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**Original Article**

# Pre- and Post-Exercise Blood Contents of HPS70 in athletes involved in different sports

VALERIAN GAVRILOV[1]*, NATALIA BORISOVA[2], VADIM ANUROV[3], ERIC CHUN-PU CHU[4]
[1] Department of Sport Management and Economy, Lesgaft National State University of Physical Education, Sport and Health, RUSSIAN FEDERATION
[2] Department of Normal and Pathological Physiology, North-Eastern Federal University named after M. K. Ammosov, RUSSIAN FEDERATION
[3] Department of Physical Education, Financial University under the Government of the Russian Federation RUSSIAN FEDERATION
[4] New York Chiropractic and Physiotherapy Center EC Healthcare, CHINA

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**Abstract**:
*Problem Statement.* Moderate physical activity is known to be beneficial to physical and mental health. It prolongs life and reduces the risk of mortality. *Purpose.* The purpose of the study is to explore the changes in the blood levels of HPS70 in athletes engaged in different sport disciplines and non-athletes. *Approach.* The measurements were taken before and after physical activity in order to evaluate the rate of the muscle adaptation processes. The study involved 120 apparently healthy people aged 18 to 30 years (60 Chinese and 60 Russians). Of them, 47 (39.2%) were women and 73 (60.8%) were men. Group 1 (n = 40) included apparently healthy young people who led a sedentary lifestyle and were physically inactive. Group 2 (n = 40) consisted of professional athletes who were engaged in rowing. Group 3 (n = 40) involved professional athletes who practiced artistic gymnastics. *Results.* There were higher basal levels of HSP70 in the blood of athletes as compared to non-athletes. The values registered for rowing athletes exceeded those registered for non-athletes by 2.83 times (13.08 ± 1.15 ng/mL versus 4.62 ± 0.73 ng/mL, $p < 0.05$). In gymnasts, the basal levels of HSP70 exceeded those measured in non-athletes by 4.20 times (19.40 ± 1.86 ng/mL versus 4.62 ± 0.73 ng/mL, $p < 0.05$). After submaximal physical exercise, the concentrations of HSP70 and total anti-HSP70 in the blood of athletes returned to the baseline values within 24 hours. As to non-athletes, they still followed an increasing trend at 24 hours post-exercise. This is indicative of impairment of the adaptive processes in the body of non-athletes and inability of their body to restore cellular homeostasis in due time. *Conclusions.* Thus, the blood levels of HSP70 may be regarded as a marker of the rate and plasticity of the adaptive processes occurring in the body after physical exercise.
**Key Words***:* **heat shock protein 70, adaptation, muscle damage, physical exercise, cellular homeostasis**.

## Introduction

Moderate physical activity is known to be beneficial to physical and mental health. It prolongs life and reduces the risk of mortality (Cairney et al., 2019; Vedøy et al., 2020). The positive effect of exercise is achieved when a person practices sports regularly and sets optimal physical activity objectives (Bull et al., 2020; Singh et al., 2020). In recent years, the health status of professional athletes has been of increasing concern. This is because high performance sports do not have much in common with health recommendations (Anisimov & Zharinov, 2017; Kettunen et al., 2015). Over the past 20 years, there has been a significant increase in the number of cases of serious diseases/pathologies and sudden deaths among professional athletes, which are clearly linked to the schedule of high-level competitions (Anisimov & Zharinov, 2017; Antero-Jacquemin et al., 2015; Braber et al., 2016). This is due to the fact that high-intensity and high-volume workouts done by athletes to achieve top results lead to changes in multiple organs and organ systems and affect the fundamental processes occurring in cells and body tissues. In particular, this refers to skeletal muscles, as structural muscle changes cause changes in their functional state (Cadegiani, 2020; Park et al., 2021). Intense physical activity leads to a significant increase in the functional activity of skeletal muscles and various organs. This causes intensification and acceleration of the metabolic processes. In parallel, a significant increase in oxygen and energy consumption is observed. If there is a lack of oxygen and energy, cellular hypoxia develops, which disrupts the key metabolic processes, and free radical lipid oxidation (FRLO) occurs, followed by the damage to cell membranes and development of irreversible changes (Seifi-Skishahr et al., 2016; Stellingwerff et al., 2021). Considering the above, it is extremely important to understand the pathophysiological mechanisms that trigger training-related dysfunction and changes in the tissues and organs of athletes. In addition, there is a need to elucidate the rate and features of adaptation to high-intensity physical activity.


Corresponding Author: VALERIAN GAVRILOV, E-mail: gavrilov789@rambler.ru

Among the most important markers of overwork and rate of the adaptive processes in the human body, heat shock proteins (HSPs) are distinguished, which are highly conserved proteins that maintain basic cellular functions (Haslbeck et al., 2019; Krüger et al., 2019). HPSs are chaperone proteins. They facilitate the formation of the secondary and tertiary structures of cellular proteins and maintain their stability. Besides, these proteins play an important role in the processes of repair, elimination of denatured proteins, etc. There are constitutive and inducible HSPs. The former are synthesized in the cell constantly irrespectively of the stressor presence, while the expression of the latter increases sharply in response to a damaging agent (with small amounts of inducible HSPs expressed under normal conditions) (Haslbeck et al., 2019; Ikwegbue et al., 2018; Krüger et al., 2019). The most important inducible HSPs include HSP70. This protein maintains and restores the normal functional activity of cells and promotes their survival (Haslbeck et al., 2019; Krüger et al., 2019). Scientists established that an increase in HSP70 expression drives the synthesis of adenosine triphosphate (ATP), enhancing cellular energy supply. The expression levels of HSP70 are the highest in heart tissues and skeletal muscles. The lowest levels of HSP70 have been reported for brain neurons (moreover, some brain neurons do not express HSP70 in response to stress). Following exposure to a damaging agent, HSP70 was seen to accumulate in the most vulnerable cell areas: initially (during the first 4 hours) in the nucleus (to protect DNA), then in the perisarcolemmal and perinuclear zones, along actin filaments (Krüger et al., 2019).

The physiological role of HSP70 was extensively investigated using a multitude of different models under conditions of hypoxia (Zhai et al., 2019), ischemia (Song et al., 2019; Song et al., 2020), hypertension (Zheng et al., 2018), hyperthermia (Annamaneni et al., 2019), apoptosis (Zhai et al., 2019), autoimmune conditions (Tukaj, 2020), etc. A number of researchers demonstrated that physical activity is the strongest trigger leading to enhanced expression of HSP70 in skeletal and cardiac muscles, as well as immune system cells (Cheng et al., 2020; Harahap et al., 2021). However, there appears to be insufficient research into the relationship between the blood contents of HSP70 and the type, specifics, and intensity of the physical activity practiced by athletes. The authors assume that this research is of immense importance since it can help objectively assess the processes of adaptation to intense and regular physical exercise and improve knowledge about training-induced changes in the body of professional athletes observed on the molecular and biochemical levels. In addition to the above, determination of the HSP70 contents in the blood of professional athletes can serve as a reliable laboratory method for monitoring the safety of their sports activities.

The purpose of this study is to explore the changes in the blood levels of HPS70 in athletes engaged in different sport disciplines and non-athletes. The measurements were taken before and after physical activity in order to evaluate the rate of the muscle adaptation processes.

## Material & methods

### *Participants*

The study involved 120 apparently healthy people aged 18 to 30 years (mean age 25.91 ± 1.39 years). Of them, 47 (39.2%) were women and 73 (60.8%) were men. Considering the national origin, 60 study subjects were Russians, and 60 study subjects were Chinese. The participants were divided into three comparison groups of 40 persons each. Group 1 (control group) included apparently healthy young people who led a sedentary lifestyle and were physically inactive. Group 2 (rowing group) consisted of professional athletes who were engaged in rowing. Group 3 (artistic gymnastics group) involved professional athletes who practiced artistic gymnastics.

The study protocol and informed consent form for participation in the research study were approved at the meeting of the Ethics Committee at Lesgaft National State University of Physical Education, Sport and Health (minutes No. 36 dated November 5, 2019) and Ethics Committee at New York Chiropractic and Physiotherapy Center EC Healthcare (minutes No. 21 dated October 16, 2019). The study followed international norms and principles of biomedical ethics approved by the International Conference on Harmonization (ICH) guidelines for Good Clinical Practice (1996), the Declaration of Helsinki (1964-2013), and the Council of Europe Convention on Human Rights and Biomedicine (dated April 04, 1997).

Enrollment in the study occurred for apparently healthy individuals aged from 18 to 30 years. To be included in group 1, participants were to lead a sedentary lifestyle and did not follow any regular sports activity program over the past 5 years. Groups 2 and 3 subjects were professionals engaged in rowing or artistic gymnastics. All participants were required to consume no caffeine within 1 month before the start date of the study. Finally, they had to provide a signed informed consent form for participation in the study.

The exclusion criteria included the use of vitamins, biologically active substances, energy drinks, anabolics, etc. within 1 month before the start date of the study. The study subjects were not allowed to receive any drugs within 3 months before the start date of the study. Exclusion from the study occurred for persons who smoked or suffered from alcohol and substance abuse. Further, ineligible individuals were those who suffered from any acute or chronic pathologies, congenital malformations, diseases, hormonal disorders, mental disorders/deviations. Lastly, body mass index (BMI) < 20.0 kg/m2 or > 25.0 kg/m2, pregnancy/lactation, and lack of compliance made it inappropriate for a candidate to be enrolled.

All study participants underwent clinical laboratory blood tests. Blood samples were taken in triplicate: before the exercise, immediately after the exercise, and 24 hours after the exercise.

A non-inclined treadmill was used as a mode of submaximal exercise testing. After warming up for 3-5 minutes, each subject walked on a treadmill at 80% of the maximum heart rate for 30 minutes. Then each participant had 3 to 5 minutes of recovery.

***Procedure/Test protocol/Skill test trial/Measure/Instruments***

The key laboratory parameters analyzed were the blood levels of HSP70 and total antibodies to HSP70 (anti-HSP70 IgG/A/M), the activity of total creatine phosphokinase (CPK), the blood levels of myoglobin, the contents of malonic dialdehyde (MDA, which is one of the main FRLO parameters), reduced glutathione (GSH) and catalase activity (which are parameters of the antioxidant defense system (ADS)).

The blood levels of HSP70 and anti-HSP70 IgG/A/M were determined by enzyme immunoassay using standard HSP70 ELISA Kits (Abcam, Cambridge, UK; measurement range: 0.78-50.0 ng/mL) and Human anti-HSP70 IgG/A/M ELISA Kits (Abcam, Cambridge, UK; measurement range: 31.25-1,000 ng/mL).

The CPK activity was quantified by using test strips for rapid analysis Roshe and a portable biochemical analyzer Reflotron Plus (Roshe Diagnostics, Switzerland). The blood contents of myoglobin were determined by the turbiometric method (COBAS 6000, Roche, Burges Hill, UK).

The intensity of the FRLO processes was assessed by spectrophotometry based on the erythrocyte MDA levels. The ADS status was explored by spectrophotometry based on the blood contents of GSH and catalase activity.

***Data collection and analysis / Statistical analysis***

Statistical data were processed using SPSS 13 and Microsoft Excel (2013). The values are presented as mean (M) ± standard deviation (SD). The Mann-Whitney U-test was used to compare the means. The difference was considered statistically significant at $p < 0.05$.

**Results**

General characteristics of the study population are shown below (Table 1). The study subjects had similar age, gender, ethnicity, and anthropometry ($p > 0.05$).

**Table 1**. General study subjects characteristics

| Characteristics | Study group | | |
|---|---|---|---|
| | Control (non-athletes) (n = 40) | Rowing (n = 40) | Artistic gymnastics (n = 40) |
| Age, years | 25.41 ± 1.36 | 26.38 ± 1.29 | 25.94 ± 1.52 |
| Sex | | | |
| female | 15 (37.5%) | 14 (35.0%) | 18 (45.0%) |
| male | 25 (62.5%) | 26 (65.0%) | 22 (55.0%) |
| Nationality | | | |
| Russians | 20 (50.0%) | 20 (50.0%) | 20 (50.0%) |
| Chinese | 20 (50.0%) | 20 (50.0%) | 20 (50.0%) |
| Body height, cm | 172.81 ± 3.06 | 176.25 ± 2.13 | 175.40 ± 2.56 |
| Body mass, kg | 68.54 ± 2.70 | 72.63 ± 2.29 | 70.89 ± 3.04 |
| BMI, kg/m$^2$ | 22.95 ± 0.85 | 23.38 ± 1.01 | 23.04 ± 0.96 |

The basal levels of HSP70 measured in the blood of athletes were found to exceed significantly those in the control group (non-athletes). The values registered for rowing athletes exceeded those registered for non-athletes by 2.83 times ($p < 0.05$). In gymnasts, the basal levels of HSP70 exceeded those measured in non-athletes by 4.20 times ($p < 0.05$). Interestingly, the levels of HSP70 in the artistic gymnastics group were 1.48 times ($p < 0.05$) higher than in the rowing group (Table 2).

**Table 2.** Baseline levels of HSP70, creatine phosphokinase, myoglobin, parameters of lipid peroxidation and antioxidant defense measured in the blood of study subjects, M ± SD

| Parameter | Study group | | |
|---|---|---|---|
| | Control (non-athletes) (n = 40) | Rowing (n = 40) | Artistic gymnastics (n = 40) |
| HSP70, ng/mL | 4.62 ± 0.73 | 13.08 ± 1.15* | 19.40 ± 1.86*/** |
| anti-HSP70 IgG/A/M, ng/mL | 52.84 ± 4.92 | 105.17 ± 7.26* | 134.38 ± 9.62*/** |
| Total CPK, U/L | 101.45 ± 12.49 | 206.51 ± 18.39* | 229.07 ± 20.53* |
| Myoglobin, ng/mL | 69.71 ± 6.80 | 82.44 ± 7.00* | 85.14 ± 7.38* |
| MDA, μmol/L | 5.03 ± 0.67 | 4.96 ± 0.72 | 5.15 ± 0.70 |
| Catalase, mmol/min×g Hb | 14.58 ± 1.35 | 14.83 ± 1.49 | 15.22 ± 1.41 |
| GSH, μmol/L | 0.90 ± 0.08 | 0.91 ± 0.09 | 0.94 ± 0.09 |

*Note. * − the differences are statistically significant in comparison with the control group (non-athletes) ($p < 0.05$); ** − the differences are statistically significant in comparison with the rowing group ($p < 0.05$).*

The baseline levels of total antibodies to HSP70 (anti-HSP70 IgG/A/M) in the rowing and artistic gymnastics groups were 1.99 and 2.54 times ($p < 0.05$) higher than those in the control group, respectively. The intergroup difference between the rowers and gymnasts was statistically significant ($p < 0.05$). No differences were revealed between the blood contents of HSP70 and total anti-HSP70 in subjects belonging to different gender and nationality subgroups ($p > 0.05$).

The study found higher basal levels of total CPK and myoglobin in athletes as compared to non-athletes. Thus, the blood contents of total CPK in the rowing and artistic gymnastics groups were 2.04 times and 2.26 times ($p < 0.05$) higher than in non-athletes, respectively. The levels of myoglobin were 1.18 and 1.22 times ($p < 0.05$) higher, respectively, as compared to non-athletes. Meanwhile, these variables did not present statistically significant intergroup differences for the rowing and artistic gymnastics groups ($p > 0.05$). In addition, the study discovered that the blood contents of total CPK in male athletes were significantly higher as compared to female athletes ($p < 0.05$). The difference was 1.26 times in the rowing group (235.18 ± 19.82 U/L in men versus 186.37 ± 17.44 U/L in women, $p < 0.05$) and 1.21 times in the artistic gymnastics group (256.40 ± 22.51 U/L in men versus 211.37 ± 19.06 U/L in women, $p < 0.05$). There were no statistically significant intergroup differences evidenced for the baseline blood levels of MDA, GSH, and catalase activity ($p > 0.05$).

Immediately after 30-minute submaximal physical exercise, all study subjects experienced an increase in their blood levels of HSP70. There was a 1.64-fold increase in the control group ($p < 0.05$), 1.55-fold increase in the rowing group ($p < 0.05$), and 1.23-fold increase in the artistic gymnastics group ($p > 0.05$). Measured 24 hours after the exercise, the blood levels of HSP70 in the control group exceeded the baseline by 2.34 times ($p < 0.05$). The rowers and gymnasts had their blood contents of HSP70 decreased within 24 hours of submaximal exercise, and their final HSP70 levels did not statistically differ from those determined before the exercise ($p > 0.05$, Table 3).

**Table 3.** The blood contents of HSP70, creatine phosphokinase, myoglobin, parameters of lipid peroxidation and antioxidant defense before and after physical exercise, M ± SD

| Parameter | Group | Before physical exercise | Immediately after physical exercise | One day (24 hours) after physical exercise |
|---|---|---|---|---|
| HSP70, ng/mL | Control | 4.62 ± 0.73 | 7.58 ± 0.90** | 10.82 ± 1.14** |
| | Rowing | 13.08 ± 1.15* | 20.34 ± 1.67*/** | 15.19 ± 1.35 |
| | Gymnastics | 19.40 ± 1.86*/*** | 24.01 ± 1.78* | 20.33 ± 1.62 |
| Anti-HSP70 IgG/A/M, ng/mL | Control | 52.84 ± 4.92 | 76.50 ± 5.33** | 81.62 ± 5.71** |
| | Rowing | 105.17 ± 7.26* | 128.47 ± 8.56*/** | 117.95 ± 8.43* |
| | Gymnastics | 134.38 ± 9.62* | 166.39 ± 10.08* | 141.30 ± 9.51* |
| CPK, U/L | Control | 101.45 ± 12.49 | 140.62 ± 14.25** | 128.70 ± 13.29 |
| | Rowing | 206.51 ± 18.39* | 290.44 ± 23.06*/** | 224.18 ± 20.57 |
| | Gymnastics | 229.07 ± 20.53* | 317.19 ± 25.30*/** | 240.50 ± 22.08 |
| Myoglobin, ng/mL | Control | 69.71 ± 6.80 | 92.95 ± 7.19** | 77.43 ± 6.40 |
| | Rowing | 82.44 ± 7.00* | 94.81 ± 10.15 | 83.45 ± 8.36 |
| | Gymnastics | 85.14 ± 7.38* | 100.35 ± 11.02 | 88.61 ± 7.90 |
| MDA, µmol/L | Control | 5.03 ± 0.67 | 12.73 ± 0.96**/***/# | 10.04 ± 0.74**/***/# |
| | Rowing | 4.96 ± 0.72 | 8.20 ± 0.81**/* | 5.17 ± 0.61* |
| | Gymnastics | 5.15 ± 0.70 | 8.09 ± 0.93**/* | 5.26 ± 0.53* |
| Catalase, mmol/min×g Hb | Control | 14.58 ± 1.35 | 30.25 ± 2.04**/***/# | 27.90 ± 1.82**/***/# |
| | Rowing | 14.83 ± 1.49 | 23.76 ± 1.64*/** | 18.73 ± 1.37* |
| | Gymnastics | 15.22 ± 1.41 | 24.37 ± 1.71*/** | 19.02 ± 1.40* |
| GSH, µmol/L | Control | 0.90 ± 0.08 | 1.13 ± 0.12** | 1.07 ± 0.09**/*** |
| | Rowing | 0.91 ± 0.09 | 1.06 ± 0.15** | 0.98 ± 0.09** |
| | Gymnastics | 0.94 ± 0.09 | 1.07 ± 0.18** | 1.00 ± 0.08** |

*Note. * − the differences are statistically significant in comparison with the control group (non-athletes, $p < 0.05$); ** − the differences are statistically significant as compared to pre-exercise ($p < 0.05$); *** − the differences are statistically significant in comparison with the rowing group ($p < 0.05$); # − the differences are statistically significant in comparison with the artistic gymnastics group ($p < 0.05$).*

In non-athletes, the levels of total antibodies to HSP70 increased by 1.45 times ($p < 0.05$) immediately after the exercise and by 1.54 times ($p < 0.05$) 24 hours after the exercise as compared to the baseline levels. In the rowing and artistic gymnastics groups, there was a 1.22- and 1.24-fold increase ($p < 0.05$) immediately after the exercise as compared to the baseline. As to the levels of anti-HSP70 IgG/A/M 24 hours after the exercise, both groups showed a decrease to the baseline values.

A similar trend was observed for exercise-induced changes in the blood levels of myoglobin and CPK activity. Thus, immediately after the exercise, the activity of total CPK increased by 1.39 times in the control group ($p < 0.05$), by 1.41 times in the rowing group ($p < 0.05$), and by 1.39 times in the artistic gymnastics group ($p < 0.05$). Then, 24 hours after the exercise, there was a significant decrease in the CPK activity ($p < 0.05$) across all study groups. Like before the exercise, there was significantly higher activity of total CPK in the rowing ($p < 0.05$) and artistic gymnastics ($p < 0.05$) groups as compared to the control. After the exercise, the blood contents of myoglobin in the control group increased by 1.33 times ($p < 0.05$). In the rowing and artistic

gymnastics groups, there was only an increasing tendency ($p > 0.05$) in myoglobin levels observed. The contents of myoglobin almost returned to the baseline in all groups within 24 hours after the exercise.

After the exercise, the control subjects had their blood MDA contents increased by 2.53 times ($p < 0.05$). The rowers had a 1.65-time increase ($p < 0.05$), and gymnasts had a 1.57-time increase ($p < 0.05$) in MDA levels. The difference was statistically significant between the control and rowing groups ($p < 0.05$), control and artistic gymnastics groups ($p < 0.05$). At 24 hours after the exercise, the control subjects had their blood levels of MDA increased by 2.00 times ($p < 0.05$) as compared to the baseline. In rowers and gymnasts, this variable almost reached the baseline level, with no statistically significant difference ($p > 0.05$) as compared to pre-exercise.

The activity of catalase (which is an antioxidant enzyme) increased after the exercise by 2.07 times ($p < 0.05$) in the control group and by 1.60 times ($p <0.05$) in the rowing and artistic gymnastic groups, with a statistically significant difference between the control and rowing groups ($p < 0.05$), control and artistic gymnastics groups ($p < 0.05$). At 24 hours after the exercise, catalase activity in the control group remained increased and exceeded the baseline value reported for this group by 1.91 times ($p < 0.05$). As to the rowing and artistic gymnastics groups, their catalase activity decreased and did not differ statistically from the baseline ($p > 0.05$). Immediately after the exercise, the blood contents of GSH increased significantly ($p <0.05$) in all comparison groups, with no statistically significant intergroup difference. At 24 hours after the exercise, the GSH levels remained increased in the control group as compared to the baseline value ($p < 0.05$), with a statistically significant difference between the control and rowing groups ($p < 0.05$). At this timepoint, the rowing and artistic gymnastics athletes had their GSH levels returned to the baseline.

**Dicussion**

The study is devoted to the exploration of changes in the levels of HSP70 and anti-HSP70 IgG/A/M in athletes and healthy non-athletes pre- and post-exercise and intends to assess their role as markers of the functional muscle state. The comparison groups were comparable in terms of age, gender, anthropometry, and nationality. The study found significantly higher basal levels of HSP70 in professional athletes as compared to non-athletes, with significantly higher variables reported for the precision sport (artistic gymnastics) as compared to the cyclic sport (rowing). To demonstrate, the basal levels of HSP70 were as follows: $4.62 \pm 0.73$ ng/mL in non-athletes, $13.08 \pm 1.15$ ng/mL in rowing athletes, and $19.40 \pm 1.86$ ng/mL in artistic gymnastics athletes. Such a difference in the basal levels of HSP70 in the blood of non-athletes and athletes can be explained by the fact that athletes are always physically active and adapted to exercise, and one of the factors of adaptation of muscle fibers to physical activity is heat shock proteins, to which HSP70 belongs. Therefore, high basal blood levels of HSP70 in professional athletes as compared to non-athletes may be regarded as an adaptive response to regular and intense physical exercise. Higher basal levels of HSP70 in the artistic gymnastics group as compared to the rowing group reflect the rate of the adaptation processes depending on the kind of sports activity undertaken. Thus, athletes involved in precision sports (as gymnasts in this study) train various muscle groups and undertake physical exercise of varying intensity and duration. At the same time, athletes engaged in cyclic sports (as rowers in this study) repeatedly use the same muscles and have similar workouts. Therefore, it can be assumed that the adaptive responses of athletes doing precision sports are more plastic.

A similar difference between the comparison groups was observed in terms of the levels of total antibodies to HSP70 (anti-HSP70 IgG/A/M), myoglobin, and CPK activity, which are also a part of the adaptive mechanism in athletes. The lower activity of total CPK in women as compared to men is explained by the protective effect of estrogen in women.

Immediately after submaximal physical exercise, the rowing athletes showed a significant increase in the blood levels of HSP70 (from $13.08 \pm 1.15$ ng/mL to $20.34 \pm 1.67$ ng/mL, $p < 0.05$), which was a response to physical activity. In the artistic gymnastics group, only a tendency for an increase in the blood contents of HSP70 (from $19.40 \pm 1.86$ ng/mL to $24.01 \pm 1.78$ ng/mL, $p > 0.05$) was observed, which might be due to the high basal levels of this protein in the artistic gymnastics group. At 24 hours after the exercise, the blood contents of HSP70 decreased almost to the baseline values, which indicated a fairly rapid muscle recovery after submaximal physical exercise in both groups of athletes and reflected the adaptive response rates. Immediately after the exercise, the blood levels of HSP70 in the control group (non-athletes) increased from $4.62 \pm 0.73$ ng/mL to $7.58 \pm 0.90$ ng/mL (by 1.64 times, $p < 0.05$, as compared to 1.55-time increase in the rowing group and 1.23-time increase in the artistic gymnastics group). A further increase to $10.82 \pm 1.14$ ng/mL (by 2.34 times, $p < 0.05$) was documented for the control group at 24 hours after submaximal physical exercise.

Thus, non-athletes were reported to have the highest percentage of increase in the blood contents of the studied protein. They experienced physiological stress caused by submaximal physical exercise and had less developed and less plastic adaptive capabilities as compared to professional athletes. Considering the quite low basal levels of HSP70, the non-athletes had the expression of this protein increased in response to a stressor (in this case, physical activity) in order to protect muscle tissue from damage. Obviously, the expression was insufficient to restore homeostasis after exposure to a stressor, and the levels of HSP70 in the blood of non-athletes continued to increase within 24 hours after the exercise. Under specific conditions, such an increase in the concentrations of HSP70 in the blood of non-athletes can serve as a marker of muscle tissue damage and

muscle overwork. This was also confirmed for the comparison groups by the dynamics of one of the main parameters of FRLO MDA, as well as antioxidant defense factors GSH and catalase activity. These variables quantified immediately after the exercise were the highest in non-athletes. A high percentage of increase in the blood contents of MDA (from 5.03 ± 0.67 μmol/L to 12.73 ± 0.96 μmol/L) indicated the activation of FRLO processes and development of oxidative stress, resulting in a significant compensatory increase in catalase activity, which was also observed within 24 hours after the exercise and confirmed low body adaptability to the effects of stressors in the form of physical activity in non-athletes. The intensive FRLO processes in the group of non-athletes, which persisted for 24 hours after the exercise, and inability of the ADS to maintain physiological homeostasis also explained the persistent increase in the blood contents of HSP70 as a stress counteracting factor. The rowers and gymnasts had significantly lower blood contents of MDA as compared to non-athletes, which indicated a higher adaptive response rate in athletes and was also associated with the relatively high contents of HSP70 in the blood of athletes as stress protection factor. The lower catalase activity and lower levels of GSH in the blood of athletes are explained by the protective effect of the protein under investigation.

The relatively high content of the latter maintains physiological homeostasis in muscle tissue and prevents the depletion of the antioxidant defense factors.

The blood contents of total anti-HSP70 changed similarly to HSP70. An increase in the blood levels of total anti-HSP70 seen in the study groups in response to physical activity may be indicative of an immunological muscle response to the stressor.

After the exercise, the most remarkable changes in the blood contents of myoglobin were observed in the group of non-athletes. The percentage of increase in the CPK activity did not differ between the comparison groups, which may be due to a fairly short observation period and indicate that the CPK activity and blood contents of myoglobin are less sensitive markers of the adaptive responses produced by muscle tissue in response to physical exercise.

The results obtained in this study are comparable with the results received by other researches investigating the changes in HSP70 expression in response to physical activity 22. (Castellani et al., 2016; Harahap et al., 2021; Murase et al., 2016; Tokizawa et al., 2016). Thus, Murase Y. et al. (2016) characterized the changes in the salivary levels of HSP70 in response to physical exercise in 16 healthy men who led a sedentary lifestyle. The study showed that physical activity causes an increase in the salivary contents of HSP70 and improves the immune function of the oral cavity, as manifested by the increased salivary IgA contents.

The study conducted by Castellani J. W. et al (2016) highlighted that physical activity warming the athlete's muscles up to 40°C causes an increase in the blood concentrations of HSP70, CPK, and myoglobin. Along with that, such activity does not result in damage to skeletal muscles or their disfunction, which may indicate a protective effect of HSP70 during increased levels of physical activity.

There was an illustrative study conducted by Harahap N. S. et al. (2021), which found that athletes doing submaximal exercise have disbalance between the processes of FRLO and antioxidant defense. This causes oxidative stress and, consequently, triggers an increase in cell expression of HSP70, which has a cytoprotective effect.

Akbulut T. et al. (2021) demonstrated that physical exercise yields an increase in the blood contents of HSP70. This parameter may be a marker of muscle damage and muscle wasting.

**Conclusions**

Thus, the study established that the basal levels of HSP70 in the blood of athletes were higher as compared to people who led a sedentary lifestyle. The values registered for rowing athletes exceeded those registered for non-athletes by 2.83 times (13.08 ± 1.15 ng/mL versus 4.62 ± 0.73 ng/mL, $p < 0.05$). In gymnasts, the basal levels of HSP70 exceeded those measured in non-athletes by 4.20 times (19.40 ± 1.86 ng/mL versus 4.62 ± 0.73 ng/mL, $p < 0.05$). Remarkably, athletes engaged in precision sports have higher basal blood levels of HSP70 as compared to those doing cyclic sports (19.40 ± 1.86 ng/mL in gymnasts versus 13.08 ± 1.15 ng/mL in rowers, $p < 0.05$). After a short submaximal exercise, the blood levels of HSP70 increase both in professional athletes and non-athletes.

This increase is associated with the protective effect of this protein in response to a stress factor, and an increase in the blood levels of total anti-HSP70 is indicative of an immune response. Within 24 hours after submaximal physical exercise, the concentrations of HSP70 and total anti-HSP70 in the blood of athletes returned to the baseline values, which indicates the achievement of homeostasis and plasticity of the adaptive processes occurring in the body of athletes in response to intense physical activity. At this timepoint, non-athletes had their blood levels of HSP70 and total anti-HSP70 increased, which indicates impairment of the adaptive processes in the body of non-athletes and inability of their body to restore cellular homeostasis in due time. Thus, before starting an intensive training program and in its course, athletes should have their blood levels of HSP70 and total anti-HSP70 checked for determination of the adaptive capabilities of their body, as well as for early verification of the adaptive process decompensation and prevention of muscle overwork.

***Prospects for further research***

Further research may focus on the effects of antioxidant mediators on the contents of HSP70 and total anti-HSP70 in the blood of professional athletes undergoing intensive preparation for sports competitions.

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**Conflict of interest**
This research has no conflict of interests.

**Availability of data and material**
Data will be available on request.

**Ethics approval statement**
The study followed international norms and principles of biomedical ethics approved by the International Conference on Harmonization (ICH) guidelines for Good Clinical Practice (1996), the Declaration of Helsinki (1964-2013), and the Council of Europe Convention on Human Rights and Biomedicine (dated April 04, 1997).

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