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Evaluation of Dynamics of Changes in Different Ways Arginine Metabolism Activity under Adaptation to Physical Stress

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Abstract. A survey of 29 trained and untrained youths aged 18 to 20 years was carried out. It has been shown that the optimal level of adaptation of a trained body to prolonged and intense physical exercise is provided by the activation of not only the constitutive calcium-dependent nitric oxide synthesis, but also by oxidative arginase metabolism of arginine, which yields a range of important low-molecular bioregulators and antioxidants, including urea. Significant reduction in the functionality of the body of trained youths at the end of competition period and as a consequence the manifestation of disadaptation signs correlates with increased oxidative calcium independent nitric oxide synthesis and its non-oxidative synthesis due to reutilization of stable circulating NO metabolites.

Key Words: *metabolism, arginine, nitric oxide, physical exercise, adaptation, youths*

Introduction. It is proved that nitric oxide plays an important role in the adaptation of the organism to physical exercise in particular through the regulation of the functional state of the cardiovascular system [4, 5, 6]. A number of authors identified the restructuring of the functional status of endothelium and nitric oxide system in humans under the influence of muscular work, which relates primarily to the increase in nitric oxide (NO) synthesis and as a consequence to the severity of vascular vasodilator responses [7-10]. It should also be noted that in almost all studies metered and nondurable physical exercises were exclusively used.

As we have shown earlier, young sportswomen performing prolonged physical work of great intensity and amount in either the mode of training, or in aggravated by psychological stress mode of competition manifested significant changes in nitric oxide synthesis: among the women, at transition from training load up the same one, but in terms of psychological stress the intensity of the oxidative degradation of arginine with the participation of cNOS reduced significantly, as well as arginase pathway of its metabolism by which such important low-molecular bioregulators as urea, polyamines and GABA are synthesized; and conversely the oxidation of arginine by calcium independent iNOS enhanced [1, 2].

The goal was to study the characteristics of changes in the exchange of L-arginine including oxidative nitric oxide synthesis in healthy persons under the influence of prolonged exercise of significant amount and intensity. It was important to examine the intensity of nitric oxide synthesis both inducible and constitutional, the intensity of its reutilization (salvage) synthesis through the restoration of stable circulating metabolites – nitrite and nitrate, as well as the intensity of a non-oxidizing degradation of arginine by arginase.

Methods. The experiment involved 17 untrained students, young men (control group) and 12 trained young men, handball players (experimental group) aged 18 to 20 years. Youths of the experimental group systematically performed muscular work of large amount and intensity during 11 months. The first three months were training period, over the next 8 months (competition period) training exercises (25 – 30% of the total load of the training period) were carried out in parallel with the competition in accordance with the schedule of a team.

In the study, biochemical parameters that characterize the rate of metabolism of L-arginine by two alternative pathways of metabolism (non-oxidative arginase way and oxidative NO synthase one) were determined in blood plasma of youths in both groups.

The intensity of non-oxidative metabolism was assessed by determining the activity of arginase and urea amount formed when using this enzyme. The intensity of the oxidative degradation of arginine to synthesize nitric oxide was assessed by the activity of different isoenzymes of NO-synthases – calcium dependent constitutive one, cNOS (it was determined as the total activity of endothelial and neuronal NOS: eNOS + nNOS = cNOS) and calcium independent inducible one (iNOS), as well as the level of stable oxidized metabolites of nitric oxide, namely: nitrite (NO_2^-) and nitrate (NO_3^-) anions.

We also estimated the intensity of nitrate anions salvage for re-synthesis of nitric oxide by determining NADPH-dependent nitrate reductase activity in the blood plasma. All those indicators were determined according to previously described methods [1, 2].

We also calculated relative changes (D,%) in biochemical parameters with respect to certain investigated period, or control according to such a formula:

$$D = 100 \cdot (X_i - X_n)/X_n,$$

where Xi is the final value of a parameter; Xn is the initial value of the parameter.

All biochemical parameters were measured in leukocyte enriched plasma of untrained young men of the control group at the beginning of survey and in trained young men of the experimental group three times: at the end of the training period (or beginning of competition period), in the middle and late period of competition period. In the process of the training period (3 months), physical activity was performed in the absence of factors of psychological stress, and within the competitive period (8 months) it was performed in both a stress-free mode, and in stressful conditions of the responsible competitions.

Chosen scheme for determining these biochemical parameters was based on the fact that we tried to evaluate the changes in the activity of different pathways of arginine and nitric oxide synthesis in the late training period (at the peak of physical fitness, achieved during the training sessions), in mid-competition period (after 7 months after the start of training or 4 months after the start of the competitions), and at the end of competition period (within 11 months after the start of training or 8 months after the start of the competitions).

In untrained young men of the control group at baseline of the survey and in trained ones of the experimental group after the training period, in the middle and late period of competition period we determined simultaneously the overall physical work capacity (PWC_{170}) by means of sub maximal test PWC_{170}, and maximal oxygen consumption (VO_2max) relative to body weight by Karpman's [3]:

$$PWC_{170} = [N_1 + (N_2 - N_1) \cdot (170 - HR_1)/(HR_2 - HR_1)]/BW,$$

where N_1 is capacity of the first load, kgm / min; N_2 is capacity of the second load, kgm / min; HR_1 is heart rate at the end of the first load, min $^{-1;}$ HR_2 is heart rate at the end of the second load, min $^{-1;}$ BW is body weight, kg.

$$VO_2max = 1.7 \cdot PWC_{170} \cdot BW + 1240,$$

where PWC_{170} is relative value of the overall physical work capacity, kgm ·min $^{-1}$ ·kg^{-1}; BW is body weight, kg; 1.7 and 1240 are constant coefficients.

All results were processed by variational statistics method, using t Student's test. Mean value (M), standard deviation (m) and coefficient of reliability (P) calculations was performed using Calc (OpenOffice.org), STATISTICA'99 ("StatSoft. Inc").

Results and their Discussion. In the investigation we analyzed the dynamics of biochemical and functional parameters of surveyed and trained youths. Table 1 shows the results of a preliminary survey of both the control and experimental youth groups. Examination of the latter group was held at the end of the preparation for the competitive season, at a kind of a peak of their functional readiness, which was confirmed by their sufficiently high values of the general physical working capacity PWC_{170} and maximal oxygen consumption (VO_2max). The young men who trained regularly and intensively for 3 months at that stage manifested values of almost all the studied biochemical parameters significantly higher than those in untrained youths of the same age in control group. This showed the importance of the studied biochemical markers of NO-synthase and arginase pathways of arginine metabolism, as well as oxidative and (by NOS) non-oxidative salvage (by nitrate reductase) synthesis of nitric oxide for youths' adaptation to prolonged and considerable physical exercises [7-10].

Table 1. Biochemical and functional parameters of the surveyed (control group n = 12) and trained (experimental group n = 17) youths (M ± m)

Parameters	Control	Trained
NO_2^-, pmol · mg^{-1} of protein	177.1 ± 18.1	323.70 ± 24.70*
NO_3^-, nmol · mg^{-1} of protein	7.74 ± 0.82	1.67 ± 0.20*
Arginase, nmol·min^{-1}· mg^{-1}of protein	1.21 ± 0.16	2.09 ± 0.25*
Urea, nmol· mg^{-1} of protein	56.68 ± 5.40	75.80 ± 6.20*
Constitutive NO-synthase, pmol·min^{-1}· mg^{-1}of protein	40.04 ± 3.24	58.6 ± 7.90*
Inducible NO-synthase, pmol·min^{-1}· mg^{-1}of protein	14.44 ± 4.08	12.3 ± 2.45
Total NO-synthase, pmol·min^{-1}· mg^{-1}of protein	54.48 ± 7.48	70.95 ± 9.85
Nitrate reductase, nmol· min^{-1}· mg^{-1} of protein	3.17 ± 0.15	3.47 ± 0.41
General physical working capacity, kgm· min^{-1}· kg^{-1}	12.86 ± 0.97	26.64 ± 1.31*
Maximal oxygen consumption,ml· min^{-1}· kg^{-1}	48.32 ± 1.24	70.24 ± 2.84*

* – P < 0,05.

However, analysis of the results made it possible to ascertain the presence of a specific hierarchical organization of arginine exchange and nitric oxide synthesis in the experimental group of surveyed youths at the highest (maximum) level of their functional readiness. So, at the end of the training period with the maximum values of PWC_{170} (twice higher than in the control) and VO_2max (statistically significant excess of VO_2max control values by 50%) the dominant role in providing the necessary content of nitric oxide and as a consequence, the maximum level of functional parameters of trained youths fully adapted to the implementation of a long and significant physical activity is provided by a sufficiently high activity of constitutive nitric oxide synthesis (+46.35% compared with the control) in combination with high levels of arginase activity (+72.73%). In addition, the importance of achieving optimal functional fitness may also have a contribution of nitric oxide re-synthesized from its circulating stable metabolites (nitrite anion content was by 82.78% higher, and nitrate anion content by 78.44% lower than those in the controls), although significant differences in nitrate reductase activity were not recorded. Significantly lower (by 14.82%) values of iNOS in young men compared with the control group also testified in favor of an optimal state of nitric oxide synthesis in young men of the experimental group at the beginning of the training period.

In general, we can say that more expressed constitutive de novo nitric oxide synthesis in trained youths, combined with a relatively moderate increase in salvage pathway, increased intensity of arginase pathway and decreased activity of calcium-independent (by iNOS) NO synthesis were observed against the background of increased values of their overall physical working capacity and aerobic efficiency as compared to control. It is a convincing proof of the important role of nitric oxide synthesized by constitutive way rather than inducible one to ensure an adequate level of functional fitness of the body.

We also evaluated the changes in nitric oxide synthesis in various stages of training and competitive activity of trained youths in the dynamics of adaptation to changes in the nature and extent of external impact on their body in the form of muscle work.

As it is seen from the results presented in Table 2, long-term combination of training and competitive pressures in trained youths of the experimental group resulted in decreased level of their physical work capacity and maximal oxygen consumption by the middle of competition period, which fully confirms the published data on this question. In this connection, significant changes were

observed in the system providing the body with nitric oxide.

It has been found that under conditions of prolonged muscular work a dominant role in ensuring the optimal level of the system of nitric oxide synthesis belongs not only to oxidative constitutive synthesis of NO but also to its non-oxidative re-synthesis from stable metabolites.

Thus, in the middle of competition period some plasma parameters reached maximum values: the content of nitrite anion (+16.77%), cNOS activity (+22.30%), and especially nitrate reductase activity (+112.68%), while on the contrary, content of the nitrate anion (-38.92%) and the activity of arginase (-37.32%) reached the minimum. It is possible that increased activity of cNOS was due to decreased activities of both arginase and iNOS (-15.20%) competing with cNOS for the common substrate – L-arginine.

Table 2. Changes in the absolute values of the biochemical and functional parameters in trained youths, depending on the duration of regular physical exercises and psychological stress during competitions (M ± m)

Parameters	Middle of competitive peruid	End of competitive period
NO_2^-, pmol · mg^{-1} of protein	378.0 ± 46.90	299.2 ± 16.00**
NO_3^-, nmol · mg^{-1} of protein	1.02 ± 0.13**	1.33 ± 0.05**
Arginase, nmol·min^{-1}· $mg^{-1}$of protein	1.31 ± 0.29*	1.89 ± 0.06
Urea, nmol· mg^{-1} of protein	57.60 ± 9.60	65.80 ± 2.70
Constitutive NO-synthase, pmol·min^{-1}· $mg^{-1}$of protein	71.67 ± 6.42	37.65 ± 3.00**
Inducible NO-synthase, pmol·min^{-1}· $mg^{-1}$of protein	10.43 ± 1.36	20.20 ± 1.30**
Total NO-synthase, pmol·min^{-1}· $mg^{-1}$of protein	82.1 ± 2.42	57.85 ± 8.03
Nitrate reductase, nmol· min^{-1}· mg^{-1} of protein	7.38 ± 0.87***	4.80 ± 0.45**
General physical working capacity, kgm· min^{-1}· kg^{-1}	23.77 ± 0.57	19.85 ± 0.61*
Maximal oxygen consumption, ml· min^{-1}· kg^{-1}	63.61 ± 1.20	60.25 ± 1.92**

* – P < 0,05; ** – P < 0,01; *** – P < 0,001.

It is known that the end of competition period in sportsmen is characterized by a powerful growth of the natural fatigue, decreased physical ability and physical fitness. In accordance with the results presented in Table 2, in trained youths at the final stage of the study we actually showed marked significant decrease in PWC_{170} (by 25.49% compared to baseline values) and VO_2max (-14.22%). At the same time, the need for continued competitive activity and performance of physical activities at the appropriate level requires the mobilization of functional reserves and the formation of new physiological mechanisms of its adequate adaptation to regular muscular work.

As it was shown by the results of our study, adaptive adjustments in the synthesis of nitric oxide in the boundary phase and even above-threshold external influences are reflected in the change in the hierarchy of the individual components. In the survey of youths in the experimental group at the end of competition period, we found significant changes in the intensity of oxidative de novo and non-oxidative "salvage" nitric oxide synthesis. At that time, the main role in the synthesis of nitric oxide was owned by its calcium-independent synthesis (significant increase in iNOS activity from baseline values was 64.23%). Thus, the calcium-dependent synthesis of NO decreased reciprocally (-35.76%).

The results of survey made it possible to identify some significant features in the organization of nitric oxide synthesis in young men aged 18 – 20 during their prolonged training and competitive activities. It has been shown that the transition from the relatively favorable conditions of the training period to the extreme conditions of the competitions was accompanied by a change in the values of the main ways to ensure their body's nitric oxide – from the dominant role of oxidative constitutive de novo NO synthesis from L-arginine and non-oxidative arginase metabolism of the latter with the formation of intermediate low-molecular bioregulators and antioxidants (urea, polyamines, GABA) to the predominance of oxidative calcium-independent inducible nitric oxide de novo synthesis and its non-oxidative "salvage" synthesis. Thus, we can assume the paradoxical possibility of participation of non-oxidative arginase pathway of arginine degradation (arginine + H2O → urea + ornithine → putrescine → GABA → guanidine butyric acid (GBA) → NO or arginine + H2O → urea + ornithine → glutamate →GABA→ GBA → NO), and not just oxidative NO synthase degradation (arginine + H2O → citrulline + NO) in maintaining high levels of nitric oxide synthesis as evidenced by a high content of nitrite anion in the blood plasma of untrained young men. Perhaps it is a necessary and important condition for their effective adaptation to the implementation of large amount of training loads for a long time. Obviously, high arginase activity in trained young men may provide additional nitric oxide formation during the oxidation of different isozymes of GBA along with the classical arginine oxidation by NOS. In addition arginase metabolism of arginine can consistently provide the body with urea as an antioxidant and regulator of NO synthesis, putrescine as a regulator of cell proliferation and antioxidant, GABA as a regulator of mitochondrial pore and inhibitory neurotransmitter in the CNS, which is also very important to adapt to the intense and prolonged physical exercise of a training period in the absence of psychological stress factors. Within the competition period when the effects of these factors are enhanced, the

intensity of the non-oxidative exchange of arginine is gradually reduced to the level of untrained young men, that is, from a biochemical point of view there is almost complete body deadaptation at the end of competition period according to non-oxidative exchange of arginine.

The data obtained are not only evidence of the importance of both oxidative and non-oxidative arginine metabolism for adaptation to prolonged physical exercise of large intensity, but also the important role of non-oxidative (constitutive calcium-dependent and calcium-independent inducible ones) and non-oxidative "salvage" pathways of nitric oxide synthesis, which is an essential complement to the existing information on the subject.

Conclusion.

1. The end of training sessions within the training period for young men performing regularly muscular work is characterized by high levels of both oxidative and non-oxidative metabolism of L-arginine on account of both calcium-dependent constitutive NO synthase (synthesis of nitric oxide and citrulline) and non-oxidative metabolism of arginine (synthesis of ornithine and urea). The high portion of nitrite anion content in the total pool of circulating in plasma stable metabolites of nitric oxide corresponds to body adaptation to prolonged and intensive physical exercise.

2. An increase in the degree of adverse effects of intensive training (physical) and competitive (both physical and psychological) stress is accompanied by a pronounced adaptive changes in the hierarchy of constitutive oxidative (decrease) and inducible (increase) de novo nitric oxide synthesis, as well as non-oxidative salvage nitric oxide synthesis (increase) andnon-oxidative degradation of L-arginine (decrease).

3. Physical deconditioning of young men at the end of competition period is accompanied by a significant increase in the activity of oxidative calcium-independent de novo synthesis of NO and in a less degree

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Богдановская Н.В., Маликов Н.В. Оценка динамики изменения активности различных путей обмена аргинина при адаптации к физическим нагрузкам

Аннотация. Проведено обследование 29 тренированных и нетренированных юношей в возрасте 18-20 лет. Доказано, что оптимальный уровень адаптации их организма к продолжительным и интенсивным физическим нагрузкам обеспечивается превалированием многостадийного недоокисленного аргиназного метаболизма аргинина и его окислительного матаболизма при участии конститутивных кальцийзависимых NO-синтаз (eNOS и nNOS), при которых образовывается несколько важных низкомолекулярных биорегуляторов и антиоксидантов (мочевина, полиамины, ГАМК) над одностадийным кальцийнезависимым окислением аргинина, из которого образовывается лишь оксид азота. Значительное снижение функциональных возможностей организма тренированных юношей в конце соревновательного периода и, как следствие, проявление признаков дезадаптации, связано с экспрессией активности индуцибельной кальцийнезависимой NO-синтазы, что не компенсирует потребности организма в оксиде азота.

Ключевые слова: *метаболизм, аргинин, оксид азота, физические упражнения, адаптация, юноши*