mean scores, with a power of 0.83 and an alpha of 0.05 (assuming the SD to be 15). Assuming that subjects would attempt to achieve an average score of 60 in each event (which is higher than the score of 50 required to graduate from basic training but is also the standard to which they know they will subsequently be held), this means that our study had a power of 0.8 to detect a difference of ~20% between the best- and worst-performing groups.

RESULTS

Characteristics of the volunteers. A total of 147 subjects (85 women and 62 men) participated in this study. The subjects were young [median age of 21 yr (interquartile range of 19–24 yr); mean age of 21.7 ± 3.6 (SD) yr] and ethnically diverse [84 (57%) Caucasians, 37 (25%) African-Americans, 20 (14%) Hispanics, 5 (3%) Asians, and 1 (1%) Native American] and were drawn from 42 different US states and territories. The mean body mass index (±SD) was 23.1 ± 3.1 in the female subjects and 24.8 ± 3.0 in the male subjects at the beginning of basic training. The mean percent body fat by DEXA (±SD) at the beginning of basic training was 27.9 ± 6.1 in the female subjects and 16.4 ± 5.7 in the male subjects.

Genotype distributions and Hardy-Weinberg equilibrium. The distributions of ACE genotypes in the cohort are summarized in Table 1. The allele frequencies in the cohort were 54% D and 46% I, which are comparable to what has been reported in populations not selected for elite athletic status (5, 16, 25). The distributions of genotypes in the overall cohort and in each of the subgroups were not significantly different from those predicted by Hardy-Weinberg equilibrium (Table 2). There was no significant difference in the distribution of genotypes between genders ($P = 0.22$ by Pearson χ^2). However, there was a significant difference in the distribution of genotypes among members of different ethnic groups ($P = 0.003$ by Pearson χ^2). Fifty-four percent of subjects of African-American ethnicity had genotype DD, whereas only 23% of subjects of Caucasian or other ethnicity had this genotype.

ACE genotype and measures of aerobic performance. Measurements of aerobic power on the treadmill at maximum effort are summarized in Table 2. Only those individuals on whom a complete set of parameters were obtained, both at the beginning and end of basic training (61 women and 56 men), are included in this analysis. One-way ANOVA of peak oxygen uptakes showed no statistically significant differences among subjects of different ACE genotypes, either before or after training. Additionally, a two-way, repeated-measures ANOVA revealed that the increase in peak oxygen uptake as a result of training was statistically significant ($P < 0.001$ for the women and $P = 0.021$ for the men). However, there was no significant effect of genotype on this increase in peak oxygen uptake ($P = 0.67$ for women and $P = 0.75$ for men). The P value for the interaction between change in peak oxygen uptake and ACE genotype was 0.74 for the women and 0.63 for the men. Additionally, with the exception of posttraining respiratory exchange ratio (RER) in the women, there was no statistically significant difference among subjects of different ACE genotypes in any of the other parameters measured. In the case of the posttraining RER in the female subjects, post hoc analysis by Tukey's honestly significant difference test showed a significant difference between subjects with genotypes DI and DD but not between subjects with genotypes DD and II, nor between subjects with genotypes DI and II.

The changes in 2-mile run scores on the APFT are presented in Table 3. Because the APFT scoring system adjusts for gender and age, the data for men and women are combined. The mean 2-mile run scores achieved, both at the beginning and at the end of basic training, were highest for subjects with ACE genotype II, intermediate for subjects with genotype DI, and lowest for subjects with genotype DD. However, these differences were not statistically significant by one-way ANOVA (Table 3). Furthermore, a two-way ANOVA revealed that, although the subjects showed a

Table 1. *Distribution of ACE genotypes among subgroups and analysis of Hardy-Weinberg equilibrium*

	Total	ACE Genotype						Hardy-Weinberg
		DD		DI		II		
	n	*n*	%	*n*	%	*n*	%	*P*
All subjects	147	45	30.6	70	47.6	32	21.8	0.63
Gender								
Male	62	15	24.2	30	48.4	17	27.4	0.81
Female	85	30	35.3	40	47.1	15	17.6	0.79
Ethnic origin								
Caucasian	84	19	22.6	45	53.6	20	23.8	0.51
African-American	37	20	54.1	14	37.8	3	8.1	0.80
Other*	26	6	23.1	11	42.3	9	34.6	0.47

ACE, angiotensin I-converting enzyme; D, deletion; I, insertion. *Includes subjects of Asian, Hispanic, and Native American heritage.

Table 2. *ACE genotype and treadmill aerobic power before and after basic training*

Parameter	Genotype	Women			Men		
		n	Pre	Post	*n*	Pre	Post
Peak oxygen uptake, ml·min^{-1}·kg^{-1}	DD	22	40.2 ± 1.3	43.8 ± 1.2	13	49.8 ± 1.4	51.8 ± 1.3
	DI	29	39.7 ± 1.0	42.2 ± 0.9	26	51.8 ± 1.5	52.5 ± 1.1
	II	10	39.4 ± 1.0	42.1 ± 1.5	17	50.5 ± 1.2	52.3 ± 1.3
P			0.91	0.50		0.61	0.93
Peak oxygen uptake, l/min	DD		2.52 ± 0.12	2.76 ± 0.12		3.93 ± 0.11	4.03 ± 0.09
	DI		2.44 ± 0.09	2.62 ± 0.07		3.90 ± 0.11	3.94 ± 0.08
	II		2.47 ± 0.11	2.69 ± 0.12		4.00 ± 0.16	4.07 ± 0.13
P			0.83	0.55		0.84	0.63
Minute ventilation, l/min	DD		98.8 ± 3.8	96.7 ± 4.3		145.4 ± 3.6	137.9 ± 4.7
	DI		100.7 ± 3.5	98.0 ± 2.7		142.4 ± 3.0	138.2 ± 3.3
	II		97.4 ± 3.5	98.5 ± 3.2		140.6 ± 6.1	135.0 ± 4.3
P			0.86	0.94		0.79	0.82
Peak CO_2 output, l/min	DD		3.01 ± 0.15	2.95 ± 0.14		4.79 ± 0.14	4.60 ± 0.13
	DI		2.98 ± 0.10	3.00 ± 0.08		4.68 ± 0.11	4.46 ± 0.10
	II		3.01 ± 0.12	3.02 ± 0.09		4.84 ± 0.19	4.61 ± 0.13
P			0.99	0.90		0.71	0.54
Respiratory exchange ratio at maximum effort	DD		1.19 ± 0.02	1.07 ± 0.02*		1.22 ± 0.01	1.15 ± 0.02
	DI		1.23 ± 0.01	1.15 ± 0.01*		1.21 ± 0.02	1.13 ± 0.01
	II		1.22 ± 0.01	1.13 ± 0.03		1.21 ± 0.02	1.14 ± 0.02
P			0.21	0.003		0.95	0.92
Minute ventilation/oxygen uptake	DD		39.9 ± 1.3	35.3 ± 0.9		37.1 ± 0.7	34.4 ± 1.2
	DI		41.6 ± 1.1	37.6 ± 0.8		37.0 ± 1.0	35.3 ± 0.9
	II		39.9 ± 1.9	36.9 ± 1.0		35.6 ± 1.5	33.6 ± 1.3
P			0.54	0.13		0.61	0.51
Percent of maximum predicted heart rate achieved	DD		97.3 ± 1.3	92.9 ± 0.8		98.6 ± 0.9	94.7 ± 1.1
	DI		98.5 ± 0.8	92.3 ± 0.7		99.3 ± 0.9	93.5 ± 0.7
	II		101.8 ± 1.4	95.4 ± 1.2		99.0 ± 1.0	93.8 ± 0.8
P			0.08	0.08		0.87	0.61

Values are means ± SE; *n*, no. of subjects. Pre and Post, before and after basic training, respectively. All reported values were measured at maximum effort. *P* values were computed by one-way ANOVA. *$P < 0.05$ by Tukey's honestly significant difference test.

statistically significant increase in 2-mile run scores over the course of basic training (within subject, $P < 0.001$), the gains realized were not significantly affected by genotype (between subject, $P = 0.24$). The *P* value for the interaction between gain in 2-mile run score and ACE genotype was 0.37.

ACE genotype and measures of muscular endurance. Pre- and posttraining scores on the push-up and sit-up events of the APFT are presented in Table 3. The mean push-up and sit-up scores at baseline were highest for subjects with genotype II, with a 10–13% difference in mean scores between subjects with genotype II and those with genotype DD at the beginning of basic training. However, this difference was not statistically significant. At the end of basic training, the differences among the scores achieved by subjects of different genotypes were very small in magnitude (each point corresponds to a single repetition) and were not statistically significant.

A two-way ANOVA revealed that the performance gains realized in both push-up and sit-up scores were statistically significant (within subject, $P < 0.001$, Table 3). However, as with the 2-mile run data, these gains were not significantly affected by genotype. The *P* values for the interactions between gains in scores

Table 3. *Effect of ACE genotype on Army Physical Fitness Test performance gains during basic training*

Performance Measure	Genotype	*n*	APFT Event Score		Repeated-Measures 2-way ANOVA	
			Pre	Post	Training effect (within subject) *P*	Genotype effect (between subjects) *P*
2-Mile run	DD	44	37 ± 4	73 ± 2		
	DI	68	44 ± 4	75 ± 2	<0.001	0.24
	II	31	49 ± 5	76 ± 4		
*P**			0.20	0.74		
Push-ups	DD	44	47 ± 3	65 ± 3		
	DI	68	52 ± 2	66 ± 2	<0.001	0.40
	II	31	54 ± 4	66 ± 3		
*P**			0.27	0.91		
Sit-ups	DD	44	51 ± 2	67 ± 2		
	DI	68	52 ± 2	68 ± 1	<0.001	0.31
	II	31	57 ± 3	70 ± 2		
*P**			0.24	0.65		

Values are means ± SE; *n*, no. of subjects. APFT, Army Physical Fitness Test. **P* values were computed by 1-way ANOVA.

and ACE genotype were 0.55 for push-ups and 0.42 for sit-ups.

Further analysis of ethnic origin and baseline fitness. The effects of two potential confounding variables were examined in detail: ethnic origin and baseline level of fitness (as measured by the prebasic training performance scores).

As noted, the distribution of ACE genotypes was significantly different between African-Americans and members of other ethnic groups (Table 2), which could bias the results if there were an independent effect of ethnic background on the performance measures under study. We, therefore, performed a univariate analysis of the effect of ethnic origin (Caucasian, African-American, other) on pre- and posttraining APFT scores (without regard to genotype). With the exception of pretraining run scores ($P = 0.03$ by one-way ANOVA), there was no effect of ethnic origin on any of the pre- or posttraining event scores. Ethnic origin was then incorporated as a covariate in the two-way, repeated-measures ANOVA of the gains in APFT performance realized over the course of basic training. This analysis showed no effect of ethnic origin on the performance gains realized ($P = 0.22$, 0.90, and 0.86 for the run, push-up, and sit-up events, respectively). Furthermore, even after incorporating ethnic origin as a covariate in the two-way ANOVA, there was no significant effect of ACE genotype on performance gains ($P = 0.22$, 0.40, and 0.31 for the run, push-up, and sit-up events, respectively).

We also specifically examined whether ACE genotype affected our measures of muscular endurance in subjects only of Caucasian heritage (the group in which an effect of genotype has most consistently been noticed by others). As in the overall cohort, a two-way, repeated-measures ANOVA showed a statistically significant increase in push-up and sit-up scores over the course of basic training ($P < 0.001$) among subjects of Caucasian heritage but no statistically significant effect of ACE genotype ($P = 0.73$ and 0.38, respectively).

It is well established that the response to training is significantly affected by baseline fitness (18, 33); subjects who have the lowest level of fitness before training tend to show the greatest improvements in performance as a result of training. To determine whether this potential confounder was in fact present in our cohort, we stratified each event's APFT performance data by quartiles, based on the pretraining APFT score in that event (Table 4). In all three events, the differences between post- and pretraining scores decreased with increasing baseline fitness quartile. In the 2-mile run event, all but the fittest subjects (those in the highest baseline run score quartile) showed statistically significant gains in run score over the course of basic training ($P < 0.001$ by paired t-test in *quartiles 1–3* and $P = 0.70$ in *quartile 4*). A significant training effect in the push-up event was also found in all but the highest quartile ($P < 0.001$ in *quartiles 1* and *2*, $P = 0.007$ in *quartile 3*, and $P = 0.89$ in *quartile 4*). In the sit-up event, subjects in all four quartiles showed statistically significant gains over the course of basic training ($P < 0.001$ in *quartiles 1–3*, $P = 0.004$ in *quartile 4*). Thus the response to training appeared to be different depending on baseline level of performance.

Based on the above findings, we incorporated both ethnic origin and baseline performance quartile as covariates in a two-way, repeated-measures ANOVA of

Table 4. *Effect of ACE genotype and baseline APFT event score on performance gains during basic training*

Pretraining Performance Quartile	ACE Genotype	APFT Score: 2-Mile run event *n*	Pre	Post	Mean gain	Push-up event *n*	Pre	Post	Mean gain	Sit-up event *n*	Pre	Post	Mean gain
1	All	35	2 ± 1	69 ± 2	+66*	36	23 ± 2	62 ± 2	+39*	33	33 ± 2	60 ± 1	+26*
(Least fit)	DD	14	3 ± 1	64 ± 4		15	22 ± 4	62 ± 2		15	35 ± 3	58 ± 2	
	DI	15	2 ± 1	72 ± 3		15	27 ± 4	61 ± 3		15	32 ± 3	60 ± 2	
	II	6	2 ± 2	72 ± 5		6	16 ± 6	63 ± 3		3	34 ± 5	67 ± 3	
P			0.97†	0.27			0.32	0.88			0.79	0.25	
2	All	36	30 ± 2	70 ± 3	+40*	34	49 ± 1	61 ± 3	+12*	38	48 ± 0	64 ± 1	+16*
	DD	12	31 ± 3	72 ± 4		8	48 ± 1	57 ± 9		9	49 ± 1	64 ± 3	
	DI	18	29 ± 2	68 ± 5		18	48 ± 1	62 ± 4		19	47 ± 1	65 ± 2	
	II	6	31 ± 5	70 ± 5		8	50 ± 1	62 ± 4		10	47 ± 1	62 ± 3	
P			0.92	0.82			0.49	0.78			0.28	0.72	
3	All	37	58 ± 1	77 ± 2	+20*	39	59 ± 1	67 ± 3	+8*	36	57 ± 0	73 ± 1	+16*
	DD	11	60 ± 2	79 ± 4		12	57 ± 1	70 ± 2		10	57 ± 1	73 ± 3	
	DI	17	56 ± 1	76 ± 3		22	59 ± 1	66 ± 5		18	57 ± 0	73 ± 2	
	II	9	58 ± 1	78 ± 5		5	59 ± 2	66 ± 3		8	57 ± 1	71 ± 2	
P			0.14	0.88			0.26	0.79			0.73	0.86	
4	All	35	81 ± 2	83 ± 4	+1	34	73 ± 1	74 ± 3	+0	36	73 ± 1	76 ± 2	+4*
(Most fit)	DD	7	78 ± 4	83 ± 5		9	73 ± 3	69 ± 9		10	70 ± 2	78 ± 3	
	DI	18	83 ± 3	83 ± 6		13	74 ± 2	79 ± 2		16	73 ± 2	75 ± 3	
	II	10	80 ± 2	81 ± 9		12	73 ± 3	70 ± 7		10	74 ± 3	77 ± 3	
P			0.44	0.97			0.96	0.41			0.60	0.66	

Values are means ± SE; *n*, no. of subjects. *P* values were calculated by one-way ANOVA unless otherwise noted. Quartiles were determined separately for each event. *$P < 0.01$ by paired t-test. †Computed by Kruskal-Wallis ANOVA on ranks.

performance gain as a function of genotype. In each of these analyses, there was a statistically significant effect of baseline performance quartile on the performance gains realized ($P < 0.001$ in all three events). There was, however, no effect of ethnic origin ($P = 0.34$, 0.59, and 0.97 for the run, push-up, and sit-up events, respectively). Thus the baseline level of fitness, but not ethnic origin, was in fact a significant confounder in the performance gains realized by our cohort. However, even after ethnic origin and baseline fitness were included in the analyses, there was still no effect of ACE genotype on the performance gains realized ($P = 1.0$, 0.58, and 0.96, respectively).

To adjust for the confounding effects of baseline fitness, we also stratified our cohort by baseline level of performance in each event (Table 4) and examined the effect of ACE genotype on performance scores in each stratum. We found no significant effect of ACE genotype on mean performance scores, either before or after training. Importantly, and in contrast to the unstratified data (Table 3), subjects with genotype II no longer consistently displayed the best performance (Table 4). A χ^2 analysis showed no significant differences in the distributions of ACE genotypes across quartiles.

DISCUSSION

Although our subjects showed statistically significant gains in peak oxygen uptake over the course of basic training, we found no statistically significant effect of ACE genotype either on peak oxygen uptake at baseline or on the realized gains in peak oxygen uptake. Furthermore, we found no effect of ACE genotype on minute ventilation, CO_2 output, maximum heart rate achieved, or the ratio of minute ventilation to oxygen uptake, either before or after basic training. We did find a statistically significant difference in RER at maximal effort between women with genotypes DD and DI at the end of basic training. However, there was no difference in posttraining RER between subjects with genotypes DD and II nor between subjects with genotypes DI and II, nor was there any effect of ACE genotype on the ratio of minute ventilation to oxygen uptake. Therefore, this significant difference is probably an artifact of subgroup analysis.

Our treadmill aerobic power findings are consistent with the work of Rankinen et al. (25), who found no association between ACE genotype and elite athletic endurance status (defined as a $\dot{V}O_{2\,max}$ of $\geq$75 $ml \cdot kg^{-1} \cdot min^{-1}$), or between ACE genotype and increases in a variety of measures of cardiopulmonary endurance that occurred during training of sedentary subjects (24). Additionally, a recent study by Bouchard et al. (3) found no linkage between genetic markers on *chromosome 17*, the location of the ACE gene, and either baseline $\dot{V}O_{2\,max}$ or the $\dot{V}O_{2\,max}$ response to training. Other recent work (6) has failed to show an effect of ACE genotype on gains in $\dot{V}O_{2\,max}$ realized by sedentary, obese, hypertensive men during a training program, and one recent survey of elite athletes found no clustering of ACE genotype II in elite athletes participating in sports with high aerobic fitness demands (32). The preponderance of evidence thus strongly suggest that ACE genotype does not significantly affect aerobic performance.

Although individuals with genotype II in our study did have the highest mean performances on the APFT both before and after basic training, the effects of the ACE ID polymorphism were far smaller than those detected in studies performed in less ethnically diverse populations and did not achieve statistical significance. It is unlikely that we would have missed an effect of ACE genotype on physical performance as large as the one reported by Montgomery et al. (21), as our study had the power to detect a 20% difference in mean APFT event scores between the lowest performing and the highest performing groups. Furthermore, after adjusting for baseline level of physical fitness (Table 4), subjects with genotype II were no longer consistently the best performers. Our results, therefore, suggest that, for young American adults drawn from a wide variety of ethnic backgrounds, there is probably no substantial effect of the ACE ID polymorphism on physical performance, including muscular endurance.

We chose to study a cohort drawn from an ethnically diverse and geographically dispersed population. This design was intended to reduce the chances that any positive associations detected would be attributable to linkage disequilibrium, which is a confounding factor that is most likely to occur in isolated populations (2). Had we detected an association between ACE genotype and performance in this cohort, we would have concluded that the original findings made by Montgomery et al. (21, 34) could have in fact been generalized to better standardized measures of muscular endurance in a broader and more diverse population of young adults. However, we found no such association.

A disadvantage to our design is that it had limited power to detect gene effects that are specific to particular ethnic subgroups. The value of identifying a gene effect on physical performance that is limited to a specific ethnic subgroup is somewhat questionable, however, as such findings inevitably raise questions about the validity of the association (due to the methodological hazards of subgroup analysis), confounding factors such as linkage disequilibrium, and the importance of the finding to the general population.

A significant strength of our study was the fact that training occurred in the highly structured and regulated environment of Army basic training, which tends to reduce sources of environmental variation that could adversely affect training responses. For example, compliance with the exercise regimen is mandatory, and because training occurs in large groups, the duration and intensity of much of the training routine is highly consistent among individuals. Activities outside of morning physical training (including even the schedule of meals and the number of hours spent sleeping) are highly regulated and also occur in groups, which would tend to reduce the influence of external factors that might influence gains in physical performance.

There were two potentially important confounding variables in our data whose effects were amenable to statistical analysis. First, the distribution of ACE genotypes was significantly different between subjects of African-American heritage and others in the cohort (Table 2). However, incorporating ethnic origin as a covariate in our analysis did not affect our conclusions regarding the lack of effect of ACE genotype on performance. Second, the performance gains that occurred over the course of basic training in our cohort depended on the baseline level of fitness. When this confounding variable was accounted for (Table 4), subjects with genotype II no longer consistently showed the best performance in APFT events, as had been seen in the unstratified data (Table 3). Notably, the study that has most convincingly found an association between ACE genotype and improvements in muscular endurance (21) did not report its adjustments for baseline level of fitness. Our observations suggest that methodological issues can account for some of the apparent effect of ACE genotype observed by others.

A third potentially confounding variable in our data was subject motivation to perform. This is an inherent confounder in any test of physical performance that can be terminated voluntarily, including the APFT. However, we do not believe that this was a serious problem in our study. Although recruits are required only to meet a minimum performance standard on the APFT to graduate, they are nonetheless strongly encouraged to perform to the limit of their abilities during the test. This "drill sergeant" effect was evident in our posttraining performance data, which showed that the mean scores achieved were substantially higher than the minimum of 50 required to graduate as well as above the minimum of 60 that is subsequently required to remain in the Army.

The fact that we found no statistically significant association between ACE genotype and physical performance implies either that no such association exists at all or that the magnitude of the effect of ACE genotype on physical performance is smaller than the power of our study to detect it. Thus, despite our findings, it is still possible that ACE genotype is important to physical performance in selected populations or in physical performance tests different from those explored in this study. For example, in young adults, the effect of ACE genotype may be eclipsed by the importance of other biological factors, such as the hormonal environment. This might account for why a relatively strong relationship between ACE genotype and $\dot{V}O_{2\,max}$ was found in a cohort of postmenopausal women (7) but not in populations of younger individuals (24, 25). Furthermore, although $\dot{V}O_{2\,max}$ is clearly important for performance in aerobic events, it only accounts for 80–90% of the variance in subject running times, with lactate threshold and running economy accounting for most of the rest (11). It is conceivable that, under some circumstances, small effects of a genetic variant on determinants of physical performance other than $\dot{V}O_{2\,max}$ may indeed be important to athletic competition. For example, at Olympic levels of competition, a difference in race time of a few seconds that is attributable to determinants other than $\dot{V}O_{2\,max}$ (such as the ability to sprint to the finish line at the end of an endurance event) could well be the difference between medaling and finishing in the middle of the pack. A small beneficial effect of ACE genotype II could, therefore, account for the apparent clustering of this genotype in elite athletes that has been observed by others (1, 5). However, we saw no evidence of such an effect in our population of individuals of nonelite athletic ability, once we controlled for baseline level of fitness.

We conclude that, in healthy young American adults drawn from an ethnically diverse population, the ID polymorphism on *intron 16* of the ACE gene has no effect on peak oxygen uptake or running performance either before or after training, nor does it appear to have a large effect on either baseline or posttraining measures of muscular endurance. Furthermore, we found that adjustment for baseline level of physical fitness removed any apparent pattern of association between ACE genotype and physical performance. Our findings are consistent with a number of studies that have failed to find a relationship between ACE genotype and measures of aerobic power and also call into question the generalizability of the apparent association between ACE genotype and muscular endurance.

In summary, the results of this and other studies suggest that the apparent association described by some is due to either a minor effect of the ACE gene on physical performance that is of importance only under select circumstances, linkage disequilibrium with other genetic variants, or other confounding factors.

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