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Are changes in HSPA1A, HSPB1 and LDHb genes expression during physical performance ”till exhaustion” independent of their exercise possibility?

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Abstract

Background: The aim of the study was to designate changes in the expression of HSPA1A, HSPB1 and LDHb in elite rowers after completing a test “till exhaustion” on a rowing ergometer. Finally, we searched for the answer whether there are significant correlations between the expression of the genes and anaerobic threshold (AnT) or the maximal oxygen uptake (VO2max). Material/Methods: The research was conducted on the sample of 9 Polish lightweight male rowers (23.7 ±3.77 yrs, 72.7 ±1.76 kg, 183.6 ±4.58 cm). To determine AnT and VO2max, the subjects performed the test “till exhaustion” with an increasing load on a rowing ergometer. Directly before and after the test, blood samples were collected from the ulnar vein in order to isolate genetic material. RNA was extracted from white cells of venous blood by the chemical method. 2 μg RNA for the reverse transcription was used and the expression of HSPA1A, HSPB1 and LDHb was determined by Real time PCR reaction. To assess the intensity of expression, the ΔΔCt method was used. Results: The study showed an increased expression of HSPA1A and HSPB1 and a decreased one of LDHb. Moreover, post-training changes of the genes activity in white blood cells occurred immediately and could be determined directly after the termination of exertion. Conclusions: No significant correlations between the expression of the genes and anaerobic threshold (AnT), maximal oxygen uptake (VO2max) were stated.

Keywords

gene expression, rowers, physical performance, leukocytes

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Are changes in *HSPA1A*, *HSPB1* and *LDHb* genes expression during physical performance "till exhaustion" independent of their exercise possibility?

Authors' Contribution:
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection

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Introduction

An increase of the production of heat shock proteins under the influence of different stress factors has been previously proved, but less data related to genes encoding them has been studied. It is reported in the literature that the expression of genes is influenced by e.g. thermal stress [1], oxidative stress [2, 3], physical effort [4] and many more exogenous or endogenous factors [5, 6]. In the recent years, the scientists have studied the effect of physical exertion on the expression of genes including those that encode the heat shock proteins [7, 8, 9, 10]. Antiapoptotic effects of proteins that are encoded by *HSPA1A* and *HSPB1* constitute a protective mechanism of the organism during intensive physical exertion accompanied by severe homeostasis disturbances [5]. There is no consensus regarding the function of genes that encode HSP during physical exertion. This inconsistency can be caused by the specificity of muscle work (a different character of physical effort) [11], involving slow twitch fibers (ST) or fast twitch fibers (FT), or a variety of their ratio in the subjects [9], higher or lower energetic exhaustion that is caused by different energy substrates usage during the performance, etc. [6]. Moreover, Łaszczyńska and Seweryn [2] reported individual differences in the expression of the *HSP* genes. Similar studies regarding the expression of *HSPA1A* and *HSPB1* during physical effort were conducted by Maltseva et al. [12]. The results revealed an increase in the expression of *HSPA1A*, and an invariable number of copies of *HSPB1* during 30-min physical effort of moderate intensity. Moreover, Ryan et al. [13] found that in healthy men the expression of *HSPA1A* increases only modestly in white cells of venous blood during 2-hour run on a treadmill in the conditions of heat stress. However, other authors, for example Lui et al. [14] observed an increased expression of the *HSPA1A* in rowers even four weeks after the termination of the training period. Although the profile of genes expression in white cells of venous blood determined after the performance shows the excessive expression of genes that encode stress and inflammatory proteins [5], there is evidence that the type of exertion, its duration and intensity influence the strength of this process. Because there is a variation in the results regarding the genes expression, such aspects as a sports level or specificity of the discipline could be the factors. Moreover, very little research data are available concerning elite sportsmen [5], and few tests involved the maximal load and performance till exhaustion. The authors cited above studied the effect of intense exertion above AnT on the profile of expression in white cells of venous blood in skiers who performed the test on a treadmill. The findings suggest that the excessive expression of some genes, for instance those which encode HSP, occurs during the exertion performed at the intensity above AnT.

The study of gene expression in white cells of venous blood during physical exertion is convenient because of the high availability of blood samples. Furthermore, this study is justified due to the systemic character of the response of the organism to physical effort [10, 12]. According to Nielsen et al. [15], these cells have high antioxidative capacity. It means that the changes of genes expression in white blood cells could differ from the results derived from skeletal muscle cells [16]. Further, professionals show effective mechanisms of adaptation to physical effort. However, the differences in genes expression in participants of various sports are unknown. There is no detailed research data considering this problem. Most studies that allow drawing the above conclusions regard *HSPA1A* [8, 14]. According to Maltseva et al. [12], the expression of *HSPB1* during physical exertion is rarely studied. For *LDHb* expression in human leukocytes similar data have not been found. An excessive expression of the genes that encode HSP hinders oxidative phosphorylation and improves the efficiency of lactate glycolysis [17]. Therefore, analysis of *LDHb* expression in terms of excessive expression of genes that encode HSP seems to be relevant. In our study elite lightweight rowers were subjected to the test "till exhaustion" with the assumption that the expression of *HSPA1A, HSPB1* and *LDHb* occurs in white cells of venous blood. However, it is difficult to predict the range and trend of the changes especially for the *HSPB1* and *LDHb*. Therefore, we questioned whether all three genes show the increase in the expression and whether there are any correlations between them? Moreover, the physiological assessment and monitoring of the rowers require determining their anaerobic threshold (AnT) and maximal oxygen uptake (VO_2max) due to a mixed anaerobic and aerobic character of the work. Therefore, the other aim of the study was to

determine the correlations between the expression of the studied genes, relevant to physical performance among AT or VO_2max in lightweight rowers.

Material and methods

The protocol (KB-3/12) was fully approved by the Ethical Committee of the local Medical Association in Gdansk (Poland), and performed according to the principles of the Helsinki Declaration 2008. All subjects were provided with detailed information about the research procedures and signed a consent form. The research was conducted on 9 lightweight male rowers (mean age 23.7 ±3.77, mean body mass 72.7 ±1.76kg, mean height 183.6 ±4.58cm). Nine subjects were members of the Polish National Team, one participated in the Olympic Games in London, and 8 have been finalists of the Championships of Poland within the last two years. They had on average 8 years' training experience. During the experiment (conducted in the preparation period just before the competition) all the subjects were subjected to the same training program created by the coach of the Polish National Team and did not participate in top tournaments.

The test was carried out in a room with ambient air temperature of 20˚C, atmospheric pressure 991 hPa, and humidity 56%. The subjects were assigned to a certain time between 10 am and 2 pm. They performed the test "till exhaustion" on a rowing ergometer Concept II (model-C, Vermont, USA). Gas analyser Oxycon-Mobile (Erich JAEGER GmbH, Hoechberg Germany) was used to determine maximal oxygen uptake. The initial load was 170 W during the first 3 min of work. During each next 3-min interval, the load was increased by 30 W until exhaustion of the participant and termination of the performance. The load was determined electronically. The highest value of oxygen uptake that was sustained for 15 seconds obtained during the maximal effort was assumed as VO_2max [18]. AnT was determined as a value of load (W) at which lactate concentration reached 4 mmol/l of blood [19].

For the purpose of isolation of total RNA, 2 ml of peripheral blood were collected twice (before and after the test) from the ulnar vein. Prior to RNA extraction, erythrocytes were lysed and discarded using RBCL buffer (A&A Biotechnology, Poland). RNA was isolated from leukocytes using Fenozol (A&A Biotechnology, Poland) to lyse the cells and subsequently precipitate according to a method proposed by Chomczynski and Sacchi [20]. Additionally, total RNA was treated with DNaseI (Initrogen, Life Technology, Poland) in order to digest the remaining DNA and was quantified by spectrophotometry (Eppendorf Biophotometer Plus, Germany). 2 µg RNA was used for cDNA synthesis utilizing Transcript Me system, oligo dT and Random primers (Blirt, Gdańsk, Poland). Q-RT-PCR was performed using Semi Fast Sybr Green qPCR (Biolone, UK) and 2 µl of synthesized cDNA were used as a template. The reaction has been performed in Step One real-time PCR (Applied Biosystem Step One, Life Technology, Poland). Gene expression analysis was performed taking advantage of Livak's comparative method - $2^{-\Delta\Delta Ctrelative}$ [21] using tata box protein (*TBP*) as a reference gene. The reaction mixture (10 µL) included 0.2 µL cDNA, 0.4 µmol/L of each forward and reverse primer, and 5 µL of real-time PCR Sensi Fast Sybr (Bioline, UK). The amplification parameters involved initial denaturation for 2 min at 95 °C followed by 40 cycles of denaturation for 5 s at 95°C, annealing for 10 s at 60°C for all primers and extension for 20 s at 72°C. Dynamic melting curve analysis was executed for all reactions. Primers used in the reaction are summarized in Table 1. Data were collected and relative expression was analyzed with GraphPad Prism 6.

Analysis of correlations was performed with the use of Pearson correlation coefficient. Additionally, a significance test was applied for correlation coefficients. To reveal statistical significant differences, test t was used. The significance was set at $p \leq 0.05$. All figures and statistical analyses were made in Graph Pad Prism 6.0

Table1. Primers used for real time PCR reaction

Gen	Primers
TBP	Reverse primer: TCTGTCGGCTCCGCTCTGAGAT Forward primer: ACTCCCGTTGTCCCAAGGCTTC PRODUCT SIZE: 147bp
HSPA1A	Reverse primer: TTCGGAGAGTTCTGGGATTGTA Forward primer: TGGACTGTTCTTCACTCTTGGC PRODUCT SIZE: 227 bp
HSPB1	Reverse primer: GAGGAAACTTGGGTGGGGTCCA Forward primer: AAGGATGGCGTGGTGGAGATCA PRODUCT SIZE: 125bp
LDHb	Reverse primer: ACCTGCCACATTCACACCACTCC Forward primer: GAAACTAAGTGGATTACCCAAACACCGC PRODUCT SIZE: 181bp

Results

Characteristics of results obtained in the test for work "till exhaustion" within the studied groups (n = 9) are presented in Table 2.

Table 2. Mean values of AnT, VO_2max measured during the test "till exhaustion"

Parameter	Mean	SD
AnT 4mmol	247 W	5.23
VO_2max (ml/kg/min)	54.9	4.313351

The mean expression of the *HSPA1A* and *HSPB1* in the rowers who performed the test "till exhaustion" increased considerably ($p \leq 0.03$). The mean value of *LDHb* transcripts decreased in comparison with the value obtained at the rest phase (Fig. 1). Mean value of the change in activity of *LDHb* did not reach significance.

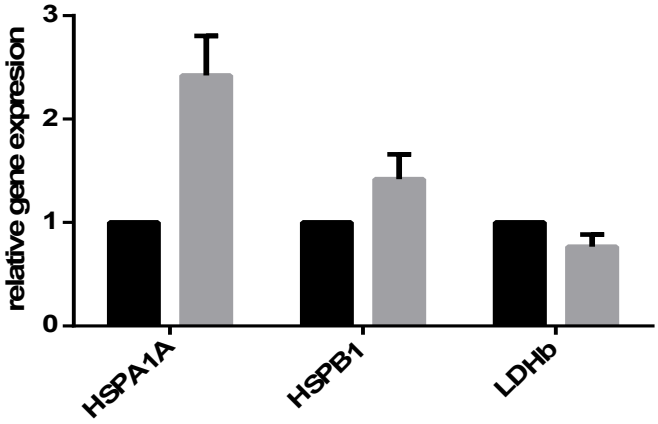

Fig. 1. Relative expression of *HSPB1*, *HSPA1A* and *LDHb* normalized to TBP. Data for three independent experiments involving rowers, measured before (dark bars) and after exercise (gray bars). To better visualize changes, the expression at rest phase is set to 1

Excessive expression of *HSPA1A* and *HSPB1* was observed. The number of *HSPA1A* transcripts increased 2.5 fold in comparison to the values obtained at the rest phase and 1.5 fold comparing to *HSPB1*. The mean expression of *LDHb* slightly decreased (by approximately 0.2). De-

spite these tendencies of an increase or a decrease in the genes expression, individual results show a considerable variation, which is caused by a different response of white cells of venous blood to disturbances of homeostasis that occur during physical performance. No significant correlations (R) between the number of transcripts and AnT and VO_2max were revealed for the rowers regardless of the number of the group.

Discussion

The results of this study show an increase in *HSPA1A* and *HSPB1* expression in white cells of venous blood of professional male lightweight rowers after the test "till exhaustion" performed on a rowing ergometer. However, there was a high diversity of the response of *LDHb* gene. There is little research data regarding the expression of all tested genes concerning a response to physical effort in professionals, whose protective mechanisms against stress are more efficient than in sedentary subjects, especially during work till exhaustion. White blood cells have high antioxidative capacity; therefore, they are supposed to be more resistant to effects of oxidative stress that occur during exertion [15]. Lui et al. [14] studied rowers during a training period and after its termination and found an increased expression of *HSPA1A* during training and even four weeks after its termination. The considerable increase in the expression of *HSPA1A* after exertion of the rowers is consistent with our study, in which significantly higher mean expression of this gene after the exertion "till exhaustion" was observed. Since relevant literature has reported that the character of muscle work (types of fibers involved and their ratio in muscles) affects the expression of genes, it is advisable to compare our results with the findings regarding the same discipline. However, there are no relevant studies except those by Lui et al. [14]. Matseva et al. [12] during 30 minutes of performance at medium intensity observed an increase in *HSPA1A* expression by approximately 40% of the initial value, and steady a level of *HSPB1*. In our study the increase in *HSPA1A* expression was considerably 2.5 times higher than the initial value. Probably the applied load ("till exhaustion") could be the reason for such a considerable increase. Ryan et al. [13] observed only slight changes in *HSPA1A* activity in white cells of venous blood in male adolescent subjects who performed on a treadmill for 2 hours in conditions of heat stress. The results by the authors cited above confirm a diversity of *HSPA1A* expression depending on the intensity of muscle work, or a physiological character of physical effort. Also Donnikow et al. [7] or Sakharov et al. [9] obtained similar results. They reported an increase in *HSPA1A* expression in human white blood cells as a result of short intensive exertion. In our study, the increase in *HSPA1A* expression was observed as an effect of performance "till exhaustion". Furthermore, it is difficult to compare the differences of genes expression between professionals and untrained subjects because the response of their organisms to physical exertion could be different [9]. This latter studied marathon runners and found an increase in *HSPA1A* activity in white cells as an effect of long-term effort. Interestingly, the authors observed decreased *HSPA1A* expression during rest in comparison to the control group as an adaptive reaction to exertion [8].

In our study, beside *HSPA1A*, the level of *HSPB1* and *LDHb* was investigated as well. The mean expression of *HSPB1* was elevated, though not as highly as for *HSPA1A*. When individual results were analysed, high diversity of expression was observed in the rowers for all of the genes, though *HSPB1* was the most homogeneous. Increased expression of *HSPA1A* occurred simultaneously with increased expression of *HSPB*1 in 8 subjects. The biggest differences in individual genes expression were revealed for *LDHb.* Therefore, no significant correlations were observed for changes for this gene although the mean value for the group decreased after exertion. Previous studies have reported that increased expression of *HSPA1A* hinders oxidative phosphorylation and simultaneously intensifies generation of energy through anaerobic glycolysis pathways [22]. However, the response of white blood cells does not confirm this correlation. Thus, it is difficult to draw far-reaching conclusions at this stage of the study. A lack of these relations could be explained by high acidification of plasma that occurred as a result of high lactic acid accumulation in skeletal muscles which was intensely removed to blood. It is likely that white blood cells play a minor role in general acidification of blood and that a decrease in anaerobic glycolysis in white cells prevents blood from further acidification.

However, the correlation between the expression of *HSPA1A* and *LDHb* genes in white cells of venous blood after exertion needs further investigation not only in trained but also in untrained subjects, and additionally in larger groups. The expression of the tested genes is not related to anaerobic threshold or maximal power measured by the test "till exhaustion". No correlations were also stated for maximal oxygen uptake. Moreover, it is obvious that gene expression in white blood cells, which have high antioxidative capacity, is not directly related to VO_2max or AnT. There were also no correlations found between either acidification and the expression of the genes that encode HSP or the expression of *LDHb*. It is likely that professionals have high tolerance of post training changes acquired with regular training. The study revealed excessive expression of *HSPA1A* and *HSPB1*, while expression of *LDHb* was decreased. There was no correlation between expression levels of the studied genes. The results confirmed that changes of expression in white blood cells occur immediately and can be measured just after termination of muscle work. The tested genes are not associated to AnT or VO_2max. Therefore, it is unlikely to determine the correlations when analyzing the genes separately, even if they are highly related to the performance. Individual changes in the expression of the tested genes were observed after a test to refuse work.

Conclusions

Our results indicate the need for individualization of the training even in the top athletes. The lack of personalized training loads can result in the appearance of chronic fatigue and even overtraining.

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