

# Angiotensin-converting enzyme ID polymorphism and fitness phenotype in the HERITAGE Family Study

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<sup>4</sup>Department of Kinesiology, Indiana University, Bloomington, Indiana 11001; <sup>5</sup>Department of Health and Kinesiology, Texas A & M University, College Station, Texas 77843-4243; and <sup>6</sup>Division of Biostatistics and Departments of Genetics and Psychiatry, Washington University Medical School, St. Louis, Missouri 63110-1093

**Rankinen, Tuomo, Louis Pérusse, Jaques Gagnon, Yvon C. Chagnon, Arthur S. Leon, James S. Skinner, Jack H. Wilmore, D. C. Rao, and Claude Bouchard.** Angiotensin-converting enzyme ID polymorphism and fitness phenotypes in the HERITAGE Family Study. *J. Appl. Physiol.* 88: 1029–1035, 2000.—It has been suggested that genetic variation in the angiotensin-converting enzyme (*ACE*) gene is associated with physical performance. We studied the association between the *ACE* insertion (I)/deletion (D) polymorphism and several fitness phenotypes measured before and after 20 wk of a standardized endurance training program in sedentary Caucasian ( $n = 476$ ) and black ( $n = 248$ ) subjects. Phenotypes measured were oxygen uptake ( $\dot{V}O_2$ ), work rate, heart rate, minute ventilation, tidal volume, and blood lactate levels during maximal and submaximal [50 W and at 60 and 80% of maximal  $\dot{V}O_2$  ( $\dot{V}O_{2\max}$ )] exercise and stroke volume and cardiac output during submaximal exercise (50 W and at 60%  $\dot{V}O_{2\max}$ ). The *ACE* ID polymorphism was typed with the three-primer PCR method. Out of 216 association tests performed on 54 phenotypes in 4 groups of participants, only 11 showed significant ( $P$  values from 0.042 to 0.0001) associations with the *ACE* ID polymorphism. In contrast to previous claims, in Caucasian offspring, the DD homozygotes showed a 14–38% greater increase with training in  $\dot{V}O_{2\max}$ ,  $\dot{V}O_2$  at 80% of  $\dot{V}O_{2\max}$ , and all work rate phenotypes and a 36% greater decrease in heart rate at 50 W than did the II homozygotes. No associations were evident in Caucasian parents or black parents or offspring. Thus these data do not support the hypothesis that the *ACE* ID polymorphism plays a major role in cardiorespiratory endurance.

candidate gene; exercise training; responsiveness; insertion/deletion polymorphism

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A HIGH LEVEL OF AEROBIC FITNESS is an essential requirement for success in endurance sports. In addition, several studies have shown that a low level of cardiorespiratory endurance is associated with an increased

risk for several degenerative diseases (2, 10, 18, 19). Cardiorespiratory fitness, for which maximal oxygen consumption ( $\dot{V}O_{2\max}$ ) is traditionally recognized as the gold standard, is a multifactorial phenotype influenced by several genetic and environmental factors. Among the environmental factors, regular physical activity is the major contributor to the  $\dot{V}O_{2\max}$  level. However, several exercise training studies have shown that there are marked interindividual differences in the trainability of cardiorespiratory endurance phenotypes after exposure to an identical training program. For example, after supervised training programs of 15–20 wk in 47 healthy young men, the training responses of  $\dot{V}O_{2\max}$  ranged from almost no change to an increase of almost 1 liter (3). Similarly, the improvements in total work output during a 90-min ergometer test ranged from 16 to 97% after 20 wk of standardized endurance training (22).

This individual variability in exercise responses has been described as a normal biological phenomenon that may reflect genetic diversity (3). Both twin and family studies support the hypothesis of a significant genetic effect on  $\dot{V}O_{2\max}$  in the sedentary state and other fitness phenotypes. The intrapair resemblance for cardiorespiratory endurance phenotypes is significantly higher in monozygotic twins than in dizygotic twins (7, 12, 23, 34), with heritability estimates ranging from 25 to 66%. The data from the family studies by using either measured (20) or estimated (21)  $\dot{V}O_{2\max}$  or both (25) have suggested a genetic effect of ~25–40% after adjusting for age, gender, and body mass or body composition. In the HERITAGE Family Study cohort, a maximal heritability of 51% was observed for the  $\dot{V}O_{2\max}$  (adjusted for age, gender, and body composition) measured in the sedentary state (5).

Because endurance performance is a multifactorial trait, the list of candidate genes that could account for human variation in related phenotypes is extensive. Thus far, associations between  $\dot{V}O_{2\max}$  and genetic variation in skeletal muscle-specific creatine kinase locus (4, 27) as well as mitochondrial DNA sequence variations (9) have been described. However, over the

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last 2 yr, significant associations between the angiotensin-converting enzyme (*ACE*) insertion (I)/deletion (D) polymorphism and performance-related phenotypes have been reported. In 78 British military recruits, an 11-times-greater training response in repetitive elbow flexions with a 15-kg barbell was observed in the II homozygotes than in the DD homozygotes after a 10-wk training period. In the same paper, it was reported that the frequency of the D allele was significantly lower in male mountaineers than in a random sample of British men (24). Also, in Australian Olympic rowers, the frequencies of the D allele and the DD genotype were lower than in nonathlete controls (15). Finally, a higher  $\dot{V}O_{2\max}$  was reported in postmenopausal women carrying the II genotype than in the DD homozygotes (16). However, in a cohort of 192 endurance athletes with  $\dot{V}O_{2\max} > 75 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  and 189 sedentary controls with  $\dot{V}O_{2\max} < 50 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , collected by our laboratory in collaboration with European and United States centers (the GENATHLETE project), no differences were found in the distribution of the *ACE* ID genotypes between the groups (26a). Further classification of the athletes on the basis of  $\dot{V}O_{2\max}$  did not provide any evidence for an excess of the I allele or the II genotype among the athletes with high  $\dot{V}O_{2\max}$  values ( $> 83 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ).

*ACE* catalyzes the conversion of angiotensin I to angiotensin II and, therefore, is an integral part of the renin-angiotensin system. *ACE* also degrades vasodilatory substances such as bradykinin. Plasma *ACE* activity is strongly influenced by genetic factors, and the ID polymorphism in the intron 16 of the *ACE* gene seems to be a marker of another variant responsible for its regulation (26, 36). However, there is no physiological explanation for the *ACE*-fitness association reported in some studies. Moreover, frequencies of the *ACE* ID alleles and genotypes vary considerably across different ethnic groups (1, 13, 28), yet populations showing greater I allele frequencies are apparently not characterized by a higher performance level, which could represent circumstantial evidence against a role for this gene.

Although the observations by Montgomery et al. (24), Gayagay et al. (15), and Hagberg et al. (16) may seem quite consistent, problems with study designs, sample sizes, and performance phenotype measurements make the interpretation of these findings extremely difficult. Thus the purpose of this study was to evaluate the above hypothesis by analyzing the associations between the *ACE* ID genotype and various cardiorespiratory endurance-related phenotypes in the sedentary state and in response to 20 wk of endurance training in 476 Caucasian and 248 black adult subjects from the HERITAGE Family Study cohort.

## METHODS

**Subjects.** The study cohort consists of 476 Caucasian subjects (229 men and 247 women) from 99 families and 248 black subjects (88 men and 160 women) from 104 families. The study design and inclusion criteria have been described previously (6). To be eligible, the individuals were required to

be in good health, i.e., free of diabetes, cardiovascular diseases, or other chronic diseases that would prevent their participation in an exercise training program. Subjects were also required to be sedentary, defined as not having engaged in regular physical activity over the previous 6 mo. Individuals with resting systolic blood pressure  $> 159 \text{ mmHg}$  and/or diastolic blood pressure  $> 99 \text{ mmHg}$  were excluded. The study protocol had been approved by each of the Institutional Review Boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant.

**Exercise training program.** The exercise intensity of the 20-wk training program was customized for each participant on the basis of the heart rate (HR)-oxygen consumption ( $\dot{V}O_2$ ) relationship measured at baseline. During the first 2 wk, the subjects trained at a HR corresponding to 55% of the baseline  $\dot{V}O_{2\max}$  for 30 min/session. Duration and intensity of the training sessions were gradually increased to 50 min and 75% of the HR associated with baseline  $\dot{V}O_{2\max}$ , which were then sustained for the last 6 wk. Training frequency was three times per week, and all training was performed on cycle ergometers in the laboratory. HR was monitored during all training sessions by a computerized cycle ergometer system (Universal FitNet System), which adjusted ergometer resistance to maintain the target HR. All exercise sessions were supervised by trained exercise specialists.

**Fitness phenotypes.** Before and after the 20-wk training program, each subject completed three cycle ergometer (SensorMedics Ergo-Metrics 800S, Yorba Linda, CA) exercise tests conducted on separate days: a maximal exercise test (Max), a submaximal exercise test (Submax), and a submaximal/maximal exercise test (Submax/Max). The Max test started at 50 W for 3 min, and the power output was increased by 25 W every 2 min thereafter to the point of exhaustion. For older, smaller, or less-fit subjects, the test was started at 40 W and increased by 10- to 20-W increments. On the basis of the results of the Max test, the Submax test was performed at 50 W and at 60% of the initial  $\dot{V}O_{2\max}$ . Finally, the Submax/Max test was started with the Submax protocol and progressed to a maximal level of exertion. During the Submax/Max test, blood samples were obtained via a venous catheter at rest; during exercise at 50 W, 60% of  $\dot{V}O_{2\max}$ , 80% of  $\dot{V}O_{2\max}$ ; and immediately on completion of Max test; and blood lactate concentrations were determined after deproteinization by using an enzymatic procedure (Sigma Diagnostics, St. Louis, MO). During the Submax and Submax/Max tests, subjects exercised for 9–12 min at each work rate, with a 4-min period of seated rest between exercise periods.

For all tests,  $\dot{V}O_2$ , carbon dioxide production, expiratory minute ventilation ( $\dot{V}E$ ), and tidal volume ( $V_T$ ) were determined every 20 s and reported as a rolling average of the three most recent 20-s values. All the respiratory phenotypes were measured by using a SensorMedics 2900 metabolic measurement cart.  $\dot{V}O_{2\max}$  was defined as the mean of the highest  $\dot{V}O_2$  values determined on each of the Max tests or the higher of the two values if they differed by  $> 5\%$ . HR was recorded by electrocardiography, and values were obtained during the last 15 s of each stage of the Max test and once steady state had been achieved at each of the submaximal work rates during the Submax and Submax/Max tests. Cardiac output was determined twice at 50 W and 60% of  $\dot{V}O_{2\max}$  by using the Collier  $\text{CO}_2$  rebreathing technique (8), as described by Wilmore et al. (38). A mean of the two measurements was used for the analyses.

**Other phenotypes.** Stature was measured to the nearest 0.1 cm with the subject standing erect on a flat surface, heels, buttocks and back pressed against the stadiometer, and the

head positioned in the Frankfort horizontal plane. Body mass was recorded to the nearest 100 g by using a balance scale with subjects clothed only in a lightweight bathing suit. Body mass index was calculated by dividing body mass (kg) by stature squared ( $m^2$ ).

**Genotype determinations.** Genomic DNA was isolated from lymphoblastoid cell lines following a standard protocol (29). The *ACE* ID polymorphism was typed with a PCR-based method using three primers as previously described (11). The final reaction mixture of 15  $\mu$ l contained 100 ng of genomic DNA, 3.0 mM  $MgCl_2$ , 200  $\mu$ M each 2'-deoxynucleoside 5'-triphosphate, 300 nM primers flanking the insertion sequence, 140 nM nested primer, 4.7% DMSO, and 1.0 U of Taq polymerase (Pharmacia Biotech, Baie d'Urfé, PQ, Canada). The PCR protocol (model 9600 thermal cycler, Perkin Elmer, Norwalk, CT) consisted of one cycle at 94°C for 3 min, 55°C for 1 min, and 72°C for 1 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 45 s, and finally one cycle at 72°C for 10 min. The PCR products were separated on 3.5% agarose gel and visualized under ultraviolet light after ethidium bromide staining.

**Statistical analyses.** A  $X^2$  test was used to confirm that the observed genotype frequencies were in a Hardy-Weinberg equilibrium. The normality of the distributions was checked with the Shapiro-Wilk statistic of the UNIVARIATE procedure of the SAS statistical software package (SAS Institute, Cary, NC). Skewed distributions were normalized with logarithmic transformations. The associations between fitness phenotypes and the genetic markers were tested with analysis of covariance by using the general linear model procedure of the SAS package. Baseline phenotypes were adjusted for age, gender, and body weight and training response phenotypes for age, gender, baseline body weight, and baseline value of the given phenotype. The results are given as means and SD for the unadjusted variables and as means and SE for the adjusted variables.

All the family members were included in the analyses. Although it is commonly believed that the relatedness of the subjects in family study cohorts may cause problems in association analyses, a recent simulation study (M. Province, T. Rice, and D. C. Rao, unpublished observations) suggests that this is not the case. In that study, the data were analyzed by four methods, where the least squares method used in the present report was one of them; the other three methods treated dependencies in different ways. The results show that, first, failure to incorporate dependencies did not induce any bias and that, second, for moderate familial correlations as seen in most family studies (including the present one), ignoring the dependencies by using ANOVA performed quite well. The only negative impact was a small reduction in power. The SEs were slightly enlarged but, most importantly type I error was unaffected. Given this, we do not believe that the dependencies or relatedness of the subjects in families causes any real problems in this type of analysis.

To explore thoroughly the associations between the *ACE* ID polymorphism and cardiorespiratory endurance traits, we selected a large number of phenotypes representing its cardiovascular, respiratory, and metabolic aspects. However, to facilitate the reporting and interpreting of the results, the phenotypes were divided into two groups: the primary ( $\dot{V}O_2$  and HR at 50 W, 60 and 80% of  $\dot{V}O_{2max}$ , and at maximal exercise; work rate at 60% and 80% of  $\dot{V}O_{2max}$ , and at maximal exercise) and the secondary (cardiac output and stroke volume at 50 W and 60% of  $\dot{V}O_{2max}$ ,  $\dot{V}E$ ,  $V_T$ , and blood lactate levels at 50 W, 60% and 80% of  $\dot{V}O_{2max}$ , and at maximal exercise) phenotypes. Thus altogether 11 primary and 16 secondary phenotypes, both in the sedentary state and for the

training response, were analyzed. Although the number of phenotypes tested is large, we did not make any adjustment for multiple testing because the purpose of the study is to explore the possible *ACE*-cardiorespiratory endurance associations, and we believe that for this purpose it is more informative to report all the actual results. However, we do recognize the issue of multiple testing and it has been taken into consideration in the interpretation of the results.

## RESULTS

The baseline characteristics of the subjects are presented in Table 1. The exercise training program increased  $\dot{V}O_{2max}$  by  $16.7 \pm$  (SD)  $9.4$ ,  $17.0 \pm 8.9$ ,  $22.6 \pm 11.3$ , and  $17.4 \pm 8.9$  % and increased maximal work rate by  $28.6 \pm 14.7$ ,  $28.5 \pm 13.4$ ,  $36.1 \pm 25.3$ , and  $30.9 \pm 15.0$  % in Caucasian parents, Caucasian offspring, black parents, and black offspring, respectively. The frequencies of the insertion and deletion alleles of the *ACE* marker were 0.468 and 0.532 in Caucasian subjects and were 0.416 and 0.584 in black subjects. In both races, the genotype frequencies were in a Hardy-Weinberg equilibrium.

The associations between baseline cardiorespiratory endurance phenotypes and the *ACE* ID genotypes are summarized in Tables 2 and 3. None of the primary fitness phenotypes were associated with the *ACE* ID polymorphism in Caucasians. In black parents, baseline HR at 60 and 80% of  $\dot{V}O_{2max}$  showed a significant association with the *ACE* marker, but the significance was due to the higher values seen in the ID heterozygotes than in the II and DD homozygotes. Of the secondary fitness phenotypes, only baseline lactate levels at 60 and 80% of  $\dot{V}O_{2max}$  workloads were associated with the *ACE* ID polymorphism in the Caucasian offspring but not in other subgroups. The DD homozygotes showed significantly lower blood lactate levels at both submaximal exercise workloads than in the other genotypes (Fig. 1). Lactate levels measured at 50 W or at maximal exercise were not associated with the *ACE* ID polymorphism.

The responses to the 20-wk exercise training program of the primary cardiorespiratory endurance phenotypes were similar across the *ACE* ID genotypes in Caucasian and black parents and in black offspring

Table 1. Baseline characteristics of the subjects

	Blacks		Caucasians	
	Parents	Offspring	Parents	Offspring
Age, yr	48.4 $\pm$ 7.1	28.0 $\pm$ 7.4	52.9 $\pm$ 5.2	25.4 $\pm$ 6.2
Gender, male/ female	25/45	63/115	92/86	139/157
Height, cm	166.6 $\pm$ 8.4	167.1 $\pm$ 9.7	169.1 $\pm$ 9.2	171.4 $\pm$ 9.6
Body mass, kg	79.1 $\pm$ 13.8	77.1 $\pm$ 19.1	80.2 $\pm$ 16.2	72.8 $\pm$ 17.4
Body mass index, $kg/m^2$	28.6 $\pm$ 5.2	27.6 $\pm$ 6.2	27.9 $\pm$ 4.6	24.6 $\pm$ 4.7
$\dot{V}O_{2max}$ , ml/min	1,780 $\pm$ 413	2,180 $\pm$ 647	2,148 $\pm$ 615	2,641 $\pm$ 736
$W_{max}$ , W	127 $\pm$ 36	156 $\pm$ 49	158 $\pm$ 50	199 $\pm$ 60
$HR_{max}$ , beats/min	171.9 $\pm$ 15.8	185.4 $\pm$ 12.2	174.2 $\pm$ 12.9	192.2 $\pm$ 9.0

Values are means  $\pm$  SD.  $\dot{V}O_{2max}$ , maximal  $O_2$  uptake;  $W_{max}$ , maximal power output;  $HR_{max}$ , maximal heart rate.

**Table 2. Baseline cardiorespiratory fitness phenotypes according to the ACE ID genotype in Caucasian subjects of the HERITAGE Family Study**

	ACE Genotype			P Value
	II	ID	DD	
<i>Parents</i>				
<i>n</i>	47	82	59	
$\dot{V}O_2$ , ml/min				
Maximum	2,059 ± 46	2,194 ± 35	2,150 ± 42	0.071
80%	1,674 ± 40	1,766 ± 30	1,767 ± 36	0.136
60%	1,257 ± 30	1,321 ± 22	1,311 ± 26	0.219
50 W	1,039 ± 12	1,038 ± 9	1,039 ± 11	0.996
Power output, W				
Maximum	155.4 ± 4.2	160.4 ± 3.2	157.6 ± 3.9	0.634
80%	112.4 ± 3.6	117.0 ± 2.7	115.8 ± 3.3	0.594
60%	71.4 ± 2.9	76.5 ± 2.2	75.0 ± 2.6	0.364
Heart rate, beats/min				
Maximum	173.4 ± 1.7	174.9 ± 1.3	173.9 ± 1.6	0.763
80%	152.5 ± 2.2	153.0 ± 1.7	153.2 ± 3.3	0.972
60%	128.7 ± 2.1	129.8 ± 1.6	128.6 ± 1.9	0.873
50 W	118.2 ± 2.2	116.8 ± 1.6	114.9 ± 2.0	0.521
<i>Offspring</i>				
<i>n</i>	72	149	82	
$\dot{V}O_2$ , ml/min				
Maximum	2,597 ± 43	2,637 ± 29	2,683 ± 40	0.350
80%	2,119 ± 36	2,132 ± 24	2,156 ± 33	0.748
60%	1,607 ± 27	1,600 ± 19	1,621 ± 25	0.803
50 W	1,018 ± 9	1,023 ± 6	1,017 ± 9	0.804
Power output, W				
Maximum	196.3 ± 4.4	198.9 ± 3.0	201.8 ± 4.0	0.659
80%	152.2 ± 3.5	151.0 ± 2.4	152.2 ± 3.3	0.938
60%	104.0 ± 2.7	103.6 ± 1.8	105.8 ± 2.5	0.766
Heart rate, beats/min				
Maximum	192.9 ± 1.0	191.7 ± 0.7	192.7 ± 0.9	0.505
80%	176.8 ± 1.4	173.8 ± 0.9	172.5 ± 1.3	0.069
60%	151.3 ± 1.5	147.9 ± 1.0	147.8 ± 1.4	0.144
50 W	120.4 ± 1.5	118.1 ± 1.0	116.4 ± 1.5	0.182

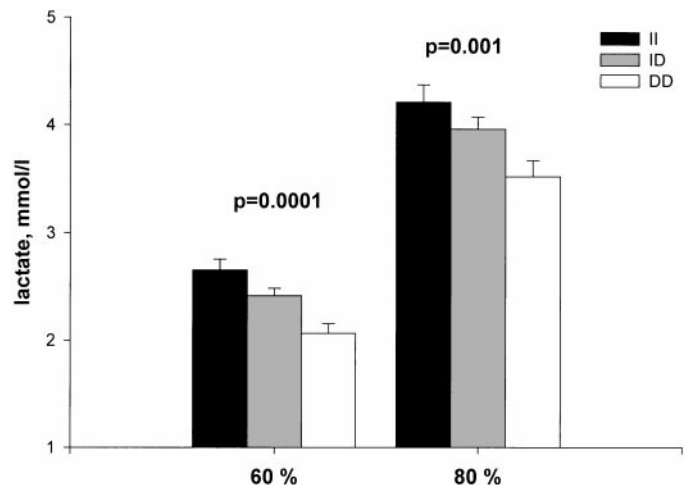
Values are means ± SE and are adjusted for age, gender, and body mass; *n*, no. of subjects. ACE, angiotensin-converting enzyme; I, insertion; D, deletion;  $\dot{V}O_2$ , O<sub>2</sub> uptake.

**Table 3. Baseline cardiorespiratory fitness phenotypes according to the ACE ID genotype in black subjects of the HERITAGE Family Study**

	ACE Genotype			P Value
	II	ID	DD	
<i>Parents</i>				
<i>n</i>	14	34	27	
$\dot{V}O_2$ , ml/min				
Maximum	1,669 ± 68	1,847 ± 44	1,751 ± 48	0.081
80%	1,424 ± 68	1,537 ± 39	1,516 ± 42	0.371
60%	988 ± 47	1,111 ± 29	1,057 ± 32	0.086
50 W	1,042 ± 26	1,065 ± 17	1,052 ± 18	0.733
Power output, W				
Maximum	120.6 ± 6.3	133.5 ± 4.0	122 ± 4.4	0.099
80%	89.1 ± 5.7	94.9 ± 3.2	89.2 ± 3.6	0.442
60%	44.3 ± 4.1	53.1 ± 2.6	49.4 ± 2.8	0.198
Heart rate, beats/min				
Maximum	168.5 ± 4.2	176.6 ± 2.7	167.7 ± 3.0	0.070
80%	142.6 ± 5.6	159.0 ± 3.2	149.2 ± 3.5	0.027
60%	121.0 ± 4.3	132.4 ± 2.7	124.3 ± 2.9	0.042
50 W	126.8 ± 4.7	131.5 ± 3.0	125.7 ± 3.3	0.414
<i>Offspring</i>				
<i>n</i>	29	109	58	
$\dot{V}O_2$ , ml/min				
Maximum	2,133 ± 61	2,187 ± 31	2,166 ± 43	0.725
80%	1,700 ± 55	1,794 ± 27	1,779 ± 37	0.318
60%	1,286 ± 40	1,335 ± 20	1,316 ± 28	0.530
50 W	1,055 ± 16	1,037 ± 8	1,050 ± 11	0.444
Power output, W				
Maximum	151.6 ± 5.4	158.8 ± 2.8	152.3 ± 3.8	0.275
80%	111.4 ± 4.5	118.6 ± 2.2	113.6 ± 3.1	0.230
60%	73.0 ± 3.7	76.2 ± 1.9	72.8 ± 2.6	0.489
Heart rate, beats/min				
Maximum	182.2 ± 2.0	187.2 ± 1.0	184.6 ± 1.5	0.074
80%	161.4 ± 2.6	167.5 ± 1.3	164.1 ± 1.8	0.069
60%	137.8 ± 2.4	140.5 ± 1.2	137.6 ± 1.7	0.323
50 W	128.2 ± 2.6	125.9 ± 1.3	124.7 ± 1.8	0.551

Values are means ± SE and are adjusted for age, gender, and body mass; *n*, no. of subjects.

(Tables 4 and 5). In Caucasian offspring, the DD homozygotes showed the greatest increases in  $\dot{V}O_{2max}$ ,  $\dot{V}O_2$  at 80% of  $\dot{V}O_{2max}$ , maximal work rate, and work rate at 80 and 60% of  $\dot{V}O_{2max}$  and the greatest decreases in HR at 50 W (*P* values from 0.042 to 0.0001, adjusted for age, gender, baseline body mass, and baseline value of the phenotype). Of the secondary fitness phenotypes, the ventilation phenotypes showed some associations with the ACE ID marker (data not shown). In Caucasian offspring, the DD homozygotes showed a greater (*P* = 0.032) increase in  $\dot{V}E$  60 (+4.2 ± 0.6 l/min) than did the II homozygotes (+1.9 ± 0.6 l/min) and the ID heterozygotes (+3.0 ± 0.6 l/min). Also the training response of  $\dot{V}T$  at 60% of  $\dot{V}O_{2max}$  followed a similar pattern (+0.12 ± 0.02, +0.05 ± 0.02 and +0.06 ± 0.02 liter in the DD, ID, and II genotypes, respectively; *P* = 0.036). In addition, the  $\dot{V}E$  at 80% of  $\dot{V}O_{2max}$  training response tended to be greater in the DD homozygotes (*P* = 0.063). In the Caucasian parents, the  $\dot{V}E$  at  $\dot{V}O_{2max}$  training response was the greatest (*P* = 0.026) in the DD homozygotes (+13.7 ± 1.3 vs. +8.6 ± 1.5 and 9.7 ± 1.2 l/min in the II and ID genotypes, respectively). However, in black parents, the II homozygotes had



**Fig. 1.** Pretraining blood lactate levels during submaximal exercise at 60 and 80% of maximal oxygen consumption, according to angiotensin-converting enzyme (ACE) insertion (I)/deletion (D) genotype in Caucasian offspring of the HERITAGE Family Study. Values are means ± SE and are adjusted for age, gender, and body weight.

Table 4. Training responses of the cardiorespiratory fitness phenotypes according to the ACE ID genotype in Caucasian subjects of the HERITAGE Family Study

	ACE Genotype			P Value
	II	ID	DD	
<i>Parents</i>				
<i>n</i>	47	73	57	
$\dot{V}O_2$ , ml/min				
Maximum	+349.6 ± 28.0	+338.2 ± 22.2	+331.1 ± 25.2	0.889
80%	+243.5 ± 30.8	+260.2 ± 24.7	+242.6 ± 28.5	0.870
60%	+155.8 ± 22.8	+163.6 ± 17.6	+147.1 ± 20.0	0.826
50 W	-34.9 ± 9.3	-31.3 ± 7.2	-43.2 ± 8.3	0.553
Power output, W				
Maximum	+40.8 ± 3.0	+45.7 ± 2.4	+40.4 ± 2.7	0.261
80%	+27.7 ± 2.2	+30.1 ± 1.7	+28.8 ± 2.0	0.679
60%	+18.9 ± 1.9	+20.4 ± 1.4	+19.7 ± 2.4	0.814
Heart rate, beats/min				
Maximum	-0.2 ± 0.8	-1.1 ± 0.6	-0.5 ± 0.7	0.643
80%	-4.0 ± 1.4	-2.3 ± 1.1	-4.1 ± 1.3	0.532
60%	-6.3 ± 1.2	-5.5 ± 1.0	-5.6 ± 1.1	0.861
50 W	-11.3 ± 1.1	-11.5 ± 0.9	-11.4 ± 1.0	0.988
<i>Offspring</i>				
<i>n</i>	68	145	81	
$\dot{V}O_2$ , ml/min				
Maximum	+417.5 ± 25.6	+403.2 ± 17.2	+476.2 ± 23.4	0.042
80%	+293.4 ± 25.9	+301.5 ± 17.7	+390.1 ± 24.7	0.008
60%	+199.2 ± 19.6	+216.8 ± 13.2	+252.5 ± 18.1	0.122
50 W	-34.1 ± 7.6	-33.5 ± 5.1	-50.0 ± 7.1	0.150
Power output, W				
Maximum	+50.5 ± 3.0	+53.2 ± 2.0	+62.3 ± 2.8	0.009
80%	+34.9 ± 2.3	+38.8 ± 1.5	+48.2 ± 2.2	0.0001
60%	+25.3 ± 1.8	+26.7 ± 1.2	+32.8 ± 1.6	0.003
Heart rate, beats/min				
Maximum	-1.0 ± 0.6	-1.3 ± 0.4	-2.3 ± 0.6	0.237
80%	-2.6 ± 1.0	-2.9 ± 0.7	-2.8 ± 1.0	0.984
60%	-4.7 ± 1.0	-4.7 ± 0.7	-6.3 ± 1.0	0.351
50 W	-9.8 ± 0.9	-9.5 ± 0.6	-13.4 ± 0.8	0.0006

Values are means ± SE and are adjusted for age, gender, baseline body mass, and baseline value of the phenotype; *n*, no. of subjects.

increase of  $9.6 \pm 2.5$  l/min in  $\dot{V}E$  at 80%  $\dot{V}O_{2max}$ , whereas the response of the DD and ID genotypes were  $+0.6 \pm 1.6$  and  $+6.0 \pm 1.4$  l/min, respectively ( $P = 0.007$ ). Cardiac output, stroke volume, and blood lactate training responses were not associated with the ACE ID polymorphism.

## DISCUSSION

Some previous studies have reported that the I allele of the ACE I/D polymorphism is associated with enhanced physical performance (15, 16, 24). However, the results of the present study, based on data from 476 Caucasian and 248 black subjects, do not support the earlier findings derived from considerably smaller cohorts. In the sedentary state, none of the cardiorespiratory endurance-related phenotypes were associated with the ACE ID polymorphism. Moreover, the associations between training responses and the ACE marker were seen only in Caucasian offspring. Furthermore,

unlike previous reports, the homozygotes for the D allele showed the most favorable changes.

The results from the present study and those reported earlier are conflicting. However, differences in study designs and subject selection strategies, sample sizes, and measurements of phenotypes may explain most of the differences. First, the greater frequency of the I allele observed among the athletes in the case-control studies (15, 24) could be simply explained by a selection bias or a simple sampling problem. If the I allele is associated with a healthier cardiovascular system as some studies suggest (33), it is possible that the requirement of a healthy cardiovascular system for endurance sports favors the selection of the II homozygotes among endurance athletes. However, this is not supported by the data from the GENATHLETE project based on a much larger sample of athletes and matched controls (26a). The selection bias may also have affected the results derived from a cohort of British soldiers (24). The frequency of the I allele (0.551) among the

Table 5. Training responses of the cardiorespiratory fitness phenotypes according to the ACE ID genotype in black subjects of the HERITAGE Family Study

	ACE Genotype			P Value
	II	ID	DD	
<i>Parents</i>				
<i>n</i>	11	30	24	
$\dot{V}O_2$ , ml/min				
Maximum	+398.6 ± 37.5	+379.7 ± 23.3	+347.1 ± 26.0	0.471
80%	+348.0 ± 56.3	+255.3 ± 32.2	+227.4 ± 35.6	0.210
60%	+238.0 ± 39.9	+217.5 ± 24.0	+179.6 ± 26.4	0.392
50 W	-26.1 ± 19.3	-20.0 ± 11.7	-47.6 ± 13.3	0.299
Power output, W				
Maximum	+45.2 ± 5.6	+40.8 ± 3.5	+38.5 ± 4.0	0.623
80%	+32.7 ± 4.1	+28.8 ± 2.3	+29.2 ± 2.6	0.713
60%	+26.4 ± 3.6	+22.8 ± 2.2	+23.2 ± 2.4	0.703
Heart rate, beats/min				
Maximum	+1.9 ± 2.0	+1.7 ± 1.3	+1.7 ± 1.4	0.997
80%	+5.7 ± 3.3	-0.8 ± 1.9	-2.1 ± 2.1	0.135
60%	-1.5 ± 2.7	-0.2 ± 1.6	-2.9 ± 1.8	0.548
50 W	-14.5 ± 2.2	-13.3 ± 1.3	-14.5 ± 1.5	0.796
<i>Offspring</i>				
<i>n</i>	28	97	57	
$\dot{V}O_2$ , ml/min				
Maximum	+319.7 ± 31.3	+369.6 ± 16.7	+356.8 ± 22.7	0.380
80%	+228.6 ± 37.0	+276.2 ± 18.6	+223.6 ± 25.1	0.193
60%	+181.3 ± 26.2	+195.1 ± 13.9	+201.9 ± 18.2	0.814
50 W	-28.5 ± 12.7	-43.0 ± 6.7	-36.6 ± 8.9	0.580
Power output, W				
Maximum	+45.8 ± 3.5	+47.0 ± 1.9	+45.1 ± 2.6	0.828
80%	+31.9 ± 2.9	+31.7 ± 1.5	+31.2 ± 2.0	0.968
60%	+21.9 ± 2.3	+24.4 ± 1.2	+23.6 ± 1.6	0.482
Heart rate, beats/min				
Maximum	+1.4 ± 1.1	-0.4 ± 0.6	-0.2 ± 0.8	0.358
80%	-2.2 ± 2.1	-2.2 ± 1.1	-1.6 ± 1.4	0.950
60%	-2.7 ± 1.5	-2.8 ± 0.8	-1.8 ± 1.1	0.775
50 W	-10.6 ± 1.3	-11.7 ± 0.7	-11.3 ± 0.9	0.745

Values are means ± SE and are adjusted for age, gender, baseline body mass, and baseline value of the phenotype; *n*, no. of subjects.

male military recruits is significantly higher than that reported in other Caucasian populations (32), including the HERITAGE cohort (0.468).

The performance phenotypes used in the previous studies is another factor hampering the interpretation of the results. The repetitive elbow flexion test with a 15-kg barbell used by Montgomery et al. (24) is not a standard test of physical performance. Because the authors did not give any repeatability estimates for the test, it is difficult to evaluate how reliable the results derived from this test are. In addition, the elbow flexion test employs mainly the biceps, a fairly small muscle group. Thus it is unclear whether this test is a valid surrogate for skeletal muscle performance capacity or whether it has any relationship with cardiorespiratory endurance. In the HERITAGE Family Study, we paid close attention to the quality of the phenotype measurements (14). For example, both maximal and submaximal cardiorespiratory endurance phenotypes are derived from two separate tests both before and after the training program. As reported previously (30, 39), the intraclass correlation coefficients for repeated measurements of the fitness phenotypes are high, ranging from 0.76 to 0.99. The coefficient for  $\dot{V}O_{2\max}$  reached 0.97 (30).

A common feature of the papers on the associations between the *ACE* ID polymorphism and performance phenotypes published so far is that the sample sizes have been relatively small, varying from 25 to 78. The present study, which is by far the intervention study with the largest sample size and the most rigorously controlled, and the GENATHLETE case-control study from our laboratory with 192 endurance athletes and 189 sedentary controls, both do not support the notion that the I allele is associated with greater performance capacity. In fact, a similar effect of sample size has been reported for the hypothesis of an increased cardiovascular disease risk associated with the D allele. Staessen and co-workers (33) reported in a meta-analysis of 49,959 subjects from 145 studies that the estimates of increased risk of coronary heart disease and nephropathy tend to decrease as a function of the sample size among these studies. Moreover, the results revealed a publication bias; i.e., smaller studies with negative findings were not reported in the literature (33). It is important to remember that a small sample size implies not only low statistical power but also a greater risk of spurious positive findings due to the contribution of outliers. Therefore, the replication of findings derived from small cohorts in studies with larger sample sizes is of the utmost importance.

At present, a physiological explanation for any association between the endurance-related cardiorespiratory phenotypes and the *ACE* polymorphism is missing. Although local renin-angiotensin systems (RAS) have been identified in several tissues, including skeletal muscle, it is unlikely that the effect of ACE is mediated by the RAS. There is no indication that an increased ACE activity per se leads to enhanced angiotensin II production. In fact, an increment in ACE activity associated with an increase in the number of functional *ACE* genes does not affect angiotensin II levels (31).

However, the increased ACE activity in the DD homozygotes could have physiologically significant effects if the substrate delivery (angiotensinogen, angiotensin I, bradykinin) was also increased (35, 37). On the other hand, we cannot exclude the possibility that the putative effects of the *ACE* gene could be mediated independently of the RAS. ACE is able to degrade vasodilatory substances such as bradykinin, and the resulting impaired vasodilation in the *ACE* DD homozygotes may theoretically influence the peripheral circulation and thereby oxygen and substrate delivery to the working muscles. However, we believe that the findings of the present study do not provide support for this hypothesis. Finally, it is possible that some or all of the associations reported between physical performance and the *ACE* gene polymorphism could be due to linkage disequilibrium with other gene or genes in close proximity to the *ACE* locus and not to the *ACE* gene per se. One such potential candidate is the human growth hormone gene, which is located in vicinity of the *ACE* gene as shown by a close linkage and a lack of recombination with the *ACE* locus. (17).

In summary, these data from the HERITAGE Family Study do not support the concept that genetic variation at the *ACE* locus is a major contributor to the cardiorespiratory endurance-related phenotypes in the sedentary state or to their responses to regular and standardized endurance training in healthy Caucasian or black subjects.

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