GENETICS

Effect of Exercise on the Expression of *HSPBP1*, *PGLYRP1*, and *HSPA1A* Genes in Human Leukocytes

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The effects of 30-min medium-intensity exercise on the expression of genes encoding heat shock protein 70 (*HSPA1A*) and its cochaperones HSP-70-binding protein 1 (*HSPBP1*) and Tag7 (*PGLYRP1*) in human leukocytes were studied. Transcription activities of *HSPA1A* and *PGLYRP1* genes increased immediately after medium-intensity exercise, while activity of *HSPBP1* gene remained unchanged. During recovery after exercise, the expression of *HSPA1A* gene virtually did not change, while the expression of *PGLYRP1* gene continued to increase and after 90 min more than 2-fold surpassed the basal level.

Key Words: *HSPA1A; HSPBP1; PGLYRP1; exercise*

Exercise modulates transcription of genes encoding proteins responsible for regulation of physiological processes [1,11]. The blood cell transcriptome attracts special interest, which is explained by easy availability of the material. Exercises at the level of 80% of the maximum oxygen consumption (MOC) stimulate transcription activity of genes regulating the functions of NK cells, a population of immunity cells [6,12]. Short-term high-intensity exercise serves as the signal to increase of heat shock protein-70 (HSP-70) mRNA expression in leukocytes, while long-term exercise in the regular training mode (training mesocycle of 28 days) causes an increase of extracellular concentrations of HSP-70 and its cochaperone HSP-70-binding protein [2,4,10]. Importantly, short-term high-intensity exercise and exercise with oxygen consumption of 80% of MOC modify the expression of a number of genes responsible for inflammation, transmission of intracellular signal, and apoptosis processes [9].

HSP-70 is responsible for cell protection from destructive effects of stress factors with ATPase activity and regulates folding of synthesized proteins and refolding of partially denatured ones (*HSPA1A* gene). The interaction of HSP-70 with some proteins considerably modifies its physiological functions. For example, Tag7 (*PGLYRP1* gene), a peptidoglycan-recognizing protein, when binding to HSP-70, forms a stable cytotoxic complex inducing apoptosis in various target cells [3]. Regulation of this cytotoxic activity is realized by HSP-70-binding protein 1 (*HSPBP1*), which binds to HSP-70 ATP-binding domain and inhibits its ATPase activity [7]; this, in turn, inhibits the formation of active Tag7-HSP-70 complex and reduces its cytotoxicity.

The effects of exercise of different intensity on the expression of *HSPA1A* gene are sufficiently well studied. However, we found no published data on the expression of its cochaperone genes (*HSPBP1*, *PGLYRP1*) under similar conditions.

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We studied the effects of long-term exercise of medium intensity on the expression of genes encoding HSP-70 and its cochaperones.

MATERIALS AND METHODS

The study was carried out in 9 athletess (skiers) engaged in training for at least 5 years (mean age 19.3±0.7 years, height 175±7 cm, body weight 68.0±4.6 kg, MOC 59.8±1.5 ml/kg/min). All athletes gave informed consent to participation in the experiment. The experimental protocol was approved by the Ethic Committee of Federal Center of Physical Culture and Sports.

The training status (readiness to exercise) was evaluated by measuring MOC in each participant. One week before the study, all athletes were tested to the limit on a Venus treadmill (h.p.Cosmos) at a stepwise power increase. The starting running velocity was 3 m/sec, the velocity of treadmill increasing by 0.5 m/sec every 3 min. The test was continued until impossibility to maintain the preset running velocity. Analysis of exhaled air was carried out during running in the breath-by-breath mode on an Oxycon Pro ergospirometric system (Viasys Healthcare).

Treadmill running for 30 min at the velocity corresponding to 80% individual MOC served as the model exercise.

Blood from the ulnar vein was collected before exercise (T0), directly after exercise (T1), and during recovery 30 (T3) and 60 (T4) min after exercise.

Lymphocyte subpopulations were evaluated in T0 and T1 blood samples. Peripheral mononuclears were isolated by cell separation in Histopaque-1077 Ficoll density gradient (Sigma). Immunophenotyping was carried out on a FACScan Calibur flow cytofluorometer (Becton Dickinson) with FITC-labeled and PE-labeled monoclonal antibodies to differentiation markers (Sorbent): CD3 (FITC), CD4 (PE), CD8 (PE), CD16 (FITC), CD56 (PE), and CD19 (FITC). Lymphocyte gate for forward and side light scatter was used. The results were analyzed by CELLQuest Pro software (Becton Dickinson).

Isolation of RNA was carried out using PacGene Blood RNA Kit (Quiagen). The concentration of RNA was evaluated by optical density on a Nanodrop photometer (Nanodrop), degradation degree was evaluated using Agilent Bioanalyzer 2100 System (Agilent Technologies). The RNA integrity number (RIN) was >8 for all samples.

Analysis of gene expression was carried out using Affymetrix microchips. The specimens were prepared according to the instruction (Affymetrix Manual P/N 701880 Rev. 4). The resultant RNA served as the matrix for the synthesis of DNA complementary chain. Part of it was then used for the synthesis of biotin-labeled cDNA. After fragmentation biotinilated cDNA was applied onto HyGene 1.0 ST Array chip (Affymetrix) and incubated for 16 h in the hybridization oven at 45°C. The chips were then washed from free cDNA and stained with streptavidin—phycoerythrin on a Fluidics Station 450 (Affymetrix). Stained chips were scanned on a GeneChip Scanner 3000 (Affymetrix).

The data on sample intensities were imported into R medium and processed by xps library (C. Stratova; www.bioconductor.org). The expression was evaluated by RMA algorithm realized in xps [5].

RESULTS

Thirty-minute exercise at the level of 80% MOC caused an increase of *HSPA1A* gene expression by 1.3±0.2 times in comparison with the basal level. During recovery, the gene expression stabilized at the level of 14±0.2 rel. units (Fig. 1).

The model exercise did not change the expression of *HSPBP1* gene. After exercise, it surpassed the initially level by only 4% and remained at this level until the end of recovery.

Our results were in line with the previously reported data on 2.7-fold increase in *HSPA1A* expression in response to short-term high-intensity exercise (stepwise increasing power test) against the background of stable *HSPBP1* level [4]. Importantly, the volunteer experienced oxygen deficiency about one-third of time, which could be an extra stress exposure for the blood cells.

Transcription of *PGLYPR1* gene under the effect of model exercise increased by 1.2±0.3 times and continued to increase throughout the entire recovery period. By the end of recovery, the expression of *PGLYPR1* gene more than 2-fold surpassed the initial level. This was paralleled by reduction of *HSPA1A*

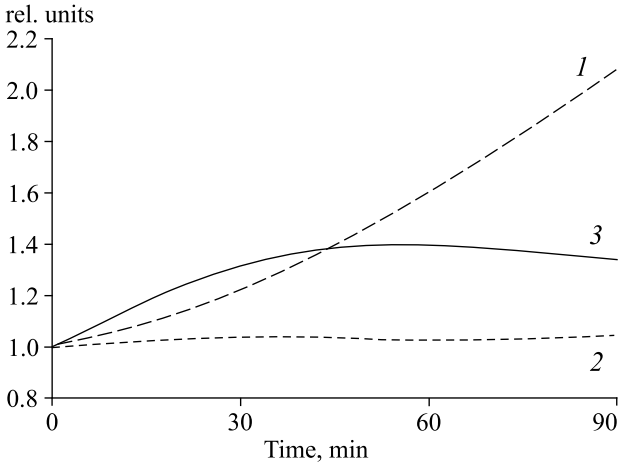

Fig. 1. Effects of exercise on expression of *PGLYRP1* (*1*), *HSPBP1* (*2*), and *HSPA1A* (*3*) genes in peripheral blood leukocytes.

TABLE 1. Effects of Model Exercise on Leukocyte Level

Population	T0 leukocytes		T1 leukocytes		Multiplicity of changes in population percentage
	cell/μl	%	cell/μl	%	
Leukocytes	5710±1170	100±20	7705±2000	100±26	1.00
Lymphocytes	2387±674	42±12	3989±932	52±12	1.24
$CD3^+$	1616±549	28±10	2349±901	30±12	1.08
$CD3^+CD4^+$	1020±413	18±7	1189±419	15±5	0.86
$CD3^+CD8^+$	596±265	10±5	1124±532	15±7	1.40
$CD3^-CD16^+$	494±203	9±4	1380±523	18±7	2.07
$CD3^-CD56^+$	393±217	7±4	1111±468	14±6	2.10
$CD19^+$	227±124	4±2	308±221	4±3	1.01

gene transcription. It was previously shown that the proportion of shifts in the *HSPBP1* and *HSPA1A* gene mRNA concentrations was an important parameter of tumor cell metabolism [4]. The molecular mechanism of interactions between proteins encoded by these genes remaines unknown, but it is known that they are co-located in the cell with PGLYPR1 protein. Presumably, the proportion of changes in the transcription of cochaperone protein genes determines the subsequent changes in the proportions of these proteins in the cells and in the serum [13].

Exercise caused redistribution of lymphocyte subpopulations [12]. In our study, the model exercise increased of the relative count of lymphocyte by 1.3 times (Table 1). The product of *PGLYPR1* gene, Tag7 protein, was produced by $CD3^+CD8^+$ lymphocytes [3]. The summary percentage of these cells increased by 1.4 times, while the expression of *PGLYPR1* increased by 1.2 times. The difference could be due to more active expression of *PGLYPR1* subpopulation of $CD8^+$ lymphocytes activated by IL-2 [14].

Hence, model exercise of medium intensity modified the expression of *HSPA1A* and *PGLYPR1* genes and did not change the expression of *HSPBP1*. Changes in the proportion of the expression of genes encoding HSP-70 and its cochaperones seemed to determine the intracellular and intercellular process of adaptation to physiological stress. The structural characteristics of the genes, proportion of quantities and size of introns and exons were inessential [8].

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