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Effect of physical load on the concentration of endothelial NO-synthase and platelet-activation factor in plasma of athletes

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ABSTRACT

Aim. To assess the effect of one-time physical load on the concentration of endothelial NO-synthase and platelet-activating factor in blood plasma of athletes training in cyclic and strength sports, as well as in untrained volunteers.

Materials and methods. The study involved 28 men aged 18–25 years, who were relatively healthy and had no disorders of the cardiovascular system. Three groups were formed according to the sports classification. Group 1 (TFG) included highly qualified athletes (Candidates for Master of Sports (CMS), Master of Sports (MS)) of cyclic sports – track and field athletics (middle-distance running, 800–1500 m), n = 10. Group 2 (WG) consisted of highly qualified athletes (CMS, MS) of strength sports – weightlifting, n = 8. Group 3 (CG) was a control group and included untrained men with no sports category, n = 10. All volunteers were examined in the morning on an empty stomach. One day before the study, the athletes were advised to stop the training process. The blood from the cubital vein was taken from all the individuals three times: before exercise (test A), immediately after performing the standard PWC_{170} test on a bicycle ergometer (test B), and 60 minutes after performing the stress test (test C). Determination of the concentration of endothelial NO-synthase (eNOS) and platelet-activating factor (PAF) in plasma was performed by enzyme immunoassay.

Results. It has been shown that the features of endothelial reactivity in athletes of various specializations in comparison with untrained volunteers are significantly associated with the level of eNOS production both at rest and in response to short-term physical exertion. Platelet-activating factor can also affect endothelial reactivity, but to a lesser extent, and is involved only in the mechanisms of adaptation to repetitive high-intensity physical loads.

Key words: endothelium, athletics, weightlifting, training.

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

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Conformity with the principles of ethics. All study participants signed an informed consent to participate in the study. The study was approved by the local Ethics Committee of the Biological Institute of NR TSU (Protocol No. 33 of 02.12.2019).

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Влияние физической нагрузки на концентрацию эндотелиальной NO-синтазы и фактора активации тромбоцитов в плазме у спортсменов

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РЕЗЮМЕ

Цель. Оценить влияние однократной физической нагрузки на концентрацию эндотелиальной NO-синтазы и фактора активации тромбоцитов в плазме крови у спортсменов, тренирующихся в циклических и силовых видах спорта, а также у нетренированных волонтеров.

Материалы и методы. В исследовании участвовали 28 мужчин в возрасте 18–25 лет, условно здоровые, без нарушений со стороны сердечно-сосудистой системы. В соответствии со спортивной классификацией было сформировано три группы. Группа 1 (ЛА): высококвалифицированные спортсмены (кандидаты в мастера спорта (КМС), мастера спорта (МС)) циклических видов спорта – легкая атлетика (бег на средние дистанции 800–1500 м), $n = 10$. Группа 2 (ТА): высококвалифицированные спортсмены (КМС, МС) силовых видов спорта – тяжелая атлетика, $n = 8$. Группа 3 (КГ): контрольная группа – нетренированные мужчины, не имеющие спортивного разряд, $n = 10$. Все волонтеры проходили обследование утром натощак. За 1 сут до исследования спортсменам было рекомендовано прекратить тренировочный процесс. У всех испытуемых трижды бралась кровь из локтевой вены: до нагрузки (проба А), сразу после выполнения стандартной пробы PWC_{170} на велоэргометре (проба В) и через 60 мин после выполнения нагрузочной пробы (проба С). Определение концентрации eNOS и PAF в плазме производилось методом иммуноферментного анализа.

Результаты. Показано, что особенности эндотелиальной реактивности у спортсменов различных специализаций в сравнении с нетренированными волонтерами в значительной степени связаны с уровнем продукции eNOS как в покое, так и в ответ на кратковременные физические нагрузки. Фактор активации тромбоцитов также может оказывать влияние на эндотелиальную реактивность, но в меньшей степени, и вовлекается только в механизмы адаптации к регулярным нагрузкам высокой интенсивности.

Ключевые слова: эндотелий, легкая атлетика, тяжелая атлетика, тренировки.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Systematic physical activity of various natures has a modulating effect on the cardiovascular system and induces the adaptation processes of all its components, including the vascular endothelium [1, 2]. These changes can have a multidirectional effect on the risk of developing hemodynamic disorders. The effect can be either positive, accompanied by the potentiation of endothelium-dependent reactions, or negative [3]. At the same time, the mechanisms of the described adaptations remain largely unclear. In our previous publications [4, 5], it was

shown that athletes of high qualification have suppression of endothelial functional activity, and its degree is determined by the intensity and type of physical activity.

The mechanisms of endothelium-dependent vascular relaxation are traditionally associated with production of nitric oxide (NO). With the participation of endothelial NO-synthase (eNOS), NO production in the endothelium is enhanced. The content of eNOS in plasma differs depending on the type of physical activity [6]. This may be one of the reasons for the opposite reactions in the cuff test in athletes training in various sports. However, information on the eNOS concentration in the blood of people during different physical activity is often contradictory. Thus, after cyclic exercise, the concentration of eNOS in plasma increases by 36%, whereas after prolonged endurance training, the content of eNOS increases by only 14% [7]. There is also evidence that acyclic loads of submaximal power are not accompanied by increases in the blood eNOS content, in contrast to long-term and intense cyclic loads [8].

Simultaneously with the influence of endothelial factors, the hemostatic system, especially its platelet link, can also participate in the modification of blood flow during physical exercise. With acute and prolonged intense physical activity, there is a tendency to hypercoagulability, especially in untrained individuals. Acute physical activity of maximum intensity induces a transient platelet increase. Platelet activation depends on the intensity of physical activity [9–11]. Platelet-activating factor (PAF) has an important role in this process [12]. Some researchers consider PAF as a compensation mechanism that protects athletes from the risk of developing thrombosis and cardiovascular diseases [13].

The aim of this study was to assess the effect of one-time physical load on the concentration of endothelial NO-synthase and platelet-activating factor in blood plasma in athletes training in cyclic and strength sports, as well as in untrained volunteers.

MATERIALS AND METHODS

The study involved 28 men aged 18–25 years, who were relatively healthy and had no disorders of the cardiovascular system. Three groups were formed according to the sports classification. Group 1 (TFG) included highly qualified athletes (Candidates for Master of Sports (CMS), Master of Sports (MS)) of cyclic sports – track and field athletics (middle-distance running, 800–1500 m), n = 10. Group 2 (WG) consisted of highly qualified athletes (CMS, MS) of strength sports – weightlifting, n = 8. Group 3 (CG) was a control group and included untrained men with no sports category, n = 10. The athletes of WG and TFG groups had been engaged in sports for more than 6 years. All athletes took part in Russian competitions and won prizes.

All volunteers were examined in the morning on an empty stomach. One day before the study, athletes were advised to stop the training process. The blood from the cubital vein was taken from all the participants three times: before exercise (test A), immediately after performing the standard PWC_{170} test on a bicycle ergometer (test B), and 60 minutes after performing the stress test (test C).

BD Vacutainer 5-ml vacuum system and 5 ml Vacuette® Premium tubes with heparin separation gel (Greiner Bio-One, Austria) were used. The heparin concentration in the tubes was 20 U / ml. After 30 min of blood collection, erythrocytes and white blood cells were sedimented for 11 minutes at 2,000 rpm using an LMC 3000 laboratory centrifuge (Biosan, Latvia). The plasma was frozen and stored in a freezer at –20 °C for no more than 30 days.

Measurement of the eNOS and PAF concentrations in plasma was performed by enzyme-linked immunosorbent assay (ELISA) using RayBio Human eNOS ELISA Kit (RayBio®, USA) and Enzyme-linked Immunosorbent Assay Kit for Platelet Activating Factor (PAF) (Cloud-Clone Corporation, USA). All samples were taken in duplicate. Microwell test strips with flat-bottomed wells (12 × 8 wells) were used for the analysis. The microwell strips were incubated on a PST-60HL microplate shaker (Biosan, Latvia) and washed using an Anthos Fluido 2 washer (Biochrom, Great Britain). The sample optical density was measured using an Anthos 2010 spectrophotometer with filters (400–750 nm) and ADAP+ software (Biochrom, Great Britain) at 450 nm and 620 nm as primary and reference wavelengths, respectively.

Statistical data processing was carried out using STATISTICA 8.0. The level of significance in testing the hypothesis that two samples belong to the same general population was assessed using the Kruskal – Wallis test (ANOVA test). Data are pre-

sented as Mean ± Standard Deviation *(M ± m)*. All the participants signed an informed consent to participate in the study and consent to blood sampling. Permission was obtained from the Ethical Committee of Tomsk State University (Protocol No. 33 of 02.12.2019).

RESULTS AND DISCUSSION

The results are shown in Table 1.

Table 1

eNOS and PAF concentrations in blood plasma of healthy volunteers (control group), weightlifters, and track and field athletes before, immediately after, and 60 minutes after the exercisee (data are presented as *M* ± *m*)

Groups	Parameter	Baseline level (sample A)	0 min post exercise (sample B)	60 min post exercise (sample C)
CG (control group) $n = 10$	eNOS (ng/ml)	4.96 ± 0.72	22.5 ± 4.11 $p_4 < 0.001$	22.25 ± 3.82 $p_4 < 0.001$
	PAF (pg/ml)	47.93 ± 3.25	52.04 ± 4.25	53.98 ± 5.37
TFG (track and field group) $n = 10$	eNOS (ng/ml)	2.23 ± 0.42 $p_3 < 0.05$	6.13 ± 0.74 $p_3 < 0.001$ $p_4 < 0.05$	4.66 ± 0.55 $p_3 < 0.001$ $p_4 < 0.05$
	PAF (pg/ml)	40.42 ± 3.27 $p_3 < 0.05$	39.25 ± 4.28 $p_3 < 0.01$	38.16 ± 4.57 $p_3 < 0.01$
WG (weightlifting group) $n = 8$	eNOS (ng/ml)	1.35 ± 0.47 $p_1 < 0.01$ $p_2 < 0.001$	1.05 ± 0.21 $p_1 < 0.001$ $p_2 < 0.001$	1.65 ± 0.07 $p_1 < 0.001$ $p_2 < 0.001$ $p_5 < 0.05$
	PAF (pg/ml)	39.67 ± 4.04 $p_2 < 0.05$	38.62 ± 3.55 $p_2 < 0.01$	45.15 ± 3.24 $p_1 < 0.001$

Note: p_1 – differences between WG and TFG; p_2 – differences between WG and CG; p_3 – differences between TFG and CG; p_4 – as opposed to sample A; p_5 – as opposed to sample B.

The control group had the eNOS concentration of 4.96 ± 0.72 ng / ml, which is significantly higher than this parameter in both groups of athletes. At the same time, in this group, the maximum increase in this parameter immediately after physical exercise and its maintenance at a constant level for 1 hour were recorded. In the athletes of both groups, the baseline level of eNOS was significantly lower: two times lower in the track and field athletes and 4 times lower in the weightlifters. In the TFG athletes, immediately after exercise, an increase in the eNOS concentration by 2.5 times was identified. After 1 hour, it decreased by about 30%. The weightlifters, on the other hand, showed a tendency to a decrease in plasma eNOS concentration immediately after exercise, which returned to the baseline level in an hour.

The results obtained show a high correlation with the previously published data on endothelial reactivity in athletes of various specializations [4, 5]. It has been shown that highly qualified athletes have signs of endothelial dysfunction, which are more significant in the group engaged in strength sports. In the track and field athletes, endothelium-dependent vasodilation is much less pronounced, and in weightlifters, it was not recorded at all, while in untrained persons, it is the most pronounced.

The PAF concentration values in the groups did not differ so significantly (Table 1). In the control group, the baseline level was 47.93 ± 3.25 pg / ml. In the athletes of both groups it was 15% lower. In all the groups, this parameter almost did not change after physical exertion. Only in the weightlifters, 1 hour after exercise, its slight increase was noted. Apparently, there is a decrease in plasma PAF concentration in athletes as a result of regular exercise. This may be a factor suppressing endothelium-dependent vasodilation. Some researchers believe that platelets can stimulate endothelial reactions due to mechanical interaction with the endothelial surface. At the same time, this mechanism is more inertial in comparison with eNOS and is not involved in short-term effects after a single exercise. In weightlifters, an increase in PAF concentration may be associated with blood stagnation, injury to muscle tissue, and microvascular injury.

Physical activity is accompanied by an increase in blood pressure [14], which may influence the growth of endothelial cells [15]. In addition, mechanical stimuli associated with changes in the shear stress and stretching of the vascular wall activate intracellular signaling cascades in the endothelium, which is accompanied by the activation of transcription factors [4].

CONCLUSION

It is obvious that the features of endothelial reactivity in athletes of various sports in comparison with untrained volunteers are significantly associated with the level of eNOS production both at rest and in response to short-term physical exertion. The platelet-activating factor can also affect endothelial reactivity, but to a lesser extent, and is involved

only in the mechanisms of adaptation to repetitive intense physical activity. The revealed patterns can serve both as a mechanism of adaptation to regular training loads and as a risk factor for acute hemodynamic dysfunctions in athletes.

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