Long-Term Exercises Increase the Concentration of HspBP1, a Co-Chaperone of 70-KDa Heat Shock Protein

E. S. Grebenyuk, T. V. Stupnikova, D. A. Sakharov, V. A. Shleptsova, L. P. Sashchenko, and E. A. Tonevitsky

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 5, pp. 574-578, May, 2010
Original article submitted February 8, 2010

Extracellular concentration of heat shock protein (Hsp) with a molecular weight of 70 kDa (Hsp70) rapidly increases in the serum in response to stress and returns to the basal level during recovery. Further regulation of its blood concentration is unclear. A possible regulator is HspBP1, a protein binding Hsp70. Binding to ATPase domain of Hsp70, HspBP1 inactivates it, thus acting as a factor of nucleotide exchange. Blood sera from athletes were examined at the beginning and end of the last mesocycle of the training period by two-staged immunoaffinity test system. The concentration of HspBP1 increased with decreasing Hsp70 concentration under conditions of long-term training. Presumably, the dynamics of Hsp70 and HspBP1 concentrations can serve as the test for evaluating the adaptation potential.

Key Words: *heat shock protein 70 kDa; co-chaperone of heat shock protein 70 kDa HspBP1; adaptation*

Exercise serves as a signal to production of heat shock proteins (Hsp). They are responsible for regulation of folding of synthesized polypeptides and prevention of protein refolding and aggregation [11] and are essential for adaptation and prevention of apoptosis in cells [9].

The best studied is the protein with a molecular weight of 70 kDa (Hsp70). It is an intracellular protein, but its concentration in the blood rapidly increases in response to physiological stress, fever, or accumulation of cytotoxic molecules. Further regulation of its concentration in the blood is unclear. It is hypothesized that increased serum level of Hsp70 is a defense reaction under stress conditions, resulting in stimulation of the immune system (macrophages, natural killers, cytokines, neutrophils, and mast cells) [8,13].

Hsp70 is an ATP-binding protein with ATPase activity. The ATP-binding form of the protein is characterized by low affinity to denaturared protein, while the ADP form tightly binds to damaged protein [2,4,12], and hence, changes in ATPase activity can modify the defense function of Hsp70. Recently, a protein binding Hsp70 was found: HspBP1. It is an Hsp70 co-chaperone and a nucleotide exchange factor. The interactions of HspBP1 with ATPase domain of Hsp70 induces its conformation changes resulting in inactivation of Hsp70-dependent protein folding [7,10]. However, the interactions of proteins in extracellular space were never described.

The effects of exercise on Hsp70 concentration were studied not once. Changes in the concentrations of HspBP1 and Hsp70 in athletes during training and the relationship between the adaptation reaction and blood concentrations of these two proteins have not been described. It is assumed that the organism is exposed to stress permanently during the training period, when the athletes are engaged in long exercises. The adaptation mechanisms start working, blood concentrations of Hsp70 and HspBP1 are changing. Studies of the new marker proteins of stress and of their regulation will provide information about the mechanisms of adaptation under conditions of stress.

Institute of Physical Culture and Sports, Moscow, Russia. ***Address for correspondence:*** katrinn.g@gmail.com. E. S. Grebenyuk

0007-4888/10/1495-0640

We studied changes in the expression of HspBP1 mRNA and in the concentration of this protein in the serum in response to short highly intense exercise and the proportion of Hsp70 and HspBP1 concentrations in the blood of athletes during adaptation to exercise.

MATERIALS AND METHODS

The study was carried out in 10 athletes engaged in academic rowing for over 5 years (anthropometric values: age 18±0.7 years, body weight 91±11 kg, body length 192±4 cm). Serum levels of Hsp70 and HspBP1 were measured in all volunteers before and after short highly intense exercise and at the beginning and end of the last mesocycle of the training period.

In order to evaluate Hsp70 mRNA (*HSPA1A* gene) and HspBP1 (*HSPBP1* gene) expression in response to short highly intense exercise, blood for isolation of mRNA from leukocytes was collected before and after exercise.

Before exercise test, all athletes were examined by cardiologist and gave written informed consent to participation in experiments and use of the data. The study was approved by the Ethic Committee of the Institute.

Serum concentration of HspBP1 was measured by two-staged immunoaffinity test system based on rabbit antibodies and affinity adsorbents by gel electrophoresis and immunoblotting. Affinity adsorbents were created using activated Sepharose (Thermo). The proteins were separated by electrophoresis by the method of Laemmli under denaturing conditions using Mini-Protein Tetra Cell device (Bio-Rad). The specimens were transferred from PAAG onto Immobilon-P polyvinylidene fluoride membrane (Millipore) by a semi-dry method using Semi Dry Trans-Blot Cell device (Bio-Rad). The specimens were then incubated with specific antibodies and goat antibodies to rabbit immunoglobulins conjugated with horseradish peroxidase (all concentrations were selected experimentally). The final chemiluminescent reaction was carried out using commercial SuperSignal WestPico kit (Pierce), the signal was detected by ChemiDoc XRS device (Bio-Rad). The results were processed using Quantity One software (Bio-Rad).

Serum level of Hsp70 in athletes was measured using EIA-based system, developed at Laboratory of Molecular Physiology of our Institute [1]. The study was carried out throughout a control training mesocycle lasting 28 days. Three days before the beginning of the mesocycle and 2 days after it, exercise tests on a Concept II rowing ergometer with a step-wise increasing power were carried out (starting power 100 W, 50-W step, duration of each step 3 min). The work was continued until the athletes were unable to maintain the preset power. Gas exchange parameters were measured on a Metalyzer II device during the test. Venous blood was collected before and after testing. Heart rate was recorded using Polar S610 heart rhythm monitor (Polar). Lactate concentration was measured using C_Line glucose and lactate automated analyzer (Biosen). Serum concentrations of biomarkers (creatine phosphokinase (CPK), ALT, AST) were measured on a HumaStar 300 automated biochemical analyzer (Human).

The expression of mRNA was evaluated by real-time semiquantitative PCR using specific reagent kits. Venous blood for mRNA isolation was collected into PAXgene Blood RNA tubes (PreAnalytiX GmbH). The mRNA was isolated according to the instruction using PAXgene Blood RNA kit (PreAnalytiX GmbH). In order to rule out primer annealing on genome DNA, the specimens were treated by DNase. The amount of the resultant mRNA RIN (RNA Integrity Number) was evaluated on an Agilent 2100 Bioanalyzer. RIN was >8 for all mRNA specimens. Reverse transcription was carried out with a reverse transcription kit (DNA-Technologies). Total RNA (100 ng) was added for reverse transcription with nonspecific primers. The reaction was carried out at 40°C for 1 h with subsequent inactivation of reverse transcriptase at 95°C for 15 min. The resultant cDNA was diluted 100-fold and used in real-time PCR. Specimens of cDNA before and after exercise were analyzed twice after reverse transcription, each test was repeated 3 times. Real-time PCR experiments were carried out with specific primers to the target gene and two house keeping genes: B2M (β_2-microglobulin) and HPRT (hypoxanthine phosphoribosyltransferase). The increase in the expression was evaluated by the difference in values before and after training, standardized by expression of B2M and HPRT genes.

Specific detection of amplification products was carried out with complementary oligonucleotides labeled with fluorophores and fluorescence quenchers (Table 1).

Amplification was carried out on a DT-96 device (DNA-Technologies) in a real-time mode with 35 µl according to the following protocol: 1 cycle: 30 sec at 80°C, 1 min at 94°C; 50 cycles: 10 sec at 94°C, 20 sec at 64°C. Fluorescence was measured during each cycle at 64°C.

The data were processed using Statistica 7.0 software (StatSoft). The significance of results was evaluated by Mann–Whitney *U* test ($p<0.05$).

RESULTS

Serum testosterone, CPK, ALT, and AST were measured in all athletes before and after short highly in-

TABLE 1. Sequences of Oligonucleotide Primers and Samples for Real-Time Quantitative PCR

Primer	Nucleotide sequence
HSPA1A-s	GTGGGGAGGACTTTGACAACA
HSPA1A-as	CCCTGGTGATGGACGTGTAG
HSPA1A-TM	(BHQ1)-5'-ACAAGAAGGACA(FDT)CAGCCAGAACAAGC-3'P
B2M-s	AGCGTACTCCAAAGATTCAGGTT
B2M-as	ATGATGCTGCTTACATGTCTCGAT
B2M-TM	(BHQ1)-5-'TCCATCCGACATTGAAGTTGACTTACTG-3'P
HPRT-s	CTCAACTTTAACTGGAAAGAATGTC
HPRT-as	TCCTTTTCACCAGCAAGCT
HPRT-TM	(BHQ1)-5'TTGCTTTCCTTGGTCAGGCAGTATAATC-3'P
HSPBP1-s	CCTCCCATGCTCTCACTCTC
HSPBP1-as	GCTCCCCAAGTCCCTTAAAC
HSPBP1-TM	(BHQ1)-5'-TTGTCATCTC(FDT)CTTTGGAGGG-3'P

Note. HSPA1A (GeneID 3303): Hsp70 1A; B2M (GeneID 567): β_2 microglobulin; HPRT (GeneID 3251): hypoxanthine phosphoribosyltransferase 1; HSPBP1: Hsp70 binding protein (GeneID 23640).

tense exercise at the beginning and end of the training cycle.

Maximum oxygen consumption (MOC) was evaluated by the results of gas analysis; its mean value was 49±4 ml/min/kg.

The initial level of total testosterone was virtually the same in athletes (20.9±8.7 nmol/liter). Basal testosterone concentration virtually did not change throughout the entire study and was 18.5±6.2 nmol/liter by the end of week 4.

Activities of ALT, AST, and CPK were normal (10.2±6.1, 21.5±9.8, and 163.5±50.5 U/liter, respectively); no appreciable differences between the athletes were detected. Enzyme activities were within the mean normal range of values, this indicating the absence of injuries in hepatocytes, cardiomyocytes, and skeletal muscles.

Serum concentrations of HspBP1 were measured by immunoaffinity method developed previously. The concentrations of Hsp70 were measured using previously developed EIA test system. Changes in serum concentrations of Hsp70 after short highly intense exercise were negligible (13.7±3.4 before and 18.8±8.4 ng/ml after exercise). However, in some athletes the concentration increased 1.5 times after exercise. The concentration of HspBP1 co-chaperone did not change after highly intense exercise and was 1.1±0.2 ng/ml.

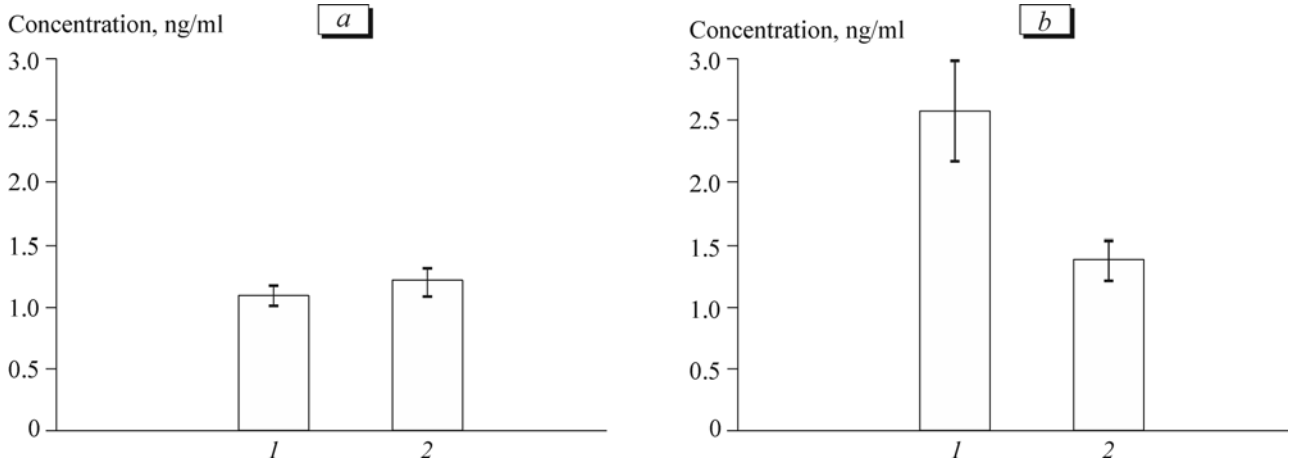

Fig. 1. Concentrations of Hsp70 co-chaperone HspBP1 in rowers of two groups. Here and in Fig. 2: *a*) at the beginning and *b*) at the end of study. *1*) group 1; *2*) group 2.

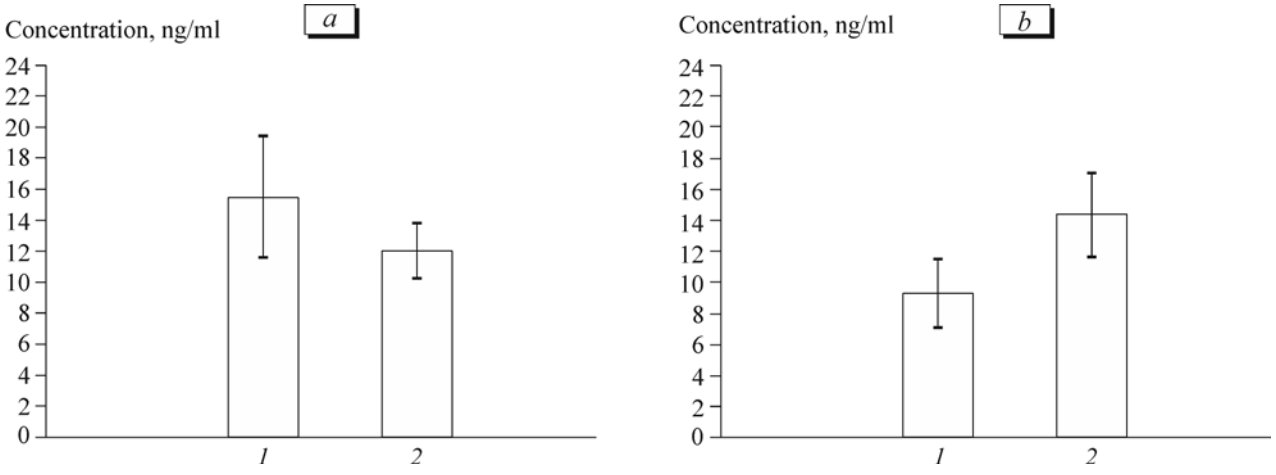

Fig. 2. Concentrations of Hsp70 in two groups of rowers.

These results suggest that HspBP1 is not a protein involved in urgent adaptation reactions.

The expression of HspBP1 and Hsp70 mRNA in blood leukocytes was studied (Fig. 3). Exercise causing physiological stress leads to an increase in *HSPA1A* gene mRNA expression, while the expression of *HSPBP1* gene did not change.

Short-term highly intense exercise led to a statistically significant ($p<0.05$) increase of Hsp70 mRNA expression in leukocytes. This fact indicates triggering of adaptive processes in cells in response to physiological stress, which is in line with numerous previous data [1,3,5,6]. No changes in HspBP1 expression were detected. These data confirm that Hsp70 is an inducible protein and the expression of its mRNA increases in response to exercise. Co-chaperone, in turn, is not involved in urgent adaptation. Presumably, it participates in more intricate and longer adaptation reactions.

After the test with step-wise increasing power, the athletes were divided into 2 groups. Group 1 consisted of better trained athletes (exercise duration over 21 min, MOC 54.1±4.7 ml/min/kg). Group 2 athletes exhibited lower values (exercise duration less than 21 min, MOC 47.5±1.9 ml/min/kg).

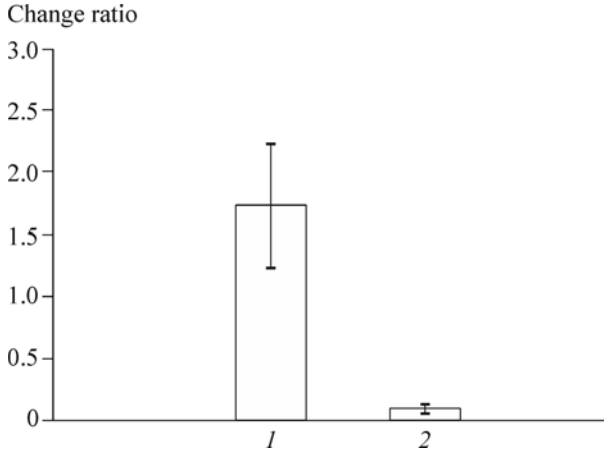

Fig. 3. Changes in HspBP1 and Hsp70 mRNA expression in response to short highly intense exercise. *1*) HSPA1A; *2*) HSPBP1.

The second step of the study was measurements of HspBP1 and Hsp70 concentrations at the beginning and end of the last mesocycle of the training period. The concentrations of HspBP1 were the same in all athletes at the beginning of the study (Fig. 1, *a*). At the end of the study, HspBP1 concentration increased significantly in group 1 and did not change in group 2 (Fig. 1, *b*). The concentrations of Hsp70 were slightly lower in group 2 at the beginning of the study compared to those in group 1 (Fig. 2, *a*). Presumably, this can be explained by the fact that group 2 athletes were not yet fully involved in the training cycle. At the end of the study, the concentrations of Hsp70 differed significantly ($p<0.05$) in athletes of groups 1 and 2 (Fig. 2, *b*).

These data suggest that Hsp70 level in better trained athletes decreased at the end of the training period and reached the basal level, while the level of HspBP1 increased. This indicates adaptation to stress exposure. In group 2 athletes, the concentration of Hsp70 increased, while that of HspBP1 did not change. This presumably indicated less active adaptive reaction to stress exposure. It seems that the time course of Hsp70 and HspBP1 concentrations can be used as the criteria of adaptation capacity.

Hence, short-term highly intense stress caused no changes in HspBP1 expression and serum concentration, while intense training period led to a significant reduction of the concentrations of Hsp70 and BP, detected at the end of the period. A relationship between serum concentrations of these proteins and the professional status of the athletes was revealed. Presumably, the autoregulatory mechanism of intracellular proteins secretion is stimulated in response to stress, blood con-

centration of Hsp70 is changed, and hence, the concentrations of its co-chaperone HspBP1 are changed as well. Binding of co-chaperone with ATPase domain of Hsp70 induces conformation changes in this domain leading to Hsp70 inactivation. Presumably, HspBP1 is a protein regulating active forms of Hsp70 circulating in the serum.

Studies of new stress marker proteins and their regulation at the immunochemical and transcription levels will contribute to our understanding of the mechanism of adaptation to stress conditions.

The study was supported by the Federal Agency for Science and Innovations within the framework of the Federal Target Program "Research and Development of Priority Trends of Scientific and Technological Complex of Russia in 2007-2012" (State contract No. 02.512.11.2318).

REFERENCES

1. D. A. Sakharov, A. V. Stepanov, M. U. Shkurnikov, and A. G. Tonevitsky, *Byull. Eksp. Biol. Med.*, **147**, No. 3, 335-340 (2009).
2. P. Bork, C. Sander, and A. Valencia, *Proc. Natl. Acad. Sci. USA*, **89**, No. 16, 7290-7294 (1992).
3. E. Fehrenbach, A. M. Niess, E. Scholtz, *et al.*, *J. Appl. Physiol.*, **89**, No. 2, 704-710 (2000).
4. K. M. Flaherty, C. DeLuca-Flaherty, and D. B. McKay, *Nature*, **346**, 623-628 (1990).
5. M. Khassaf, R. B. Child, A. McArdle, *et al.*, *J. Appl. Physiol.*, **90**, No. 3, 1031-1035 (2001).
6. Y. Liu, S. Mayr, A. Opitz-Gress, *et al.*, *Ibid.*, **86**, No. 1, 101-104 (1999).
7. C. A. McLellan, D. A. Raynes, and V. Guerriero, *J. Biol. Chem.*, **278**, No. 21, 19,017-19,022 (2003).
8. J. P. Morton, A. C. Kayani, A. McArdle, and B. Drust, *Sports Med.*, **39**, No. 8, 643-662 (2009).
9. P. Narasimhan, R. A. Swanson, S. M. Sagar, and F. R. Sharp, *Glia*, **17**, No. 2, 147-159 (1996).
10. D. A. Raynes and V. Guerriero Jr., *J. Biol. Chem.*, **273**, No. 49, 32,883-32,888 (1998).
11. H. Wegele, L. Muller, and J. Buchner, *Rev. Physiol. Biochem. Pharmacol.*, **151**, 1-44 (2004).
12. C. N. White, L. E. Hightower, and R. J. Schultze, *Mol. Biol. Evol.*, **11**, No. 1, 106-119 (1994).
13. P. Yamada, F. Amorim, P. Moseley, and S. Schneider, *Sports Med.*, **38**, No. 9, 715-733 (2008).