The following is the abstract of the article discussed in the subsequent letters:

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.

ACE Genotype and Performance

To the Editor: The angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism has previously been associated with measures of training response and human endurance performance. A recent article (4) concluded that such an association was weak or spurious. However, this conclusion is erroneous, being derived from flaws in the gene-environment model used. The application of four different training regimes to nine subgroups of race and sex in a sample of only 117 individuals will per force prevent the identification of any such association. The rationale for this contention is discussed.

Gene-environment interaction (GEI) studies relate the magnitude of physiological response to a uniform environmental stimulus with variation in a candidate gene. However, response magnitude depends on stimulus nature and scale and on the subject’s physical and genetic characteristics. The ideal GEI study thus seeks 1) subject homogeneity (similar individuals of identical race and sex), 2) stimulus homogeneity (nature, magnitude, and duration), and 3) selection of an appropriate phenotype to assess.

Sonna et al. (4) report such a GEI study: candidate gene was ACE, variant was I/D polymorphism, subjects were army recruits, environmental stimulus was exercise, and phenotypic responses were performance measures. However, their conclusion that the ACE gene exerts minor or spurious effects on performance characteristics is not justified.

Subjects were not homogeneous. The use of nine combinations of race and sex is disadvantageous. 1) Associations of phenotype with race confound association with genotype if polymorphism frequency also varies dramatically (as here) with race. 2) Phenotype is influenced by genes associated with race and gender. Left ventricular growth is greater in men and those of African descent (3), and some performance responses will also be influenced by race and sex. 3) The influence of a polymorphism on phenotypic response will, as the authors acknowledge, differ as a function of the genetic background of the population under study. Mixing such backgrounds by inclusion of diverse race and sex combinations is thus detrimental. 4) Polymorphism functionality may itself vary with race. Fundamental to this study, the ACE I/D polymorphism is used as a marker of tissue ACE activity. Such an association is unexplored and unproven among non-Caucasians. However, its association with serum ACE activity in Caucasians may be absent among African-Americans (1), thus negating the very rationale for the use of the polymorphism as a marker of ACE activity in such groups entirely.

The complex effects of race and sex on the gene-environment interaction cannot be modeled when 117 subjects are divided into nine potential combinations of race and sex themselves spread over three genotypes. Furthermore, detecting a 20% difference in phenotypic response (far greater than that reported in any gene-environment association studies of mixed race-sex cohorts so far) with 80% power requires (they suggest) 30 subjects in each group, which is far more than is found for any race-sex combination here. Thus, with only 37 African-Americans starting training (3 of II genotype, roughly one-half likely to be women) and a reported cohort dropout rate of 20.4%, even fewer subjects are left for analysis. The problem cannot be overcome by “lumping together” those of one sex (and different race) or those of different race, as performed in the Sonna et al. study (4) for the three non-African-American/Caucasian groups. Furthermore, conclusions cannot be drawn from the study of Caucasians alone. Data for only 117 (61 women) individuals were studied, of 147 “starters,” and, of the original cohort, only 57% were Caucasian. If we assume no race and sex selection in passing training (an additional confounder that we cannot judge from the data presented), then there would have been only 29 male Caucasians of three genotypes in the final analysis, which is again far too few for meaningful comparisons with other race-sex groups.

Training stimulus was inhomogeneous. Training stimulus was inhomogeneous, differing according to four abil-
ity groups whose racial or gender composition, genotype mix, and “pass rate” may have also differed.

Choice of phenotype. An association of ACE genotype with training-related change in maximal O₂ uptake has never been shown. Other measures (such as press-ups and sit-ups) may require mixed strength and endurances. The association of the I allele with the latter and the D allele with the former (2) would confound allele association with such measures.

Furthermore, raw data were adjusted for age and sex to provide a “score” in a range of 0–100, the effect being to “reduce the statistical impact of outliers without excluding them from analysis” (4), a major problem when the genetic variation being examined may account for such outliers.

In addition, lack of association of genotype with such measures (even if statistically valid) cannot be used to discount past association with different measures of performance in other more homogeneous groups. Nor can such data be used to infer anything of the validity of other studies of entirely other types (e.g., candidate gene-association studies of elite athletes).

Thus a heterogeneous (four group) environmental stimulus has been applied to nine combinations of race and sex with three genotypes among only 117 individuals. The conclusion that “the apparent association described by some is due to a minor effect of the ACE gene on physical performance that is important only under selected circumstances” (4) is thus questionable.

Putative race and sex dependence of the strength of association of a polymorphism with a given phenotypic trait remains an important issue to address. This study did not set out to (and could not) do this. In addition, analysis of mixed training in such small mixed-sex and race groups does not prove, or disprove, the impact of any given polymorphism in any population, mixed or otherwise.

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Genetic Studies of Performance

To the Editor: In a recent issue of the Journal of Applied Physiology, Sonna and colleagues (9) cite three references (1, 5, 6) that they claim “convincingly ruled out a role for ACE genotype in aerobic performance.” This conclusion is misleading.

First, the fact that “a genomewide scan for markers linked with VO₂ max found none on chromosome 17” (where VO₂ max is maximal O₂ uptake) is in no way evidence for a lack of a role for ACE genotype in aerobic performance. Linkage studies extend over substantial genetic distances and aid long-range mapping. Association studies, on the other hand, are far more powerful in the demonstration of alleles with modest phenotypic effects (8), such that “the future of the genetics of complex diseases is likely to require large-scale testing by association analysis” (7). For an allele frequency of ~50% (that of the ACE I allele) and an increase in “risk” of a fixed phenotype of 1.5, a powerful sub-pair linkage study would need to examine over 17,000 families (7). Absence of linkage marker association on a chromosome thus does not exclude the presence of a powerful gene cited there, as evidenced by the failure of linkage strategies to relate the insulin gene region with insulin-dependent diabetes mellitus (IDDM) (2) when association studies were later able to do so (3). These findings emphasized “the importance of performing careful association studies before considering any region of the genome void of IDDM-susceptibility loci” (4).

Second, the authors also cite a cross-sectional study (6) that failed to identify an excess frequency of the I allele among “elite endurance athletes.” However, these were drawn from four countries, and potentially even more races, and from diverse sporting disciplines. Such heterogeneity dramatically weakens the power of any association study in which single, not multiple, phenotypes and races should be studied. In addition, to be included, subjects also had to demonstrate a VO₂ max of >75 ml·kg⁻¹·min⁻¹. Similarly, Sonna et al. (9) also cite a suggested lack of association between the ACE I/D polymorphism and VO₂ max in one study.

Therefore, the cited data in Ref. 9 might perhaps only offer some support for a lack of association between the ACE I allele and VO₂ max. However, a lack of association with “aerobic performance” has not been demonstrated, convincingly or otherwise.

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To the Editor: Recently, Sonna and colleagues (3) concluded that the ACE gene may have no association of note with the human endurance-training response. We do not feel these conclusions are justified. Military recruits of diverse race and both sexes were subjected to a heterogeneous (four different groupings of intensity) 8-wk period of physical training. Such application of heterogeneous training to a heterogeneous group will prevent identification of candidate allele-associated changes in performance unless each race-sex combination is itself substantial, as a consistent stimulus must be applied to assess the influence of any gene on the response to an environmental stimulus. In this study, subjects were divided into groups according to their baseline level of fitness and subsequently trained at different levels of intensity.

The argument that the study of such mixed subjects reveals the “true effect” of a genotype in a population is misguided. The more diverse the genetic background and environment, the less likely is the possibility for identifying mechanisms of importance through gene-environment interactions. Thus the association of the ACE gene with left ventricular growth responses becomes clear when small uniform groups (by age and sex and race) are studied in the presence of a qualitatively similar growth stimulus, whereas such an effect is not seen in very large studies where such effects are uncontrolled (2). Quite simply, such heterogeneity instills too much white noise.

In addition, the effect on “endurance performance” was assessed by changes in sit-up and the push-up “scores.” It is debatable whether these are accurate measures of pure endurance. Indeed, the US Army National Guard define these as “measures of strength,” whereas the US Army Physical Fitness Test (APFT) protocol suggests these tests are measures of combined endurance and strength development (1), a view with which other authors concur (4). Given the potential association of the D allele with a strength-training response and the I allele with possible endurance measures, it is unsurprising that no allele association was identified.

Other problems exist. Soldiers were encouraged to obtain “scores” of over 60 with the knowledge that they had obtained (pass) scores of 50. With the incentive to continue gone, it is not unreasonable to assume that subjects might not have performed to the limit of their capabilities, blunting any identification of gene association with maximal performance. The “weighting” of scores to annul the effect of “outliers” has a similar effect in attenuating the very extremes of performance with which a genotype may be associated.

Such studies should be performed with (even multiple) similar age-sex-race cohorts being exposed to identical training stimuli and with measures of endurance and not strength or strength-endurance. Until such time, the authors’ conclusions (3) concerning the lack of influence of the ACE genotype remain unfounded.

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REPLY

To the Editor: We accept Dr. Payne’s criticism that we may have overstated the importance of previous studies that failed to detect a relationship between the ACE I/D polymorphism and aerobic performance (of which VO_{2max} is, quantitatively, the most significant determinant). He eloquently states why genetic association studies continue to be an important tool in the study of complex traits.

Drs. Montgomery and Dhamrait highlight many of the limitations that are, unfortunately, almost inescapable in any reasonably large field study of the human physical performance response to training, including their own (1). We agree that the study of physical training in military recruits has significant limitations; however, outside of a military organization, it is very hard to find a substantial cohort of subjects who will undergo a comparably regulated and enforced training program over a period of several weeks.

A few of the issues raised require clarification. First, during basic training, our subjects trained in ability groups only for running, not for training activities designed to enhance muscle strength and endurance (calisthenics, and so forth). From the perspective of
muscle strength and endurance, our subjects were subjected to a highly homogeneous physical training regime and essentially identical environmental conditions.

Second, we reported APFT data on 143 of 147 subjects, which is a 2.7% loss. Although it is true that we had paired treadmill data for only 80% of the subjects, the conclusions drawn from these data were similar to those obtained from examining the 2-mile run scores. In any case, as pointed out in their letter, Drs. Montgomery and Dhamrait agree that available evidence to date does not demonstrate an effect of ACE I/D genotype and essentially identical environmental conditions. Although it is true that we had paired treadmill data for only 80% of the subjects, which is a 2.7% loss. Although it is true that we had paired treadmill data for only 80% of the subjects, the conclusions drawn from these data were similar to those obtained from examining the 2-mile run scores. In any case, as pointed out in their letter, Drs. Montgomery and Dhamrait agree that available evidence to date does not demonstrate an effect of ACE I/D genotype on $V_O^{2\text{max}}$ in young adults. Our findings are consistent with this body of literature, but, of course, it is impossible to prove a negative finding beyond all doubt.

Third, it is true that the APFT scoring system can narrow the differences between superb athletes (i.e., athletes who score above the 100 maximum) and athletes of average ability, thus (in principle) reducing the statistical power of a genetic association study. However, in our cohort, the highest score achieved at the end of basic training was 92 in the pushup event, and only two subjects achieved a score of 100 in the sit-up event. It is therefore unlikely that underestimation of the abilities of the best-performing subjects led to a significant bias in our findings concerning muscular endurance. We believe the very substantial advantages of the APFT (namely, its ability to adjust for age and gender, based on measurements in thousands of individuals, and the fact that APFT scores vary linearly with number of repetitions and run times at all but the very lowest scores) greatly outweighed its limitations in this study.

Fourth, there is no universally accepted measure of pure muscular endurance. As in other tests of muscular endurance, we agree that performance on the APFT pushup and sit-up events are likely influenced to some extent by muscle strength (as defined by single-repetition isotonic maximums). However, few performance physiologists would argue that events involving multiple, repetitive, submaximal muscular contractions to exhaustion over a period of 2 min are primarily tests of strength. Even with a contribution of strength, we would have expected that an effect on muscular endurance as large as that reported by Montgomery et al. (1) (an 11-fold difference) would have been detectable.

Fifth, motivation to perform affects any exercise that can be terminated voluntarily, not just the APFT. Fortunately, the APFT also has a standard against which a “just good enough” effort can be judged: the minimum score required to graduate from basic training. Had our subjects been motivated to achieve a passing score and nothing more, the mean scores we reported would have been only slightly greater than the minimum passing score (equal to 50). In fact, they were substantially higher.

Sixth, by far the most important confounding variable (“white noise”) that affects the gains realized in a physical training regimen is prior level of fitness, not ethnic origin. Adjusting for these in our analysis did not affect our conclusions.

With these clarifications in mind, we certainly agree that our results must not be overinterpreted. We agree that the more subtle a gene’s contribution is to a complex trait, the more important it is to study the most homogeneous group possible, so as to minimize confounding effects. However, at the time we undertook this study, a very large effect of ACE genotype on muscular endurance had already been reported in a homogeneous group (1). Our primary objective was thus to ascertain whether this finding could be extended to a heterogeneous group. We found no evidence that it can. Nonetheless, as pointed out by Drs. Montgomery, Dhamrait, Jones, and Woods, our study had limited statistical power to detect an effect that is manifest only in specific demographic subgroups, and it is certainly plausible that ACE genotype has an effect on performance that can be detected readily only in individuals of a certain specific genetic background. One hypothetical mechanism by which this might occur would be if the effect of ACE genotype on performance is dependent on other, as of yet unidentified genetic factors that are highly prevalent in some but not other demographic groups. Our study was not designed or powered to address this interesting possibility.

The challenge, of course, is to design a prospective study that meets the methodological criteria outlined by Drs. Montgomery and Dhamrait, properly accounts for a baseline level of fitness, and yet is large enough to allow for a substantive analysis of different gender and ethnic subgroups with reasonable statistical power. We would welcome, and gladly contribute to, such a study.

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