Allelic Polymorphism of Endothelial No-Synthase (eNOS) Association with Exercise-Induced Hypoxia Adaptation

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A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
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Svitlana B. Drozdovska (B,C,D) [1], **Victor E. Dosenko** (A,B) [2], **Vladimir N. Ilyin** (A,D) [1], **Mykhailo M. Filippov** (A,D) [1], **Ludmila M. Kuzmina** (B,C,E) [1]

[1]National University of Physical Education and Sports of Ukraine, Kiev
[2]National Academy of Sciences of Ukraine, Bogomoletz Institute of Physiology, Kiev

Key words: *endothelial NO-synthase, polymorphism of the eNOS gene, hypoxia of loading, finswimming, throwing*

Abstract

Background: *The aim of the study is to define the possibility of usage of the gene eNOS polymorphisms as molecular-genetic markers of athletes' resistance to exercise-induced hypoxia.*

Material/Methods: *127 highly qualified athletes were examined (30 persons are underwater finswimmers, 41 – rowers and 56 sportsmen who practice speed and power track and field exercises). The frequency of promotor polymorphisms and the 7th exon of the eNOS gene in athletes engaged in finswimming and throwing has been studied. The comparative analysis of the obtained data with the frequency of allel variants of the eNOS gene has been carried out in athletes engaged in speed-power sports and the individuals not going in for sports. To study the influence of polymorphisms of this gene on individual values of resistance to hypoxia, a gas analysis was conducted in the athletes practicing rowing. During the study the eNOS gene expression was examined in athletes practicing short-course underwater finswimming with apneapracticing long distance swimming with a snorkel.*

Results: *The findings are evidence of a possible variant of influence of polymorphism of the eNOS gene on the phenotype formation resistant to exercise-induced hypoxia.*

Conclusions: *In the athletes practicing underwater finswimming, the occurrence of the allelic variant of C/C $T^{-786}\rightarrow C$ promoter polymorphism and T/T-variant in exon 7 of the gene of NO-synthase is lower than in the general population of Ukraine and in the athletes of speed and power type of sports, which may be the result of selection and be important for peculiarities of the development of resistance to exercise-induced hypoxia with different types of muscle activity.*

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Address for correspondence:
Svitlana B. Drozdovska, Ph.D.
National University Of Physical Education and Sports of Ukraine, Chair of Clinical and Theoretical Human Morphology
Fizkultury Str. 1, 03680 Kyiv, Phone: *+38(044) 287-54-52,* e-mail: SDrozdovska@gmail.com

Background

It is well-known that nitric oxide (NO) takes part in the processes of the organism adaptation to different types of hypoxia [1]. Its concentration in plasma and erythrocytes increases in the case of hypoxic and circulatory hypoxia [2].

NO concentration in blood depends on the activity of the enzyme of NO-synthase. Gene polymorphism of this enzyme may influence the gene expression and, as a consequence, individual capabilities of triggering the mechanisms of hypoxia compensation [3].

The eNOS gene is located on chromosome 7q35-36 and comprises 26 exons. Among 15 allelic variants of this gene three variants of polymorphism are the most influential. These are the transversion of $T^{-786}\rightarrow C$ in the gene eNOS promoter, the transversion of $G^{894}\rightarrow T$ in exon 7, resulting in the substitution of glutamine to asparagine at position 298 in the eNOS protein, and a variable nucleotide number of tandem repeats in intron 4 (4a/b).

By now, only the effect of the intron 4 a/b and G894T polymorphisms of the gene eNOS on the muscle activity and high-altitude adaptation success have been studied [4].

According to the data of Ukrainian researchers, the polymorphism in intron 4 does not considerably affect both the intensity of the eNOS gene expression or the activity of this protein, which explains its insignificant influence on the development of cardiovascular diseases [5, 6].

The allelic polymorphism in exon 7 ($G^{894}\rightarrow T$) of the gene eNOS contributes greatly to the development of essential arterial hypertension in children and teenagers. The T/T variant of this polymorphism is observed 3 times more frequently in patients than in healthy subjects. But the greatest influence on the eNOS gene expression is exerted by the allelic promoter polymorphism of this gene [6, 7]. The presence of the C allele at position 786 of the promoter results in a decrease in its activity, while a lack of eNOS, which takes place in this case, is a reason for the synthesis reduction and a release of nitric oxide and endothelium dysfunction.

The intensity of the gene expression of the endothelial NO-synthase is 35% lower in the case of the C/C promoter genotype than in the case of the T/T variant, and the activity of NO production by platelets in the subjects with the C/C promoter variant is 2,1 times lower than with the T/T genotype.

Besides, the fact of the increase in expression of the eNOS gene caused by musle activity is considered as proven [8, 9, 10].

Thus, **the aim** of our study is to define the possibility of usage of the gene eNOS polymorphisms as molecular-genetic markers of athletes' resistance to exercise-induced hypoxia.

The researchers have set the following tasks:

- to study the occurrence of the gene eNOS polymorphous variants in sportsmen adapted to exercise-induced hypoxia.
- to examine the regularities of changes of this gene expression with different variants of the gene and adaptation to different physical exercises.

Material and Methods

During the study 127 highly qualified athletes were examined (30 persons are underwater finswimmers, 41 – rowers, and 56 – sportsmen who practice speed and power track and field exercises (track and field jumps, sprints, throwings).

In all these sports types, oxygen deprivation (exercise-induced hypoxia) is observed in the muscular tissue with the intensification of the function of the neuromuscular system. However, the manifestations of hypoxia are different. In speed and power track and field exercises

the mechanism of hypoxia development is connected with complete absence of O_2. In underwater finswimming the character of exercise-induced hypoxia development is different: for a period of time there is a use of oxygen supply connected with myoglobin and haemoglobin as well as with oxygen, present in residual lung volumes. In rowing exercise-induced hypoxia development is predetermined by the cardio-respiratory system capabilities.

The control group included 147 subjects, not engaged in sports. DNA was isolated from the buccal epithelium. Using the method of polymerase chain reaction (PCR) with the following analysis of the length of restriction fragments, the $T^{-786}\rightarrow C$ polymorphism of the promoter and the $G^{894}\rightarrow T$ polymorphism of exon 7 of the eNOS gene were determined. RNA was isolated from the venous blood platelets The eNOS gene expression was determined using the method of real time polymerase chain reaction (Fast 7500 Real Time PCR System (Applied biosystems, USA)).

The adaptation to exercise-induced hypoxia was assessed using the gas analysis, where findings were received by means of an automatic gas analyzer of the “Oxycon Pro” type (Jeager, Germany). Loading tests were conducted using the ergometer Concept 2 INDOOR POWER.

Results and Discussion

The analysis of occurrence of the allelic variants of $T^{-786}\rightarrow C$ promoter polymorphism (Tab.1) has shown that the frequency of normal T/T homozygotes among the sportsmen practicing underwater finswimming, adapted to work of mainly anaerobic character of energy supply with hypercapnia development, was 33.5% higher (P=0.0016) in comparison with a similar frequency in the control group. In the sportsmen specialized in speed and power sports types, it was only 21.2% (P=0.024) higher than in the control group, and in the sportsmen practicing rowing, it was 6.5% higher. The C/C allelic variant in the group of the sportsmen practicing rowing was observed least often. The minimum frequency of the unfavourable C allele was observed in the group of athletes, practicing underwater finswimming (16.7 % lower than in the control group) (Fig. 1).

Tab. 1. Distribution of the allelic variants of $T^{-786}\rightarrow C$ promoter polymorphism of the endothelial NO-synthase in the sportsmen engaged in different types of sports (%)

Genotype	Underwater finswimming (n=30)	Athletes of speed and power kinds of sports (n=56)	Rowing (n=41)	Control group (n=147)
T/T, n (%)	22 (73)	34 (60.7)	19 (46)	58 (39.5)
T/C, n (%)	5 (17)	19 (33.9)	20 (49)	75 (51.0)
C/C, n (%)	3 (10)	3 (5.4)	2 (5)	14 (9.5)
Frequency of C allele, %	18.3	22.3	29.3	35

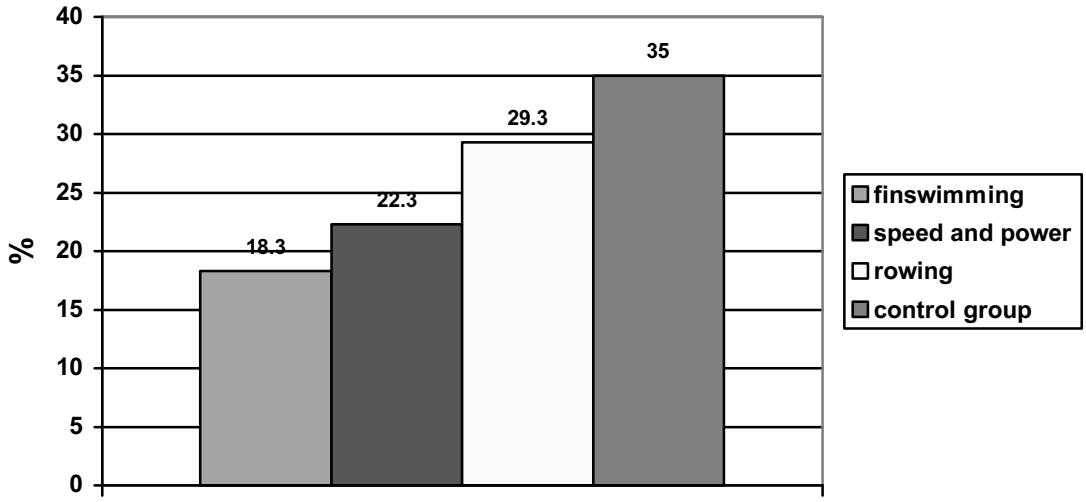

Fig. 1. Frequency of C allele of T >C promoter polymorphism of the eNOS I the athletes engaged in different kinds of sports (%)

The most of significant differences from other samplings in the distribution of the allelic variants of this polymorphism were observed in the group of athletes practicing underwater finswimming (Tab. 2).

Tab. 2. Significant differences of the samplings in distribution of the allelic variants of $T^{-786}\rightarrow C$ promoter polymorphism of the endothelial NO-synthase using χ^2 test

Group	Underwater finswimming	Rowing	Athletes of speed and power kinds of sports
Control group	0.0016*	0.54	0.024*
Rowing	0.019*	-	0.33
Speed and power types	0.205	0.33	-

**significant differences*

The analysis of occurrence of the allelic variants of $G^{894}\rightarrow T$ polymorphism in exon 7 of the eNOS gene (Tab. 3) has shown that the G/G variant of polymorphism in exon 7 was observed 1.5 times more often in the sportsmen adapted to hypoxia than in the sportsmen of speed and power types of sports. The G/T genotype was observed nearly 3 times more often in the sportsmen of both groups than in the control group. The T/T genotype in the athletes adapted to hypoxia was not detected, while in the athletes, adapted to speed and power stress it was observed twice less frequently than in the control group.

To study the presence of interdependence between polymorphous gene variants and sports achievements, all athletes were divided into 2 groups: highly qualified (world-class athletes and master of sports) and qualified (candidate master and first-class sportsmen) (Fig. 2).

Tab. 3. Distribution of the allelic variants of G^{894}→T polymorphism in exon 7 in the athletes of different kinds of sports (%)

Genotype	Underwater finswimming (n=19)	Speed and power types of sports (n=14)	Control group (n=89)	P_1	P_2	P_3
G/G, n (%)	9 (47.4)	4 (28.6)	28 (31.5)	0.3	0.9	0.3
G/T, n (%)	10 (52.6)	9 (64.3)	57 (64.0)			
T/T, n (%)	0 (0)	1 (7.1)	4 (4.5)			
Frequency of C allele,%	26.3	39.25	36.5			

P_1 − probability of absence of differences between the group of underwater finswimmers and the control group;
P_2 − probability of absence of differences between the group of speed and power types of sports and the control group;
P_3 − probability of absence of differences between the group of underwater finswimmers and the group of speed and power types of sports.
** probability of differences if $p<0.05$ using χ^2 test*

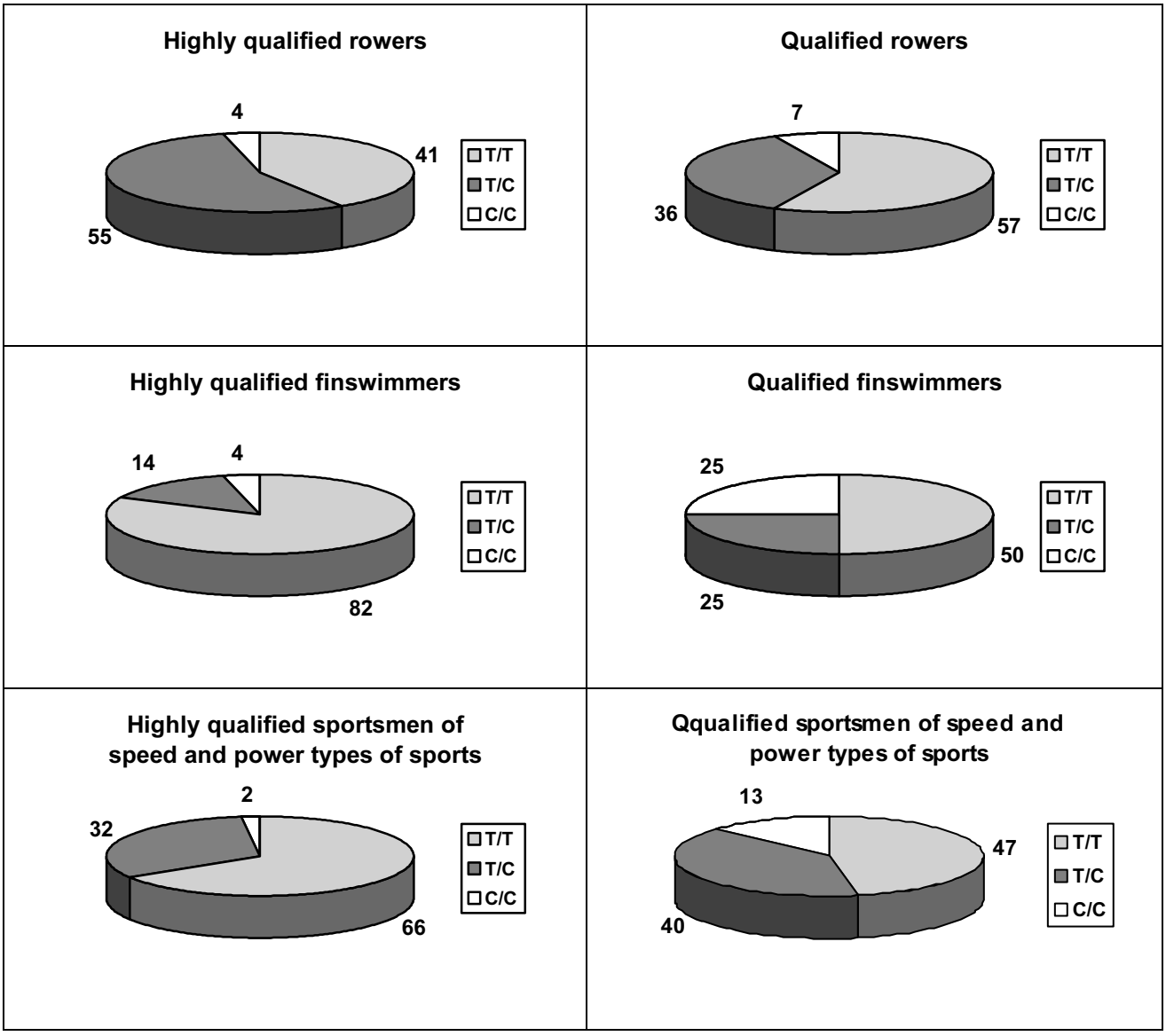

Fig. 2. Distribution of the allelic variants of T-786→C promoter polymorphism of the eNOS gene in the athletes of different qualifications (%)

In the groups of athletes practicing underwater finswimming and speed and power types of sports, one tendency may be outlined: in the groups of highly qualified sportsmen the favourable T/T variant is observed more frequently (32% more frequently in the group of underwater finswimming and 19% more frequently in the group of speed and power kinds of sports), while the unfavourable C/C variant is observed less frequently than in the groups of qualified athletes.

In the group of athletes practicing rowing, this tendency is not observed, because in this kind of sports an athlete's qualification depends not only on his/her functional cardio-respiratory system capabilities but also on power capabilities and on the capabilities of the remaining members of the boat crew.

To study the influence of polymorphisms of this gene on individual values of resistance to hypoxia, a gas analysis was conducted in the athletes practicing rowing. The effect of exercise-induced hypoxia was estimated by the capability of increasing oxygen intake, by the character of its compensation by means of cardio-respiratory system capabilities. We have also taken into account the indices of cardinality equivalence to every liter of the consumed oxygen. We have assessed the efficiency of external respiration system by a ventilation equivalent and breathing cycle oxygen effect as well as the blood circulatory system by oxygen pulse.

Insufficiency of the present material does not allow us to confirm the reliability of the results, but we have identified a tendency which proves that in the athletes with the T/T gene variant the breathing system capacity and maximum aerobic capacity are higher than in the sportsmen with T/C, but the efficiency of the cardio-respiratory system is lower.

Using a standard test with graded work capacity up to the moment of a voluntary refuse to continue the work, the maximum oxygen intake made 5371.7±419.7 mL/min. in the athlete with the T/T allelic variant, and 4974.17±407.41 mL in the athlets with the T/C variant.

Oxygen uptake per kg of body weight in the athletes with the T/T variant (58.7+2.4) was higher than in the athlets with T/C – 54.90+5.26.

Judging by the respiratory coefficient indices, the athletes with the T/C variant have a higher activity of anaerobic processes in energy supply (T/C – 1.24±0.06; T/T – 1.11±0.07).

The cardiac cycle oxygen effect (oxygen pulse), characterized by the heart activity efficiency in athletes, is higher in the athletes with the T/T variant (in the ones with the T/T variant, it corresponds to 193.25±5.74 mL·beats^{-1}, while in the sportsmen with T/C – 196.84±79.81 mL·beats^{-1}). Yet the ventilation equivalent in the sportsmen with the T/T variant is lower than in the sportsmen with the T/C variant. These questions are to be further studied, but the fact that the breathing cycle oxygen effect is higher is the evidence of lung ventilation efficiency.

During the study the eNOS gene expression was examined in 20 athletes practicing underwater finswimming. During the examination the athletes were in the contest season of training before the main competition of the season. All the subjects were divided into 2 groups: athletes adapted to anaerobic loading (practicing short course underwater finswimming with apnea) and adapted to aerobic loading (practicing long distance swimming with a snorkel).

During our study we have determined that in the athletes practicing short distance swimming with apnea, the eNOS gene expression (4.753±0.766) was higher ($p \leq 0.05$) than in the athletes practicing long distance swimming with a snorkel (0.246±0.072). The interdependence between the qualification of a sportsman and gene expression level has not been established in our work. In the athletes with the T/T genotype with $T^{-786} \rightarrow C$ promoter polymorphism, the eNOS gene expression is higher than in the group of the athletes with the T/C and C/C genotype.

The findings are evidence of a possible variant of influence of polymorphism of the eNOS gene on the phenotype formation resistant to exercise-induced hypoxia.

In the athletes adapted to exercise-induced hypoxia, the occurrence of the allelic variant of C/C T^{-786}→C promoter polymorphism and T/T-variant in exon 7 of the gene of NO-synthase is lower than in the subjects not engaged in sports, and in the athletes adapted to speed and power loading, it may be used for selection and be important in defining resistance to hypoxia.

The athletes of these groups are adapted to exercise-induced hypoxia, but the athletes practicing short course underwater finswimming with apnea are adapted to an associated influence of hypoxic hypoxia, exercise-induced hypoxia and hypercapnia. It is obvious that such triple influence is a potent inciting factor to trigger compensation mechanisms, one of which is the increase of NO production, possessing a vasodilatatant action.

Conclusions

1. In the athletes practicing underwater finswimming, the occurrence of the allelic variant of C/C T^{-786}→C promoter polymorphism and T/T-variant in exon 7 of the gene of NO-synthase is lower than in the general population of Ukraine and in the athletes of speed and power type of sports, which may be the result of selection and be important for peculiarities of the development of resistance to exercise-induced hypoxia with different types of muscle activity.
2. Systematic physical exercises, mainly of an anaerobic character, in combination with apnea result in the increase in the eNOS gene expression.
3. There is some interdependence between cardio-respiratory indices and eNOS gene polymorphic variants.

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