

Angiotensin-Converting Enzyme Genotype Predicts Cardiac and Autonomic Responses to Prolonged Exercise

Euan A. Ashley, MRCP, DPHIL,* Attila Kardos, MD, PhD, FESC,† Ewan S. Jack, MB, ChB, FRCA,‡ Walter Habenbacher, PhD,§ Mathew Wheeler, MD, PhD,|| Young M. Kim, BS,¶ Jeffrey Froning, MA,# Jonathan Myers, PhD,* Gregory Whyte, PhD, FACSM,** Victor Froelicher, MD,* Pamela Douglas, MD, FACC, FASE††

Stanford and Los Altos, California; Oxford and Manchester, United Kingdom; Glasgow, Scotland; Graz, Austria; Toronto, Canada; and Durham, North Carolina

OBJECTIVES	The purpose of this study was to investigate the phenomenon of left ventricular (LV) dysfunction after ultraendurance exercise.
BACKGROUND	Subclinical LV dysfunction in response to endurance exercise up to 24 h duration has been described, but its mechanism remains elusive.
METHODS	We tested 86 athletes before and after the Adrenalin Rush Adventure Race using echocardiography, impedance cardiography, and plasma immunoassay.
RESULTS	At baseline, athletes demonstrated physiology characteristic of extreme endurance training. After 90 to 120 h of almost-continuous exercise, LV systolic and diastolic function declined (fractional shortening before the race, $39.6 \pm 0.65\%$; after, $32.2 \pm 0.84\%$, $p < 0.001$; mitral inflow E-wave deceleration time before the race, 133 ± 5 ms; after, 160 ± 5 ms, $n = 48$, $p < 0.001$) without change in loading conditions as defined by LV end-diastolic dimension and total peripheral resistance estimated by thoracic impedance. There was a compensatory increase in heart rate (before, 55 ± 1.3 beats/min; after, 59 ± 1.5 beats/min, $p = 0.05$), which left cardiac output unchanged, as well as significant-but-subclinical increases in brain natriuretic peptide and troponin I. In addition, we found that athletes who were homozygous for the intron-16 insertion polymorphism of the angiotensin-converting enzyme (ACE) gene exhibited a significantly greater decrease in fractional shortening than athletes who were homozygous for the deletion allele. Heterozygotes showed an intermediate phenotype. In addition, the deletion group manifest an enhanced sympathovagal balance after the race, as evidenced by greater power in the low-frequency component of blood pressure variability.
CONCLUSIONS	The ACE genotype predicts the extent of reversible subclinical LV dysfunction after prolonged exercise and is associated with a differential postactivity augmentation of sympathetic nervous system function that may explain it. (J Am Coll Cardiol 2006;48:523–31) © 2006 by the American College of Cardiology Foundation

Throughout history, humans have pushed themselves to their physical limits. Although skeletal muscle fatigue is well recognized and characterized, cardiac fatigue is relatively new and less well described (1,2). Although Saltin and Stenberg (3) made reference to exercise induced cardiac dysfunction, Douglas et al. (2,4,5) characterized the phenomenon through a series of studies on athletes participating in the Hawaii Ironman. This multidiscipline triathlon comprises a 2.4-mile swim, a 112-mile bike race and a 26.2-mile run, representing 8 to 17 h of continuous exercise. After prolonged exercise, athletes were found to exhibit systolic and diastolic right and left ventricular (LV) dysfunction

with only minimal change in loading conditions (2,4–6). Although the balance of evidence was clearly in favor of this cardiac fatigue, not all authors found similar declines. In particular, studies examining shorter exercise periods showed little evidence of such dysfunction (7–9), suggesting a threshold duration of exercise. This hypothesis was supported by the Whyte et al. (10) report which noted changes in fractional shortening that were significant after full but not half Ironman-distance events. Several authors have independently described cardiac “drift,” whereby heart rate (HR) “drifts” upward as prolonged exercise continues. Although a series of experiments have suggested that this phenomenon may be explained by thermoregulatory processes (11–16), it remains possible that the increase in HR is a compensatory response to LV dysfunction. However, the only study to address this found a relationship only with diastolic function (11).

The insertion/deletion polymorphism of intron-16 of the angiotensin-converting enzyme (ACE) gene was originally found to be associated with increased risk of myocardial infarction (17,18). Although this association was not confirmed in two subsequent studies (19,20). Montgomery

From the *Division of Cardiology and ||Division of Medicine, Stanford University, Stanford, California; †Department of Cardiovascular Medicine, University of Oxford, Oxford, United Kingdom; ‡Department of Anaesthetics, University of Glasgow, Glasgow, Scotland; §CNSystems, Graz, Austria; ¶Department of Cardiovascular Medicine, University of Toronto, Toronto, Canada; #Sunnyside Biomedical, Los Altos, California; **Director of Science and Research, English Institute of Sport, Manchester, United Kingdom; and ††Ursula Geller Professor of Research in Cardiovascular Diseases and Cardiology Division, Duke University Medical Center, Durham, North Carolina.

Manuscript received October 8, 2005; revised manuscript received February 1, 2006, accepted February 21, 2006.

Abbreviations and Acronyms

ACE	=	angiotensin-converting enzyme
BRS	=	baroreceptor reflex sensitivity
BNP	=	brain natriuretic peptide
BP	=	blood pressure
HR	=	heart rate
LV	=	left ventricular

et al. (21) were the first to suggest over-representation of the insertion allele in elite mountaineers and, subsequently, other groups of aerobic endurance athletes, such as rowers and distance runners. Although some inconsistent findings emerged in respect of this “fitness” gene, the elegant follow-up work of Montgomery et al. (21) confirmed an important role for this genomic regulator of ACE activity in fitness and training (22). Given the central role of the renin-angiotensin system in LV remodeling, heart failure, and bodily fluid balance, we hypothesized a differential effect of ACE genotype on exercise induced LV dysfunction.

METHODS

Ethics. The study was approved by the University of Oxford Institutional Ethics committee. Each patient gave informed written consent. The study was performed according to the principles of the Declaration of Helsinki.

Adrenalin Rush Adventure Race. Adrenalin Rush (now the British Adventure Racing Championship) is a multidiscipline adventure race conducted annually over a distance of approximately 300 miles in the Scottish highlands or Irish dales. Teams of 4 containing at least 1 member of the opposite gender race together over the course: trekking, mountain biking, kayaking, ascending and descending fixed ropes, and swimming. The goal of the competition is to be the first team across the finish line. The event is designed to push human endurance to the limits. In many cases, athletes compete for days without rest or sleep.

Baseline measurements. Height and weight were measured in each competitor before and after the race. Skin caliper assessment of body fat was conducted by one operator according to standard techniques (23). Eighty-six athletes were consented and had blood drawn. Fifty-four athletes underwent echocardiography before the race, and 48 underwent echocardiography after the race. Of these, 27 also underwent measurements of thoracic impedance, HR variability, and blood pressure (BP) variability before and after the race. Fifty-five athletes had repeat blood draws.

Echocardiography. Echocardiography was performed by a single operator using the Acuson Cypress machine (0.5 to 3.6 MHz phased-array adult cardiac probe). Five beat loops derived from standard parasternal and apical views were stored for later offline analysis. Fractional shortening, ejection fraction, LV mass, and LV mass index were derived according the standard methods recommended by the American Society of Echocardiography (24) with leading

edge to leading edge quantification of the left ventricular cavity, posterior wall, and interventricular septum at a long-axis position just apical to the mitral valve leaflets in the parasternal view. Pulsed-wave Doppler assessment of the transmitral valve blood flow was used to provide a measurement of LV relaxation. Peak early (E) and atrial (A) filling velocities were recorded, as well as the deceleration time of the early (E) wave. Left atrial size also was measured in the parasternal long-axis view. These measurements were conducted before and within 6 h of the end of the race.

Impedance cardiography. We assessed cardiothoracic impedance and autonomic function using the Task Force Monitor (CNSystems, Graz, Austria). This integrated system includes electrocardiogram, impedance cardiography, beat-to-beat BP, and oscillometric BP recording. Stroke volume and cardiac output are estimated using impedance cardiography (25–27). In brief, a constant sinusoidal alternating current I_0 of 400 μ A and 40 kHz is passed through the thorax between short-band electrodes placed on the neck and on the lower thorax aperture. The baseline impedance (Z_0) and the maximum rate of change in impedance (dZ/dt) are used for the estimation of stroke volume by a modification of the method of Kubicek et al. (28).

BP and HR variability. Autonomic parameters were obtained by analysis of HR and BP variability derived from detected R-R intervals in the ECG and continuous BP monitoring. An adaptive autoregressive model based on a recursive least-squares algorithm is used to estimate power spectral density. Time-variant autoregressive coefficients are determined by adaptive parametric identification, which obtains weighted values of a sliding exponential window with a history of \sim 60 beats. Absolute power in the very low-frequency (0.003 to 0.04 Hz), low-frequency (0.04 to 0.15 Hz), and high-frequency (0.15 to 0.4 Hz) bands are calculated according to the European Society of Cardiology Task Force recommendations (26).

Spontaneous baroreceptor activity is determined using the sequence method, which detects increasing sequences (increasing systolic BP, longer RR interval) and decreasing sequences (decreasing systolic BP, shorter RR interval) from the continuous beat-to-beat measurement of RR interval and systolic BP. If 3 or more consecutive beats show an increase (or decrease) of systolic BP (≥ 1 mm Hg per beat), a BP “ramp” is detected. If this ramp is matched by an increase (or decrease) of ≥ 4 ms in the RR interval, a baroreceptor sequence “event” is detected. Sequences with an increase of BP and RR interval are called baroreceptor “up” sequences, whereas sequences with a decrease of BP and RR interval are called baroreceptor “down” sequences. Baroreceptor reflex sensitivity (BRS) is then computed as follows:

$$BRS = \frac{\Delta RRI}{\Delta Sys} \left[\frac{ms}{mm\ Hg} \right]$$

The baroreceptor effectiveness index (BEI) is the ratio of baroreceptor sequences to the number of BP ramps:

$$\text{BEI} = \frac{\text{No. of sequences}}{\text{No. of ramps}} [\%]$$

Plasma markers. Blood was drawn using a standard vacutainer system. One sample was immediately frozen as whole blood, and a second was centrifuged after clotting. Serum supernatant was pipetted into a plain Eppendorf for later assessment of electrolytes and cardiac troponin I. A third blood sample was collected in an ethylene diamine tetraacetic acid tube, spun down, and the plasma supernatant pipetted into a second Eppendorf tube containing a protease inhibitor. This second sample was used for measurement of brain natriuretic peptide (BNP). The assessment of blood electrolytes was conducted using a standardized clinical system. Cardiac troponin I and BNP were quantified using standard enzyme immunoassay kits (Troponin I: AccuTnI assay, Beckman Coulter, Fullerton, California, lower limit of detection of troponin was 0.01 $\mu\text{g/l}$; BNP: Bayer, Newbury, United Kingdom, lower limit of detection 0.6 pmol/l).

Genotyping of the ACE locus. One blood sample was stored and deoxyribonucleic acid extracted from 200 μl of whole blood using the Qiagen DNA mini kit (Qiagen, Crawley, United Kingdom). Genotyping for the insertion/deletion polymorphism (intron 16) of the ACE gene was conducted using the 3 primer method of Humphries (ACE1: CAT CCT TTC TCC CAT TTC TC; ACE2: TGGGATTACAGGCGTGATAC; ACE3: ATTTCA-GAGCTGGAATAAAA) (29). Primer ratios correspond to the 50-pmol ACE1 and 3- and 15-pmol ACE2 used in a 50- μl reaction, giving amplification products of 84 bp for allele ACE D and 65 bp for allele ACE I. We used touchdown cycling for amplification: 1 cycle at 95°C for 2 min; 10 cycles of 95°C for 30 s, 62°C for 30 s, 72°C for 2 min followed by 20 cycles of 95°C for 30 s, 57°C for 30 s, and 72°C for 2 min, followed by a final 10-min hold at 72°C. This method yields amplification products of 65 bp (I allele) and 84 bp (D allele). Products were separated by electrophoresis on a 3% agarose ethidium bromide gel.

Data analysis. Data were analyzed using NCSS (NCSS, Kaysville, Utah). A three-by-two (genotype \times pre/post) repeated-measures analysis of variance was used. Exact p values for pre-post main effects are reported except where a genotype main effect or an interaction is specifically noted. Because there were a large number of samples with below detection levels of troponin and BNP, these data were coded as categorical (0 = undetectable, 1 = detectable) and included in the general linear model.

RESULTS

Participant and race demographics. Participants in the race were in teams of 4, with each team including at least one member of the opposite gender. The group was highly diverse and ranged from national class elite athletes with body fat percentages as low as 6% and resting HRs as low as

Table 1. Demographic Characteristics of the Athletes (Men, n = 62; Women, n = 23)

	Mean	SEM
Age (yrs)	34	5
Weight (kg)	74.6	1.2
Height (cm)	177.7	0.8
BSA (m^2)	1.91	0.025
Fat % by skin caliper	19.9	0.56
LVPW (diastole, cm)	1.2	0.03
IVS (diastole, cm)	1.37	0.04
LV mass (g)	322.53	9.43
LV mass index (g/m^2)	168.51	5.59

BSA = body surface area; IVS = inter ventricular septum; LV = left ventricular; LVPW = left ventricular posterior wall.

28 beats/min to recreational athletes (Table 1). The race was conducted over approximately 300 miles of Scottish countryside and involved the following disciplines: trekking, running, cycling, kayaking, swimming, rope maneuvers such as abseiling, and horse riding. The leaders completed the race in 84 h, 7 min, and the last team tested crossed the line more than 24 h later at 109 h, 58 min. Most teams slept approximately 2 h per night, with a small number of competitors sleeping up to 4 h per night.

Hemodynamics and LV function. Baseline echocardiography demonstrated characteristics typical for endurance athletes (Table 2, Fig. 1) and similar to those found in previous studies (30,31). Continuous HR monitoring was conducted in a small number of competitors during the race (n = 8, Fig. 1E) and demonstrated consistent and sustained tachycardia, in some cases with mean HRs >100 beats/min for 100 h. After the race, significant decrements were noted in systolic and diastolic function in the absence of changes in loading conditions (Fig. 1, LV end diastolic diameter, p = 0.19; mean arterial pressure p > 0.5). Total peripheral resistance showed a downward trend (p = 0.09) whereas HR was slightly higher after the race (p = 0.05). Despite these changes, which would tend to augment ventricular function, systolic function measured by fractional shortening declined by 7.43% (p < 0.0001). In addition, the deceleration time of the early (E) wave increased significantly (+ 27 ms; p < 0.001). The left atrial diameter was also mildly but significantly greater after the race (+ 0.17 cm; p = 0.02).

In the subset of competitors who underwent impedance cardiography, stroke volume did not change (Table 3). However, base impedance was significantly decreased after the race (−2.6 ohms, p = 0.001), and the rapid ejection period (time from opening of aortic valve to maximum rate of change of impedance) was increased (+0.005 ms; p < 0.001), which is consistent with a more sluggish LV ejection.

Resources did not allow us to scan a large number of competitors at time points distant from the end of the race. However, we did have the opportunity to rescan one of the top teams at both 24 and 48 h (n = 4) (Fig. 2A). In these competitors, partial recovery of the systolic function was demonstrated at 48 h. The decrements in systolic function

Table 2. Echocardiography (n = 54) and Blood Chemistry (n = 55) Analysis Before and After the Race

	Before	SEM	After	SEM	p Value
LVEDD, (cm)	5.12	0.07	5.05	0.06	0.19
LVESD, (cm)	3.09	0.07	3.43	0.06	<0.0001*
Fractional shortening, (%)	39.63	0.65	32.20	0.84	<0.0001*
Ejection fraction, (%)	77.52	0.71	67.92	1.18	<0.0001*
HR, (beats/min)	55	1.3	59	1.5	0.05
SBP (mm Hg)	124	1.5	127	2.0	0.09
DBP (mm Hg)	73	1.3	73	1.3	0.8
MAP (mm Hg)	91	1.3	92	1.3	0.52
Mitral E peak (m/s)	0.86	0.03	0.90	0.02	0.2
Mitral A peak (m/s)	0.42	0.02	0.45	0.02	0.6
Mitral E _{decel} (ms)	133	5	160	5	<0.001*
Left atrium (cm)	3.93	0.07	4.10	0.05	0.02
Sodium (mmol)	138	0.6	138	0.3	0.8
Potassium (mmol)	4.2	0.04	3.7	0.04	<0.0001*
Urea (mmol)	5.5	0.16	5.5	0.24	0.8
Creatinine (mmol)	83.6	1.9	81.2	1.4	0.06
Calcium (mmol)	2.3	0.02	2.1	0.01	<0.001*
Magnesium (mmol)	0.87	0.001	0.87	0.001	0.6
Albumin (mmol)	44.4	0.73	41.7	0.36	<0.001
ALT (mmol)	9.80	0.48	24.0	2.40	<0.0001
AST (mmol)	14.0	3.6	70.0	7.5	<0.0001
LDH (mmol)	132.6	4.9	320	15.7	<0.0001
CK (mmol)	108	7.4	1358	181.2	<0.0001

*Indicates a p value <0.05.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK = creatine kinase; DBP = diastolic blood pressure; HR = heart rate; LDH = lactate dehydrogenase; LVEDD = left ventricular end diastolic diameter; LVESD = left ventricular end systolic diameter; MAP = mean arterial pressure; SBP = systolic blood pressure.

observed were greater than those previously reported (Fig. 2B).

Autonomic function. Markers of autonomic function were derived from beat-to-beat measurement of RR interval and BP. Resolving small changes in the frequency domain by Fourier analysis leads to derivation of components traditionally recognized to represent sympathovagal activation. We saw changes in several components of these frequency domains as well as in total power (Table 4). The more consistent results were found in BP variation when normalized to total power. Specifically, the very low frequency component was diminished ($p = 0.001$) whereas the low- and high-frequency components were increased ($p = 0.07$ and $p = 0.03$, respectively). Very low frequency components, which are thought to relate to humoral or thermoregulatory influences, were changed in both HR variability (increased, along with the total power, $p = 0.02$ and $p = 0.04$, respectively) and BP variability (decreased, $p = 0.001$).

The spontaneous activity of the baroreceptor reflex is estimated by increasing or decreasing sequences if sustained over three beats or more. The quantity of sequences sustained over three beats or more ("ramps") was little changed after the event, but the when the number of sequences were expressed as a function of the number of ramps (the baroreceptor effectiveness index) a significant increase was found ($p = 0.02$).

Plasma markers. Plasma levels of electrolytes were normal in athletes before the race. After the event, plasma potassium was reduced (Table 2, $p < 0.0001$). Both calcium and

albumin were significantly lower ($p < 0.001$, the latter could cause the former). Other markers of liver function, as well as creatine kinase (+1,250 mmol) were highly significantly increased after the race. Although most athletes had undetectable levels of troponin I both before and after the race, a significantly greater proportion had detectable levels afterwards (detectable range before the race, 7 of 54 [13%]; after the race, 22 of 54 [41%] $p < 0.0001$). One athlete had a troponin level in the clinical range (0.36), which was associated with a 24% decrease in fractional shortening, a 94-ms increase in time E_{decal}, a 528-mmol increase in creatine kinase, and the appearance of incomplete right bundle branch block. This athlete was the only one to have an after-race ejection fraction in the pathological range (45%).

Most athletes had undetectable levels of BNP before the race (13 of 54 detectable, 24%), but a much greater proportion were in the detectable range after the race (48 of 54, 89%; mean level 6.5 ± 0.66 with a peak of 20.4 pmol/l; $p < 0.0001$). No competitor had a BNP level in the pathological range.

Effect of ACE genotype. The ACE genotype was distributed in a fashion consistent with the Hardy-Weinberg equilibrium (DD genotype 31%, II genotype 23%, ID heterozygotes 46%). There was no excess of the I allele in this population, as has been previously reported in mountaineers (21). In addition, we found no effect of ACE genotype on LV mass or LV mass corrected for body surface area, as has been described in clinical populations (Fig. 3B). However, the ACE genotype did predict a differential decline

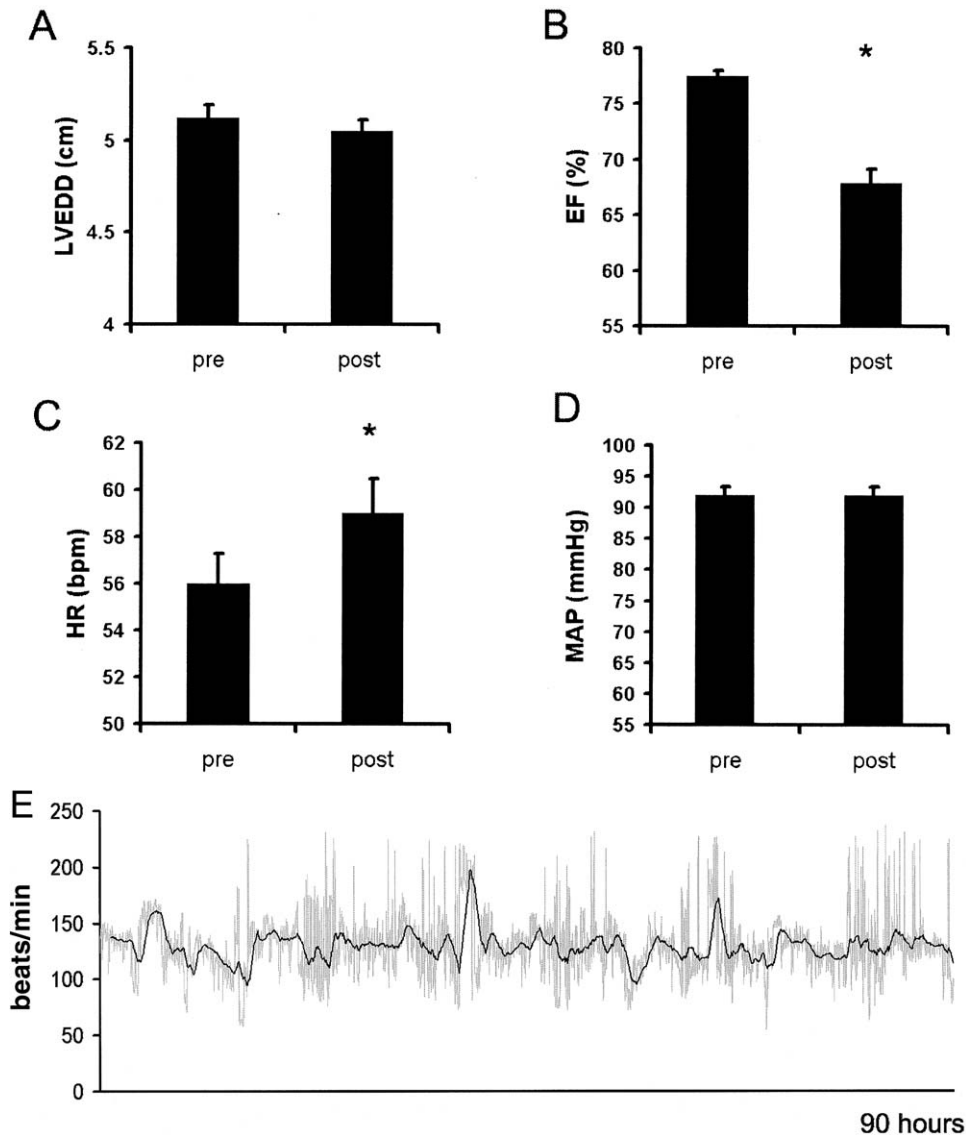


Figure 1. Echocardiographic and hemodynamic variables. Echocardiographic and hemodynamic variables (n = 48) before and after the race (mean \pm SE) (A) Preload represented by left ventricular end diastolic diameter was unchanged (p = 0.19). (B) Ejection fraction decreased significantly after the race (p < 0.001). (C) Heart rate increased significantly after the race (p = 0.05). (D) Afterload represented by mean arterial pressure did not change (p = 0.52). (E) Continuous heart rate tracing from lead competitor. bpm = beats/minute; EF = ejection fraction; HR = heart rate; LVED = left ventricular end-diastolic diameter; MAP = mean arterial pressure.

in systolic function as measured by fractional shortening and ejection fraction. Specifically, competitors homozygous for the insertion allele had a significantly greater decline than those homozygous for the deletion allele (Fig. 3A, p = 0.017). Heterozygotes fell in an intermediate range. No such differential effect was found for diastolic function. In addition, there was no association of genotype with possible confounders such as exercise time. We found that ACE genotype also predicted differential effects in autonomic function (Fig. 4). Specifically, the low-frequency domain of BPV increased dramatically in DD genotype individuals after the race whereas those with II or ID genotypes showed little change (p = 0.06). In a similar fashion, a significant decline the high-frequency domain was observed only in DD-genotype individuals (p < 0.01). These individual

results explain a significant increase in sympathovagal balance (low-frequency:high-frequency ratio) concentrated in competitors with DD genotype (p = 0.02). The BEI also increased more in this group (p = 0.06).

DISCUSSION

A decline in cardiac function after prolonged exercise has been recognized for some time. Its nature, however, has remained elusive. In the most extensive examination of this phenomenon to date, we show that a competitor's genotype at the ACE locus can predict the extent of their exercise induced decline in systolic but not diastolic function and that this may be explained through enhanced sympathovagal balance in DD-genotype individuals.

Table 3. Impedance Cardiography (n = 27)

Variable	Before	SEM	After	SEM	p Value
RR interval (ms)	1.08	0.03	1.06	0.04	0.60
SV (ml)	101.5	3.6	104.9	4.3	0.42
SI (ml/m ²)	52.4	1.9	54.5	2.1	0.32
CO (l/min)	5.7	0.21	6.1	0.31	0.21
CI, l/(min·m ²)	3.0	0.10	3.2	0.16	0.16
TPR (dyne·s/cm ⁵)	1536	109	1328	66	0.09
LVET (ms)	0.32	0.004	0.32	0.004	0.9
PEP (ms)	0.11	0.002	0.10	0.004	0.08
RZ (ms)	0.18	0.003	0.18	0.003	0.70
REP (ms)	0.071	0.0009	0.076	0.002	<0.001*
ZO (ohm)	31.9	0.60	29.3	0.54	0.001*
dZ-dt _{max} (ohm·ms ⁻¹)	1.70	0.13	1.84	0.09	0.24

*Indicates a p value <0.05.

CI = cardiac index; CO = cardiac output; dZ-dt_{max} = maximum rate of change of thoracic impedance; LVET = left ventricular ejection time; PEP = pre-ejection period; REP = rapid ejection period (time interval from the opening of the aortic valve to dz/dt_{max}); RZ = time interval from ECG R-wave peak to maximum rate of change of thoracic impedance; SI = stroke index; SV = stroke volume; TPR = total peripheral resistance; ZO = mean base impedance.

The most extensive period of exercise in which cardiac fatigue has been previously studied was 24 h. Niemela et al. (32) studied 12 marathon runners who completed a 146- to 227-km race and found reversible changes in fractional shortening and prolongation of early diastolic filling, the latter of which correlated with the total distance covered. However, most studies have focused on shorter race duration. In the present study, we found a greater decline in systolic function that previously reported. In the Douglas et al. (2) study, fractional shortening decreased in 21 athletes, from 39 ± 5% to 35 ± 5% (±SD) whereas in the Whyte et al. (10) study, fractional shortening decreased from 40 ± 3% to 37 ± 2%. Over the course of 24 h (32), the decline was 38 ± 5% to 32 ± 5%. Our reduction from 40 ± 5% to 32 ± 6% presents the intriguing question as to whether even more prolonged exertion could result in further decreases in fractional shortening into the clinical range. Although the shape of the curve in Fig. 2B suggests

a leveling of effect, in the current study, one participant's fractional shortening decreased dramatically, which was associated with an increase in troponin in the clinical range (0.35). Investigators have previously reported isolated examples of pulmonary edema in athletes (33) that are consistent with deficient forward flow from LV dysfunction (overhydration notwithstanding) (34).

Cardiac drift describes the tendency of HR to drift upwards during prolonged exercise (12,14,16). Debate has been ongoing regarding the cause of this phenomenon, which is clearly linked to a reduction in stroke volume, with most suggesting it relates to changes in thermoregulation and specifically hyperthermia (13,15). One recent study demonstrated a link with diastolic parameters (reduction in E:A ratio) in 16 athletes (11). However, in that study, the exercise challenge was only 2 h and, accordingly, no systolic dysfunction was demonstrated. Further, the presence of a drift of 9 beats/min at constant work rate indicates that

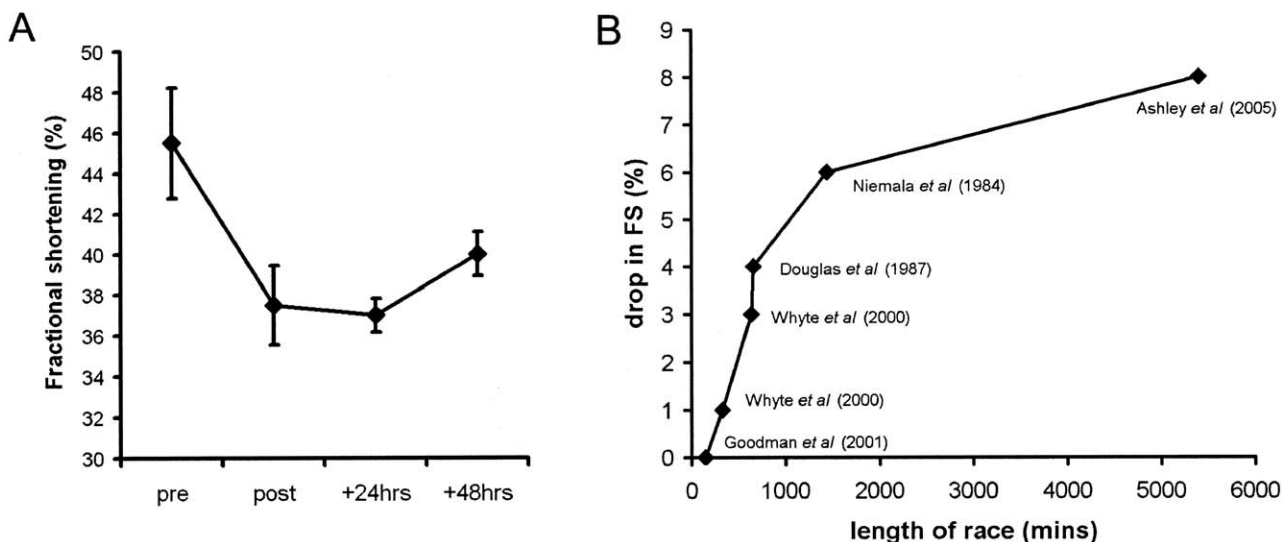


Figure 2. Changes in fractional shortening. (A) Four competitors were rescanned at 24 and 48 h after the race. Partial recovery of systolic function was demonstrated. (B) Decline in fractional shortening plotted against length of race as reported for key studies. In the current study, the exercise challenge and drop in fractional shortening were greater than previously reported. FS = fractional shortening.

Table 4. Changes in Frequency Components of HR and BP Variability

Variable	Before	SEM	After	SEM	p Value
BP VLFnu (%)	50.9	3.1	37.6	2.3	0.001*
BP LFNu (%)	30.4	3.1	39.2	3.3	0.07
BP HFnu (%)	18.7	2.6	25.9	3.2	0.03*
BP VLF (mm Hg ²)	484	46	454	50	0.66
BP LF (mm Hg ²)	380	60	694	111	0.03*
BP HF (mm Hg ²)	246	53	291	39	0.43
BP power (mm Hg ²)	1,201	150	1,952	360	0.07
BP LF/HF (ratiometric)	2.28	0.34	2.39	0.41	0.84
HR VLFnu (%)	25.9	2.4	27.3	2.54	0.68
HR LFNu (%)	40.5	3.2	38.0	2.4	0.50
HR HFnu (%)	33.4	2.6	33.6	2.6	0.96
HR VLF (ms ²)	0.11	0.02	0.16	0.02	0.02*
HR LF (ms ²)	0.18	0.03	0.24	0.03	0.16
HR HF (ms ²)	0.16	0.03	0.25	0.04	0.07
HR Power (ms ²)	0.45	0.06	0.68	0.1	0.04*
HR LF/HF (ratiometric)	1.92	0.37	1.56	0.21	0.42
BRS slope total (ms/mm Hg)	19.0	2.36	21.9	2.79	0.33
BEI total (%)	13.3	1.73	19.7	1.91	0.02*

*Indicates a p value <0.05.

BEI = baroreceptor effectiveness index; BP = blood pressure; BRS = baroreceptor sensitivity; HF = high frequency; HR = heart rate; LF = low frequency; nu = units normalized to overall power; VLF = very low frequency. (n = 27).

systolic dysfunction is not necessary to explain cardiac drift, but it may yet be sufficient in more prolonged exercise and is likely contributory. In our study, we found no change in preload, a trend toward a decrease in afterload (both of which alone would augment function), and a decline in fractional shortening, all of which suggests that the increase in HR is a compensatory measure to maintain cardiac output, something we observed in our study participants.

A unique aspect of our study was the investigation of modulatory effects of the insertion deletion polymorphism of the ACE gene on long-term cardiovascular performance. The ability of ACE genotype to predict the extent of exercise-induced decline in systolic LV function is a novel finding. Despite its intronic location, the insertion allele has been shown to be associated with a lower serum ACE

activity, which is believed to correlate with more “efficient” muscle activity (35). As such, our findings could relate to changes in enzyme function (although we might expect less “efficient”—albeit skeletal—muscle to fatigue more easily). A linked possibility is that although no overrepresentation of the I allele was found in the overall group of athletes (our population was more diverse than that of Montgomery et al. [21], with some elite and some recreational athletes), the I allele was overrepresented in the elite athletes or those nearer the front of the race. In fact, participants with the I allele were spread throughout the rankings (data not shown). An alternative mechanism is suggested by our investigation of HR and BP variability in this population. Sympathovagal balance, although not changed after the race in the group overall, was differentially modulated according

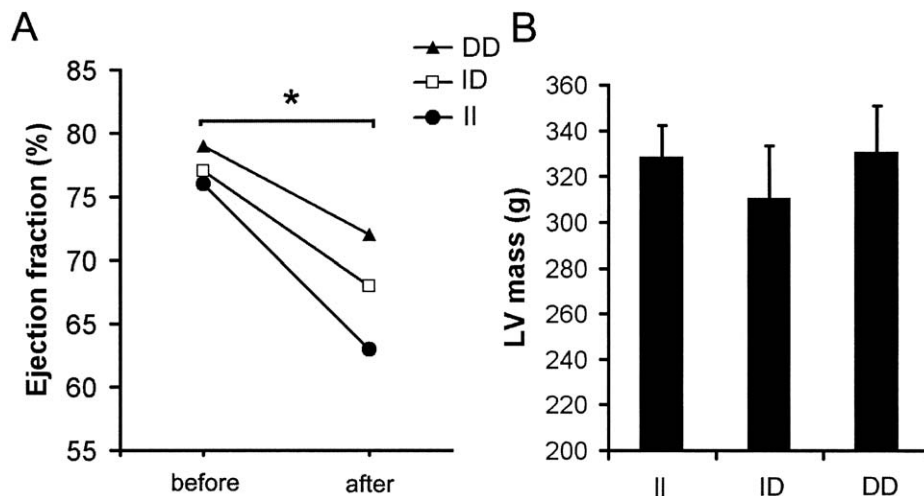


Figure 3. Effect of angiotensin-converting enzyme (ACE) genotype. There was a differential decline in systolic function according to ACE genotype (A, p = 0.017, n = 22 [ID], 11 [II], 15 [DD]). Individuals homozygous for the insertion allele exhibited greater declines in systolic function than those homozygous for the deletion allele. Heterozygous individuals exhibited an intermediate phenotype. In contrast, ACE genotype did not predict athletic hypertrophy (B, p = 0.8). LV = left ventricular.

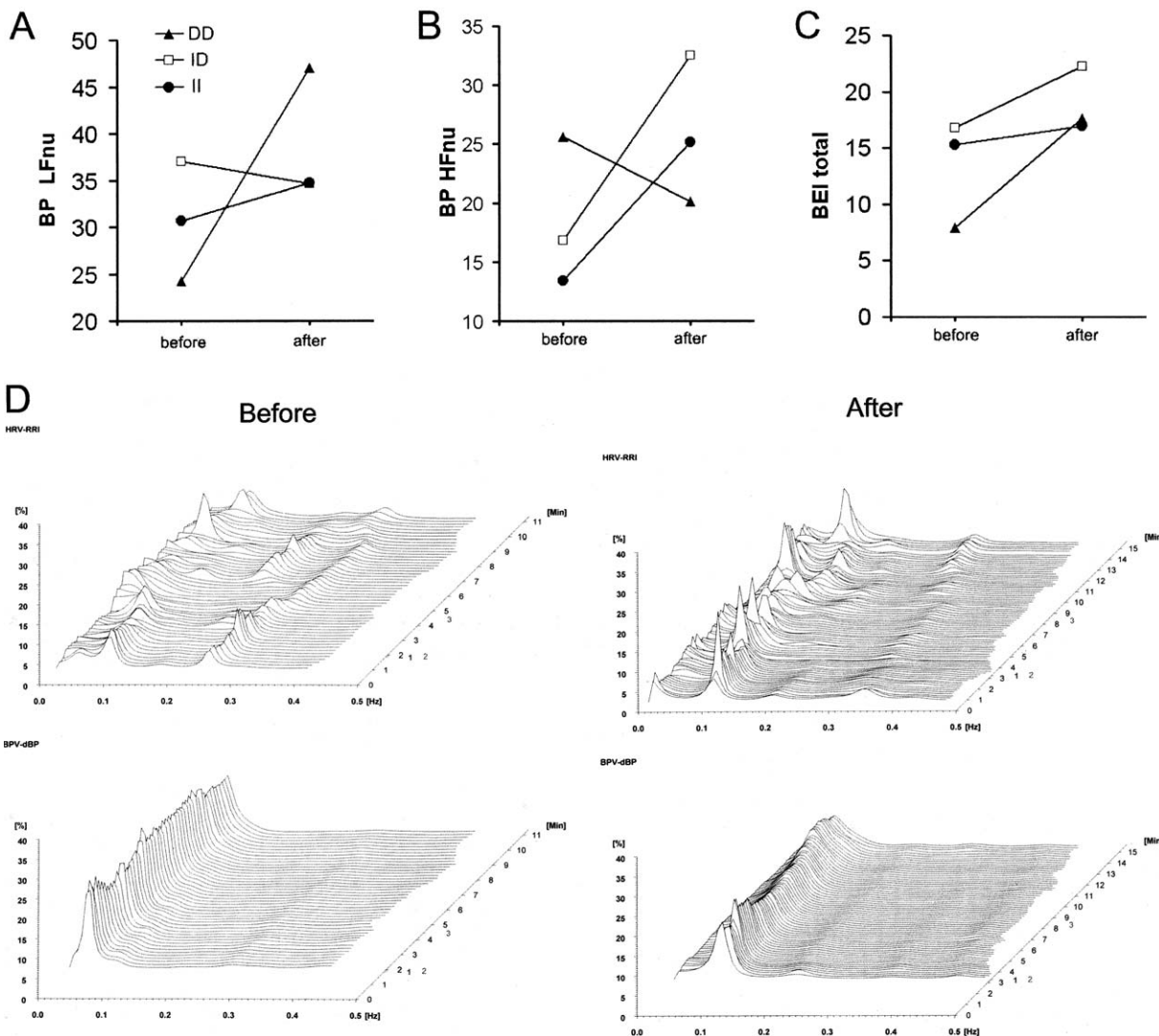


Figure 4. Heart rate and blood pressure (BP) variability. There was a differential effect of angiotensin-converting enzyme genotype on both the low- and high-frequency (units normalized to overall power; LFnu, HFnu) components of BP variability (**A and B**) and an overall significant enhancement of the sympathovagal balance in participants homozygous for the deletion allele. (**C**) In addition, there was a more dramatic increase (from a lower initial value) in these individuals in the baroreceptor effectiveness index (BEI). (**D**) Shown are raw tracings for one competitor (heart rate variability [HRV] is signified by upper tracings, blood pressure variability [BPV] by lower tracings) illustrating the strongest overall signals (decrease in very low frequency component of BPV, increase in low- and high-frequency; increase in very low frequency component of HRV). dBP = diastolic blood pressure; RRI = R-R interval.

to genotype with participants homozygous for the deletion allele exhibiting an increased sympathovagal balance not found in the other groups. This surprising finding suggests a greater sympathetic activation in the DD-allele participants after the race that may have served to limit the extent of measured decline in LV systolic function. Of importance, this observation does not speak to the level of activation during the race. Greater within-race activation might portend greater rather than lesser cardiac fatigue. In fact, changes in beta-adrenergic responsiveness have been suggested previously in the etiology of exercise-induced LV dysfunction. Eysmann et al. (36) demonstrated a decline in chronotropic responsiveness in sedentary individuals and in athletes after the Hawaii Ironman (6) (in the former study, the change correlated with functional declines). These findings, in combination with our report, clearly implicate

differential modulation of autonomic nervous system function in the phenomenon of cardiac fatigue but do not preclude alternative mechanisms, such as prolonged increased HRs, transient ischemia (37), or a decline in local or change in circulating substrate (38). The relative contribution of each of these remains to be illuminated by future study.

Conclusions. We present a comprehensive evaluation of cardiovascular physiology in a group of athletes participating in exercise at the limit of aerobic endurance. We confirm the phenomenon of cardiac fatigue in response to prolonged exercise and report more extensive declines in LV function than previously observed. In addition, we show a differential decline according to ACE genotype and, by demonstrating corresponding changes in autonomic function, suggest one possible mechanism. Contractile failure in the context of substrate depletion is an important phenomenon which may

advance our understanding of cardiac physiology, cardiomyopathy and athletic performance.

Acknowledgments

The authors would like to thank the athletes, Brian Elliott and Gary Tompsett, Angus Ashley, Fiona Ashley, Jonathan Kay, and Brian Shine. These studies were featured in the BBC Science documentary "Tomorrow's World."

Reprint requests and correspondence: Dr. Euan A. Ashley, Division of Cardiovascular Medicine, Falk CVRC, Stanford University, 300 Pasteur Drive, Stanford, California 94305. E-mail: euan@stanford.edu.

REFERENCES

1. Dawson E, George K, Shave R, Whyte G, Ball D. Does the human heart fatigue subsequent to prolonged exercise? *Sports Med* 2003;33:365-80.
2. Douglas PS, O'Toole ML, Hiller WD, Hackney K, Reichek N. Cardiac fatigue after prolonged exercise. *Circulation* 1987;76:1206-13.
3. Saltin B, Stenberg J. Circulatory response to prolonged severe exercise. *J Appl Physiol* 1964;19:833-8.
4. Douglas PS, O'Toole ML, Woolard J. Regional wall motion abnormalities after prolonged exercise in the normal left ventricle. *Circulation* 1990;82:2108-14.
5. Douglas PS, O'Toole ML, Hiller WD, Reichek N. Different effects of prolonged exercise on the right and left ventricles. *J Am Coll Cardiol* 1990;15:64-9.
6. Douglas PS, O'Toole ML, Katz SE. Prolonged exercise alters cardiac chronotropic responsiveness in endurance athletes. *J Sports Med Phys Fitness* 1998;38:158-63.
7. Palatini P, Bongiovi S, Macor F, et al. Left ventricular performance during prolonged exercise and early recovery in healthy subjects. *Eur J Appl Physiol Occup Physiol* 1994;69:396-401.
8. Goodman JM, McLaughlin PR, Liu PP. Left ventricular performance during prolonged exercise: absence of systolic dysfunction. *Clin Sci (Lond)* 2001;100:529-37.
9. Upton MT, Rerych SK, Roebuck JR Jr, et al. Effect of brief and prolonged exercise on left ventricular function. *Am J Cardiol* 1980;45:1154-60.
10. Whyte GP, George K, Sharma S, et al. Cardiac fatigue following prolonged endurance exercise of differing distances. *Med Sci Sports Exerc* 2000;32:1067-72.
11. Dawson EA, Shave R, George K, et al. Cardiac drift during prolonged exercise with echocardiographic evidence of reduced diastolic function of the heart. *Eur J Appl Physiol* 2005;94:305-9.
12. Coyle EF. Cardiovascular drift during prolonged exercise and the effects of dehydration. *Int J Sports Med* 1998;19 Suppl 2:S121-4.
13. Coyle EF, Gonzalez-Alonso J. Cardiovascular drift during prolonged exercise: new perspectives. *Exerc Sport Sci Rev* 2001;29:88-92.
14. Heaps CL, Gonzalez-Alonso J, Coyle EF. Hypohydration causes cardiovascular drift without reducing blood volume. *Int J Sports Med* 1994;15:74-9.
15. Montain SJ, Coyle EF. Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. *J Appl Physiol* 1992;73:1340-50.
16. Hamilton MT, Gonzalez-Alonso J, Montain SJ, Coyle EF. Fluid replacement and glucose infusion during exercise prevent cardiovascular drift. *J Appl Physiol* 1991;71:871-7.
17. Cambien F, Poirier O, Lecerc L, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992;359:641-4.
18. Tiret L, Rigat B, Visvikis S, et al. Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. *Am J Hum Genet* 1992;51:197-205.
19. Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Sorensen TI, Jensen G, Tybjaerg-Hansen A. ACE gene polymorphism: ischemic heart disease and longevity in 10,150 individuals. A case-referent and retrospective cohort study based on the Copenhagen City Heart Study. *Circulation* 1997;95:2358-67.
20. Lindpaintner K, Pfeiffer MA, Kreutz R, et al. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. *N Engl J Med* 1995;332:706-11.
21. Montgomery HE, Marshall R, Hemingway H, et al. Human gene for physical performance. *Nature* 1998;393:221-2.
22. Williams AG, Rayson MP, Jubbs M, et al. The ACE gene and muscle performance. *Nature* 2000;403:614.
23. Williams SR, Jones E, Bell W, Davies B, Bourne MW. Body habitus and coronary heart disease in men. A review with reference to methods of body habitus assessment. *Eur Heart J* 1997;18:376-93.
24. Gottdiener JS, Bednarz J, Devereux R, et al. American Society of Echocardiography recommendations for use of echocardiography in clinical trials. *J Am Soc Echocardiogr* 2004;17:1086-119.
25. Winker R, Barth A, Bidmon D, et al. Endurance exercise training in orthostatic intolerance: a randomized, controlled trial. *Hypertension* 2005;45:391-8.
26. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Eur Heart J* 1996;17:354-81.
27. Fortin J, Habenbacher W, Gruellenberger R, Wach P, Skrabal F. Real-time monitor for hemodynamic beat-to-beat parameters and power spectra analysis of the biosignals. Presented at: 20th Annual International Conference of the IEEE Engineering in Medicine and Biology Society; April 19-25, 1998.
28. Kubicek WG, Karnegis JN, Patterson RP, Witsoe DA, Mattson RH. Development and evaluation of an impedance cardiac output system. *Aerosp Med* 1966;37:1208-12.
29. O'Dell SD, Humphries SE, Day IN. Rapid methods for population-scale analysis for gene polymorphisms: the ACE gene as an example. *Br Heart J* 1995;73:368-71.
30. Sharma S, Maron BJ, Whyte G, Firoozi S, Elliott PM, McKenna WJ. Physiologic limits of left ventricular hypertrophy in elite junior athletes: relevance to differential diagnosis of athlete's heart and hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2002;40:1431-6.
31. Spirito P, Pelliccia A, Proschan MA, et al. Morphology of the "athlete's heart" assessed by echocardiography in 947 elite athletes representing 27 sports. *Am J Cardiol* 1994;74:802-6.
32. Niemela KO, Palatsi JJ, Ikaheimo MJ, Takkunen JT, Vuori JJ. Evidence of impaired left ventricular performance after an uninterrupted competitive 24 hour run. *Circulation* 1984;70:350-6.
33. McKechnie JK, Leary WP, Noakes TD, Kallmeyer JC, MacSearraigh ET, Olivier LR. Acute pulmonary oedema in two athletes during a 90-km running race. *S Afr Med J* 1979;56:261-5.
34. Noakes T. Fluid replacement during marathon running. *Clin J Sport Med* 2003;13:309-18.
35. Williams AG, Day SH, Folland JP, Gohlke P, Dhamrait S, Montgomery HE. Circulating angiotensin converting enzyme activity is correlated with muscle strength. *Med Sci Sports Exerc* 2005;37:944-8.
36. Eysmann SB, Gervino E, Vatner DE, Katz SE, Decker L, Douglas PS. Prolonged exercise alters beta-adrenergic responsiveness in healthy sedentary humans. *J Appl Physiol* 1996;80:616-22.
37. Starnes JW, Bowles DK. Role of exercise in the cause and prevention of cardiac dysfunction. *Exerc Sport Sci Rev* 1995;23:349-73.
38. Seals DR, Rogers MA, Hagberg JM, Yamamoto C, Cryer PE, Ehsani AA. Left ventricular dysfunction after prolonged strenuous exercise in healthy subjects. *Am J Cardiol* 1988;61:875-9.