Muscle-specific creatine kinase gene polymorphism and $\dot{V}O_{2max}$ in the HERITAGE Family Study

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ABSTRACT

RIVERA, M. A., F. T. DIONNE, J.-A. SIMONEAU, L. PÉRUSSE, M. CHAGNON, Y. CHAGNON, J. GAGNON, A. S. LEON, D. C. RAO, J. S. SKINNER, J. H. WILMORE, and C. BOUCHARD. Muscle-specific creatine kinase gene polymorphism and $\dot{V}O_{2max}$ in the HERITAGE Family Study. *Med. Sci. Sports Exerc.* Vol. 29, No. 10, pp. 1311–1317, 1997. This study examined the association between a DNA polymorphism in the muscle-specific creatine kinase (CKMM) gene and $\dot{V}O_{2max}$ in the sedentary state, as well as its response ($\Delta\dot{V}O_{2max}$) to a standardized 20-wk endurance training program. The subjects were 160 biologically unrelated Caucasian parents (80 women, 80 men) and 80 biologically unrelated adult offspring of the HERITAGE Family Study. The CKMM polymorphism was detected by PCR and digestion with the *NcoI* restriction enzyme. $\dot{V}O_{2max}$ was measured during maximal cycle ergometer tests. $\dot{V}O_{2max}$ was 2119 ± 45 mL·min^{-1} (mean ± SE) or 26 ± 0.4 mL·kg^{-1}·min^{-1}. Both sexes had a significant ($P < 0.05$) increase in the $\Delta\dot{V}O_{2max}$ (women = 283 ± 20 mL·min^{-1} and men = 363 ± 25 mL·min^{-1}). Allele and genotype frequencies were not significantly different ($P > 0.05$) between sexes. Age and sex adjusted $\dot{V}O_{2max}$ was significantly ($P = 0.007$) associated with the CKMM genotype in the parents, whereas no association ($P > 0.05$) was observed in the offspring. $\Delta\dot{V}O_{2max}$ values adjusted for age, sex, $\dot{V}O_{2max}$, and body mass were characterized by genotype differences in both parents ($P = 0.0004$) and offspring ($P = 0.0025$). A significantly ($P < 0.05$) lower $\Delta\dot{V}O_{2max}$ to endurance training was detected in both parents and offspring homozygotes for the rare allele. The genotype accounted for at least 9% of the variance in $\Delta\dot{V}O_{2max}$. These results indicate that the *NcoI* polymorphism in the 3′ untranslated region of the muscle-specific creatine kinase gene is associated with the $\Delta\dot{V}O_{2max}$ to endurance training.

DNA, POLYMERASE CHAIN REACTION, GENETIC VARIATION, EXERCISE, RESTRICTION ENZYMES

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Endurance training of sufficient duration and intensity induces increases in maximal oxygen uptake (17,24). However, one cannot yet predict accurately the response of a given sedentary person to a standardized training program. Indeed, it is now well documented that there are large interindividual differences in the response of the maximal oxygen uptake ($\Delta\dot{V}O_{2max}$) to endurance training in young adults (11,17,20) as well as in older men and women (14). Training studies with monozygotic twins have shown that the heterogeneity in trainability is likely the consequence of genetic differences (20). Thus, some genotypes are thought to be more responsive to aerobic training than others (6).

In the effort to define the molecular basis of the genotypic effect observed in the maximal oxygen uptake both in the untrained state ($\dot{V}O_{2max}$) and in the $\Delta\dot{V}O_{2max}$ to endurance training, candidate genes may provide useful information. In this context, we are currently studying a panel of genes involved in the process of ATP regeneration and in other metabolic pathways potentially related to aerobic performance. The present study examines the relationship between a marker of the gene coding for skeletal muscle-specific creatine kinase (CKMM) and $\dot{V}O_{2max}$, as well as in the $\Delta\dot{V}O_{2max}$ to an endurance training program.

The cytosolic muscle isoform of the enzyme is one of at least four subunits of CK that are known for their tissue-specific expression (13,28,33). The CKMM gene has been mapped to the q13.2-q13.3 region of chromosome 19 (19). This gene extends over 17.5 kilobase pairs and contains 8 exons and 7 introns (28). The CKMM isozyme generates high concentrations of ATP in the

TABLE 1. Characteristics of 160 biologically unrelated sedentary Caucasian parents and 80 biologically unrelated sedentary adult offspring of the HERITAGE Family Study.

Variables	Parents		Adult Offspring	
	Men (N = 80)	Women (N = 80)	Men (N = 34)	Women (N = 46)
Age (yr.)	53 ± 0.6	52 ± 0.6	21 ± 1	25 ± 0.8
Body mass (kg)	88 ± 2*	73 ± 2	82 ± 3*	65 ± 2
BMI (kg/m^2)	28 ± 0.4	28 ± 0.6	25 ± 0.8	24 ± 0.6
$\dot{V}O_{2max}$ (mL·min^{-1})	2607 ± 48*	1631 ± 31	3245 ± 84*	2028 ± 46
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	30 ± 0.6*	23 ± 0.6	40 ± 1*	32 ± 0.8
$\Delta\dot{V}O_{2max}$ (mL·min^{-1})	363 ± 25*	283 ± 20	583 ± 40*	370 ± 25
$\%\Delta\dot{V}O_{2max}$	14 ± 0.8*	18 ± 0.8	18 ± 1	19 ± 0.9
$\Delta\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	4 ± 0.3	4 ± 0.2	7 ± 0.5*	6 ± 0.4

* Mean significantly different from women; both sexes had a significant ($P < 0.05$) increase in $\Delta\dot{V}O_{2max}$; Values are means ± SE; BMI, body mass index; $\dot{V}O_{2max}$ (mL·min^{-1}) = baseline $\dot{V}O_{2max}$ (mL·min^{-1}); $\dot{V}O_{2max}$ (mL·kg^{-1}· min^{-1}) = baseline $\dot{V}O_{2max}$ (mL·kg−1·min^{-1}); $\Delta\dot{V}O_{2max}$ (mL·min^{-1}) = after training $\dot{V}O_{2max}$ (mL·min^{-1})−$\dot{V}O_{2max}$ (mL·min^{-1}); $\%\Delta\dot{V}O_{2max}$ (mL) = percent change in $\dot{V}O_{2max}$ (mL); $\Delta\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1}) = after training $\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1}) − $\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1}).

region of the myosin heads (31). It is bound specifically to the M-line of the myofibril (29) subfragment one of heavy meromyosin in the vicinity of the myosin ATPase (4), and to the sarcoplasmic reticulum outer membrane and vesicles (22). In addition, CKMM is expressed in human cardiac muscle (28).

Type I (slow-twitch) skeletal muscle fibers and Type II (fast-twitch) fibers have been shown to differ in their CKMM activities (34), where Type I fibers demonstrate at least a two-fold lower CKMM activity. Type I fibers are known for their high activity levels of marker enzymes of oxidative metabolism (25). There are now several lines of evidence that suggest that genetic factors are responsible for some of the variations observed in muscle fiber type distribution and enzyme activities (8,26). Studies in mice indicate that, with respect to wild type (controls), a marked increase in the capacity to synthesize ATP and improved endurance performance during low intensity exercise are observed when the CKMM gene is knocked out (30). Moreover, earlier work indicated that a CKMM protein charge variant, detected by isoelectric focusing and apparently in a coding region of the gene, was weakly associated with the ability to perform during a 90-min endurance test (5). Collectively, these observations support the notion that CKMM is a reasonable candidate gene to investigate in relation to $\dot{V}O_{2max}$ and the $\Delta\dot{V}O_{2max}$ to endurance training. Therefore, the present study tested the hypothesis of association between a *NcoI* polymorphism in the 3′ untranslated region of the CKMM gene (10) and $\dot{V}O_{2max}$ in the sedentary state and in response ($\Delta\dot{V}O_{2max}$) to an endurance training program in subjects of the HERITAGE Family Study.

METHODS

Subjects. The sample consists of 160 biologically unrelated sedentary Caucasians parents (80 men, 80 women) and 80 biologically unrelated sedentary adult offspring randomly selected from 80 nuclear families of the HERITAGE Family Study. Their characteristics are presented in Table 1. All the subjects were in good health according to the HERITAGE Family Study protocol and met the inclusion criteria (7). The HERITAGE Family Study design and methods have been described before (7). The study protocol had been previously approved by each of the Institutional Review Boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant.

HERITAGE endurance exercise training program. Briefly, subjects exercised an average of three times per week during 20 wk following a standardized protocol that required the use of a cycle ergometer (Universal Aerobicycle IV, Cedar Rapids, IA) in the sitting position. The cycle ergometer was connected to a computer system (Universal Mednet, Cedar Rapids, IA) that adjusted the power output of the ergometers to maintain constant training heart rates. During the initial 2 wk, subjects trained at a heart rate associated with 55% of $\dot{V}O_{2max}$ for 30 min per session. This was gradually increased to 50 min at the end of 14 wk at the heart rate associated with 75% $\dot{V}O_{2max}$. These intensity and duration were maintained through the remaining 6 wk.

Maximum oxygen uptake ($\dot{V}O_{2max}$) measurements. Before and after the training program, two maximal exercise tests were performed on separate days on a cycle ergometer (SensorMedics Ergo-Metrics 800S, Yorba Linda, CA) connected to a metabolic measurement cart (SensorMedics 2900). The exercise tests were conducted at almost the same time of day with at least 48 h between tests. In the first test, initial power output was 50 W for 3 min, followed by increases of 25 W each 2 min until volitional exhaustion. In special circumstances (older, smaller, or less fit individuals), the test was started at 40 W, with increases of 10 to 20 W each 2 min thereafter. During the first two stages of the second maximal exercise test, subjects exercised for 10–12 min at an absolute (50 W) and at a relative power output equivalent to 60% of their $\dot{V}O_{2max}$. They then exercised for 3 min at a relative power output that was 80% of their $\dot{V}O_{2max}$, after which resistance was increased to the highest power output attained in the first maximal test. If the subjects were able to pedal after 2 min, power output was increased each 2 min thereafter until they reached volitional fatigue. Throughout each exercise test, $\dot{V}O_2$, car-

bon dioxide production, minute ventilation, and the respiratory exchange ratio were recorded as a rolling average of three 20-s intervals using the SensorMedics 2900 metabolic measurement cart. Gas analyzers were calibrated before each maximal test with gases of certified concentration. Posttest calibration verifications were also conducted after each maximal test. Heart rate was determined from an electrocardiogram and values recorded during the last 15 s of each stage. The criteria for $\dot{V}O_{2max}$ were: respiratory exchange ratio >1.1, heart rate within 10 beats·min^{-1} of the predicted maximal for age, and a plateau in $\dot{V}O_2$ (Δ <100 mL·min^{-1} in the last three consecutive 20-s averages). After the two maximal tests were completed both tests were averaged, if $\dot{V}O_2$ was within 5% of each other. Otherwise, the test with the higher $\dot{V}O_2$ was chosen.

Genotype determinations. DNA was extracted from lymphoblastoid cell lines after a standard protocol of digestion by proteinase K and purification with phenol-chloroform (11). The polymerase chain reaction (PCR) was performed in a DNA thermal cycler (Perkin Elmer Cetus GeneAmp 9600, Norwalk, CT). Primers for the *NcoI* RFLP of the CKMM gene were as follows: 5′ GTG-CGG-TGG-ACA-CAG-CTG-CCG 3′ and 5′ CAG-CTT-GGT-CAA-AGA-CAT-TGA-GG 3′ (12). These provided a product of 1170 base pairs (bp). Total volume of the PCR was 25-μL of a reaction mixture containing 10% DMSO, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 μM of each dATP, dCTP, dGTP, and dTTP, 0.3 μM of each forward and backward primers, 0.75 U of Taq polymerase (Perkin Elmer Cetus), and 500 ng of DNA. The amplification protocol was: 1) one cycle of denaturation at 95°C for 5 min; 2) 30 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 45 s; and 3) one final 5 min elongation cycle at 72°C. Preventive contamination measures were taken by the inclusion of PCR reaction mixture without DNA (negative control) in every run of amplification.

RFLP analysis. After each amplification, the PCR product was digested with 10 U of *NcoI*. Restriction digest conditions were those recommended by the enzyme manufacturer (New England Biolabs, Mississauga, Ontario, Canada). The resulting fragments were separated by horizontal electrophoresis on 1.5% agarose gels. Each gel was run for 60 min at 150 mA while refrigerated at 10°C, stained with ethidium bromide, and photographed under UV transmitted lights. The ϕX174 DNA, digested with *HaeIII*, was used as length marker to estimate the size of the digested DNA fragments. The allele without the *NcoI* restriction site was designated as allele 1170 bp, whereas the allele with the polymorphic *NcoI* site was designated as allele 985 + 185 bp.

Statistical analysis. A chi-square test was used to examine sex differences in allele and genotype frequencies and to determine whether the observed genotype frequencies were in Hardy-Weinberg equilibrium. Pearson coefficients of correlation were calculated to quantify the degree of linear association between two variables. $\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1}) was adjusted for age and sex, while the $\Delta\dot{V}O_{2max}$ (mL·min^{-1}) was adjusted for age, sex, body mass (BM), and $\dot{V}O_{2max}$. All adjustments for covariates were made by linear regression procedures. Differences in the unadjusted and adjusted $\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1}) and $\Delta\dot{V}O_{2max}$ (mL·min^{-1}) between the three genotypes (homozygotes for the rare 1170 bp allele, heterozygotes, and homozygotes for the common 985 + 185 bp allele) were tested by ANOVA. When a statistically significant genotypic effect was detected, significance in mean differences was assessed with the Duncan *post hoc* test. These tests were performed using the General Linear Model program of SAS software package. Moreover, comparisons of allelic and genotypic differences were undertaken between the lowest (≤10th) and highest (≥90th) deciles of the covariate-adjusted $\Delta\dot{V}O_{2max}$ distribution. These same analyses were repeated in the randomly chosen sample of one adult offspring from each of the 80 participating families. P values <0.05 were considered statistically significant. The results are presented as means ± SE.

RESULTS

Subject characteristics. Sex specific values for the physical characteristics and $\dot{V}O_{2max}$ data for the parents and adult offspring are presented in Table 1. Statistically significant sex differences were observed in BM (kg), $\dot{V}O_{2max}$ (mL·min^{-1} and mL·kg^{-1}·min^{-1}), and $\Delta\dot{V}O_{2max}$ (mL·min^{-1}) for both parents and adult offsprings. Additional significant differences between sexes were observed for the percent change in $\Delta\dot{V}O_{2max}$ (%$\Delta\dot{V}O_{2max}$) in the parental generation and the $\Delta\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1}) in the adult offspring. Each sex, in both parents and adult offspring, demonstrated a significant increase in $\Delta\dot{V}O_{2max}$. Individual $\Delta\dot{V}O_{2max}$ in the response to training ranged from −187 to 1089 (mL·min^{-1}) or −8 to 39% for the men in the parents group. For the women in the parents group, the $\Delta\dot{V}O_{2max}$ ranged from −275 to 1026 (mL·min^{-1}) or −12 to 51%. In the adult offspring, the range of the individual $\Delta\dot{V}O_{2max}$ was 30 to 1083 (mL·min^{-1}) or 1 to 43%. No significant correlations (Pearson r) between age, body mass, and $\dot{V}O_{2max}$ with $\Delta\dot{V}O_{2max}$ or between age and body mass with $\dot{V}O_{2max}$ were observed in both men and women.

Allele and genotype frequencies. The allelic frequencies of the CKMM *NcoI* 1170 bp and 985 +185 bp alleles are presented in Table 2. These allele frequencies were not significantly different (χ^2 = 0.60, df = 1, P = 0.44) between men and women. In both sexes the allele designated as 1170 bp was the less frequently observed. Table 2 also shows that the three expected genotypes

TABLE 2. Sex specific allele and genotype frequencies for the NcoI polymorphism in the 3′ untranslated region of the muscle-specific creatine kinase gene for 160 biologically unrelated sedentary Caucasian parents of the HERITAGE Family Study.

	Allele Frequencies*		Genotype in bp†		
	1170	985 + 185	1170/1170	1170/985 + 185	985 + 185/985 + 185
Men (N = 80)	0.32	0.68	0.07	0.51	0.42
Women (N = 80)	0.27	0.73	0.09	0.36	0.55
Total (N = 160)	0.30	0.70	0.07	0.44	0.49

* χ^2 = 0.60, df = 1, P = 0.44.
† χ^2 = 3.7, df = 2, P = 0.16.

were observed in both sexes. Chi-square tests revealed that the observed genotypic distributions within each sex were in agreement ($P > 0.05$) with those expected under Hardy-Weinberg equilibrium and were not significantly ($\chi^2 = 3.7$, $df = 2$, $P = 0.16$) different between sexes. Since no significant genotype-sex interaction effect was detected (not shown) for the variables under study, and given that there were similar allelic and genotypic frequency distributions in men and women parents, the data for both sexes were pooled for subsequent analyses.

Association studies. Table 3 shows the unadjusted and covariate-adjusted values of the $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) by CKMM *NcoI* genotypes for the parents. After adjusting $\dot{V}O_{2max}$ for age and sex, the genotype effect was statistically significant. ($F = 5.1$, $P = 0.007$). A closer examination of the means by the Duncan *post hoc* test revealed that the heterozygotes had a statistically significant ($P < 0.05$) higher adjusted $\dot{V}O_{2max}$ than the homozygotes for the rare 1170 bp allele or the common 985 + 185 bp allele. The association between the CKMM *NcoI* genotype and $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) adjusted for age and sex in parents and offspring is summarized in Figure 1. No significant association was detected between the CKMM *NcoI* genotype and $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) in adult offspring (Fig. 1).

The summary of the results evaluating the association between $\Delta\dot{V}O_{2max}$ under the various schemes of adjustment and CKMM *NcoI* genotypes in parents are presented in Table 4. The unadjusted $\Delta\dot{V}O_{2max}$ was not significantly associated with the genotype ($F = 2.07$, $P = 0.13$). However, when the analysis was carried out

TABLE 3. $\dot{V}O_{2max}$ by muscle-specific creatine kinase NcoI genotypes for 160 biologically unrelated sedentary Caucasian parents of the HERITAGE Family Study.

	Genotype in bp				
	1170/1170	1170/985 + 185	985 + 185/985 + 185	F	P value
N	12	70	78		
Unadjusted $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	25 ± 1	27 ± 0.7	26 ± 0.7	1.38	0.25
*Sex	25 ± 1	28 ± 0.6	26 ± 0.6	3.64	0.03
*Age, sex	24 ± 1	28 ± 0.5	26 ± 0.6	5.08	0.007

Values are means ± SE; $\dot{V}O_{2max}$ = baseline $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$);
* Adjustment of $\dot{V}O_{2max}$ by linear regression procedures with age + age^2 + age^3 and sex as covariates.

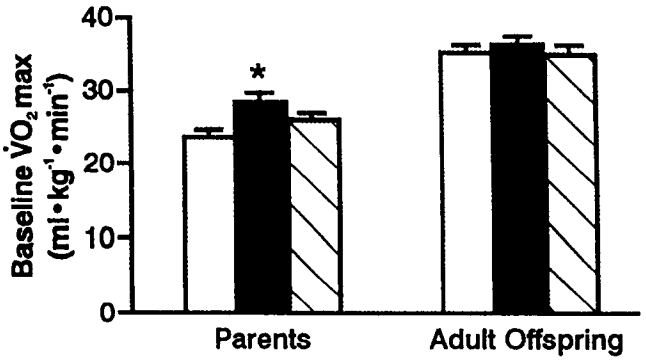

Figure 1—Covariate-adjusted (age + age^2 + age^3 and sex) $\dot{V}O_{2max}$ by muscle-specific creatine kinase *NcoI* genotype of 160 biologically unrelated sedentary Caucasian parents and 80 biologically unrelated sedentary adult offspring of the HERITAGE Family Study. Open bars, homozygotes for the rare 1170 bp allele; filled bars, heterozygotes; hatched bars, homozygotes for the common 985 + 185 bp allele. Values are means ± SE * Significantly ($P < 0.05$) different from the other two genotypes.

with sex, age, BM, and $\dot{V}O_{2max}$ as covariates, a highly significant association ($F = 8.37$, $P = 0.0004$) became apparent between the $\Delta\dot{V}O_{2max}$ and the CKMM *NcoI* genotype. This latter accounted for 10% of the variation in $\Delta\dot{V}O_{2max}$. The homozygotes for the rare 1170 bp allele had a significantly lower $\Delta\dot{V}O_{2max}$ than the heterozygotes or the homozygotes for the common 985 + 185 bp allele. The association between the CKMM *NcoI* genotype and the covariate adjusted (sex, age, $\dot{V}O_{2max}$, and BM) $\Delta\dot{V}O_{2max}$, in parents and offspring are summarized in Figure 2. As in the parental generation, a significant association ($F = 3.87$, $P < 0.025$) was observed between genotype and the offspring covariate-adjusted $\Delta\dot{V}O_{2max}$, with the homozygotes for the 1170 bp allele exhibiting a lower trainability than the other two genotypes (Fig. 2). In this subsample, the genotype accounted for 9% ($P = 0.0025$) of the variation in the $\Delta\dot{V}O_{2max}$.

Figure 3 illustrates the prevalence of the CKMM *NcoI* genotypes among low and high responders to training (lowest and highest deciles, respectively), of the covariate-adjusted $\Delta\dot{V}O_{2max}$. Thirty-three percent (33%) of all homozygotes for the rare 1170 bp allele were low responders to training, while this genotype was not seen among the high responders to training. Moreover, the homozygotes for the rare 1170 bp allele were at least three times more prevalent than the other genotypes among the low responders to training.

DISCUSSION

The present study examined the association between a *NcoI* polymorphism in the 3′ untranslated region of the CKMM gene (10) and both $\dot{V}O_{2max}$ in the sedentary state and the $\Delta\dot{V}O_{2max}$ to an endurance training program. The most important finding of this study was a significant association between the CKMM *NcoI* genotype and the covariate-adjusted $\Delta\dot{V}O_{2max}$. To our knowledge, this is

TABLE 4. Response to training by muscle-specific creatine kinase NcoI genotypes for 160 biologically unrelated sedentary Caucasian parents of the HERITAGE Family Study.

	Genotype in bp				
	1170/1170	1170/ 985 + 185	985 + 185/ 985 + 185	*F*	*P* value
N	12	70	78		
Unadjusted $\Delta\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	210 ± 57	339 ± 28	327 ± 20	2.07	0.13
*Sex	103 ± 53	347 ± 26	335 ± 20	7.91	0.0005
*Age, Sex	109 ± 54	349 ± 26	333 ± 21	7.63	0.0007
*Age, Sex, $\dot{V}O_{2max}$	107 ± 53	349 ± 26	333 ± 21	7.86	0.0006
*Age, Sex, $\dot{V}O_{2max}$, BM	103 ± 58	352 ± 26	331 ± 20	8.37	0.0004

Values are means ± SE; $\Delta\dot{V}O_{2max}$ = after training $\dot{V}O_{2max}$ ($mL \cdot min^{-1}$) − baseline $\dot{V}O_{2max}$ ($mL \cdot min^{-1}$); $\dot{V}O_{2max}$ = baseline $\dot{V}O_{2max}$ ($mL \cdot min^{-1}$); BM = body mass.
* Adjustment of $\Delta\dot{V}O_{2max}$ by linear regression procedures with age + age^2 + age^3, sex, $\dot{V}O_{2max}$, and BM as covariates.

the first quantification in a training study of a significant association between a DNA polymorphism in a candidate gene and $\dot{V}O_{2max}$ in a relatively large sample size (N = 160) of biologically unrelated sedentary Caucasian adults. Additional support was provided by the genotypic distribution among the high and low responders to training. In this instance, one-third of all the homozygotes for the rare 1170 bp allele were observed in the low responders (lowest decile of the response), while this genotype was not evident in the high responders (upper decile of the response). Furthermore, the hypothesis of an association was verified in a group of 80 biologically unrelated adult offspring in which the adjusted $\Delta\dot{V}O_{2max}$ was also found to be significantly different among genotypes. As in the parental generation, the homozygotes for the rare 1170 bp allele in the adult offspring demonstrated a lower adjusted $\Delta\dot{V}O_{2max}$ than for the other two genotypes. The magnitude of the difference in the $\Delta\dot{V}O_{2max}$ to endurance training between the homozygotes for the rare allele and the other two phenotypes was at least three-fold lower in the parents and 1.5 times lower in the adult offspring.

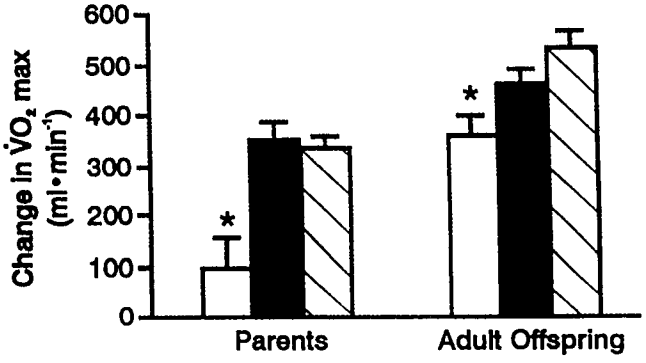

Figure 2—Covariate-adjusted (sex, age + age^2 + age^3, $\dot{V}O_{2max}$, and body mass) changes in $\dot{V}O_{2max}$ after 20-wk of endurance training by muscle-specific creatine kinase *NcoI* genotype in 160 biologically unrelated sedentary Caucasian parents and 80 biologically unrelated sedentary adult offsprings of the HERITAGE Family Study. Open bars, homozygotes for the rare 1170 bp allele; filled bars, heterozygotes; hatched bars, homozygotes for the common 985 + 185 bp allele. Values are means ± SE. *Significantly ($P < 0.05$) different from other two genotypes.

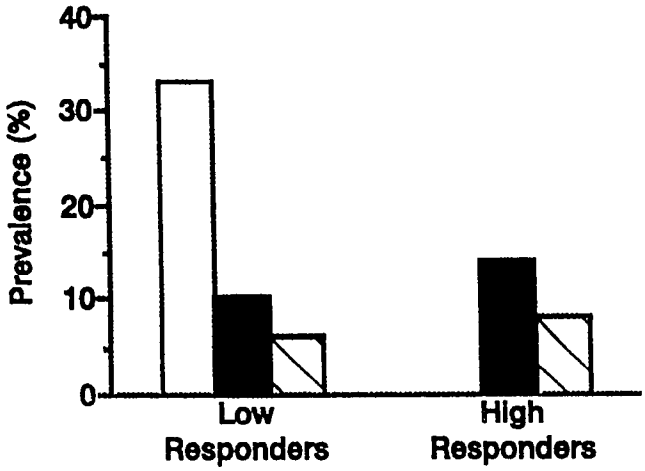

Figure 3—Prevalence of CKMM *NcoI* genotype in low and high responders to training for 160 biologically unrelated sedentary Caucasian parents of the HERITAGE Family Study. Low and high response to training were defined as the lowest and highest deciles, respectively, of the distribution for the covariate-adjusted (sex, age + age^2 + age^3, $\dot{V}O_{2max}$ and body mass) changes in $\dot{V}O_{2max}$. Prevalence of homozygotes for the rare 1170 bp allele is at least three times that of the other two genotypes. No case of homozygotes for the rare 1170 bp allele was observed in the high responders to training. Open bars, homozygotes for rare 1170 bp allele; filled bars, heterozygotes; hatched bars, homozygotes for the common 985 + 185 bp allele.

Moreover, the genotype accounted for 9 and 10% of the variance in the $\Delta\dot{V}O_{2max}$ for the adult offspring and parents, respectively

Previous research has indicated that: 1) the genetic heritability of $\dot{V}O_{2max}$ and several determinants of endurance performance was statistically significant (6); 2) there were large individual differences in the response of $\dot{V}O_{2max}$ to endurance training (16) but much of the heterogeneity in response was likely accounted for by genetic differences in trainability (20); and 3) $\dot{V}O_{2max}$ was likely characterized by a maternal effect (15).

Three lines of evidence currently provide an explanation of the role of CK in cells, particularly in the brain, skeletal and heart muscle, and smooth muscle. These three lines of evidence have been the subject of recent reviews (23,33). One considers CK as a "temporal energy buffer system" with a role in preserving a suitable ATP/ADP ratio in cellular regions of high energy demand (18). A second states that CK acts as an energy carrier from sites of production to those of utilization. This hypothesis is known as the phosphorylcreatine (3) or creatine phosphate (2) shuttle or circuit (32). The third line of evidence describes the role of CK as a metabolic capacity system (27).

The genotypic effects underlying individual differences in trainability may arise from DNA sequence polymorphisms that translate in gene product polymorphism or variation in gene expression. The CKMM gene *NcoI* polymorphism examined in this study lies in the 3′ untranslated region (10), which is outside the coding and the usual 5′ regulatory sector of the gene. For this reason the

probability of this mutation been the direct cause of the association is low. The observed results could be the effect of another mutation within the CKMM gene. In fact, a close linkage disequilibrium between the *NcoI* and a *TaqI* polymorphism within the CKMM gene has been reported (9,12). The *TaqI* polymorphism is located in codon 463 (1). However, it does not result in an amino acid substitution. Therefore, the observed effect in this study is probably associated with another mutation in the CKMM gene or in a closely linked gene. More research is needed on this topic.

There are two possible explanations for the role of CKMM in the expression of $\dot{V}O_{2max}$. First, it might be that CKMM activity has no direct role in $\dot{V}O_{2max}$ but just covaries with another trait that is important for $\dot{V}O_{2max}$. A second possibility, however, is that the enzymatic activity of CKMM plays a role in the determination of $\dot{V}O_{2max}$. Our results are to some extent compatible with the notion that the enzymatic activity of CKMM in the myocardium and skeletal muscle tissue plays a role in the determination of the $\dot{V}O_{2max}$. The traditional concept of individual variation in $\dot{V}O_{2max}$ considers that its major determinants are a high central cardiovascular and peripheral vascular capacities, skeletal muscle fiber type profile and metabolic properties, and level of training (21). Along these lines, $\dot{V}O_{2max}$ is influenced by slow twitch motor units (Type I fibers) known for their high activity level of oxidative enzymes (25), along with a low CKMM activity (34). Recent evidence suggests that genetic factors partially mediate these skeletal muscle attributes (8,26). In addition, research on transgenic mice indicates an improved endurance performance in the CKMM knock-out mice compared with that seen in control animals (30). Thus, there is some indirect support for the hypothesis that CKMM activity plays a role in limiting $\dot{V}O_{2max}$ and perhaps in its responsiveness to endurance training.

In conclusion, the results of this study have shown a significant association between CKMM genotype and the $\dot{V}O_{2max}$ response to endurance training in both biologically unrelated sedentary adult Caucasians and a subsample of adult offspring of the HERITAGE Family Study. At least 9% of the variation observed in the $\Delta\dot{V}O_{2max}$ was explained by the genotype. Moreover, the $\Delta\dot{V}O_{2max}$ of the homozygotes for the rare 1170 base pair allele was 1.5 to 3 times lower than that observed for the other two genotypes. The consistency in the results in both parents and offspring provides strong evidence for the first time that a genetic polymorphism may account for some of the individual differences observed in the $\Delta\dot{V}O_{2max}$ to endurance training. Additional research is needed to examine mutations in the CKMM gene and in other candidate genes in an effort to understand the genetic contribution to $\dot{V}O_{2max}$ in the sedentary state and in its response to training.

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