Tohoku J. Exp. Med., 2017, **243**, 311-320

Polymorphisms in *ACE* and *ACTN3* Genes and Blood Pressure Response to Acute Exercise in Elite Male Athletes from Serbia

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Physiological adaptations to various types of prolonged and intensive physical activity, as seen in elite athletes from different sports, include changes in blood pressure (BP) response to acute exercise. Also, functional polymorphisms of the angiotensin I converting enzyme (*ACE*) and alfa-actinin-3 (*ACTN3*) genes are shown to be associated with BP parameters changes, both in athletes and sedentary population. In this study, an Alu insertion (I)/deletion (D) polymorphism in *ACE* gene, as well as nonsense mutation in the gene encoding *ACTN3* have been scored in 107 elite Serbian athletes classified according to their sporting discipline to power/sprint (short distance runners/swimmers), endurance (rowers, footballers, middle-distance swimmers) or mixed sports (water polo, handball, volleyball players). Presence of nonfunctional allele in *ACTN3* is associated with significantly increased maximal systolic BP (SBPmax, $p = 0.04$). Athletes with Alu insertion in *ACE* had significantly ($p = 0.006$) larger decline of systolic BP after 3 minutes of recovery (SBPR3), calculated as the percentage of maximal SBP response during exercise stress testing. Concomitant presence of non-functional variant in *ACTN3* gene decreased this beneficiary effect of *ACE* mutation on SBPR3. Long term enrollment in power/sprint sports significantly increased resting diastolic BP (DBPrest: 74 mmHg) and SBPmax (197 mmHg) and improved SBPR3 (74.8%) compared to enrolment in endurance (72 mmHg; 178mmHg; 81.1%) and mixed sports (69 mmHg; 185 mmHg; 80.0%). Lack of the effect of genotype by sport interaction on BP parameters suggests that the long-term effects of different disciplines on BP are not mediated by these two genes.

Keywords: *ACE*; *ACTN3*; blood pressure; elite athletes; gene polymorphisms
Tohoku J. Exp. Med., 2017 December, **243** (4), 311-320.

Introduction

Elite sport performance represents a complex phenotype, composed of various intricately intertwined biological traits, some of which are manifested at the morpho-anatomical, biochemical or psychological level, with a great influence of various physiological features. Regular and intense physical activity leads to several morphological and functional adaptive heart modifications that have been known as "athlete's heart" (Król et al. 2016). The extent of these physiological cardiovascular "adaptive" changes is predominantly related to the intensity and type of sport activity and highly depends on the type of physical training (Santoro et al. 2014).

On the other hand, the field of genetics in elite sport performance has made a notable progress in the last several years. Nowadays, the main question is no longer whether genes are associated with the elite athletic status, but rather with the genetic profile contributing most to extraordinary results in sports. The majority of studies have concentrated on genotyping of individuals at physiological end-points of the athletic performance - in "endurance" and "power" sport disciplines, overlooking the fact that the majority of sport disciplines nowadays can be labelled as "mixed energy system" sports (Milioni et al. 2017). Although multiple genetic variants in various genes are thought to influence muscle function and physical performance, here we focus on two common gene polymorphisms trying to

Received September 20, 2017; revised and accepted November 28, 2017. Published online December 19, 2017; doi: 10.1620/tjem.243.311.
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explain blood pressure variations in a group of Serbian elite male athletes from either endurance, power or mixed sports.

Angiotensin converting enzyme (ACE) is an important component of the renin-angiotensin-aldosterone system (RAS) with the central role in blood pressure (BP) regulation. The main function of this metalopeptidase is to convert angiotensin I to angiotensin II, the vasoconstrictor hormone, and also to degrade the vasodilator kinins (He et al. 2015). A large inter-individual, together with a small intra-individual variation in the plasma ACE level, suggest that the activity of this enzyme is under strong genetic control. *ACE* gene, which contains 26 exons and 25 introns, is located on chromosome 17q23 (Tiret et al. 1992). Although there are many identified polymorphic sites in *ACE* gene, some of which located in the coding regions of this gene, polymorphism rs4646994 defined by the presence (insertion, I allele), or the absence (deletion, D allele) of 287-base pair (bp) Alu fragment in intron 16 of the gene, is by far the best studied (Folland et al. 2000; Ay et al. 2007). This polymorphism, unlikely functional due to its location, can explain nearly a half of the inter-individual variation in production and activity of ACE, both in tissues and in the circulation. Increased ACE level/activity, observed in persons with the DD genotype, is associated with increased blood pressure, and also with a higher risk for many other cardiovascular disorders (Wang and Staessen 2000). Additionally, a number of studies have suggested that *ACE* insertion is associated with better endurance performance in elite athletes (Ma et al. 2013; Gunel et al. 2014; Saber-Ayad et al. 2014).

The other popular candidate gene for elite performance is 16,934-bp long, α-actinin-3 *(ACTN3)* gene, located on chromosome 11q13.2, encoding skeletal muscle α-actinin-3 protein, a member of the family of actin-binding proteins (Vincent et al. 2007). Namely, four genes for α-actinin are found in humans: *ACTN1, ACTN2, ACTN3*, and *ACTN4*. While *ACTN1* and *ACTN4* are non-muscle proteins (Roth et al. 2008), *ACTN2* and *ACTN3* are myofibrillar proteins localized at the Z disk. *ACTN3* gene is highly conserved through evolution and almost exclusively expressed in type 2 fast twitch muscle fibres (Vincent et al. 2007), responsible for rapid and strong muscle contractions, mainly in sprint and power activities (Clarkson et al. 2005). A very common genetic variation in *ACTN3* gene leads to the replacement of amino acid arginine (R) with a stop codon (X) at the position 577 (R577X, rs1815739). The nonsense allele (X) produces no detectable α-actinin-3 protein. Although it is well known that this variant, which leads to α-actinin-3 protein deficiency, does not cause any muscular functional impairment, there are a lot of studies confirming positive association between high power muscle contractions and the presence of the R allele (Clarkson et al. 2005; Vincent et al. 2007; Roth et al. 2008). Recent data (Bernardez-Pereira et al. 2014) associate *ACTN3* R577 allele with increased survival time in patients with chronic heart failure. The possible explanation could be the finding of extra-sarcomeric ACTN3 expression in pulmonary artery, suggesting its potential role in the maintenance of vascular tone, and influencing the arterial BP (Deschamps et al. 2015). Additionally, it is documented that the presence of X allele may lead to better endurance performance (Vincent et al. 2007; Roth et al. 2008).

Researchers agree that recreational/moderate physical activity decreases BP and slows down the progression of hypertension (Fuentes et al. 2002). On the other hand, intensive and continuous physical activity in elite athletes affects BP, depending on the type of sport, as well as on the intensity and duration of training (training hours per week). However, hypertension in elite athletes is very rare, due to their cardiovascular adaptive changes. Literature data suggest that power athletes have higher BP values than endurance athletes (McArdle et al. 2010). Although the exact physiological basis of this association is not yet clear enough, the most probable cause is the autonomic nervous system modification, along with baroreceptors resetting and vasoactive substances activity modification (Varga-Pintér et al. 2011; Berge et al. 2015). All mentioned adaptive modifications decrease the two most important BP-determining factors - the cardiac output and total peripheral resistance.

Although a number of studies deal with the effect of *ACE* I/D and *ACTN3* R577X polymorphisms on cardiovascular adaptation in athletes (De Moor et al. 2007; Ma et al. 2013; Ahmetov et al. 2014), their results often provided conflicting data. By clarifying the association of these polymorphisms with resting, maximal and recovery BP in elite athletes from power, endurance and mixed sport disciplines, we want to shed more light on this controversy.

Material and Methods

Subjects

In this study, 107 unrelated Caucasian male elite athletes: 17 sprint/power (short-distance runners, swimmers competing in events < 200 m), 36 endurance athletes (rowers, football players, middle distance swimmers), and 54 athletes from mixed sports (water polo, handball and volleyball) participating in annual screening tests at the Serbian Institute of Sport and Sports Medicine, were enrolled. The including criteria were: 1) being a minimum national level athlete; 2) 15 or more hours of training per week; 3) lack of any history of structural cardiac, cerebrovascular, chronic renal or hepatic diseases; and 4) restraining from taking any medication at the time of testing. Each subject gave the written informed consent for participation in the study. All procedures were reviewed and approved by the Ethics Committee of the School of Medicine, University of Belgrade. All subjects whose data on any measured variables were missing, were excluded.

Anthropometric data

The body weight (BW) and body fat percentage (BF %) were measured on a scale with 0.01 kg readability (InBody 370, InBody, Seoul, Korea), with participants wearing minimal clothes and being barefoot. Body height (BH) was assessed to the nearest 0.1 cm using a portable stadiometer fixed to the wall. Body mass index (BMI) was calculated for all the participants as the ratio of body mass (kilo-

grams) and height (meters) squared.

Resting blood pressure measurement

Resting blood pressure (BPrest) was measured at each annual pre-participation physical evaluation, at the same time as complete pre-participation screening. After 15 minutes of rest in a sitting position, brachial systolic pressure (SBP) and diastolic blood pressure (DBP) were measured using a mercury sphygmomanometer with an appropriately sized cuff. Three consecutive BP recordings at 5-minute intervals were taken and the average of these values was included for the present study. Rate pressure product (RPP) was calculated for all the participants as the product of heart rate and maximal systolic arterial pressure.

Cardiopulmonary exercise testing and blood pressure measurement

Incremental exercise testing was performed on an electronical treadmill ergometer (Treadmill T200, Cosmed, Italy). All the ventilatory parameters were measured continuously, using a breath by breath automated ergospirometry system (Cosmed, Quark CPET, Rome, Italy). Heart rate was constantly monitored using a 12-canal ECG monitoring system. Ergospirometry exercise test consisted of three phases. During the first (resting) phase, which lasted for 3 minutes, athletes were in a standing position. The second (exercise) phase starts with the treadmill strip speed set at 4 km/h, and increased by 1 km/h at every subsequent start-minute. The testing phase was completed when at least three out of the four following criteria were met: 1) oxygen consumption plateau, 2) attainment of age predicted maximal heart rate (220 - age), 3) respiratory exchange ratio higher than 1.1 or 4) athletes' subjective reasons. The third (recovery) phase lasted for 3 minutes and consisted of 1 min of active cool-down on the treadmill speed of 4 km/h and 0% elevation, after which subjects sat on a chair for 2 min.

Blood pressure was measured manually before the exercise, as well as on every 3 min during this phase. During the recovery period, heart rate, systolic blood pressure and diastolic blood pressure were measured in the first and the third minute. Peak systolic and diastolic BPs (SBP/DBP max) were defined as the BP measurement at the last stage of exercise, just before or immediately after the interruption. It has been suggested that BP measurement during the peak of the graded exercise treadmill test is less accurate due to the constant movements of the arm of the tested individual. Therefore, peak exercise BP measured immediately after the test completion, during the earliest phase of post-exercise recovery, avoids the inaccuracy associated with exercise BP measurement (Taylor and Beller 1998). The third minute post exercise systolic and diastolic BP ratios (SBPR3 and DBPR3, respectively) were calculated as (third minute recovery SBP/peak exercise SBP) × 100 and (third minute recovery DBP/peak exercise DBP) × 100, respectively. These two parameters were preferable to peak exercise BP values, because they reflect more precisely cardiovascular fitness recovery. Namely, a delayed decrease in the BP during the third minute after acute exercise, which is represented by higher SBPR3 and DBPR3, is a powerful predictor of overall cardiovascular pathophysiology (McArdle et al. 2010).

DNA extraction and genotyping

Using sterile swabs, buccal cells samples were collected from all tested subjects, upon obtaining their written consent, in accordance with the principles of World Medical Association's Declaration of Helsinki. Genomic deoxyribonucleic acid (DNA) was extracted manually, using the commercial kit (PureLink genomic DNA, Invitrogen). Concentration and purity of DNA samples were assessed spectrophotometrically. All DNA samples were normalized to 1 ng of DNA per 1 microliter. Regions of *ACNT3* and *ACE* genes containing studied polymorphisms (rs1815739 and rs4646994, respectively) were amplified by polymerase chain reaction (PCR), using 1 nanogram of DNA sample, previously published flanking primers (Mills et al. 2001; Cieszczyk et al. 2010) at a final concentration of 0.3 μM each, and MeltDoctor™ HRM Master Mix (Thermo Fisher Scientific) in a total volume of 20 microliters. PCR amplification and melting of the PCR products were performed in ViiA 7 machine (Applied Biosystems), running QuantStudio Real Time PCR software, ver. 3.2 (Applied Biosystems). After 40 cycles of amplification (10 seconds of denaturation at 95°C and 30 seconds of annealing/extension at 55°C), PCR products were heated at 95°C for 10 seconds, annealed for 1 minute at 55°C, and then melted by increasing the temperature from 55 to 95°C, at a rate of 0.02°C/sec. Drop in fluorescence of the dye detached from the melted DNA was continuously monitored. Melting profiles were assessed using high Resolution Melt (HRM) Software Module for ViiA™ 7 System (Applied Biosystems™), able to identify gene variants based on the differences in the shape of the melt curves and the differences in the values of melting temperature of amplicons. Accuracy of the HRM genotyping was checked by comparing results to those obtained for a subset of samples genotyped by conventional methods (gel electrophoresis for I/D polymorphism in *ACE* gene, according to the previously published protocol (Cieszczyk et al. 2010), and restriction fragment length polymorphism (RFLP) analysis for R577X mutation in *ACTN3* gene (Mills et al. 2001). Perfect concordance between genotyping results obtained using different methods proved 100% analytical specificity of this, in house developed, HRM method for accurate genotyping of these polymorphisms.

Statistical analyses

Statistical analysis was performed using SPSS software version 15.0 (SPSS, Inc., Chicago, Illinois). Continuous data were expressed as mean ± SD, while categorical data were expressed as frequencies. To access the differences between athletes according to the types of sports in which they participated, we have used one-way analysis of variance with multiple Bonferroni post hoc tests, with necessary BH and BW adjustments. For these analyses, dominant, additive and recessive models for the allele D in the *ACE* gene and allele R in the *ACTN3* gene were assumed. Statistical significance was set for a 2-tailed p value < 0.05.

Results

Anthropometrical measures of all athletes are reported in Table 1. Athletes from the mixed group were significantly taller and weighed more compared to athletes from the power/sprint and endurance groups ($p < 0.01$). There was no difference in body mass indices and body fat percentages between groups (Table 1).

The results of cardiopulmonary exercise testing showed that maximal oxygen consumption was similar among different groups of athletes. The power/sprint group had the highest resting diastolic BP (DBPrest), compared to the mixed group ($p < 0.01$). Our results point to the values of resting SBP being higher and the maximum rate of oxy-

Table 1. The anthropometrical characteristics, basal cardiovascular variables and cardiopulmonary exercise testing parameters in elite male athletes.

	Power/Sprint (n = 17)	**Endurance** (n = 36)	**Mixed** (n = 54)
Age (years)	25.6 ± 4.7	24.7 ± 4.3	24.0 ± 3.9
Body height (cm)	184.3 ± 8.4	181.2 ± 7.8	**193.6 ± 8.1** [a]
Body weight (kg)	83.0 ± 16.1	78.8 ± 9.9	**93.2 ± 10.2** [a]
Body mass index (kg/m2)	24.3 ± 3.9	23.9 ± 2.3	24.8 ± 2.2
Body fat percentage (%)	12.7 ± 3.1	11.8 ± 4.8	11.8 ± 4.1
HRrest (beats/min)	62.9 ± 16.1	60.5 ± 11.1	59.14 ± 9.8
SBPrest (mm Hg)	114.5 ± 10.3	113.1 ± 10.5	113.9 ± 10.8
DBPrest (mm Hg)	**75.9 ± 5.8** [b]	71.9 ± 8.0	68.8 ± 7.5
VO_2max (mL/min/kg)	47.8 ± 5.7	51.9 ± 7.1	48.9 ± 9.2
HRmax (beats/min)	187.1 ± 7.9	187.3 ± 14.1	184.5 ± 10.1
SBPmax (mm Hg)	192.1 ± 23.6	174.7 ± 17.8	188.2 ± 22.9
DPBmax (mm Hg)	59.5 ± 9.2	59.8 ± 10.1	56.6 ± 18.0
HRrec1 (beats/min)	161.8 ± 22.8	166.8 ± 13.2	157.3 ± 16.7
SBPrec1 (mm Hg)	175.7 ± 22.5	**170.0 ± 15.1** [c]	185.6 ± 22.9
DBPrec1 (mm Hg)	61.1 ± 7.6	61.4 ± 10.1	55.6 ± 19.7
HRrec3 (beats/min)	111.1 ± 19.8	115.4 ± 11.6	107.5 ± 10.7
SBPrec3 (mm Hg)	142.3 ± 15.8	141.6 ± 15.2	149.6 ± 17.8
DBPrec3 (mm Hg)	71.1 ± 5.5	70.5 ± 6.4	68.9 ± 13.6
RPP (mm Hg/min)	35,802 ± 4,257	32,708 ± 3,888	34,636 ± 4,472

Values are shown as mean ± SD. HRrest, resting heart rate; SBPrest, resting systolic blood pressure; DBPrest, resting diastolic blood pressure; VO2max, maximal oxygen consumption; HRmax, maximal heart rate; SBPmax, maximal systolic blood pressure; DPBmax, maximal diastolic blood pressure; HRrec1, heart rate recovery first minute; HRrec3, heart rate recovery third minute; HRRindex, hear rate recovery index; SBPrec1, first minute recovery systolic blood pressure; DBPrec1, first minute recovery diastolic blood pressure; SBPrec3, third minute recovery systolic blood pressure; DBPrec3, third minute recovery diastolic blood pressure; RPP, rate pressure product.
[a]$p < 0.05$ compare mixed to power/ sprint and endurance, [b]$p < 0.05$ compare power/sprint to mixed, [c]$p < 0.05$ compare endurance to mixed.

gen consumption (VO_2 max) values being lower, although not significantly, in power/sprint athletes compared to the other two groups. On the other hand, the endurance subgroup had the best recovery BP values, with significantly lower first minute recovery systolic BP (SBPrec1) compared to the power/sprint group ($p < 0.01$) (Table 1). Of all the tested parameters, the most striking results were obtained for SBPR3 and SBPmax. Since previous studies (Taylor and Beller 1998) pointed to the fact that BP recordings during exercise may be inaccurate due to the vigorous movements of the arm to which the cuff was attached, with an error as much as 40 mmHg for SBP at peak exercise, in our study we measured maximal BP values immediately after the cessation of the exercise, when the arms were relaxed. As the measure of cardiovascular fitness we used the percentage decline of SBP and DBP (SBPR3 and DBPR3, respectively) calculated as (third minute recovery SBP/peak exercise SBP) × 100 and (third minute recovery DBP/peak exercise DBP) × 100, respectively.

The *ACE* I/D and *ACTN3* R577X genotype frequencies in all subgroups of athletes are shown in Table 2. The genotype distributions were in accord with Hardy–Weinberg equilibrium (HWE) ($p > 0.05$) in each group. In addition, statistically significant differences in *ACE* (I/D) and *ACTN3* (R577X) allele/genotype frequencies were not observed between any tested group (Table 2).

The design of the present study has enabled us to evaluate the effects of genotypes (of *ACE* and *ACTN3* genes), type of sports (endurance, power/sprint and mixed) as well as the effect of genotype caused by sport interaction on the variation of cardiovascular adaptation parameters in tested subjects. The results from two-way ANOVA (Table 3) indi-

Table 2. Genotype and allele frequencies of *ACE* (I/D), *ACTN3* (R577X) polymorphisms for the athletes' groups.

	Power/Sprint n = 17		Endurance n = 36		Mixed n = 54		All athletes n = 107	
	Freq	**%**	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**
ACE								
II	3	17.7	8	22.2	9	16.7	20	18.7
ID	4	23.5	12	33.3	8	14.8	24	22.4
DD	10	58.8	16	44.4	37	68.5	63	58.9
I allele	10	29.4	28	38.9	26	24.1	64	29.9
D allele	24	70.6	44	61.1	82	75.9	150	70.1
ACTN3								
RR	6	35.3	12	33.3	20	37.1	38	35.5
RX	10	58.8	16	44.4	26	48.1	52	48.6
XX	1	5.9	8	22.2	8	14.8	17	15.9
R allele	22	64.7	40	55.5	66	61.1	128	59.8
X allele	12	35.3	32	44.5	42	38.9	86	40.2

ACE, angiotensin-converting enzyme; *ACTN3*, *α*-actinin 3.

cate that athletes with two fully functional copies of *ACE* gene (DD genotypes) had significantly ($p = 0.006$) higher percentage of SBPR3 values, compared to carriers of alleles with reduced expression (genotypes II or ID). Also, athletes from the power/sprint group had lower values of SBPR3 ($p = 0.03$), compared to the endurance and mixed sport groups. However, no significant effect of the interaction between genotype and sport on any of the measured parameters was detected ($p > 0.05$).

According to *ACTN3* dominant model, two-way ANOVA indicated a significant effect of the genotype (XX + RX vs. RR, $p = 0.04$) and the type of sport ($p = 0.04$) on peak SBP values, but not of their interaction ($p > 0.05$). Peak systolic BP was significantly lower in RR genotypes, compared to RX + XX group in all sports groups. On the other hand, as expected, power/sprint athletes had the highest (197 ± 7 mmHg) and endurance athletes had the lowest (178 ± 3 mmHg) maximal systolic blood pressure (Table 3).

The combined genetic effect of *ACE* and *ACTN3* genes (DDRR vs. DDXX + DDRX vs. IDRR + IIRR vs. IDXX + IDRX + IIXX + IIRX) on SBPR3 values, was also significant ($p = 0.03$), but again the interaction between the genotype and the type of sport was not significant ($p > 0.05$). The best recovery (lowest SBPR3) values were found in athletes with IDRR/IIRR genotypes (76 ± 7%) and the athletes with both functional copies of *ACE* and *ACTN3* alleles (genotype DDRR) had the slowest BP recovery (SBPR3 = 86 ± 8%), irrespective of the type of the sport (Table 4).

Discussion

The present study is the first report investigating the association of *ACE* I/D and *ACTN3* R577X polymorphisms with BP values in elite male athletes according to their respective type of sport. The results of our study showed that the systolic BP recovery pattern depends not only on the type of sport, but also on polymorphisms in *ACE* and *ACTN3* genes. For some investigated parameters of BP (SBPmax, SBPR3), we have shown significant influence of the analyzed genes, under the dominant model, as well as the long term effect of the type of physical activity, but not of their interaction. Our results show that the DD genotype of the *ACE* gene was associated with a lesser decline of systolic BP measured at the third minute after the stress test, suggesting that these athletes have slower BP recovery, regardless of the type of physical activity that they are generally exposed to during their regular training. Additionally, the RR genotype of the *ACTN3* gene was found to be associated with lower maximal SBP values in elite athletes. Also, we showed that combined IDRR/IIRR genotypes were associated with the lowest SBPR3 values after the stress test, suggesting that the best BP recovery is expected in athletes with both functional copies of *ACTN3* gene and at least one copy of *ACE* gene with reduced function, regardless of the predominant type of physical activity associated with their sport discipline.

Our study has clearly confirmed previously established physiological connection between a predominant physical activity in elite athletes and higher maximal and recovery BP values: power/sprint athletes had the highest values of these parameters, endurance athletes had the lowest, while mixed sports athletes showed intermediate values. Namely, power training, particularly the concentric and static phase of muscle contraction, mechanically compresses the peripheral arterial blood vessels that supply active muscles, lead-

Table 3. Effect of *ACE/ACTN3* genotype and type of sport on blood pressure parameters.

	Gene / Type of sport	*ACE*			*ACTN3*			
		DD	ID + II	ANOVA (p)	RR	RX + XX	ANOVA (p)	Any genotype
SBP rest (mmHg)	**Endurance**	114.8 ± 3.3	110.9 ± 2.3	G: 0.70 S: 0.94 GxS: 0.57	112.5 ± 5.0	112.4 ± 1.8	G: 0.70 S: 0.94 GxS: 0.57	112.4 ± 1.9
	Power/Sprint	114.2 ± 2.8	113.7 ± 2.4		115.0 ± 2.6	113.2 ± 3.1		114.0 ± 2.0
	Mixed	112.8 ± 1.8	114.1 ± 2.2		113.7 ± 1.4	112.3 ± 2.1		113.2 ± 1.4
	All sports	113.5 ± 1.4	112.5 ± 1.5		112.7 ± 1.6	113.9 ± 1.4		112.9 ± 1.0
DBP rest (mmHg)	**Endurance**	74.2 ± 2.1	70.6 ± 1.5	G: 0.20 **S: 0.01** GxS: 0.82	73.0 ± 3.0	71.6 ± 1.3	G: 0.19 **S: 0.01** GxS: 0.82	72.0 ± 1.3
	Power/Sprint	74.2 ± 2.4	73.7 ± 2.4		75.0 ± 2.6	73.2 ± 2.5		74.0 ± 1.8
	Mixed	69.7 ± 1.2	66.5 ± 2.1		68.7 ± 1.5	68.5 ± 1.5		68.7 ± 1.0
	All sports	71.3 ± 1.0	69.2 ± 1.2		69.9 ± 1.4	70.3 ± 1.0		70.4 ± 0.8
SBP max (mmHg)	**Endurance**	175.9 ± 2.1	179.7 ± 4.5	G: 0.93 **S: 0.01** GxS: 0.72	177.5 ± 6.3	178.3 ± 2.4	G: 0.93 **S: 0.01** GxS: 0.72	178.0 ± 2.6
	Power/Sprint	199.7 ± 10.1	193.3 ± 9.5		181.0 ± 11.0	205.8 ± 7.6		197.0 ± 6.9
	Mixed	184.8 ± 3.9	186.2 ± 4.6		183.1 ± 5.2	185.8 ± 3.6		185.3 ± 3.0
	All sports	184.4 ± 2.8	184.2 ± 3.1		180.8 ± 3.7	185.9 ± 2.5		184.0 ± 2.1
DBP max (mmHg)	**Endurance**	58.4 ± 2.4	58.3 ± 2.6	G: 0.36 S: 0.47 GxS: 0.26	52.1 ± 4.0	61.6 ± 1.3	G: 0.36 S: 0.47 GxS: 0.26	58.3 ± 1.8
	Power/Sprint	54.1 ± 2.9	64.6 ± 3.3		62.5 ± 2.7	56.4 ± 3.6		58.6 ± 2.5
	Mixed	56.5 ± 2.8	54.3 ± 2.9		52.2 ± 3.4	58.4 ± 2.7		55.8 ± 2.1
	All sports	56.7 ± 1.8	57.7 ± 1.8		54.5 ± 2.1	59.0 ± 1.6		57.2 ± 1.3
SBPrec1 (mmHg)	**Endurance**	171.9 ± 3.1	175.8 ± 3.1	G: 0.68 S: 0.16 GxS: 0.51	172.9 ± 4.0	174.6 ± 2.7	G: 0.68 S: 0.16 GxS: 0.50	174.0 ± 2.2
	Power/Sprint	182.8 ± 9.2	173.3 ± 6.1		169.0 ± 5.6	184.0 ± 8.3		179.0 ± 6.0
	Mixed	181.9 ± 3.9	182.2 ± 4.0		181.4 ± 4.7	181.4 ± 3.7		182.0 ± 3.0
	All sports	179.2 ± 2.7	177.8 ± 2.3		177.6 ± 3.3	179.5 ± 2.2		178.3 ± 1.8
SBPrec3 (mmHg)	**Endurance**	145.3 ± 3.5	143.1 ± 3.6	G: 0.59 S: 0.41 GxS: 0.31	143.5 ± 4.8	144.4 ± 2.9	G: 0.59 S: 0.41 GxS: 0.31	144.1 ± 2.5
	Power/Sprint	147.2 ± 5.8	139.2 ± 4.9		141.0 ± 6.4	145.5 ± 5.3		144.0 ± 4.0
	Mixed	145.6 ± 2.2	150.3 ± 4.2		144.4 ± 2.6	148.5 ± 3.0		147.2 ± 2.0
	All sports	145.8 ± 1.8	145.2 ± 2.5		140.3 ± 1.8	148.7 ± 1.9		145.4 ± 1.5
DBPrec1 (mmHg)	**Endurance**	60.3 ± 2.8	60.3 ± 1.9	G: 0.25 S: 0.36 GxS: 0.58	55.8 ± 3.6	62.6 ± 1.4	G: 0.25 S: 0.36 GxS: 0.58	60.3 ± 1.6
	Power/Sprint	56.4 ± 3.1	64.6 ± 3.4		62.5 ± 2.7	58.2 ± 3.4		59.7 ± 2.5
	Mixed	55.5 ± 2.8	57.5 ± 3.4		54.3 ± 3.8	57.6 ± 2.8		56.1 ± 2.2
	All sports	57.0 ± 1.8	59.9 ± 1.6		56.5 ± 2.4	59.3 ± 1.5		58.3 ± 1.3
DBPrec3 (mmHg)	**Endurance**	71.7 ± 1.2	67.8 ± 1.4	G: 0.95 S: 0.96 GxS: 0.25	68.9 ± 2.2	69.9 ± 1.0	G: 0.95 S: 0.96 GxS: 0.25	69.6 ± 1.0
	Power/Sprint	67.5 ± 3.0	71.2 ± 1.2		68.5 ± 3.7	69.2 ± 2.3		69.0 ± 1.9
	Mixed	69.3 ± 1.4	69.2 ± 2.9		68.9 ± 2.1	69.4 ± 1.8		69.2 ± 1.3
	All sports	69.7 ± 1.0	68.8 ± 1.3		69.8 ± 1.5	69.0 ± 0.9		69.3 ± 0.8
DBPR3 (%)	**Endurance**	125.8 ± 0.1	127.7 ± 0.1	G: 0.93 S: 0.13 GxS: 0.57	145.3 ± 10.9	117.3 ± 4.6	G: 0.93 S: 0.13 GxS: 0.57	126.9 ± 5.3
	Power/Sprint	129.8 ± 0.1	112.1 ± 0.1		110.2 ± 3.8	128.9 ± 7.1		122.4 ± 5.3
	Mixed	138.6 ± 1.4	151.4 ± 0.1		145.6 ± 12.1	141.0 ± 4.6		142.8 ± 8.9
	All sports	134.5 ± 6.6	132.9 ± 6.9		131.6 ± 5.5	135.2 ± 6.9		133.9 ± 4.8
SBPR3 (%)	**Endurance**	86.3 ± 0.04	78.9 ± 0.01	**G: 0.01 S: 0.03** GxS: 0.34	77.1 ± 2.8	82.7 ± 2.1	**G: 0.01 S: 0.03** GxS: 0.34	81.1 ± 1.1
	Power/Sprint	78.1 ± 0.03	67.0 ± 0.08		74.8 ± 9.0	75.0 ± 1.1		74.8 ± 2.5
	Mixed	83.1 ± 0.02	79.3 ± 0.02		81.3 ± 2.4	81.8 ± 1.8		80.0 ± 1.2
	All sports	79.9 ± 1.1	79.2 ± 1.2		78.3 ± 1.3	80.4 ± 1.0		80.5 ± 1.1
Sample size	**Endurance**	16	20		12	24		36
	Power/Sprint	10	7		6	11		17
	Mixed	37	17		20	33		54
	All sports	63	44		38	68		107

Effects from two-way ANOVA with interactions (G, genotype; S, sport; GxS, genotype and sport interaction). Values are shown as mean ± SE.

SBPrest, resting systolic blood pressure; DBPrest, resting diastolic blood pressure; SPBmax, maximal systolic blood pressure; DBPmax, maximal diastolic blood pressure; SBPrec1, systolic blood pressure recovery in 1st minute; SBPrec3, systolic blood pressure recovery in 3rd minute; DBPrec1, diastolic blood pressure recovery in 1st minute; DBPrec3, diastolic blood pressure recovery in 3rd minute; DBPR3, DBPrec3/max × 100 (%); SBPR3, SBPrec3/max × 100 (%).

Table 4. Effect of combined *ACE/ACTN3* genotype and type of sport on blood pressure parameters.

		DD/RR	DD XX + RX	ID + II RR	ID + II XX + RX	Any genotype	ANOVA (p)
SBP rest (mmHg)	**Endurance**	120.0 ± 13.2	113.1 ± 2.0	108.7 ± 4.2	111.9 ± 2.9	112.4 ± 1.9	G: 0.71 S: 0.95 GxS: 0.70
	Power/Sprint	117.5 ± 7.5	113.2 ± 3.1	113.7 ± 2.4	114.1 ± 1.8	114.0 ± 2.0	
	Mixed	112.7 ± 1.6	111.9 ± 2.7	115.7 ± 2.8	113.0 ± 3.3	113.2 ± 1.4	
	All sports	114.4 ± 10.0	112.4 ± 10.5	112.8 ± 8.2	112.4 ± 10.2	112.9 ± 1.0	
DBP rest (mmHg)	**Endurance**	76.7 ± 7.3	73.3 ± 1.8	71.2 ± 2.9	70.4 ± 1.9	72.0 ± 1.3	G: 0.28 **S: 0.01** GxS: 0.98
	Power/Sprint	77.5 ± 7.5	73.2 ± 2.5	73.7 ± 2.4	73.6 ± 2.3	74.0 ± 1.8	
	Mixed	69.8 ± 1.3	69.5 ± 1.8	66.8 ± 3.7	66.2 ± 2.6	68.7 ± 1.0	
	All sports	71.8 ± 7.3	71.1 ± 7.5	70.0 ± 8.1	68.6 ± 7.5	70.4 ± 0.8	
SBP max (mmHg)	**Endurance**	168.0 ± 3.7	179.5 ± 1.7	184.3 ± 10.0	177.1 ± 4.4	178.0 ± 2.6	G: 0.12 S: 0.06 GxS: 0.26
	Power/Sprint	172.5 ± 12.5	208.7 ± 10.7	186.7 ± 17.6	200.0 ± 10.0	197.0 ± 6.9	
	Mixed	185.0 ± 7.4	183.4 ± 4.6	180.0 ± 7.4	191.6 ± 5.6	185.3 ± 3.0	
	All sports	178.9 ± 21.3	186.4 ± 20.4	182.9 ± 23.0	185.1 ± 17.4	184.0 ± 2.1	
DBP max (mmHg)	**Endurance**	54.0 ± 7.5	60.4 ± 1.2	71.2 ± 2.9	62.7 ± 2.2	58.3 ± 1.8	G: 0.65 S: 0.44 GxS: 0.24
	Power/Sprint	57.5 ± 2.5	52.9 ± 3.8	65.8 ± 3.0	63.3 ± 6.7	58.6 ± 2.5	
	Mixed	50.4 ± 5.1	60.4 ± 3.3	55.0 ± 3.7	53.7 ± 4.7	55.8 ± 2.1	
	All sports	52.2 ± 15.6	59.2 ± 11.5	55.1 ± 11.4	59.7 ± 10.8	57.2 ± 1.3	
SBPrec1 (mmHg)	**Endurance**	168.0 ± 3.7	173.6 ± 4.1	176.4 ± 6.1	175.4 ± 3.6	174.0 ± 2.2	G: 0.62 S: 0.17 GxS: 0.70
	Power/Sprint	172.5 ± 12.5	185.7 ± 11.5	166.7 ± 6.7	180.0 ± 10.0	179.0 ± 6.0	
	Mixed	184.1 ± 7.2	179.2 ± 4.6	177.1 ± 4.7	186.6 ± 6.2	182.0 ± 3.0	
	All sports	178.3 ± 20.6	178.8 ± 20.6	175.0 ± 13.8	179.9 ± 15.2	178.3 ± 1.8	
SBPrec3 (mmHg)	**Endurance**	144.0 ± 6.0	145.9 ± 4.4	143.2 ± 7.5	143.1 ± 4.1	144.1 ± 2.5	G: 0.53 S: 0.54 GxS: 0.59
	Power/Sprint	150.0 ± 10.0	146.4 ± 7.3	135.0 ± 7.6	143.3 ± 6.7	144.0 ± 4.0	
	Mixed	145.4 ± 3.1	145.0 ± 4.6	142.8 ± 4.7	156.9 ± 6.0	147.2 ± 2.0	
	All sports	145.5 ± 11.0	145.5 ± 14.7	141.6 ± 15.4	147.9 ± 15.7	145.4 ± 1.5	
DBPrec1 (mmHg)	**Endurance**	54.0 ± 7.5	63.2 ± 2.1	57.1 ± 3.6	62.1 ± 2.1	60.3 ± 1.6	G: 0.57 S: 0.46 GxS: 0.83
	Power/Sprint	57.5 ± 2.5	56.1 ± 4.1	65.8 ± 3.0	63.3 ± 6.7	59.7 ± 2.5	
	Mixed	51.4 ± 5.0	58.1 ± 3.7	58.9 ± 5.8	56.2 ± 4.3	56.1 ± 2.2	
	All sports	52.8 ± 15.4	59.2 ± 13.0	59.4 ± 11.7	60.2 ± 9.7	58.3 ± 1.3	
DBPrec3 (mmHg)	**Endurance**	71.5 ± 2.4	71.8 ± 1.4	67.1 ± 3.3	68.1 ± 1.3	69.6 ± 1.0	G: 0.72 S: 0.90 GxS: 0.72
	Power/Sprint	62.5 ± 7.5	68.9 ± 3.3	72.5 ± 2.5	70.0 ± 1.0	69.0 ± 1.9	
	Mixed	67.9 ± 1.8	70.0 ± 2.1	70.3 ± 4.9	68.1 ± 3.6	69.2 ± 1.3	
	All sports	68.3 ± 6.5	70.3 ± 7.8	69.4 ± 9.9	68.4 ± 6.7	69.3 ± 0.8	
DBPR3 (%)	**Endurance**	139.7 ± 13.6	119.5 ± 3.2	149.3 ± 16.9	151.2 ± 8.4	126.9 ± 5.3	G: 0.98 S: 0.13 GxS: 0.27
	Power/Sprint	109.2 ± 7.5	136.7 ± 6.9	110.8 ± 5.4	133.3 ± 1.3	122.4 ± 5.3	
	Mixed	154.6 ± 18.9	129.2 ± 13.2	131.4 ± 9.1	168.9 ± 2.8	142.8 ± 8.9	
	All sports	1.4 ± 0.5	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.5	133.9 ± 4.8	
SBPR3 (%)	**Endurance**	72.2 ± 0.1	88.7 ± 4.3	77.9 ± 3.2	79.4 ± 1.8	81.1 ± 1.1	**G: 0.03** S: 0.33 GxS: 0.08
	Power/Sprint	90.3 ± 0.9	75.0 ± 1.1	67.0 ± 7.9	74.1 ± 1.6	74.8 ± 2.5	
	Mixed	88.1 ± 2.8	82.0 ± 2.5	76.7 ± 1.8	81.5 ± 2.8	80.0 ± 1.2	
	All sports	85.8 ± 8.0	82.6 ± 9.3	75.8 ± 7.4	80.2 ± 6.5	80.5 ± 1.1	
Sample size	**Endurance**	5	11	7	13	36	
	Power/Sprint	2	8	4	3	17	
	Mixed	13	23	7	10	53	
	All sports	20	42	18	26	106	

Effects from two-way ANOVA with interactions (G, genotype; S, sport; GxS, genotype and sport interaction). Values are shown as mean ± SE.

SBPrest, resting systolic blood pressure; DBPrest, resting diastolic blood pressure; SPBmax, maximal systolic blood pressure; DBPmax, maximal diastolic blood pressure; SBPrec1, systolic blood pressure recovery in 1st minute; SBPrec3, systolic blood pressure recovery in 3rd minute; DBPrec1, diastolic blood pressure recovery in 1st minute; DBPrec3, diastolic blood pressure recovery in 3rd minute; DBPR3, DBPrec3/max ×100 (%); SBPR3, SBPrec3/max ×100 (%).

ing to significant increase of total peripheral resistance and reduction of muscle perfusion. These changes induce the sympathetic nervous system activation, along with cardiac output and the increase of mean arterial pressure. Additionally, upper-body physical activity produces significantly higher systolic and diastolic BP, due to greater cardiovascular strain. Thus, the BP response during power physical activity depends directly on the intensity of physical activity and quantity of activated muscle mass (McArdle et al. 2010).

On the other hand, during endurance physical activity, resistance to peripheral blood flow significantly decreases, while systolic BP increases considerably more than diastolic BP. After an initial rapid rise, systolic BP continues to rise proportionally with the intensity of physical activity. Diastolic BP remains relatively stable or decreases slightly at the higher activity levels. The previously described BP changing pattern is the consequence of increased blood flow during endurance activity, which rapidly increases systolic BP during the first few minutes, but, as activity continues, systolic BP gradually declines, while diastolic BP remains relatively constant.

There is a considerable number of studies investigating the relationship between the *ACE* gene variants, BP and hypertension, since RAS plays an important role in blood pressure regulation, fluid volume and sodium–potassium balance. Also, it is well known that the imbalance of RAS contributes significantly to hypertension development (Mottl et al. 2008; Imbalzano et al. 2017). Some population studies found that D allele of the *ACE* gene is significantly associated with hypertension (Fuentes et al. 2002; Gard 2010), but, to the best of the author's knowledge, there are no studies associating BP parameters with specific *ACE* and *ACTN3* polymorphisms in elite athletes.

It has been shown that the I/D polymorphism of the *ACE* gene is responsible for almost half of the phenotypic variance of serum *ACE* (Mottl et al. 2008). Namely, the *ACE* DD genotype is associated with higher levels of serum ACE (Ay et al. 2007). A number of studies have confirmed that individuals with the DD genotype have a higher incidence of myocardial infarction and other cardiovascular disorders, and even sudden cardiac death (Gard 2010). Additionally, recent studies showed that Caucasians carrying the *ACE* DD genotype have more than 50% increased atherosclerotic risk and endothelium-dependent vasodilatation disability (Imbalzano et al. 2017), and higher carotid stiffness (Sie et al. 2009), which all contribute to cardiovascular disorders.

Some authors proposed that the *ACE* I/D polymorphism may be considered accountable for differences in anabolic response and specific cardiovascular adaptation in athletes subjected to different types of physical activity (Montgomery et al. 1997; Charbonneau et al. 2008). Athletes involved in power sports have lower BP compared to sedentary population, although this decrease is not as large as in other sports, and specifically not as large in individuals with DD genotype, compared to individuals with other genotypes.

A possible mechanism through which the *ACE* DD genotype could influence BP is through the increased expression of this gene, and subsequent increased production of angiotensin II, which, as a potent vasoconstrictor, leads to powerful increase of total peripheral resistance and directly acts to increase BP. Also, increased BP attained during exercise recovers more slowly in athletes with DD genotype, due to physiological mechanisms mediated by increased and prolonged vasoconstriction.

Alpha-actinin-3 is structural protein responsible for fast and powerful muscle contractions, and therefore at least one copy of the 577X allele is advantageous in sport disciplines associated with power/sprint type of physical activity (Ma et al. 2013; Deschamps et al. 2015). Also, the latest scientific data describe a role of this polymorphism, not only in all-cause mortality in humans, but also with increased survival time in patients with chronic heart failure, suggesting a potential extra-sarcomeric role for α-actinin-3 (Bernardez-Pereira et al. 2014). Observed widespread expression of ACTN3, not only in skeletal muscles, but in many other tissues, and especially in pulmonary artery, may be responsible for changes in vascular myogenic tone, and consequently could explain observed differences in BP in athletes in different sports, as well as different vascular response in various types of physical activity (Kim et al. 2014). A recent study from Bernardez-Pereira et al. (2014) confirmed that the R577X polymorphism in the *ACTN3* gene was independently associated with worse survival in patients with chronic heart failure.

The possible mechanism underlying the association of the *ACTN3* RR genotypes and a faster BP recovery pattern may be based on the rate of blood oxygenation after the maximal stress test. Namely, α-actinin-3 is important for maintaining the mechanical structure of myofibrillar array within the muscle fiber. Deficiency of this protein, due to R577X mutation, may influence the arrangement of other contractile proteins and have a negative effect on myogenic tone of pulmonary artery, affecting the rate of blood oxygenation in the lungs. We suppose that the highest values of SBPR3 in RX/XX group, i.e. the slowest recovery observed in our study could be explained by this difference in the contractility of pulmonary artery.

Also, our results are even more important, keeping in mind the fact that the monitoring of the BP recovery pattern in elite athletes is nowadays widely used in clinical practice as one of the inexpensive, valid and, the most importantly, simple indicators of the autonomic nervous system activity (Henríquez et al. 2013). Several studies emphasize that measuring the three-minute recovery BP to calculate the post exercise systolic BP ratio provides the best discrimination between normal and pathological cardiovascular response to physical activity. Namely, values higher than 0.90 reflect an abnormal delay in post exercise recovery systolic BP. This pathological finding could be seen in

exercise-induced ischemia and ischemic LV dysfunction highly susceptible for coronary artery disease (CAD) (Nishiyama et al. 2014). This parameter is easily obtainable, and although hypertension and CAD in athletes are rarely seen, compared to other individuals, this parameter should be taken into account regularly, as it can pinpoint athletes with potentially fatal heart condition (Caselli et al. 2017).

The importance of our study also lies in the fact that our allele frequency data provide useful background information for future studies of the association between BP and examined polymorphisms in elite athletes. Our data also suggest that, during the course of clinical care of elite athletes, genetic screening should be implemented, not only to identify individuals with best prospects for top results in sports, but rather to identify subjects prone to cardiovascular disorders, when exposed to prolonged and intensive training.

In conclusion, athletes with the *ACE* DD genotype showed higher percentage of the systolic BP decline after maximal incremental stress test (SBPR3), while those with the *ACTN3* RR polymorphism showed the lowest maximal systolic BP values. On the other hand, power athletes had the most "unfavourable" BP recovery pattern compared to all other athletes, with the highest values of resting and maximal systolic BP. Observed differences in BP in athletes in different sports, not only according to the type of sport, but also according to the different genetic variants, impose a question of what contributes more to the cardiovascular status in elite athletes, perpetuating the old "nature vs. nurture" dilemma. Obviously, the genetic effect on BP relies not only on *ACE* and *ACTN3* gene, but on the handful of other genes, some of which have the major effect, and some with minor effect. Thus, it would be important to study the combined effect of all these genes on BP, not only in sedentary population, but also in the group of elite athletes. On the other hand, further studies are called for to explain the differences in cardiac features of athletes in elite sports, compared to those involved only in recreational activity, or to the ones with the sedentary lifestyle.

To sum up, we have identified a novel association between *ACE* and *ACTN3* polymorphisms and BP values in elite athletes. We also suggested a plausible mechanism by which these polymorphisms, along with the specific type of physical activity, may influence the BP recovery pattern. Finally, we attempted to link specific genetic variations with BP adaptations in elite athletes subjected to different types of prolonged and intense physical activity. Also, our study could be important not only for coaches who can individually adapt the training regime to the genetic constitution of an athlete, but also for physicians who take care of athletes' health, especially keeping in mind that elite athletes have been exposed to extraordinary intensity and duration of physical activity, usually from a very early age.

Acknowledgments

The authors express their deepest thanks to The Serbian Institute of Sport and Sports Medicine for their effort to accomplish this study. This work was supported by the Ministry of Education and Science of the Republic of Serbia (N° III 41022 and OI 175093).

Conflict of Interest

The authors declare no conflict of interest.

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