The NOS3 -786 T/C polymorphism is associated with power performance in adolescent male basketball players

Original Investigation

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Preferred running head: NOS3 -786 T/C polymorphism and power performance

Abstract word count: 238 words

Text-only word count: 3352 words

Number of tables: 4 tables

Abstract

Purpose: The -786 T/C polymorphism of the NOS3 gene (rs2070744) has been previously linked to athletic performance with the T allele found to be present in higher frequency in power-oriented athletes. Still, what remains elusive is the putative effect of this polymorphism on specific measures of physical fitness and technical condition. In this study we examined whether the NOS3-786 T/C genotype is correlated with specific physical or technical skills in a cohort of adolescent basketball players.

Methods: 85 young basketball players (59 boys and 26 girls) were evaluated in various physical fitness parameters (agility, flexibility, speed, jumping ability, and aerobic capacity) as well as technical skills (dribbling, shooting). All players were also genotyped for the NOS3 -786 T/C polymorphism.

Results: The frequency of the TT genotype was positively correlated with better performance in flying sprints times (10-20 secs; $p=0.05$, 5-10 secs; $p=0.02$, and 5-20 secs; $p=0.01$) as well as with Counter Movement Jump (CMJ) ($p=0.03$) in boys. The heterozygous CT genotype was correlated with better performance in the agility test for girls. In both sexes, no differences were found between the other physical fitness parameters evaluated ($p>0.05$).

Conclusions: The NOS3 -786 TT genotype appears to be correlated with better performance in speeds and jumps in boys. These data agree with current literature associating the -786 TT NOS3 genotype with power elite athletes. The molecular mechanism by which the TT genotype may confer an advantage in power exercises is still under investigation.

Abstract 239 words

Key words: NOS3 gene, -786 T/C polymorphism, basketball, power sports

Introduction

The endothelial nitric oxide synthase gene (NOS3) is located on chromosome 7 (7q36) and encodes NOS3, an enzyme responsible for nitric oxide (NO) production via the conversion of L-arginine to L-citrulline [1]. NO has been shown to have an effect in various physiological processes including being the most potent vasodilator produced by the endothelium. Related to exercise, various studies show that NO is involved in the control of skeletal muscle function, human skeletal muscle glucose uptake during exercise [2] as well as mitochondrial ATP production [3]. All the aforementioned processes can control muscle strength and modulate oxygen consumption in skeletal muscles [4]. Therefore, the NOS3 gene and its genetic variations have been investigated for their putative association with power/sprint performance in individuals [5].

Several genetic variations have been found in the NOS3 gene adding to human individual variability related to exercise. Of those, the NOS3 -786 T/C polymorphism (rs2070744) in the 5'-flanking region of the gene has been found to be associated with changes in gene transcription levels. Specifically, the -786C allele has been shown to result in significantly reduced gene promoter activity, NOS3 mRNA levels, and consequently reduced endothelial NO synthesis [6]. These findings were confirmed by another study where the NOS3 TT genotype was shown to result in higher expression of NOS3 mRNA as well as increased NOS3 activity in isolated platelets [7].

Based on these findings, it is hypothesized that the frequency of the NOS3 -786T/C polymorphism could be different in power vs. endurance athletes or controls. Indeed, data confirmed that the TT genotype is associated with elite power-oriented athletes compared to either endurance or non-athletic controls in Spanish [8,9], Italian [10] and Ukrainian cohorts [11]. Still, what remains elusive is the effect of this polymorphism on specific measures of physical and technical conditions as well as the study of this polymorphism in adolescent athletes.

Basketball is a highly physically demanding game that requires players performing repeated bouts of power actions such as sprinting, shuffling and jumping [12,13]. Therefore, for young basketball players, besides their technical and tactical skills, muscle strength, especially neuromuscular explosiveness and speeds are among the key factors contributing to their future success as elite basketball players [14]. Since the NOS3 -786T/C polymorphism has been linked to elite power-oriented athletes, it could be a candidate to explain, at least partly, individual variations in basketball performance.

The aim of this study was to determine the influence of the NOS3 -786T/C genotype on individual athletic performance as assessed by specific fitness, strength, technical and endurance tests in a cohort of adolescent male and female basketball players. We hypothesized that the NOS3 -786T/C polymorphism would be associated with power performance in our cohort, with the TT genotype posing a favourable influence compared with the CC and/or the CT genotype.

Methods

Participants

Eighty-five (n=85) basketball players (59 male and 26 female), between 14-16 years old (**Table 1**), were recruited from the National Scheme of Youth Development Plan of Cyprus. All participants and their parents signed a consent form and the study has been approved by the National Bioethics Committee of Cyprus (Registry number: ΕΕΒΚ/ΕΠ/2016/23) and conforms to the Code of Ethics of the Worlds Medical Association (Declaration of Helsinki).

Athletes were requested to refrain from strenuous exercise session for at least 48 hours before the field-testing session. Additionally, players that had experienced an injury or were restrained from workouts, at least 15 days prior to measurements, were not included into the evaluation process. Each player was instructed and verbally encouraged to give a maximal effort during all physical fitness tests. A standardized warm-up, consisting of jogging calisthenics and a series of increasing intensity sprints, was performed prior to testing as well as dynamic stretching. Prior to the main trials, all participants had previously performed all the physical-fitness (agility, flexibility, speed, jumping ability, and aerobic capacity) and basketball-skills tests (dribbling, shooting) several times. However, all participants had performed at least 5 supervised familiarization trials in order to further become familiar with the nature of the tests and with the experimental procedure. In all tests, all participants had received equal encouragement and motivation. The evaluation took place in the middle of the competitive season.

Anthropometry

Prior to the initiation of the physical fitness evaluations, height (m), body mass (kg), and body fat percentage were measured. Body mass was obtained to the nearest 0.1 kg using an electronic scale (Seca Instruments Ltd, Hamburg, Germany). Height was measured to the nearest 0.1 cm using a stadiometer (Holtain Ltd, Crymych, United Kingdom). Skinfold thickness was measured at 2 sites (triceps and gastrocnemius) on the left side of the body. Measurements were carried out using a Harpenden caliper (Harpenden Skinfold Caliper, 0.2 mm precision). Measurements were carried out by the same person for all athletes. Body fat percentage was calculated based on the following equation: $\%BF=0,735(\Sigma SKF)+1,0$ where SKF= sum of several skinfolds from the triceps and gastrocnemius muscles [15].

Basketball technique and skills

Dribbling test. Dribbling drills test how a player handles the ball. Players performed a 10,5-m slalom course with cones inside the backcourt using the cross-over dribble. Performance times were measured using photoelectric cells (Brower Timing Systems, Salt Lake City, Utah USA), recording the best of three trials as previously described [16].

Shooting test: All players executed free shots from 5 different spots within 5 meters from the rim of the basket and for a period of one minute. All shots on target were recorded. After each shot, the player would take the rebound himself and move to the next point, in random order [17] (modified from Pojskic et al. 2015).

Functional Capacities

T-Drill Agility Test: Four 35cm collapsible agility cones were arranged as outlined in Semenick et. al (Semenick, 1990). Subjects sprinted forward for 9.14m and touched the tip of the cone (B) with their right hand. Then they performed a lateral shuffle to the left 4.57 m and touched the cone (C) with the left hand. Subjects then changed direction and shuffled 9.14m to the right to touch the cone (D) with their right hand. They then shuffled 4.57m to the left to touch point (B) with their left hand. Finally, the subjects run backwards 9.14m, passing through the finish at point A. Times were measured using an electronic timing system (Brower Timing Systems, Salt Lake City, Utah USA). Each participant performed one sample test and three trials, with the best of these trials recorded.

Standing Long Jump: Leg-Explosiveness was measured using a standing horizontal-jump test (Board Jump: Gill Athletics Standing Long Jump Testing-Mat). Prior to each jump-trial, the participants were asked to jump as long as possible where an arm swing and countermovement was used for supporting the maximum jump. The take-off will from two feet with no preliminary steps or shuffling. Each participant performed one sample test and three trials, with the best of these trials recorded.

Neuromuscular explosiveness. Neuromuscular explosive was estimated through the counter movement jump (CMJ) and the Counter Movement Jump with Arm Swing (CMJ-AS) using an optical measurement system (Optojump Next, Italy). Counter Movement Jump (CMJ) test involves the participant jumping, starting from an upright position, with arms on the waist and was conducted following a protocol, as in Bosco et al, [18], recording the best of two jumps and Counter Movement Jump with Arm Swing (CMJ-AS) test involves the participant jumping, starting from an upright position, with arms free to move recording the best of three jumps.

Speed. Participants also performed a 20m sprint test on the athletics track. Sprint times were measured using photoelectric cells. Photoelectric cells were placed at the 5m, 10m, and 20m distance. Times recorded were at the spot positions of all three distances but also the time interval of 5-20 m. and 10-20 m. respectively. The best time of two trials were recorded (Brower Timing Systems, Salt Lake City, Utah USA). The subjects performed 3 maximal 20-m sprints (with 5- 10- and 20-m split times also recorded) on an indoor synthetic track. During the recovery period between 20-m sprints (2–3 minutes), the subjects walked back to the starting line and then waited for their next sprint. Time was recorded using photocell gates (Brower Timing Systems, Salt Lake City, Utah USA). When ready, the subjects commenced the sprint from a standing start 0.5 m behind the first timing gate. Stance for the start was consistent for each subject. The run with the lowest 20-m time (and corresponding 5- 10- and 20-m split times) was selected for analysis.

Endurance test (VO2max). The players performed a multistage fitness test (Bleep test) to their limit of tolerance [19]. Briefly, the Bleep-test involves continuous shuttle running, between two parallel lines set exactly at 20m apart, in time to recorded bleeps being indicated by signals produced from a commercially available pre-recorded CD (Coachwise Ltd, UK). The participants' initial speed dictated at $2.36ms^{-1}$ and the running speed increased by $0.14ms^{-1}$ each minute. For adding a competition element and aiding of achieving maximum effort, groups of four to six players (based on their playing position) performed the Bleep-test simultaneously. The test was terminated when the participant could no longer maintain the speed-pace, for three consecutive bleeps, as indicated by the audio signal.

The test-retest reliability [20] for performance tests maximum speeds 5, 10 and 20 m, CMJ, CMJ-AS, Broad-jump, T-test agility and Lane drill agility test were $r = 0.88$, $r = 0.97$, $r = 0.98$, $r = 0.96$, $r = 0.99$, $r = 0.94$, $r = 0.94$, $r = 0.94$ respectively.

DNA extraction and genotyping

For each participant that provided consent form, buccal cells were collected by rinsing the mouth for 60s with 15 mL of saline and expectorating the rinse in a 50 mL propylene tube. DNA was extracted using the NucleoSpin® Blood kit (Macherey-Nagel, Germany) and following manufacturer's instructions for salivary DNA extraction. Genomic DNA was then checked for purity. For the identification of the NOS -786 T/C genotype, samples underwent PCR followed by Sanger sequencing. Primers were designed with Primer3 (http://frodo.wi.mit.edu/) software. Primers used were: forward "CTCTGAGGTCTCGAAATCACG" and reverse "GGGACACAAAAGAGCAGGAA". Product size was 338bp and was sequenced using an 3130×l Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) as previously described [21]. Genotyping was successful for all but one participant.

Statistical analysis

Analysis was performed using the IBM SPSS (ver.24) package and the lever of significance was set at $p<0.05$. We compared genotype frequencies in our population as well as between men and women using the x^2 test. We searched for correlations between each NOS3 -786 C/T genotype (CC, CT or TT) and the individual physical and technical parameters examined using ANOVA followed by Tuckey's Post Hoc tests.

Results

NOS3 genotype distributions were in Hardy-Weinberg equilibrium (HWE) in our population ($p = 0.377$) making selection bias less likely. The various genotype frequencies of the studied polymorphism in our cohort of adolescent basketball players compared to "World" and "European" data are depicted in **table 1**. Characteristics of the study participants by sex are shown on **table 2**. The association between the NOS3 -786 T/C polymorphism and studied phenotypes (physical and technical exercises) within each group are presented in **table 3** and **table 4** for men and women, respectively.

Statistically significant differences between each genotype are depicted in bold letters. Results show that the frequency of the TT genotype was positively correlated with better flying sprint times in boys. Specifically, the TT genotype carriers demonstrated a better performance as compared to the CC genotype for the 10-20m ($p=0.05$). For the 5-20m flying sprints the TT genotype carriers demonstrated better performance both compared to the CC ($p=0.05$) and the heterozygous CT carriers ($p=0.04$) and for the 5-20m flying sprints, the TT genotype carriers performed better as compared both to the CC ($p=0.04$) and the CT carriers ($p=0.01$). Additionally, the frequency of the TT genotype was positively correlated with better performance in the CMJ ($p=0.02$) as compared to the CC genotype carriers. In girls we found no correlation between performance in speeds or jumps and the NOS3 -786T/C polymorphism. In girls, it appears to be a correlation between better performance in the agility test with heterozygous CT carriers ($p=0.03$). No other significant correlations were observed in girls. Finally, in boys, the frequency of the TT genotype was positively correlated with better performance in shoots as compared to the CT genotype ($p = 0.013$) but there was no difference as compared to the CC genotype ($p=0.35$). In both sexes, no other differences were found between the other physical and technical training parameters studied ($p>0.05$) **(Tables 3 & 4)**.

Discussion

In the present study we investigated the association between the NOS3 -786 T/C polymorphism and various physical fitness parameters (agility, flexibility, speed, neuromuscular explosiveness, and aerobic capacity) as well as basketball technical skills (dribbling, shooting) in adolescent basketball players. The major finding in our study is the correlation of the TT genotype with improved performance in power exercises in our cohort such as sprints and jumps. This result even though in agreement with our initial hypothesis was found to be true only to the male population. Additionally, some rather surprising results evolved from our study such as that in boys the frequency of the TT genotype was positively correlated with improved performance in shoots as compared to the CT genotype. Additionally, we found a positive correlation between the heterozygous TC genotype and improved performance in the agility tests in our female population; results that are currently not easy to be interpreted. This correlation however, might instigate in the differential physiological parameters between male and females.

The NOS3 -786 T/C polymorphism has been previously linked to power-oriented athletic performance with the T allele being overrepresented in power athletes vs. endurance athletes or controls in various ethnic populations [8,10,11]. Therefore, this is in agreement with our data showing that the TT genotype is correlated with better performance in power exercises in our cohort such as sprints and jumps. Previous studies have shown that the TT genotype results in higher NO production compared to the CC one [6,7,22]. Still, it is important to mention that other studies have tried to link specific genetic variations to power exercises in basketball players with negative results. For example, the ACTN3 R577X polymorphism failed to show any association with explosive power performance in Spanish elite basketball players [14]. Additionally, Guidry et al. examined the influence of two NOS3 SNPs on muscle strength response to resistance training (RT) in adults but found no correlation between the SNPs and response to RT. The team measured dynamic strength of the elbow flexor muscles after a 12w resistance training program[23].

In our data, correlation results for boys and girls differed massively. It is interesting that previous studies have suggested that there are gender differences in NO production due to ovarian hormones such as estrogens [24] and this effect could explain in part the difference in our data between the male and female population.

One strength of our study is that our cohort consisted of U16 basketball athletes. Few studies have examined the association between genetic variation and athletic performance phenotypes in child or adolescent athletes [25-27]. This is unsurprising given the potential ethical considerations of genetic testing in young people. Still, determining genotypic variance and its effect on particular physical parameters could have a practical benefit in young athletes as a personalized approach to training in reaching their full potential.

In our study, no link was found between endurance (VO2 max) and the NOS3 -786 T/C polymorphism, even though NOS3 has been implicated in the past to endurance-oriented athletes. Saunders et al. (2006) for example, found an association between another NOS3 polymorphism (G894T) and the actual performance during the Ironman Triathlons [28], whereas Drozdovska et al. have found significant differences in the frequency of the NOS3 -786 T/C polymorphism allele between 71 endurance-oriented Ukrainian athletes (30 underwater fin swimmers, 41 rowers) and 147 controls[11]. Still, our data are in agreement with other studies showing no correlation between the NOS3 -786 T/C polymorphism and endurance training. Gomez-Gallego et al. for example did not find any differences in the frequency of the NOS3 rs2070744 T allele between 100 Spanish world-class endurance athletes and 100 controls [8] and

Wolfarth et al (2008) examined the contribution of three polymorphisms in the endothelial nitric oxide synthase (NOS3) gene including the -786 T/C polymorphism to discriminate elite endurance athletes from sedentary controls and found no association between this variation and elite endurance performance [29], Therefore, the role of the NOS3 -786 T/C polymorphism in endurance performance is still elusive and requires further investigation.

Mechanistically wise, increased NO has also been shown to play a role in the hypertrophy of skeletal muscles [30] which could explain in part the improved performance in power exercises found in this study. Additionally, it has been shown in animal models that inhibition of NOS activity interferes with normal muscle fiber hypertrophy [31] as well as with the physiological up-regulation of contractile protein gene expression in response to chronic skeletal muscle overload [32]. Therefore, higher NO production (in the TT genotype individuals) could be implicated in improving muscle hypertrophy and hence better performance in sprints and jumps in the individuals carrying this polymorphism. Since, greater amounts of NO may influence muscle hypertrophy, which therefore may be the causal link between these NOS3 genotypes and power performance.

There are limitations in this study that need to be mentioned. Results from genetic association studies should be interpreted with caution as results could always be due to chance especially when the sample size of the population is small. Also, the genotypic distribution varies from one ethnic population to another concerning Caucasians and in Cyprus we do not have data for this polymorphism. We cannot exclude the fact that the results we observe could be attributed to the specificities of the Cyprus population *per se*. The lack of data from a replication cohort of a different ethnic background is also to be kept in mind.

The relatively small sample size of the population tested also limits the “external validity” (and therefore generalization) of our data [33]. It is important to note though, that given the unique characteristics of Cyprus with a population of <1million the total amount of players in the U16 category is 264 (boys) and 156 (in girls), our sample consisted of 22.3% and 16.7% of the total population for boys and girls respectively. Therefore, we believe that the sample size of our basketball cohort is justifiable. Having said that, we plan to repeat this analysis in the future using other athletic cohorts as well as non-athletic populations. Therefore, more research is required to replicate current findings in larger cohorts of basketball players and on other separate cohorts.

Practical Applications

Our data suggest that the NOS3 -786 TT genotype is related to power performance in young (U16) elite male basketball players, so if these data are verified by other studies they could be considered when training programs are designed by basketball coaches and trainers. In addition, our results indicate that the study of this complex phenomenon should proceed through the prism of multidisciplinary big data approach. Use of artificial intelligence hyper-modelling that can handle big data collected from various athletes’ cohorts, might provide strong predictive clues about the development of new athletic training schemes and better management of young athletes.

Conclusions

In summary, in our cohort of young basketball players we identified an association between the presence of the T allele and better performance in dynamic speeds and power exercises in boys only. However, power performance is set by many factors and one sole genetic marker should be interpreted with caution, not being a diagnostic but rather an informative factor. Further research is vital in order to confirm present findings. Importantly, the mechanism behind this association remains elusive and more studies would shed light on the putative role of the NOS3-786 TT genotype in specific power phenotypes.

Acknowledgements

The authors would like to thank the participants and their parents for their excellent collaboration throughout the study period. The authors also are grateful to all participants who kindly provided their samples for DNA analysis. The study was partly funded by the Department of Life and Health Sciences, University of Nicosia. Funding for the DNA sequencing is provided by NIPD.

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Table 1. Genotypic Frequencies [%(n)] of NOS3 -786 T/C polymorphism (rs2070744) in Cypriot (Caucasian, male and female) adolescent basketball players versus frequencies reported in Europe and the World.

NOS3 -786 T/C	**CC**	**CT**	**TT**
Men n=59	0.15 (n=9)	0.37 (n=22)	0.48 (n=28)
Women n=26	0.27 (n=7)	0.38 (n=10)	0.35 (n=9)
Total n=85	0.19 (n=16)	0.38 (n=32)	0.44 (n=37)
World*	0.06 (n=167)	0.34 (n=840)	0.60 (n=1497)
Europe*	0.18 (n=92)	0.51 (n=257)	0.31 (n=154)

* Data are taken from HapMap Project

Table 2. Physical characteristics among the total sample [Mean $\pm$ SD]

Characteristics	Boys n=59	Girls n=26
Age (years)	15.3 ± 0.87	14.46 ± 2.83
Height (m)	1.82 ± 0.07	1.68 ± 0.09
Weight (kg)	72.23 ± 11.56	56.62 ± 16.28
Body Fat (%)	14.80 ± 5.20	22.06 ± 5.03
Aerobic (ml·kg^{-1}·min^{-1})	49.7 ± 5.12	39.7 ± 9.83

Table 3: Means of physical fitness and technical values based on their NOS3 -786 T/C polymorphism (rs2070744) for male basketball players

	CC (n=9)		CT (n=22)		TT (n=28)		ANOVA	
BOYS	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**F**	**Sig.**
Sit and reach test (cm)	19.83	9.06	20.91	7.28	23.57	6.88	1.27	0.29
CMJ (cm)	**32.31**	**4.00**	**35.98**	**5.34**	**37.60**	**4.95**	**3.88**	**0.03**
CMJ-AS (cm)	39.74	4.97	41.47	5.16	44.29	6.03	2.91	0.06
Long Jump (m)	2.12	0.17	2.23	0.19	2.29	0.22	2.40	0.10
T-test agility (sec)	9.54	0.47	9.27	0.56	9.11	0.45	2.58	0.09
Lane drill agility test (sec)	12.44	0.81	12.22	0.92	11.95	0.79	0.85	0.44
Sprint 0-5m (sec):	1.03	0.03	1.02	0.08	1.02	0.08	0.16	0.85
Flying Sprint time 10-20m (sec):	**1.36**	**0.05**	**1.36**	**0.05**	**1.32**	**0.05**	**3.14**	**0.05**
Sprint 0-10m (sec):	1.83	0.04	1.81	0.09	1.78	0.09	1.76	0.18
Sprint 0-20m (sec):	3.19	0.08	3.17	0.13	3.10	0.13	2.84	0.07
Flying Sprint time 5-10m (sec)	**0.80**	**0.03**	**0.79**	**0.05**	**0.76**	**0.04**	**4.53**	**0.02**
Flying Sprint time 5-20m (sec)	**2.15**	**0.07**	**2.15**	**0.08**	**2.08**	**0.07**	**5.49**	**0.01**
Beep Test 20m (VO2max ml/kg/min):	47.48	4.56	50.46	5.14	50.34	4.90	1.20	0.31
Dribbling (sec)	4.05	0.25	3.95	0.39	3.99	0.40	0.23	0.79
Shooting 5 m. (1 min.):	**4.67**	**2.12**	**5.27**	**1.96**	**3.59**	**1.99**	**4.40**	**0.02**

Abbreviations: CMJ: Counter Movement Jump. CMJ-AS: Counter Movement Jump with Arm Swing

Bold values are statistically significant.

Table 4: Means of physical fitness and technical values based on their NOS3 -786 T/C polymorphism (rs2070744) for female basketball players

GIRLS	CC (n=7)		CT(n=10)		TT(n=9)		ANOVA	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**F**	**Sig.**
Sit and reach test (cm)	21.14	6.18	25.10	7.64	24.22	7.16	0.67	0.52
CMJ (cm)	25.89	2.27	25.81	5.44	24.78	3.83	0.19	0.83
CMJ-AS (cm)	30.24	3.13	30.97	6.23	29.83	3.38	0.15	0.87
Long Jump (m)	1.74	0.15	1.78	0.28	1.75	0.16	0.10	0.91
T-test agility (sec)	**10.32**	**0.34**	**10.06**	**0.57**	**10.87**	**0.77**	**4.35**	**0.03**
Lane drill agility test (sec)	13.11	0.37	13.17	0.65	14.43	1.16	2.55	0.15
Sprint 0-5m (sec):	1.11	0.06	1.12	0.07	1.15	0.05	0.79	0.47
Flying Sprint time 10-20m (sec):	1.51	0.04	1.53	0.11	1.52	0.08	0.18	0.83
Sprint 0-10m (sec):	1.96	0.08	1.99	0.12	2.01	0.08	0.60	0.56
Sprint 0-20m (sec):	3.47	0.11	3.53	0.22	3.54	0.14	0.34	0.71
Flying Sprint time 5-10m (sec)	0.85	0.07	0.87	0.06	0.87	0.06	0.17	0.84
Flying Sprint time 5-20m (sec)	2.36	0.10	2.40	0.16	2.39	0.12	0.21	0.81
Beep Test 20m (VO2max ml/kg/min):	43.86	4.69	41.41	6.84	40.54	6.43	0.59	0.56
Dribbling (sec)	4.19	0.35	4.19	0.45	4.44	0.48	0.95	0.40
Shooting 5 m. (1 min.):	3.43	1.62	3.90	1.52	2.22	1.86	2.49	0.11

Abbreviations: CMJ: Counter Movement Jump. CMJ-AS: Counter Movement Jump with Arm Swing

Bold values are statistically significant.