

highlighted topics

The Na⁺-K⁺-ATPase α 2 gene and trainability of cardiorespiratory endurance: the HERITAGE Family Study

TUOMO RANKINEN,¹ LOUIS PÉRUSSE,² INGRID BORECKI,³ YVON C. CHAGNON,² JACQUES GAGNON,² ARTHUR S. LEON,⁴ JAMES S. SKINNER,⁵ JACK H. WILMORE,⁶ D. C. RAO,³ AND CLAUDE BOUCHARD¹

¹Pennington Biomedical Research Center, Human Genomics Laboratory, Baton Rouge, Louisiana 70808-4124; ²Physical Activity Sciences Laboratory, Laval University, Ste-Foy G1K 7P4, Québec, Canada; ³Division of Biostatistics and Departments of Genetics and Psychiatry, Washington University Medical School, St. Louis, Missouri 63110-1093; ⁴School of Kinesiology and Leisure Studies, University of Minnesota, Minneapolis, Minnesota 55455; ⁵Department of Kinesiology, Indiana University, Bloomington, Indiana 11001; and ⁶Department of Health and Kinesiology, Texas A&M University, College Station, Texas 77843-4243

Rankinen, Tuomo, Louis Pérusse, Ingrid Borecki, Yvon C. Chagnon, Jacques Gagnon, Arthur S. Leon, James S. Skinner, Jack H. Wilmore, D. C. Rao, and Claude Bouchard. The Na⁺-K⁺-ATPase α 2 gene and trainability of cardiorespiratory endurance: the HERITAGE Family Study. *J. Appl. Physiol.* 88: 346–351, 2000.—The Na⁺-K⁺-ATPase plays an important role in the maintenance of electrolyte balance in the working muscle and thus may contribute to endurance performance. This study aimed to investigate the associations between genetic variants at the Na⁺-K⁺-ATPase α 2 locus and the response (Δ) of maximal oxygen consumption ($\dot{V}O_{2\max}$) and maximal power output (\dot{W}_{\max}) to 20 wk of endurance training in 472 sedentary Caucasian subjects from 99 families. $\dot{V}O_{2\max}$ and \dot{W}_{\max} were measured during two maximal cycle ergometer exercise tests before and again after the training program, and restriction fragment length polymorphisms at the Na⁺-K⁺-ATPase α 2 (exons 1 and 21–22 with *Bgl*II) gene were typed. Sibling-pair linkage analysis revealed marginal evidence for linkage between the α 2 haplotype and $\Delta\dot{V}O_{2\max}$ ($P = 0.054$) and stronger linkages between the α 2 exon 21–22 marker ($P = 0.005$) and α 2 haplotype ($P = 0.003$) and $\Delta\dot{W}_{\max}$. In the whole cohort, $\Delta\dot{V}O_{2\max}$ in the 3.3-kb homozygotes of the exon 1 marker ($n = 5$) was 41% lower than in the 8.0/3.3-kb heterozygotes ($n = 87$) and 48% lower than in the 8.0-kb homozygotes ($n = 380$; $P = 0.018$, adjusted for age, gender, baseline $\dot{V}O_{2\max}$, and body weight). Among offspring, 10.5/10.5-kb homozygotes ($n = 14$) of the exon 21–22 marker showed a 571 ± 56 (SE) ml O₂/min increase in $\dot{V}O_{2\max}$, whereas the increases in the 10.5/4.3-kb ($n = 93$) and 4.3/4.3-kb ($n = 187$) genotypes were 442 ± 22 and 410 ± 15 ml O₂/min, respectively ($P = 0.017$). These data suggest that

genetic variation at the Na⁺-K⁺-ATPase α 2 locus influences the trainability of $\dot{V}O_{2\max}$ in sedentary Caucasian subjects.

exercise training; sodium-potassium pump; family study

THE CAPACITY TO PERFORM high-intensity exercise over an extended period of time is influenced by several factors. The availability of oxygen and energy substrates, as well as the efficiency of muscle energy production mechanisms, is fundamental to maintain a given exercise intensity level. The potential of respiratory and cardiovascular systems to adapt to regular exercise training is also of importance. One factor critically influencing the excitability of skeletal muscles is the concentration gradients for Na⁺ and K⁺ across sarcolemma and t-tubular membranes (30). The membrane potential, created by active transport of Na⁺ out of the fibers and of K⁺ into the fibers, is essential for the spread of action potentials along the sarcolemma and the t-tubular membranes and, subsequently, for muscle contraction. After contraction, the membrane potential must be restored to facilitate the spread of a new action potential. During exercise, K⁺ efflux and Na⁺ influx increase drastically in skeletal muscle, leading to depolarization of the cell membrane and, if not corrected, to a decrease in muscle contractility.

The sarcolemmal Na⁺-K⁺-ATPase is a key enzyme for the maintenance of cation-concentration gradients across the cell membrane by transporting three Na⁺ out of the cell and two K⁺ into the cell for each molecule of ATP used to drive the process (9). The activity of Na⁺-K⁺-ATPase in working skeletal muscles is markedly increased during an acute bout of exercise, mainly because of activation of inactive molecules already present in the sarcolemma (10, 28). However, during

Original submission in response to a special call for papers on "Molecular and Cellular Basis of Exercise Adaptations."

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

high-intensity exercise, the rate of cation fluxes seems to exceed the capacity of the sarcolemmal Na⁺-K⁺-ATPase, resulting in a lower membrane potential (10, 17). The activity level of the enzyme remains elevated after cessation of exercise to facilitate the recovery of cation gradients in muscles (27, 28). Regular exercise training has been shown to increase Na⁺-K⁺-ATPase concentration in the plasma membrane of trained muscles in sedentary subjects (15, 16, 25), moderately endurance-trained men (22), and endurance athletes (11); this pattern is observed regardless of the mode of exercise training (24). On the other hand, inactivation of the Na⁺-K⁺-ATPase by ouabain or by K⁺ depletion significantly decreases contractile endurance in animal models (18, 26), whereas activation of the Na⁺-K⁺-ATPase with insulin or catecholamines or by electrical stimulation restores hyperpolarization of the sarcolemma and force production of rat soleus muscle exposed to altered Na⁺ and K⁺ gradients (29, 31).

Genetic factors have been shown to influence the cardiorespiratory fitness level, both in the sedentary state and in response to regular endurance training. Results from twin and family studies have yielded significant heritability estimates for maximal oxygen consumption ($\dot{V}O_{2\max}$) in sedentary subjects (7, 12, 21, 23). Similarly, the response of $\dot{V}O_{2\max}$ to endurance training programs exhibits greater variability between than within pairs of monozygotic twins, with intraclass correlations varying from 0.60 to 0.77 (5, 8, 33). The effects of the genotype on responsiveness to regular endurance training have been investigated in the HERITAGE Family Study, and maximal heritability estimates of 51 and 47% were derived for $\dot{V}O_{2\max}$ in the sedentary state and in response to training, respectively (3, 4).

Considering the potential role of Na⁺-K⁺-ATPase in the contractility of skeletal muscle and the heritability of cardiorespiratory fitness and Na⁺-K⁺-ATPase phenotypes, the purpose of this study was to investigate the associations between the Na⁺-K⁺-ATPase $\alpha 2$ gene markers (a gene expressed mainly in skeletal muscle) and the responsiveness of $\dot{V}O_{2\max}$ and maximal power output (W_{\max}) to a 20-wk endurance training program in 472 Caucasian subjects of the HERITAGE Family Study.

METHODS

Subjects. The study cohort consisted of 472 Caucasian subjects (230 men and 242 women) from 99 families. The study design and inclusion criteria have been described previously (6). The individuals were required to be in good health (i.e., free of diabetes, cardiovascular diseases, or other chronic diseases) and to be sedentary at baseline (defined as not having engaged in regular physical activity over the previous 6 mo) to be eligible for the study. The study protocol was 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 based on the heart rate-oxygen consumption ($\dot{V}O_2$) relationship measured at baseline. During the first 2 wk, the subjects

trained at a heart rate 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 at a heart rate associated with 75% of the baseline $\dot{V}O_{2\max}$; these were sustained for the last 6 wk. Training frequency was three times per week, and all training was performed on cycle ergometers in the laboratory. Heart rate was monitored during all training sessions by a computerized cycle ergometer system (Universal Gym Mednet, Cedar Rapids, IA), which adjusted ergometer resistance to maintain the target heart rate. All exercise sessions were supervised by trained exercise specialists.

Fitness phenotypes. Before and after the 20-wk training program, each subject completed two maximal cycle ergometer (SensorMedics Ergo-Metrics 800S, Yorba Linda, CA) exercise tests conducted on separate days. The first test started at 50 W for 3 min, and the rate of work 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. The second test started with a submaximal exercise period, performed at 50 W and at 60% of the initial $\dot{V}O_{2\max}$ for 12–15 min at each work rate with a 4-min period of seated rest between work rates, and progressed to a maximal level of exertion (4, 36).

For both tests, $\dot{V}O_2$ was determined every 20 s and is 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 in each of the maximal tests, or the higher of the two values if they differed by >5%. The intraclass correlation coefficient for repeated measurements of the $\dot{V}O_{2\max}$ reached 0.97 (36).

Other phenotypes. Stature was measured to the nearest 0.1 cm with the subject standing erect on a flat surface, with 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 light-weight bathing suit.

Genotype determination. Genomic DNA samples isolated from lymphoblastoid cell lines were digested with *Bgl* II restriction enzyme, and the resulting DNA fragments were separated by agarose gel electrophoresis. DNA fragments were transferred to nylon filters, hybridized with ³²P-labeled genomic probes, and visualized with autoradiograms after 1–7 days of exposure at –80°C. Phage lambda DNA digested with *Hind* III and *Eco*R I was used as size standard. The genomic probes used were a 2.5-kb DNA fragment of the 5' end of the $\alpha 2$ gene that includes exon 1 ($\alpha 2$ exon 1 marker) and a 1.0-kb DNA fragment of the 3' portion of the $\alpha 2$ gene that includes exons 21 and 22 ($\alpha 2$ exon 21–22 marker) (35).

Statistical analyses. A χ^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.

For the sibling-pair linkage analyses, the phenotypes were adjusted for potential confounders by using a stepwise regression procedure separately in four gender-by-generation subgroups, and the standardized (mean = 0, SD = 1) residuals were used for the linkage analyses. The covariates used included age, gender, and baseline body weight for the baseline phenotypes and age, gender, and baseline values of the phenotype and of body weight for the training-response

variables. Linkage between the fitness phenotypes and the Na⁺-K⁺-ATPase $\alpha 2$ markers was investigated with the sibling-pair linkage procedure (1, 19). Briefly, if there was a linkage between the marker locus and a putative gene influencing the phenotype, siblings sharing a greater proportion of alleles identical by descent at the marker locus would also show a greater resemblance in the phenotype. The squared sibling-pair phenotypic difference was regressed on the expected proportion of marker alleles identical by descent at the locus. A one-sided *t*-test was then used to test whether the regression coefficient was <0. A significant inverse relationship between the squared sibling-pair phenotypic difference and allele sharing at the marker locus was taken as evidence of linkage. The linkage analysis was performed by using the SIBPAL program of the SAGE Statistical Package (34).

Associations between fitness phenotypes and the genetic markers were tested with analysis of covariance by using the GLM 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 phenotypes. Possible generation-by-genotype interaction effects were tested by introducing an interaction term in the GLM model in addition to the genotype and generation main effects. If the interaction term was significant, or if a significant sibling-pair linkage was observed between an $\alpha 2$ marker and a phenotype, association analyses were performed separately in parents and offspring. In addition to the fully adjusted models, analyses were also performed without adjustment, by adjusting for each of the covariates separately and by using various combinations of covariates. The results of all of these analyses were globally identical to those of the full model, and, therefore, only the data from the full models are reported in the present study. The results are expressed as means \pm SE.

All the family members were included in the association analyses. Although it is commonly believed that the relatedness of the subjects in family studies may cause problems in association analyses, recent evidence (M. Province, T. Rice, and D. C. Rao, unpublished observations) suggests that this is not the case. In that study, simulated data were analyzed by four methods: the least squares method ignored relatedness in the present study, and the other three methods treated the dependencies in different ways. The results showed that, first, failure to incorporate dependencies did not induce any bias and, second, for moderate familial correlations as seen in most family studies (including the present one), ignoring the dependencies in an ANOVA performed quite well. The only negative impact was a small reduction in power. The SEs were slightly larger, 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. However, we repeated the analyses after randomly selecting only one offspring per family, and the results were basically the same as with the whole cohort (see RESULTS).

RESULTS

The basic characteristics of the subjects are presented in Table 1. The endurance training program increased $\dot{V}O_{2\max}$ by 16.9 ± 0.7 and $17.0 \pm 0.5\%$ and W_{\max} by 28.6 ± 1.1 and $28.5 \pm 0.8\%$ in parents and offspring, respectively. The allele frequencies were 0.89 (8.0-kb allele) and 0.11 (3.3 kb) for the $\alpha 2$ exon 1 marker and 0.79 (4.3 kb) and 0.21 (10.5 kb) for the $\alpha 2$ exon 21–22 marker. Genotype frequencies were in Hardy-Weinberg equilibrium for each marker. Body weight

Table 1. Characteristics of 472 Caucasian subjects in the HERITAGE Family Study

	Parents	Offspring
Males/females	92/85	138/157
Age, yr	52.8 \pm 0.4	25.5 \pm 0.4
Height, cm	169.2 \pm 0.7	171.4 \pm 0.5
Weight, kg	80.0 \pm 1.2	72.8 \pm 1.0
Body mass index, kg/m ²	27.8 \pm 0.3	24.6 \pm 0.3
$\dot{V}O_{2\max}$, ml/min	2,146 \pm 45	2,647 \pm 42
W_{\max} , W	159 \pm 3.7	200 \pm 3.4

Values are means \pm SE. $\dot{V}O_{2\max}$, maximal oxygen consumption; W_{\max} , maximal power output.

and body composition at baseline were similar across the genotypes (data not shown).

Sibling-pair analysis revealed marginal evidence for linkage between the $\alpha 2$ haplotype and training response in $\dot{V}O_{2\max}$ (Table 2). The linkage evidence was much stronger between the $\alpha 2$ exon 21–22 marker and the $\alpha 2$ haplotype and ΔW_{\max} . The $\alpha 2$ exon 1 marker also showed a suggestive linkage with ΔW_{\max} . The distribution of the squared intrapair differences for each significant phenotype-genotype combination was checked, and no isolated outliers were detected, i.e., there was no sibling pair at least 3 SD above the sibling pair with the next highest value for the squared intrapair difference of a given phenotype. Thus the results of the linkage analyses were not influenced by extreme phenotype values. No linkages were observed between the $\alpha 2$ markers and $\dot{V}O_{2\max}$ and W_{\max} in the sedentary state.

A significant association between the $\alpha 2$ exon 1 marker and $\Delta \dot{V}O_{2\max}$ was observed in the whole cohort (Table 3). The association was characterized by a smaller training response in the homozygotes for the rarer 3.3-kb allele than in the heterozygotes or the wild-type homozygotes. The $\alpha 2$ exon 21–22 marker was not associated with $\Delta \dot{V}O_{2\max}$ or ΔW_{\max} in the whole cohort, but, because of the significant findings from the sibling-pair linkage analyses and a suggestive trend for a generation-by-genotype interaction effect for $\Delta \dot{V}O_{2\max}$ ($P = 0.075$), the analyses were repeated separately in parents and offspring. In offspring, the homozygotes for the 10.5-kb allele showed 29 and 39% greater $\dot{V}O_{2\max}$ training responses than did the heterozygotes and the wild-type homozygotes, respectively (Table 4). Neither $\Delta \dot{V}O_{2\max}$ nor ΔW_{\max} was associated with the $\alpha 2$ exon 21–22 genotypes in parents. Baseline $\dot{V}O_{2\max}$ and W_{\max}

Table 2. Sibling-pair linkage analysis, results for the Na⁺-K⁺-ATPase $\alpha 2$ markers, and changes in cardiorespiratory fitness in Caucasian subjects of the HERITAGE Family Study

	$\alpha 2$ Exon 1			$\alpha 2$ Exon 21–22			$\alpha 2$ Haplotype		
	<i>n</i>	<i>t</i>	<i>P</i>	<i>n</i>	<i>t</i>	<i>P</i>	<i>n</i>	<i>t</i>	<i>P</i>
$\Delta \dot{V}O_{2\max}$	318	-0.78	0.217	316	-1.14	0.128	309	-1.61	0.054
ΔW_{\max}	318	-1.94	0.027	316	-2.59	0.005	309	-2.77	0.003

Δ , change; *n*, no. of sibling-pairs; *t*, *t* ratio derived from *t*-test. Phenotypes are adjusted for age, gender, baseline body mass, and baseline phenotype value.

Table 3. Associations between the Na⁺-K⁺-ATPase α2 exon 1 polymorphism and $\dot{V}O_{2max}$ and \dot{W}_{max} in sedentary state and in response to 20-wk endurance training program in 472 Caucasian subjects of the HERITAGE Family Study

	8.0/8.0 kb	8.0/3.3 kb	3.3/3.3 kb	P Value
<i>n</i>	380	87	5	
$\dot{V}O_{2max}$, ml/min				
Baseline	2,452 ± 18	2,496 ± 38	2,436 ± 156	0.562
Response	+406 ± 10	+361 ± 21	+211 ± 89	0.018
\dot{W}_{max} , W				
Baseline	184 ± 1.8	185 ± 3.7	177 ± 15	0.882
Response	+51 ± 1.2	+49 ± 2.5	+40 ± 10.6	0.431

Values are means ± SE of *n* subjects.

showed no associations with either of the α2 markers. Finally, the analyses were repeated after randomly selecting only one offspring per family. The results were similar to those obtained with the whole cohort, except for the association between the α2 exon 21–22 marker and $\Delta\dot{W}_{max}$ in offspring, which was statistically significant (*P* = 0.011) in the partial cohort (increases of 86.7 ± 10.4, 60.6 ± 4.9, and 53.8 ± 3.5 W in the 10.5/10.5-, 10.5/4.3-, and 4.3/4.3-kb genotypes, respectively).

DISCUSSION

The results of the present study suggest that the Na⁺-K⁺-ATPase α2 gene locus is associated with the training responses of endurance performance phenotypes in healthy, previously sedentary Caucasian subjects. This is the first time such an association is reported. However, data from human and animal studies support the role of sarcolemmal Na⁺-K⁺-ATPase in

the skeletal muscle contractility, development of fatigue, and, ultimately, endurance performance (30).

High-intensity exercise leads to increased passive leak of Na⁺ into and K⁺ out of the muscle fibers, and K⁺ concentration in the interstitial fluid can rise two- to fourfold during exercise (30). Loss of cation concentration gradient across the cell membrane leads to depolarization of the sarcolemma, which hinders the spread of new action potentials. To restore the contractility of the muscle, K⁺ and Na⁺ concentration gradients must be regenerated, and the sarcolemmal Na⁺-K⁺-ATPase is essential in this process. Significantly greater concentrations of Na⁺-K⁺-ATPase in vastus lateralis muscle have been reported in endurance-trained subjects than in sedentary age-matched controls (20). Animal studies have shown that inhibition of Na⁺-K⁺-ATPase reduces the performance of skeletal muscle (18, 26). However, when the Na⁺-K⁺-ATPase was activated with insulin, catecholamines, or electrical stimulation, both membrane potential of the sarcolemma and force production of the muscle were restored (29, 31). Several studies have also shown that exercise training increases the Na⁺-K⁺-ATPase concentration in skeletal muscle (11, 15, 16, 22, 25), and the increases take place independently of the changes in oxidative potential of the muscle (15).

Our results suggest that the homozygotes for the variant allele of the α2 exon 1 decreased the $\dot{V}O_{2max}$ response to regular endurance training, whereas the variant allele of the α2 exon 21–22 is associated with greater $\dot{V}O_{2max}$ responsiveness. Although these two markers are in significant linkage disequilibrium in the HERITAGE cohort, it is unlikely that the variant alleles reflect the same underlying functional gene variant. A more detailed inspection of the markers revealed that all of the 10.5-kb homozygotes of the α2 exon 21–22 marker were also α2 exon 1 wild-type homozygotes, whereas 87% of the α2 exon 1 variant allele carriers and all of the variant allele homozygotes were α2 exon 21–22 wild-type homozygotes. Thus it seems plausible that these two markers index two different mutations: one associated with a decreased and the other with an increased responsiveness to endurance training. However, these markers explain a relatively small proportion of the variance in $\Delta\dot{V}O_{2max}$ (1.5–2.4%), which is in line with the polygenic nature of cardiorespiratory fitness phenotypes.

A role for the Na⁺-K⁺-ATPase α2 locus in the training responsiveness was further supported by the sibling-pair linkage results. Although neither of the individual markers showed significant linkages with $\Delta\dot{V}O_{2max}$, the haplotype revealed a suggestive linkage. This is most likely due to the low information content of the individual markers. With the use of the haplotype, it was possible to increase the polymorphic information content value of the marker from 0.174 (exon 1) and 0.306 (exon 21–22) to 0.411.

Although Na⁺-K⁺-ATPase α2 gene markers used in the present study were described 10 years ago (35), it is still unclear whether these variants have any effect on the function of the Na⁺-K⁺-ATPase α2 subunit. How-

Table 4. Associations between Na⁺-K⁺-ATPase α2 exon 21–22 polymorphism and $\dot{V}O_{2max}$ and \dot{W}_{max} in sedentary state and in response to 20-wk endurance training program in Caucasian parents and offspring of the HERITAGE Family Study

	10.5/10.5 kb	10.5/4.3 kb	4.3/4.3 kb	P Value
<i>Parents</i>				
<i>n</i>	11	55	111	
$\dot{V}O_{2max}$, ml/min				
Baseline	2,045 ± 97	2,142 ± 43	2,158 ± 30	0.537
Response	+307 ± 55	+356 ± 25	+338 ± 17	0.686
\dot{W}_{max} , W				
Baseline	162 ± 8.8	160 ± 3.9	157 ± 2.7	0.769
Response	+32 ± 6.1	+43 ± 2.8	+43 ± 1.9	0.203
<i>Offspring</i>				
<i>n</i>	14	93	187	
$\dot{V}O_{2max}$, ml/min				
Baseline	2,667 ± 98	2,592 ± 38	2,678 ± 27	0.182
Response	+570 ± 56	+442 ± 22	+410 ± 15	0.017
\dot{W}_{max} , W				
Baseline	208 ± 9.9	196 ± 3.8	202 ± 2.7	0.339
Response	+68 ± 6.7	+55 ± 2.6	+54 ± 1.8	0.157

Values are means ± SE.

ever, exons 19–22 of the Na⁺-K⁺-ATPase α 2 gene encode hydrophobic, probably transmembrane, domains (35). In vitro studies have shown that artificially induced mutations in this region alter the pump current of the rat α 2 subunit (38) and decrease catalytic function and affinities of Na⁺ and K⁺ in the sheep α 1 subunit (2, 13, 37).

We observed an association between the α 2 exon 21–22 marker and Δ VO_{2max} in offspring but not in parents. The exact explanation for this difference is unclear, but it is possible that the skeletal muscle cation balance is a less important determinant of endurance capacity in older individuals. One could speculate that the energy production pathways in skeletal muscle, the availability of oxygen and substrates for energy production, or the ability of the cardiorespiratory system to adapt to increased levels of physical activity become more dominant performance-limiting factors with advancing age. Some studies have shown that the erythrocyte Na⁺-K⁺-ATPase activity decreases with age (14, 32) and that this decrease is associated with age-related reduction in resting metabolic rate (32). However, it is not known whether a similar phenomenon takes place in skeletal muscles or if it has any relevance to the generation difference observed in the present study.

In summary, these data from the HERITAGE Family Study suggest that DNA sequence variation at the Na⁺-K⁺-ATPase α 2 locus, or a locus in close proximity, is associated with the responsiveness of VO_{2max} and W_{max} to a 20-wk endurance training program in healthy, sedentary Caucasian subjects.

The HERITAGE Family Study is supported by the National Heart, Lung, and Blood Institute through Grants HL-45670 (to C. Bouchard), HL-47323 (to A. S. Leon), HL-47317 (to D. C. Rao), HL-47327 (to J. S. Skinner), and HL-47321 (to J. H. Wilmore). A. S. Leon is partially supported by the Henry L. Taylor endowed Professorship in Exercise Science and Health Enhancement. C. Bouchard is partially supported by the Donald B. Brown Research Chair on Obesity funded by the Medical Research Council of Canada and Hoffmann-La Roche Canada. T. Rankinen is a fellow from the Medical Research Council of Canada. Some of the results of this paper were obtained by using the program package SAGE, which is supported by US Public Health Service Resource Grant 1 P41 RR0365 from the National Center for Research Resources.

Address for reprint requests and other correspondence: C. Bouchard, Pennington Biomedical Research Center, 6400 Perkins Rd., Baton Rouge, LA 70808–4124 (E-mail: bouchac@pbrc.edu).

Received 13 July 1999; accepted in final form 27 August 1999.

REFERENCES

- Amos, C. I., R. C. Elston, A. F. Wilson, and J. E. Bailey-Wilson. A more powerful test of linkage for quantitative traits. *Genet. Epidemiol.* 6: 435–439, 1989.
- Arguello, J. M., and J. B. Lingrel. Substitutions of serine 775 in the alpha subunit of the Na,K-ATPase selectively disrupt K⁺ high affinity activation without affecting Na⁺ interaction. *J. Biol. Chem.* 270: 22764–22771, 1995.
- Bouchard, C., P. An, T. Rice, J. S. Skinner, J. H. Wilmore, J. Gagnon, L. Pérusse, A. S. Leon, and D. C. Rao. Familial aggregation of VO_{2max} response to exercise training: results from the HERITAGE Family Study. *J. Appl. Physiol.* 87: 1003–1008, 1999.
- Bouchard, C., E. W. Daw, T. Rice, L. Pérusse, J. Gagnon, M. A. Province, A. S. Leon, D. C. Rao, J. S. Skinner, and J. H. Wilmore. Familial resemblance for VO_{2max} in the sedentary state: the HERITAGE family study. *Med. Sci. Sports Exerc.* 30: 252–258, 1998.
- Bouchard, C., F. T. Dionne, J. A. Simoneau, and M. R. Boulay. Genetics of aerobic and anaerobic performances. *Exerc. Sport Sci. Rev.* 20: 27–58, 1992.
- Bouchard, C., A. S. Leon, D. C. Rao, J. S. Skinner, J. H. Wilmore, and J. Gagnon. The HERITAGE Family Study: aims, design, and measurement protocol. *Med. Sci. Sports Exerc.* 27: 721–729, 1995.
- Bouchard, C., R. Lesage, G. Lortie, J. A. Simoneau, P. Hamel, M. R. Boulay, L. Pérusse, and G. Theriault. Aerobic performance in brothers, dizygotic and monozygotic twins. *Med. Sci. Sports Exerc.* 18: 639–646, 1986.
- Bouchard, C., A. Tremblay, J. P. Després, G. Thériault, A. Nadeau, P. J. Lupien, S. Moorjani, D. Prud'homme, and G. Fournier. The response to exercise with constant energy intake in identical twins. *Obes. Res.* 2: 400–410, 1994.
- Clausen, T. Clinical and therapeutic significance of the Na⁺,K⁺ pump. *Clin. Sci. (Colch.)* 95: 1–17, 1998.
- Clausen, T., O. B. Nielsen, A. P. Harrison, J. A. Flatman, and K. Overgaard. The Na⁺,K⁺ pump and muscle excitability. *Acta Physiol. Scand.* 162: 183–190, 1998.
- Evertsen, F., J. I. Medbø, E. Jebens, and K. Nicolaysen. Hard training for 5 mo increases Na⁺-K⁺ pump concentration in skeletal muscle of cross-country skiers. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 272: R1417–R1424, 1997.
- Fagard, R., E. Bielen, and A. Amery. Heritability of aerobic power and anaerobic energy generation during exercise. *J. Appl. Physiol.* 70: 352–362, 1991.
- Feng, J., and J. B. Lingrel. Functional consequences of substitutions of the carboxyl residue glutamate 779 of the Na,K-ATPase. *Cell. Mol. Biol. Res.* 41: 29–37, 1995.
- Gambert, S. R., and E. H. Duthie. Effect of age on red cell membrane sodium-potassium dependent adenosine triphosphate (Na⁺,K⁺-ATPase) activity in healthy men. *J. Gerontol.* 38: 23–25, 1983.
- Green, H., A. Dahly, K. Shoemaker, C. Goreham, E. Bombardier, and M. Ball-Brunett. Serial effects of high-resistance and prolonged endurance training on Na⁺-K⁺ pump concentration and enzymatic activities in human vastus lateralis. *Acta Physiol. Scand.* 165: 177–184, 1999.
- Green, H. J., E. R. Chin, M. Ball-Brunett, and D. Ranney. Increases in human skeletal muscle Na⁺-K⁺-ATPase concentration with short-term training. *Am. J. Physiol. Cell Physiol.* 264: C1538–C1541, 1993.
- Hallen, J., L. Gullestad, and O. M. Sejersted. K⁺ shifts of skeletal muscle during stepwise bicycle exercise with and without beta-adrenoceptor blockade. *J. Physiol. (Lond.)* 477: 149–159, 1994.
- Harrison, A. P., O. B. Nielsen, and T. Clausen. Role of Na⁺-K⁺ pump and Na⁺ channel concentrations in the contractility of rat soleus muscle. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 272: R1402–R1408, 1997.
- Haseman, J. K., and R. C. Elston. The investigation of linkage between a quantitative trait and a marker locus. *Behav. Genet.* 2: 3–19, 1972.
- Klitgaard, H., and T. Clausen. Increased total concentration of Na-K pumps in vastus lateralis muscle of old trained human subjects. *J. Appl. Physiol.* 67: 2491–2494, 1989.
- Lesage, R., J. A. Simoneau, J. Jobin, J. Leblanc, and C. Bouchard. Familial resemblance in maximal heart rate, blood lactate and aerobic power. *Hum. Hered.* 35: 182–189, 1985.
- Madsen, K., J. Franch, and T. Clausen. Effects of intensified endurance training on the concentration of Na,K-ATPase and Ca-ATPase in human skeletal muscle. *Acta Physiol. Scand.* 150: 251–258, 1994.
- Maes, H. H., G. P. Beunen, R. F. Vlietinck, M. C. Neale, M. Thomis, B. Vanden Eynde, R. Lysens, J. Simons, C. Derom, and R. Derom. Inheritance of physical fitness in 10-yr-old twins and their parents. *Med. Sci. Sports Exerc.* 28: 1479–1491, 1996.
- McKenna, M. J., A. R. Harmer, S. F. Fraser, and J. L. Li. Effects of training on potassium, calcium and hydrogen ion regulation in skeletal muscle and blood during exercise. *Acta Physiol. Scand.* 156: 335–346, 1996.

25. **McKenna, M. J., T. A. Schmidt, M. Hargreaves, L. Cameron, S. L. Skinner, and K. Kjeldsen.** Sprint training increases human skeletal muscle Na⁺-K⁺-ATPase concentration and improves K⁺ regulation. *J. Appl. Physiol.* 75: 173–180, 1993.
26. **Nielsen, O. B., and T. Clausen.** The significance of active Na⁺, K⁺ transport in the maintenance of contractility in rat skeletal muscle. *Acta Physiol. Scand.* 157: 199–209, 1996.
27. **Nielsen, O. B., and T. Clausen.** Regulation of Na⁺-K⁺ pump activity in contracting rat muscle. *J. Physiol. (Lond.)* 503: 571–581, 1997.
28. **Nielsen, O. B., and A. P. Harrison.** The regulation of the Na⁺,K⁺ pump in contracting skeletal muscle. *Acta Physiol. Scand.* 162: 191–200, 1998.
29. **Nielsen, O. B., L. Hilsted, and T. Clausen.** Excitation-induced force recovery in potassium-inhibited rat soleus muscle. *J. Physiol. (Lond.)* 512: 819–829, 1998.
30. **Nielsen, O. B., and K. Overgaard.** Ion gradients and contractility in skeletal muscle: the role of active Na⁺, K⁺ transport. *Acta Physiol. Scand.* 156: 247–256, 1996.
31. **Overgaard, K., O. B. Nielsen, and T. Clausen.** Effects of reduced electrochemical Na⁺ gradient on contractility in skeletal muscle: role of the Na⁺-K⁺ pump. *Pflügers Arch.* 434: 457–465, 1997.
32. **Poehlman, E. T., M. J. Toth, and G. D. Webb.** Sodium-potassium pump activity contributes to the age-related decline in resting metabolic rate. *J. Clin. Endocrinol. Metab.* 76: 1054–1057, 1993.
33. **Prud'homme, D., C. Bouchard, C. Leblanc, F. Landry, and E. Fontaine.** Sensitivity of maximal aerobic power to training is genotype-dependent. *Med. Sci. Sports Exerc.* 16: 489–493, 1984.
34. **Rammelkamp Center for Education and Research.** 1997. SAGE (statistical analysis for genetic epidemiology). Release 3.0. Cleveland, OH: Dept. of Epidemiology and Biostatistics, Rammelkamp Center for Education and Research, Case Western Reserve University. Computer program package.
35. **Shull, M. M., D. G. Pugh, and J. B. Lingrel.** Characterization of the human Na,K-ATPase $\alpha 2$ gene and identification of intragenic restriction fragment length polymorphisms. *J. Biol. Chem.* 264: 17532–17543, 1989.
36. **Skinner, J. S., K. M. Wilmore, A. Jaskolska, A. Jaskolski, E. W. Daw, T. Rice, J. Gagnon, A. S. Leon, J. H. Wilmore, D. C. Rao, and C. Bouchard.** Reproducibility of maximal exercise test data in the HERITAGE Family Study. *Med. Sci. Sports Exerc.* In press.
37. **Van Huysse, J. W., T. A. Kuntzweiler, and J. B. Lingrel.** Critical effects on catalytic function produced by amino acid substitutions at Asp⁸⁰⁴ and Asp⁸⁰⁸ of the $\alpha 1$ isoform of Na,K-ATPase. *FEBS Lett.* 389: 179–185, 1996.
38. **Yamamoto, S., T. A. Kuntzweiler, E. T. Wallick, N. Spelrelakis, and A. Yatani.** Amino acid substitutions in the rat Na⁺,K⁺-ATPase alpha-2-subunit alter the cation regulation of pump current expressed in HeLa cells. *J. Physiol. (Lond.)* 495: 733–742, 1996.

