The NOS3 G894T (rs1799983) and -786T/C (rs2070744) polymorphisms are associated with elite swimmer status

INTRODUCTION

Nitric oxide (NO) is a gaseous free radical that is the most potent endothelium-derived relaxation factor [1]. NO is synthesized from arginine by a family of three distinct isoforms of nitric oxide synthase (NOS) enzymes. Both neuronal NOS (nNOS, NOS1) and endothelial NOS (eNOS, NOS3) are constitutively expressed, while inducible NOS (iNOS, NOS2) is not expressed under normal circumstances although it may be induced under stress conditions [2]. A growing body of evidence suggests that NO is involved in glucose metabolism (human skeletal muscle glucose uptake during exercise), control of skeletal muscle structure and function, skeletal muscle fiber type conversion as well as mitochondrial ATP production and oxygen consumption in skeletal muscles [3,4] which are all crucial for aerobic and anaerobic performance. NO also plays an important role in repairing and regeneration of myocardium and in the modulation of oxygen consumption in the myocardium [5].

There are two NOS isoforms that have been identified in skeletal muscle – nNOS and eNOS. nNOS is the primary isofrom to be found in skeletal muscle, whereas eNOS is mainly expressed in endothelial cells and mostly contribute to control of vascular tone. The endothelial nitric oxide synthase (eNOS or NOS3) is encoded by NOS3 gene that is located on chromosome 7 (7q36). The gene spans roughly 21 kb of genomic DNA and comprises 26 exons that encode...
a 1203 amino acids protein [6]. The NOS3 has been extensively screened for polymorphisms and several polymorphic sites have been identified. The most examined and functionally related common variants of the NOS3 are single nucleotide polymorphisms (SNP): promoter -786T/C (rs2070744), G894T (Glu298Asp or, E298D or rs1799983) in exon 7, as well as the variable number tandem repeats (VNTR, microsatellite (CA)n repeats in intron 13 and 27 bp repeats in intron 4) [2].

Several investigations have shown that NOS3 polymorphic variants can lead to altered transcription of eNOS and/or processing rates, and in that way interfering with normal enzyme function [7]. On the basis of these facts, the eNOS gene variants have been extensively investigated to determine their relevance in various diseases such as myocardial infarction, hypertension, coronary artery disease, stroke, and evidence supports a major role of these variants in increasing susceptibility to cardiovascular disease [2].

Numerous candidate gene studies that have been performed also indicate that NOS3 gene -786T/C, G894T and VNTR polymorphisms are associated with several health/fitness, training or exercise response phenotypes, e.g. adaptation of parasympathetic modulation response to exercise training [8], cardiovascular traits such as blood pressure [9] and heart rate responses [10], cardio-biochemical parameters [11], vascular reactivity [12], exercise-induced adaptation to hypoxia [13] and aerobic capacity of athletes [14]. Collectively, these data suggest that NOS3 and its polymorphisms are logical candidates for association with athlete status and physical performance.

In fact, -786T/C and G894T variants of NOS3 have been associated with endurance performance, elite endurance athlete [13,15], power athlete [16,17] and soccer player’s [18] statuses, as well as with the differentiation of elite power from endurance athletes [19]. More specifically, significant differences in the frequency of the NOS3 786T/C T allele between Spanish world-class endurance athletes and controls [16]. As to the power athlete status, Drozdovska et al. (2012) have found that the frequency of the NOS3 786T/C T allele was significantly higher in Ukrainian power-oriented athletes than healthy controls [20]. These results were confirmed in two independent studies of Spanish elite power-oriented athletes and non-athletes [16] and Italian power-oriented athletes [17].

With respect to the NOS3 G894T polymorphism and its relation to athletic performance and/or athletes' status, results across various studies have been inconsistent. Saunders et al. reported an excess of the G894T G allele (combined with a BDKRB2-9/-9 genotype) in the fastest finishing Caucasian Ironman triathletes when compared with control subjects [15]. However, Wolfarth et al. in the Genathlete study found no difference in allele and genotype frequencies of the G894T polymorphism between elite endurance athletes and controls [21]. Likewise, no differences in allele and genotype frequencies of the G894T were found by Buxens et al. between elite Spanish Caucasian elite endurance and power-oriented athletes [22]. On the other hand, Sessa et al. have shown that the G894T G allele was over-represented in Italian power-oriented athletes compared to controls [17].

Taken together, although inconsistent results exist, previous findings indicate that more common NOS3 alleles (i.e., -786T/C T with frequency of ~ 56% in European populations and G894T G with frequency of ~ 66% in European populations) may be favorable for both power and endurance performance [23].

There are some behavioral variability and motor performance differences as an effect of practice specialization in swimming between competitive short distance swimming vs. long distance swimmers [24]. However regular swimming modulate oxidant-antioxidant balance via the involvement of the endothelial NO system [25] where vascular adaptive response involved an increase in endothelial nitric oxide is induced by swimming exercises [26]. Previously we suggested that the response to long-term exercise training could be modulated by the BDKRB2 polymorphism in competitive male swimmers [27]. In this article we hypothesize that the NOS3 G894T and -786T/C polymorphisms may influence swimming performance in Polish competitive swimmers.

The purpose of this study was to determine the association between the NOS3 G894T and -786T/C polymorphisms with elite swimmer status in Polish athletes.

MATERIALS AND METHODS

**Ethics Committee**

The Pomeranian Medical University Ethics Committee, Poland, approved the study and an informed consent form was completed by each participant. The study complied with the guidelines set out in the Declaration of Helsinki.

**Participants**

One hundred and ninety-six Polish swimmers (104 males and 93 females; 20.31 ± 2.67 years), who competed in national and international events, were recruited for this study. All participants were Caucasians to minimize the influence of racial genetic skew and to remove any potential population stratification problems. The swimmers were divided into two groups, based on their competitive distance and values of relative contribution of the aerobic or/anaerobic energy systems: long distance swimmers (LDS; n=49; 24 males, 25 females), more than 500 m (mainly aerobic events) and short distance swimmers (SDS; n=147; 80 males, 67 females), between 50 and 200 m (mainly anaerobic events).

All investigated swimmers had been finalists of the Polish National Championships. Additionally, 7 of them had participated in the Olympic Games and 48 of them had taken part in the World Championships or European Championships. The whole group of swimmers included 8 World Championship medalists, 15 European Championship medalists and 128 Polish Championship medalists.
### Table 1. Frequencies of the NOS3 gene G894T and -786T/C genotypes, alleles and haplotypes in Polish swimmers and controls.

| Group | n  | NOS3 G894T genotypes | Glu298 allele, % | P  | NOS3 -786T/C genotypes | HWE T allele, % | P  | NOS3 haplotypes |
|-------|----|----------------------|-------------------|----|------------------------|-----------------|----|----------------|
|       |    | GG (%) | GT (%) | TT (%) | HWE (%) | % | GG (%) | GT (%) | TT (%) | HWE (%) | % | G-T (%) | G-C (%) | T-C (%) | T-T (%) | p$ |
| MALES |    |        |        |        |        |    |        |        |        |        |    |        |        |        |        |     |
| Controls | 222 | 113 (50.9) | 89 (40.1) | 20 (9.0) | 1.000 | 0.744 | 71.0 | 1.000 | 92 (41.4) | 87 (39.2) | 43 (19.4) | 1.000 | 0.011* | 61.0 | 1.000 | 47.4 | 23.6 | 15.4 | 13.6 | 1.000 |
| SDS | 80 | 46 (57.5) | 27 (33.8) | 7 (8.7) | 0.575 | 0.376 | 74.4 | 0.408 | 0.469 | 38 (47.5) | 29 (36.3) | 13 (16.2) | 0.624 | 0.085 | 65.6 | 0.305 | 0.351 | 0.087† | 0.596† | 0.042† | 0.005 |
| LDS | 24 | 14 (58.3) | 9 (37.5) | 1 (4.2) | 0.649 | 1.000 | 77.1 | 0.371 | 0.467 | 12 (50.0) | 11 (45.8) | 1 (4.2) | 0.181 | 0.636 | 72.9 | 0.107 | 0.145 | 0.110† | 0.347† | 0.232† | 0.990† | 0.453 |
| FEMALES |    |        |        |        |        |    |        |        |        |        |    |        |        |        |        |     |
| Controls | 157 | 104 (66.2) | 48 (30.6) | 5 (3.2) | 1.000 | 1.000 | 81.5 | 1.000 | 63 (40.1) | 81 (51.6) | 13 (8.3) | 1.000 | 0.077 | 65.9 | 1.000 | 58.7 | 22.8 | 11.3 | 7.2 | 1.000 |
| SDS | 67 | 30 (44.8) | 28 (41.8) | 9 (13.4) | 0.001 | 0.590 | 65.7 | 0.00027 | 0.00043 | 37.3 | 0.109 | 0.613 | 59.7 | 0.209 | 48.3 | 0.250 | 0.198† | 0.001† | 0.062† | 0.005 |
| LDS | 26 | 17 (65.4) | 7 (26.9) | 2 (7.7) | 0.526 | 0.288 | 78.9 | 0.647 | 0.790 | 16 (61.5) | 10 (38.5) | 0 (0.0) | 0.071 | 0.545 | 80.8 | 0.033 | 67.6 | 0.049 | 0.195† | 0.035† | 0.427† | 0.112† | 0.079 |
| TOTAL |    |        |        |        |        |    |        |        |        |        |    |        |        |        |        |     |
| Controls | 379 | 217 (57.3) | 137 (36.1) | 25 (6.6) | 1.000 | 0.582 | 75.3 | 1.000 | 155 (40.9) | 168 (44.3) | 56 (14.8) | 1.000 | 0.377 | 63.1 | 1.000 | 52.0 | 23.3 | 13.6 | 11.1 | 1.000 |
| SDS | 147 | 76 (51.7) | 55 (37.4) | 16 (10.9) | 0.209 | 0.235 | 70.4 | 0.103 | 0.120 | 63 (42.9) | 59 (40.1) | 25 (17.0) | 0.647 | 0.111 | 62.9 | 0.964 | 54.3 | 16.1 | 21.0 | 8.6 | 0.007 |
| LDS | 50 | 31 (62.0) | 16 (32.0) | 3 (6.0) | 0.815 | 0.680 | 78.0 | 0.559 | 0.645 | 28 (56.0) | 21 (42.0) | 1 (2.0) | 0.020* | 0.419 | 77.0 | 0.0061 | 63.7 | 14.3 | 8.7 | 13.3 | 0.064 |

*p values were calculated by $\chi^2$ test and $\chi^2$ test with Yates' correction for comparisons between groups of athletes and control group. HWE – $\chi^2$ test for Hardy-Weinberg equilibrium, †global score statistic, additive haplotype effect, ‡versus Controls.
A control group of healthy individuals (n = 379; 222 males and
157 females; 22.6 ± 2.8 years) was also selected from the Polish
population (college students) with no background in swimming.

Genetic analyses
The buccal cells donated by the participants were collected in Re-
suspension Solution (GenElute Mammalian Genomic DNA Miniprep
Kit, Sigma, Germany) using sterile foam-tipped applicators (Puntan,
USA). DNA was extracted from the buccal cells using a GenElute
Mammalian Genomic DNA Miniprep Kit according to the manufac-
turer’s protocol.

All samples were genotyped using an allelic discrimination assay
on a CFX96 Touch™ Real-Time PCR Detection System (Biorad, USA)
with TaqMan probes. For discrimination between the NOS3 G894T
(rs1799983) G and T as well as NOS3 -786T/C (rs2070744) T and
C alleles, TaqMan Pre-Designed SNP Genotyping Assays were used
(Applied Biosystems, USA), including primers and fluorecently la-
belled (FAM and VIC) MGB probes. The Glu298Asp and rs2070744
T/C genotypes were determined for 197 out of 198 DNA samples of
athletes and 379 out of 381 DNA samples of controls. Genotyping
error was assessed as 1%, while the call rate (the proportion of
samples in which the genotyping provided unambiguous reading)
was above 95%.

Statistical analysis
The STATISTICA statistical package (version 8.0, StatSoft Inc.,
Tulsa, Oklahoma, USA) was used to perform all analyses. The Har-
dy-Weinberg equilibrium (HWE) for NOS3 G894T and -786T/C
genotypes was assessed separately in swimmers and control subjects
with a \( \chi^2 \) test. Genotype distributions as well as allele frequencies
were determined and \( \chi^2 \)tests were used to compare the NOS3 G894T
and -786T/C alleles and genotypes between the groups of
athletes and control participants. The analysis of the individual
effects of these variants was based on three genetic models: gen-
eral, dominant and recessive. The odds ratio (OR) with 95% confi-
dence intervals were calculated. For haplotype analysis, haplo.stats
package for R (http://cran.r-project.org) was used. Global and hap-
lotype-specific tests for associations were conducted using haplo.
score function, while haplo.group function was used to estimate
haplotype frequencies. Bonferroni’s correction for multiple testing
was applied, and the alpha level for determining statistical signifi-
cance was set at \( P< 0.025 \).

RESULTS
Genotype distributions of all athletes including LDS group and SDS
group as well as sedentary controls met Hardy-Weinberg equilibri-
um (all \( P > 0.05 \)). The results of the distribution of alleles and genotypes
for both the NOS3 G894T and -786T/C polymorphic sites in Polish
athletes compared with the non-athletic controls (all subjects strat-
ified by gender) are presented in Table 1.

The genotype as well as allele distributions of the G894T poly-
morphic site were not significantly different when long distance swim-
mers and short distance swimmers were compared to sedentary
controls (Table 1). However, a sex-specific analysis indicated sig-
ificant differences in genotype distribution between female athletes
and female controls. The data showed that the genotype distribution
and allele frequencies amongst the female SDS (n = 67): 44.8% GG,
41.8% GT, 13.4% TT, the Asp allele frequency 34.3%, differed
significantly from female control subjects (n = 157): 66.2% GG,
30.6% GT, 3.2% TT, the T allele frequency 18.5%; \( p = 0.0013 \)
(Chi2=13.2, df=2), \( p = 0.00043 \) (Chi2=12.4, df=1), for genotype
and allele frequencies respectively (Table 1).

There were significant NOS3 G894T genotype-dependent differ-
ces in the likelihood of being classified as a short distance swim-
ner under general, dominant and recessive models in female SDS
group. Assuming general model, relative to female control subjects,
carriers of the GG genotype of G894T polymorphism were less like-
ly to be found in the SDS group than TT homozygotes (OR 0.16
[CI=0.05-0.514], \( p = 0.0021 \)). On the anther hand, the chance of
being a female short distance swimmer was 6.24 times higher
(Chi2=1.94-20.03, \( p = 0.0021 \)) for the TT than in a female control.
These results indicate that the T allele of the G894T may be benefi-
cial for short distance swimmers compared to control subjects.

In reference to the second SNP analyzed in the presented study,
a \( \chi^2 \) test revealed that the genotype as well as allele frequencies
of the -786T/C polymorphic site were significantly different when long
distance swimmers group (n = 50): 56.0% TT, 42.0% TC, 2% CC,
the T allele frequency 77.0%, was compared to sedentary controls
(n = 379): 40.9% TT, 44.3% TC, 14.8% CC, the T allele frequency
63.1%; \( p = 0.019 (\chi^2 = 7.8, df=2), p = 0.0085 (\chi^2 = 6.9, df=1) \), for
genoype and allele frequencies respectively (Table 1). Further ana-
lyzes showed that the frequency of the T allele of the -786T/C SNP
was higher, however not statistically significant, in the female LDS
group that in the female controls (80.8% vs. 65.9%); \( p = 0.049 \)
(\( \chi^2 = 3.7, df=1, \) Table 1). The athletes from LDS group were more
likely than controls to possess TT genotype (TT genotype compared
to the CC [OR 10.11, CI=1.34–76.10], \( p = 0.026 \); TT compared
to both TC and CC genotypes [OR 1.84, CI=1.01–3.33], \( p = 0.047 \),
both \( p \) values statistically not significant). It was also observed
that the T allele carriers (TT+TC) were over-represented in LDS
mer under general, dominant and recessive models in female SDS
group. Assuming general model, relative to female control subjects,
carriers of the GG genotype of G894T polymorphism were less like-
ly to be found in the SDS group than TT homozygotes (OR 0.16
[CI=0.05-0.514], \( p = 0.0021 \)). On the anther hand, the chance of
being a female short distance swimmer was 6.24 times higher
(Chi2=1.94-20.03, \( p = 0.0021 \)) for the TT than in a female control.
These results indicate that the T allele of the G894T may be benefi-
cial for short distance swimmers compared to control subjects.

In reference to the second SNP analyzed in the presented study,
a \( \chi^2 \) test revealed that the genotype as well as allele frequencies
of the -786T/C polymorphic site were significantly different when long
distance swimmers group (n = 50): 56.0% TT, 42.0% TC, 2% CC,
the T allele frequency 77.0%, was compared to sedentary controls
(n = 379): 40.9% TT, 44.3% TC, 14.8% CC, the T allele frequency
63.1%; \( p = 0.019 (\chi^2 = 7.8, df=2), p = 0.0085 (\chi^2 = 6.9, df=1) \), for
genoype and allele frequencies respectively (Table 1). Further ana-
lyzes showed that the frequency of the T allele of the -786T/C SNP
was higher, however not statistically significant, in the female LDS
group that in the female controls (80.8% vs. 65.9%); \( p = 0.049 \)
(\( \chi^2 = 3.7, df=1, \) Table 1). The athletes from LDS group were more
likely than controls to possess TT genotype (TT genotype compared
to the CC [OR 10.11, CI=1.34–76.10], \( p = 0.026 \); TT compared
to both TC and CC genotypes [OR 1.84, CI=1.01–3.33], \( p = 0.047 \),
both \( p \) values statistically not significant). It was also observed
that the T allele carriers (TT+TC) were over-represented in LDS
swimmers group. Specifically, when compared with the CC genotype,
the chance of being a long distance swimmer was 8.49 times higher
(Chi2=1.94-20.03, \( p = 0.023 \)) for the carriers of T allele than in
control subjects. Further data analyzes showed that the allele fre-
quency amongst the female LDS (n = 26), the T allele frequency
19.2%, differed from female control subjects (n = 157), the T allele
frequency 34.1%; \( p = 0.049 (\text{Chi}^2=3.9, df=1), \) Table 1). There
was also NOS3 -786T/C genotype-dependent differences, however
not statistically significant, in the likelihood of being classified as
a long distance swimmer under dominant mode in female LDS group.
It was observed that the chance of being a long distance swimmer
was 2.39 times higher (Chi2=1.02-5.60, \( p = 0.045 \)) for the holders
of TT genotype than in a female control subjects. Presented results
The NOS3 G894T (rs1799983) and -786T/C (rs2070744) polymorphisms demonstrate that the T allele of the -786T/C may be beneficial for long distance swimmers compared to control subjects. The haplotype analysis revealed that in presented study four haplotypes (G-T, G-C, T-C and T-T) occur in both the athletes and sedentary controls (Table 1). The linkage disequilibrium between analysed loci was D’=0.287 and D’=0.474 for control subjects and all athletes, respectively. A statistically significant difference was observed in the frequency of the all four haplotypes between SDS (both men and women) and control (p=0.007), with excess of the T-C haplotype observed in the SDS subgroup compared with the controls (21.0% vs. 13.6%, p=0.015). A sex-specific analysis indicated, that haplotype frequencies amongst the female SDS differed significantly from female control subjects (p=0.005), and T-C haplotype was over-represented in the female SDS compared to the female controls (22.9% vs. 11.3%, p=0.001). We also identified that the G-T haplotype was over-represented in the LDS group compared with controls (63.7 vs. 52.0, p=0.025).

DISCUSSION

Previously we observed a significant over-representation of the GG genotype and the G allele of the G894T polymorphic site irrespective of athletes’ status, i.e. type and intensity of exercise performed (power-oriented, endurance-oriented, or “mixed”) [28]. Comparison of sedentary control subject and whole athlete cohort led us to the conclusion that G894T polymorphism may be associated with overall physical fitness and physical ability, no matter what type of sports activity the athletes are involved in [28]. Additionally, we observed a tendency towards an increase in both the GG genotype and the G allele frequency in relation to a smaller aerobic component of physical ability. We interpret it that the G allele promotes power-oriented sport events.

In this paper the G894T genotype was determined for 197 simmers and 379 control individuals. HWE tested for genotype distributions of LDL and SDL athletes for both the NOS3 G894T polymorphic site in Polish athletes compared with the non-athletic controls allow to state there is no divergence from the expected values (in all p > 0.05.) One could expect this as the Poland become still highly genetically constant and migration as well as transnational marriage is still seldom also among athletes.

In this study we observed that the G894T G allele is disadvantageous in female SDS swimmers since the chance of being a female short distance swimmer was 6.24 times higher for the TT than in a female control. Additionally, GG homozygotes were significantly under-represented in female SDS swimmers group.

Previous studies delivered inconsistent results on the association between the G894T NOS3 polymorphism and athletic performance. Until today some research group do not confirm such dependencies. Among them, the Genathlete study did not find any differences in allele and genotype frequencies of the G894T between elite endurance athletes and controls [21]. Also when we examined elite Polish rowers we found no evidence of association between the G894T and endurance performance [29]. Likewise, no differences in allele and genotype frequencies of the G894T were found between elite Spanish Caucasian elite endurance and power-oriented athletes [22]. The missense type G894T polymorphism within exon 7 NOS3 gene is one of the most analyzed polymorphism within this gene among several others like, microsatellite (CA)n repeats in intron 13, and 27-bp repeats in intron 4 (4B/4A) variations [30].

The promising expectations are based on facts that NOS3 G894T is associated with the BP [9], HR, and stroke volume responses to submaximal and maximal aerobic exercise [10], the no exercising muscle vasodilation response to isometric handgrip exercise [12], and parasympathetic modulation response to submaximal aerobic exercise [8].

Physical training causes changes of arterial tension and increasing the cross section area of the medium and small arteries [31]. In large vessels a vascular resistance to blood flow is small. In the aorta, adaptive changes associated with training are less visible than in the smaller arterioles located in skeletal muscle [32]. Physical training causes an increase in blood flow in the vessel and a consequent increase shear stress, causing deformation of the mechanosensitive channel of endothelial cells [33]. Following separation cascade is initiated a series of compounds that affect the size of the local movement. Nitric oxide is one of the most important factors dishes diastolic produced by the endothelium [34]. Adjusting the flow of blood by increasing the production of NO increases the speed of transport endothelium, and thus supply the necessary ingredients myocytes [35]. Remodeling of the arteries in athletes, is also largely related with increased release of NO by the endothelium [36].

For the -786T/C polymorphic site genotypes and allele frequencies were significantly different when long distance swimmers vs. short distance swimmers where compared. Frequency of the T allele of the -786T/C SNP and TT genotype was over-represented in the female LDS group. It means that the T allele of the -786T/C may be beneficial for long distance swimmers compared to control subjects since chance of being a long distance swimmer was 2.39 times higher for the TT genotype than in a female control subjects. In the opposite, T-C haplotype was over-represented in the female SDS compared to the female controls. Interesting conclusions may be brought in studies by Gomez-Gallego et al., who found a significant association between an intronic polymorphism -786T/C, and elite performance in strength/ power sports [16]. Expression of eNOS is subject to fluctuations in response to growth factors, hormones, cytokines released during exercise [37]. Depending on the intensity and applied volume of physical exercise, they are induced in a vessel different pattern of shear forces and transient hypoxia [36].

It was shown that shear forces vary depending on whether the physical effort is of the aerobic or anaerobic, and which ultimately results in specific changes among the endothelial function [34]. Adaptive changes in endothelial function associated with athletic training are dependent on the activity of endothelial nitric oxide synthase [33] and environmental redox prevailing in the vessel. The
level of oxidative damage to cells, production of free radicals or the efficiency of the antioxidant system [34].

Increased blood flow in a blood vessel associated with physical training exercises while induced increase in the level of mRNA and protein expression, and therefore an increase in eNOS activity and production of nitric oxide [38]. Increase in eNOS activity also occurs after the training [39], but is independent of intracellular Ca²⁺ and triggered by some phosphorylation of the enzyme molecule.

It can therefore be assumed that the increased trend to reduce the activity of eNOS was associated with decreased antioxidant capacity of blood plasma [40] pointed out that the very intense, longstanding training can cause adverse changes in endothelial function correlated with lower levels of antioxidant system and the increase in oxidative stress.

**CONCLUSION**

In conclusion, although our study is restricted to two SNPs and the statistical power suffers from low sample size, it offers suggestion that the Glu allele of the NOS3 G894T polymorphic site is disadvantageous in female SDS swimmers and the T allele of the -786T/C polymorphism may be beneficial for long distance swimmers.

**REFERENCES**

1. Féletou M. The Endothelium, Part I: Multiple Functions of the Endothelial Cells – Focus on Endothelium-Derived Vasoactive Mediators. Colloq Ser Integr Syst Physiol From Mol to Funct. 2011;3(4):1–306.

2. Vecoli C. Endothelial Nitric Oxide Synthase Gene Polymorphisms in Cardiovascular Disease. Vitamins and hormones. 2014. p. 387–406.

3. Gao Y. The multiple actions of NO. Pflugers Arch. 2010;459(6):829–39.

4. Martins KJB, St-Louis M, Murdoch GK, MacLean IM, McDonald P, Dixon WT, Putman CT, Michel RN. Endothelial nitric oxide synthase inhibition prevents activity-induced calcineurin-NFATC1 signalling and fast-to-slow skeletal muscle fibre type conversions. J Physiol. 2012;596(6):1427–42.

5. Lokke KE, Laycock SK, Mital S, Wolin MS, Bernstein R, Oz M, Addonizio L, Kaley G, Hintze TH. Nitric oxide modulates mitochondrial respiration in failing human heart. Circulation. 1999;100(12):1291–7.

6. Marsden PA, Heng HH, Scherer SW, Stewart RJ, Hall A V, Shi XM, Tsui LC, Schappert KT. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. J Biol Chem. 1993;268(23):17478–86.

7. Nakayama M, Yasue H, Yoshimura M, Shimasaki Y, Kugiyama K, Ogawa H, Nakayama M, Yasue H, Yoshimura M, 268(23):17478–88.

8. Silva BM, Neves FJ, Negrão M V, Alves CR, Dias RG, Alves GB, Pereira AC, Rondon MUPB, Dos Santos MR, Krieger MH, Negrão CE, DA Nóbrega ACL. Endothelial nitric oxide synthase polymorphisms and adaptation of parasympathetic modulation to exercise training. Med Sci Sports Exerc. 2011;43(9):1611–8.

9. Rankinen T, Rice T, Pérusse L, Chagnon YC, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C. NOS3 Glu298Asp genotype and blood pressure response to endurance training: the HERITAGE family study. Hypertens (Dallas, Tex 1979). 2000;36(5):885–9.

10. Hand BD, McCole SD, Brown MD, Park JJ, Ferrell RE, Huberty A, Douglass LW, Hagberg JM. NOS3 gene polymorphisms and exercise hemodynamics in postmenopausal women. Int J Sports Med. 2006;27(12):951–8.

11. Rezende TM, Sponton CHG, Malagrinho PA, Bezerra MAC, Penteado CFF, Zanesco A. Effect of exercise training on the cardiovascular and biochemical parameters in women with eNOS gene polymorphism. Arch Physiol Biochem. 2011;117(5):265–9.

12. Dias RG, Alves M-JNN, Pereira AC, Rondon MUPB, Dos Santos MR, Krieger JE, Krieger MH, Negrão CE. Glu298Asp eNOS gene polymorphism causes attenuation in nonexercising muscle vasodilatation. Physiol Genomics. 2009;37(2):99–107.

13. Drozdovska S, Dosenko V, Ilyin V, Filipovov M, Kuzmina L. Allelic Polymorphism of Endothelial No-Synthase (eNOS) Association with Exercise-Induced Hypoxia Adaptation. Bait J Heal Phys Act. 2009;11(1):13–9.

14. Ahmetov II, Khakimullina AM, Popov D V, Missina SS, Vinogradova OI, Rogozkin VA. Polymorphism of the vascular endothelial growth factor gene (VEGF) and aerobic performance in athletes. Hum Physiol. 2008;34(4):477–81.

15. Saunders CJ, Xenophontos SL, Carloulu M a, Anastassiades LC, Noakes TD, Collins M. The bradykinin beta 2 receptor (BDKRB2) and endothelial nitric oxide synthase 3 (NOS3) genes and endurance performance during Ironman Triathlons.

16. Gómez-Gallego F, Ruiz JR, Buxens A, Artieda M, Arteta D, Santiago C, Rodriguez-Romo G, Lao Ji, Lucia A. The -786 T/C polymorphism of the NOS3 gene is associated with elite performance in power sports. Eur J Appl Physiol. 2009;107(5):565–9.

17. Sessa F, Chetta M, Petito A, Franzetti M, Bafunno V, Pisanelli D, Sarno M, Iuso S, Margaglione M. Gene polymorphisms and sport attitude in Italian athletes. Genet Test Mol Biomarkers. 2011;15(4):285–90.

18. Eynon N, Ruiz JR, Yvert T, Santiago C, Gómez-Gallego F, Lucia A, Birg R. The C allele in NOS3 -786 T/C polymorphism is associated with elite soccer player's status. Int J Sports Med. 2012;33(7):521–4.

19. Gómez-Gallego F, Ruiz JR, Buxens A, Altmae S, Artieda M, Santiago C, Gonzalez-Freire M, Verde Z, Arteta D, Martinez A, Tejedor D, Lao Ji, Arenas J, Lucia A. Are elite endurance athletes genetically predisposed to lower disease risk? Physiol Genomics. 2010;41(1):82–90.

20. Drozdovska SB, Dosenko VE, Ahmetov II, Iljin VN. The association of gene polymorphisms with athlete status in Ukrainians. Biol Sport. 2013; 30(3):163–7.

21. Wolfarth B, Rankinen T, Mühlbauer S, Dude M, Rauamara R, Boulay MR, Pérusse L, Bouchard C. Endothelial nitric oxide synthase gene polymorphism and elite endurance athlete status: the Genathlete study. Scand J Med Sci Sports. 2008;18(4):485–90.

22. Buxens A, Ruiz JR, Arteta D, Artieda M, Santiago C, Gonzalez-Freire M, Martinez A, Tejedor D, Lao Ji, Gómez-Gallego F, Lucia A. Can we predict top-level sports performance in power vs endurance events? A genetic approach. Scand J Med Sci Sports. 2011;21(4):570–9.

23. Ahmetov II, Fedotovskaya ON. Current
Progress in Sports Genomics. Adv Clin Chem. 2015;70:247–314.

24. Seifert L, De Jesus K, Komar J, Ribeiro J, Abraldes JA, Figueiredo P, Vilas-Boas JP, Fernandes RJ. Behavioural variability and motor performance: Effect of practice specialization in front crawl swimming. Hum Mov Sci. 2016;47:141–50.

25. Kumral ZNO, Sener G, Ozgur S, Koc M, Suleymanoglu S, Hurdag C, Yegen BC. Regular exercise alleviates renovascular hypertension-induced cardiac/endothelial dysfunction and oxidative injury in rats. J Physiol Pharmacol. 2016;67(1):45–55.

26. Bruder-Nascimento T, Silva ST, Boer PA, Cordellini S. Effects of exercise training on stress-induced vascular reactivity alterations: role of nitric oxide and prostanoids. Brazilian J Phys Ther. 2015;19(3):177–85.

27. Zmiijewski P, Grenda A, Leoriska-Duniec A, Ahmetov I, Orysiak J, Cięszczyk P. Effect of BDKRB2 Gene -9/+9 Polymorphism on Training Improvements in Competitive Swimmers. J strength Cond Res. 2016;30(3):665–71.

28. Eider J, Ficek K, Kaczmarczyk M, Maciejewska-Kartowska A, Sawczuk M, Cięszczyk P. Endothelial nitric oxide synthase g894t (rs1799983) gene polymorphism in polish athletes. Open Life Sci. 2014;9(3):260–7.

29. Cięszczyk P, Sawczuk M, Maciejewska A, Jascaniene N, Eider J. Do G894T Polymorphisms of Endothelial Nitric Oxide Synthase 3 (NOS3) Influence Endurance Phenotypes? J Hum Kinet. 2010;24(1):73–80.

30. Ahmetov II, Egorova ES, Gabdrakhmanova LJ, Fedotovskaya ON. Genes and Athletic Performance: An Update. Medicine and Sport Science. 2016. p. 41–54.

31. McAllister RM, Albarracin I, Price EM, Smith TK, Turk JR, Wyatt KD. Thyroid status and nitric oxide in rat arterial vessels. J Endocrinol. 2005;185(1):111–9.

32. Huonker M, Schmid A, Schmidt-Trucksass A, Grathwohl D, Keul J. Size and blood flow of central and peripheral arteries in highly trained able-bodied and disabled athletes. J Appl Physiol. 2003;95(2):685–91.

33. Hambrecht R. Regular Physical Activity Improves Endothelial Function in Patients With Coronary Artery Disease by Increasing Phosphorylation of Endothelial Nitric Oxide Synthase. Circulation. 2003;107(25):3152–8.

34. Tinken TM, Thijssen DHJ, Hopkins N, Dawson EA, Cable NT, Green DJ. Shear stress mediates endothelial adaptations to exercise training in humans. Hypertens (Dallas, Tex 1979). 2010;55(2):312–8.

35. Chang YS, Yaccino JA, Lakshminarayanan S, Frangos JA, Tarbell JM. Shear-induced increase in hydraulic conductivity in endothelial cells is mediated by a nitric oxide-dependent mechanism. Arterioscler Thromb Vasc Biol. 2000;20(1):35–42.

36. Green DJ, Spence A, Rowley N, Thijssen DHJ, Naylor LH. Vascular adaptation in athletes: is there an “athlete’s artery”? Exp Physiol. 2012;97(3):295–304.

37. Ren W, Yang X, Jiang X, Li Z, Zhang Z. Chronic hypoxia and exercise training affect the NO content and NOS activity of rat skeletal muscle. Int Sport J. 2010;11(1):244–57.

38. Gielen S, Sandri M, Erbs S, Adams V. Exercise-induced modulation of endothelial nitric oxide production. Curr Pharm Biotechnol. 2011;12(9):1375–84.

39. Harris MB, Mitchell BM, Sood SG, Webb RC, Venema RC. Increased nitric oxide synthase activity and Hsp90 association in skeletal muscle following chronic exercise. Eur J Appl Physiol. 2008;104(5):795–802.

40. Goto S, Radáč Z. Hormetic effects of reactive oxygen species by exercise: a view from animal studies for successful aging in human. Dose Response. 2009;8(1):68–72.