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ACE I/D Polymorphism and Cardiac Adaptations in Adolescent Athletes

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ABSTRACT

RIZZO, M., F. GENSINI, C. FATINI, P. MANETTI, N. PUCCI, A. CAPALBO, M.C. R. VONO, AND G. GALANTI ACE I/D Polymorphism and Cardiac Adaptations in Adolescent Athletes. *Med. Sci. Sports Exerc.*, Vol. 35, No. 12, pp. 1986–1990, 2003. **Purpose:** The aim of this cross-sectional study was to determine whether there is a correlation between left ventricular hypertrophy (LVH) and angiotensin converting enzyme (ACE) insertion/deletion (I/D) polymorphism in adolescent athletes. **Methods:** Seventy-five competitive soccer players (aged 15 ± 1.2 yr) and 52 untrained control subjects (aged 15 ± 1.6 yr) were examined with echocardiography (echo) and bioelectrical impedance analysis. The ACE genotype of all subjects was determined by PCR and correlated with left ventricular mass (LVM) indices. **Results:** Allele frequencies were comparable between athletes and controls. Body surface area (BSA), fat-free mass (FFM), and all mean echo measurements were significantly greater in athletes than in controls. LVM and LVM indices for both BSA and FFM were all significantly greater in athletes than in controls (LVM 195.3 ± 32 g vs 165.3 ± 37.6 g; LVM/BSA 115.5 ± 18.9 $g \cdot mq^{-1}$ vs 95 ± 18.2 $g \cdot mq^{-1}$; LVM/FFM 3.5 ± 0.5 vs 3 ± 0.54, $P < 0.001$ for the three variables). Left ventricular hypertrophy was found in 17 (23%) athletes. There was no correlation between ACE I/D polymorphism and athletes with LVH as the II and DD genotype frequencies were identical (41%). However, in athletes with LVH, the presence of the D allele was associated with a greater LVM index than compared to homozygous II genotype (LVM = 145 ± 7.6 $g \cdot mq^{-1}$ in DD+ID group vs 135 ± 2.9 $g \cdot mq^{-1}$ in II group, $P = 0.008$). **Conclusions:** The results of the study show that significant changes occur in cardiac morphology and function in adolescent athletes. Interestingly, the ACE I/D polymorphism was associated with the degree of cardiac hypertrophy but not with the occurrence of LVH itself. **Key Words:** ECHOCARDIOGRAPHY, LEFT VENTRICULAR MASS, LEFT VENTRICULAR HYPERTROPHY, ACE I/D POLYMORPHISM

Long-term physical training induces morphological and functional cardiac adaptations that are usually described as "athlete's heart" (21). The predominant changes include increased left ventricular mass (LVM) and decreased rest and submaximal heart rate. These adaptations are beneficial and allow the heart to work as a more efficient and powerful pump (5).

Exercise-induced cardiac morphological adaptations have been thoroughly examined in adult athletes, whereas very few data are available about adolescents.

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A number of factors modulate LVM in athletes. These include both intrinsic factors—gender, age, blood pressure, body weight and composition, and hormonal influences—and extrinsic factors, which particularly concern the type (resistance or endurance), intensity, and length of training (7,9,16). However, in view of the variability observed in the LVM of similarly trained athletes, the influence of genetic factors on cardiac mass development may be postulated.

The insertion/deletion (I/D) polymorphism of the gene encoding for the angiotensin converting enzyme (ACE) is known to affect changes in LVM in response to physical training in adult athletes. In several previous studies, different authors documented the association of the DD genotype with physiological left ventricular hypertrophy (LVH) in endurance athletes (4,8,12,13). This association was correlated to the enhanced cardiac renin-angiotensin system (RAS) activity in subjects who were homozygotes for the D allele. The final effector of RAS is angiotensin II (AT II), a powerful myocardial growth factor that results from ACE proteolytic action. Enhanced activity of cardiac AT II in

response to mechanical stimuli is a better explanation of the correlation between allele D and exercise-induced left ventricular hypertrophy.

At present, no data are available on the influence of the ACE genotype on LVM changes in response to physical training in teenage athletes.

The aim of this study was to determine whether there is a correlation between LVH and ACE I/D polymorphism in adolescent athletes.

MATERIALS AND METHODS

Sample selection. Over a period of 2 months, 300 male soccer players, aged 12–18 yr, underwent a preparticipation screening for competitive activity at the Sports Medicine Reference Center. All subjects were members of the same athletic club (A.C. Fiorentina) and had been previously divided into teams according to date of birth.

A single evaluation of all subjects was performed in the morning, under fasting conditions, and a minimum of 36 h from the last training session.

The first level of examination for all the athletes included physical assessment, rest-ECG, and echocardiography. The pubertal development stage was assessed in all the athletes by physical examination, as defined by Marshall and Tanner (11). According to these authors, we considered five stages of pubertal development: T1, prepubertal stage; T2, early puberty; T3, middle puberty; T4, late puberty; and T5, postpubertal stage or sexual maturity. Only athletes classified as T5 were included in this study.

Athletes with valvular alteration, as assessed by echocardiography (bicuspid aortic valve and mitral valve prolapse) were also included, in the presence of trivial valvular regurgitation.

Subjects. The study population consisted of 75 male competitive soccer players aged 14–18 (mean age $\pm$ SD = 15 $\pm$ 1.2 yr), from the A.C. Fiorentina juvenile teams, and 52 healthy volunteer age- and sex-matched controls (mean age $\pm$ SD = 15 $\pm$ 1.6 yr) and comparable for pubertal stage. At the time of the evaluation, athletes had usually been training for over 2 yr.

The training program consisted of five sessions shared as follows: 1) Four sessions of 2 h of endurance training including 15 min of warm-up, 40 min of aerobic exercises, 40 min of technical exercises performed with ball (shots, dribbling, and crossings), and 15 min of cool-down and stretching. 2) One session of low- to moderate-intensity resistance training including a single set of different exercises (e.g., shoulder press, abdominal crunch, quadriceps extension or leg press, leg curls, and calf rise). Each training session lasted 30 min, and the intensity of strength training was 60% of one-repetition maximum.

The control subjects were recruited from Florentine high schools. Their physical activity was limited to 2 $h \cdot wk^{-1}$ of low-level training performed at school, according to the educational program applied in Italians schools. Moreover, no control subject had been involved in any other sport activity.

The study was approved by the local Ethics Committee. Written informed consent was obtained from all the subjects before their participation in the study. For subjects under 18 yr of age, parental consent was also obtained.

General measurements. Height and weight were assessed in all the subjects. Body surface area (BSA) was calculated using the Dubois formula (6).

Systolic and diastolic blood pressure were measured with a Riva-Rocci sphygmomanometer. Blood pressure was calculated as the average of three measurements obtained after 5 min of supine rest at 1-min intervals.

Bioimpedance analysis. Body composition was measured with tetrapolar BIA RJL-Akern 101 (Florence, Italy) in the supine position, according to the NIH Consensus Statement (15). This method allowed us to evaluate fat-free mass (FFM; Bodycomp 2.55 program with BIA analyzer), which correlates directly to muscle mass.

Echocardiographic assessment. Studies were performed with Esaote Biosound Megas (Florence, Italy) phased-array echocardiography with transducer frequencies that were appropriate for body size, using standard techniques. According to the recommendations of the American Society of Echocardiography, two-dimensionally guided M-mode tracings of left ventricular (LV) end-systolic and end-diastolic diameters were made, and interventricular septum and posterior wall thickness were measured (19). LV mass was calculated according to the equation reported by Devereux (3). The early to atrial wave ratio in left ventricular peak inflow velocities (E/A ratio) was obtained according to Benjamin (1), wall stress was calculated according to the equation reported by Reichek et al. (17), and the ejection fraction was calculated with a formula derived from Teicholz et al. (22).

DNA analysis. Genomic DNA was extracted from peripheral blood and I/D polymorphism was determined by PCR using primers and cycling conditions as previously described by Rigat et al. (18). DNA was amplified in the presence of 5% dimethylsulphoxide (DMSO) in the reaction mixture, at an annealing temperature of 60°C, in order to reduce the mistyping of ID as DD. Fragments of 190 bp (deletion allele, D) and 490 bp (insertion allele, I) were separated on a 2% agarose gel, stained with ethidium bromide, and viewed on a UV transilluminator. Each DD genotype was moreover subjected to a second PCR amplification without the 5% DMSO, at an annealing temperature of 67°C, and using a primer pair that recognizes an insertion-specific sequence in order to reduce underestimation of heterozygotes.

Statistical analysis. Statistical analyses were performed with the GB-STAT program. Data are presented as mean $\pm$ SD for quantitative variables. Study population characteristics were compared using Student's test when Gaussian distribution was observed. Significance for all statistical analyses was set at $P < 0.05$.

RESULTS

Genotype distribution was in agreement with Hardy-Weinberg equilibrium, and the allele frequencies found in

TABLE 1. ACE Genotype distribution and allele frequency in athletes and controls.

	Athletes ($N = 75$)	Controls ($N = 52$)
ACE genotype distribution		
II	28%	17%
DD	29%	32%
ID	43%	51%
ACE allele frequency		
I	49%	42%
D	51%	58%

athletes were comparable to those of controls (Table 1). The anthropometric measurements and FFM were significantly different between athletes and controls (Table 2).

Tables 3 and 4 show mean, SD, and 5th to 95th percentile ranges of the echocardiographic measurements of the soccer player cohort and those of the sedentary control group.

LV internal dimensions, septal and LV posterior wall thicknesses, LVM and LVM corrected for both BSA and FFM, left atrial chamber size, and right ventricular diastolic diameter were all significantly greater in the soccer players than in sedentary controls (LVM 195.3 ± 32 g vs 165.3 ± 37.6 g; LVM/BSA 115.5 ± 18.9 g·mq^{-1} vs 95 ± 18.2 g·mq^{-1}; LVM/FFM 3.5 ± 0.5 vs 3 ± 0.54. $P < 0.001$ for all the variables).

Soccer players had greater LVM, resulting from greater chamber dimensions and wall thickness, in comparison with untrained controls. In particular, we observed an increase in septal thickness (41% of the athletes exceeded the 95th percentile of the values observed in sedentary controls, see Table 4) and in LV end-diastolic dimensions (21% of athletes exceeded the 95th percentile of the values observed in sedentary controls, see Table 4). LV systolic and diastolic functions were not affected by increased LVM; the lower ejection fraction observed in athletes had no clinical relevance.

Left ventricular hypertrophy (LVH, defined as LVM/BSA >132 g·mq^{-1}) was found in 17 athletes (23%). No relationship between the ACE I/D polymorphism and the development of exercise-induced cardiac hypertrophy was found. The prevalence of the DD genotype was in fact comparable to that of the II genotype in the hypertrophic athletes (II = 41%, ID = 18%, DD = 41%). In these athletes, the level of LVH was significantly different between ACE D allele carriers and II homozygote athletes (LVMI = 145 ± 7.6 g·mq^{-1} in ID+DD group vs 135 ± 2.9 g·mq^{-1} in II group, $P = 0.008$).

TABLE 2. General data in athletes and controls.

	Athletes ($N = 75$)	Controls ($N = 52$)	P
Age (yr)	15 ± 1.2	15 ± 1.6	NS
HR (bpm)	68.3 ± 10.4	73.7 ± 10	<0.001
Height (cm)	175.7 ± 6.5	171.3 ± 11.5	<0.001
Weight (kg)	66.4 ± 8.2	67 ± 8.5	<0.05
BSA (mq)	1.8 ± 0.1	1.7 ± 0.2	<0.001
FFM (kg)	58.6 ± 7	52 ± 8.5	<0.001
BPs (mm Hg)	117.6 ± 8.6	118 ± 7.4	<0.05
BPd (mm Hg)	71 ± 7.2	74.5 ± 7.5	<0.001

HR, heart rate; BSA, body surface area; FFM, fat-free mass; BPs, systolic blood pressure; BPd, diastolic blood pressure.

TABLE 3. Echocardiographic measurements in athletes and controls.

	Athletes ($N = 75$)	Controls ($N = 52$)	P
AO root (mm)	29.3 ± 2.2	28.3 ± 2.9	<0.05
LA size (mm)	34.7 ± 3.1	32.2 ± 3.4	<0.001
RVDd (mm)	21 ± 3.7	19.6 ± 3	<0.001
IVSd (mm)	9.5 ± 0.1	8.5 ± 1	<0.001
LVPWd (mm)	9.5 ± 1.1	8.3 ± 0.9	<0.001
LVIDd (mm)	51.4 ± 4.4	49.4 ± 3.5	<0.001
LVIDs (mm)	32.1 ± 3.6	30 ± 3.3	<0.001
LVM (g)	195.3 ± 32	165.3 ± 37.6	<0.001
LVM/BSA (g·mq^{-1})	115.5 ± 18.9	95 ± 18.2	<0.001
LVM/FFM (g·kg^{-1})	3.5 ± 0.5	3 ± 0.4	<0.001
FE%	66.6 ± 6.9	69.1 ± 4.7	0.03
D%	38.3 ± 5.8	38.8 ± 3.9	NS
E/A	2 ± 0.5	2.2 ± 0.6	NS

AO root, aortic root; LA size, left atrium end systolic dimension; RVDd, right ventricular end diastolic dimension; ISVd, interventricular septal end diastolic dimension; LVPWd, left ventricular end diastolic posterior wall dimension; LVIDd, left ventricular end diastolic internal dimension; LVIDs, left ventricular end systolic internal dimension; LVM, left ventricular mass.

DISCUSSION

Long-term physical training is associated with physiological cardiac hypertrophy. A number of studies have investigated exercise-induced cardiac adaptations in adults, whereas little information is available about teenagers.

In this study a group of adolescent athletes was evaluated. The sample was homogeneous for gender, stage of sexual maturation, lifestyle, and form of training (type, intensity, and frequency of training sessions). Regarding the training, soccer players undergo a prevalently isotonic type with a small isometric component.

Athletes showed significantly higher LVM and LVM indices than sedentary controls. It is unlikely that these differences between the two groups were due to a previous selection to the specific sport because soccer requires technical ability more than superior cardiovascular performance.

The greater LVM resulted from greater chamber dimensions and wall thickness. Thus, from a morphological point of view, this pattern of cardiac hypertrophy was in the middle of the two extremes described in previous echocardiographic studies: eccentric hypertrophy, characterized by increased LV internal dimensions with proportional parietal thickening, and concentric hypertrophy, characterized by increased wall thickness with no alterations of chamber dimensions. The eccentric pattern has been described in high-endurance trained athletes such as marathon runners, cyclists, and rowers as the adaptation to the physiological volume overload related to the isotonic exercise. On the other hand, the concentric pattern has been described in strength-trained athletes, such as weightlifters, as the adaptation to the repetitive pressure overload related to the isometric exercise (5,7,21).

The pattern of LVH that we observed in the young soccer players reflected the characteristics of the training program these athletes undertook, i.e., a combined endurance and strength training inducing a physiological repetitive volume and pressure cardiac overload. The results of the current study are in agreement with the findings of Somauroo et al. (20), in spite of the fact that their subjects were more

TABLE 4. Lower and upper limits of echocardiographic measurements in athletes and controls.

	Athletes ($N = 75$)	5th–95th Percentile (Athletes)	Controls ($N = 52$)	5th–95th Percentile (Controls)	Athletes: Number (%) Greater than Upper Limit of Controls
AO root (mm)	29.3 ± 2.2	25–32.8	28.3 ± 2.9	30–32	6.6
LA size (mm)	34.7 ± 3.1	30–39.5	32.2 ± 3.4	25.7–38.9	9.3
RVDd (mm)	21 ± 3.7	8–11	19.6 ± 3	7.9–10.1	20
IVSd (mm)	9.5 ± 0.1	8–11	8.5 ± 1	6.3–9.6	41.3
LVPWd (mm)	9.5 ± 1.1	15.6–26	8.3 ± 0.9	15–24	12
LVIDd (mm)	51.4 ± 4.4	46.4–57.3	49.4 ± 3.5	43–54.9	21.3
LVIDs (mm)	32.1 ± 3.6	26.7–37	30 ± 3.3	24.6–35.2	8
LVM (g)	195.3 ± 32	146.4–247	165.3 ± 37.6	111–222.9	20
LVM/BSA ($g \cdot mq^{-1}$)	115.5 ± 18.9	86.7–146	95 ± 18.2	68.2–126.1	23
LVM/FFM	3.5 ± 0.5	2.7–4.4	3 ± 0.4	2.3–3.7	17

See Table 3 for key to abbreviation.

heterogeneous with respect to age and stage of sexual maturation.

Apart from the pattern of cardiac hypertrophy, which was similar throughout the athletic sample, we found a wide range of LVM and LVM indices within the same group (the 5th and the 95th percentile were LVM = 146.4–247 g; LVMI = 86.7–146 $g \cdot mq^{-1}$), in spite of their similar athletic training. These findings suggest that different factors affect LVM response to athletic training in teenagers. The athletes of this study had been recruited from the juvenile teams of the same athletic society, in order to minimize the differences related to the type of training and to lifestyle, both of these being extrinsic factors that could well affect the impact of exercise on the cardiovascular system. So, given the relatively high degree of environmental homogeneity of the athletic sample, we postulated that the observed variance in LVM values among athletes was due to some underlying genetic difference.

To our knowledge, this is the first study to examine the relationship between ACE I/D polymorphism and LVH in adolescent athletes. Though no relationship between LVH in adolescent athletes and ACE I/D polymorphism was observed, the D allele was associated with the degree of LVH.

Over the past few years, the I/D polymorphism of the ACE gene has been the most investigated genetic variant for affecting exercise-induced left ventricular hypertrophy in adult athletes. In previous studies, different authors described the association of the DD genotype with LVH in athletes (4,8,12,13). Two hypotheses have been advanced to explain this association: an increased activity of AT II, a powerful cell growth factor, and/or increased degradation of bradykinin, a cellular growth inhibitor, both resulting from the enhanced myocardial ACE activity in subjects who are homozygotes for ACE D allele (2).

Our results, regarding no influence of ACE I/D polymorphism on the presence of LVH, are at variance with those found in adults by various authors. Montgomery et al. (12), Diet et al. (4), and Nagashima et al. (13) reported a strong association between the DD genotype and LVH in long-term endurance-trained adult athletes. Moreover in a previous study we ourselves described the same association in a group of adult professional soccer players (8).

The absence of a relationship between LVH and ACE genotype in the current study highlights the fact that LVM is influenced by a large number of environmental and biological factors, such as age, sex, race, blood pressure, genetic factors, and physical training. Moreover, LVM can be considered the result of an interaction between physiological stimuli and transducing systems. One possible hypothesis is that in adolescent athletes the influence of ACE I/D polymorphism on the development of LVH is masked by some other factor. In particular, insulin-like growth factor-1 (IGF-1) is an attractive candidate because: 1) IGF-1 plasma levels have been shown to be age-dependent (10) and 2) IGF-1 is known to be associated with cardiac hypertrophy in trained soccer players (14). Collectively, these data provide a possible explanation for the discrepancy between adolescent and adult athletes and the impact of ACE polymorphism on LVH.

CONCLUSIONS

This study describes the morphological features of young soccer players' hearts. Soccer players have a significantly greater left ventricular mass, resulting from the greater chamber size and wall thickness, in comparison with that observed in untrained controls. The pattern of this cardiac enlargement represents an adaptation to the physiological volume and pressure overload, which the above-mentioned type of training involves. Thus, these findings suggest that the main features of the athlete's heart already described in adults are observed in teenagers too. The ACE I/D polymorphism did not influence the development of LVH but did influence the LVH levels.

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