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Effects of 6-week specific low-intensity training on selected aerobic capacity parameters and *HSPA1A*, *HSPB1*, and *LDHb* gene expression in high-level rowers

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ABSTRACT. The aim of this study was to demonstrate the effects of 6-week low-intensity training on changes in indicators of aerobic capacity and on *HSPA1A*, *HSPB1*, and *LDHb* expression in white blood cells in high level rowers. We hypothesized that the type of training would have an impact not only on the adaptation of athletes to the aerobic nature of the exercises, but also on the expression of genes, designated during exercises "until refusal". Nine Polish lightweight male rowers (21.8 ± 3.77 years of age, 74.2 ± 1.7 6 kg, 184.8 ± 4.58 cm) of high level participated in the experiment. To determine the anaerobic threshold (AnT) and peak oxygen uptake (VO_{2max}) at the beginning and end of the 6-week training period, the subjects performed the test "till exhaustion", with increasing load. Directly before and after the test, blood samples were collected from the ulnar vein for isolation of RNA. Consecutively, reverse transcription and real time polymerase chain reaction amplification was performed. A significant influence of applied training on physiological parameters such as VO_{2max} (P = 0.0001),

AnT (W/AT) (P = 0.0007), and maximal acid lactate concentration (P = 0.018) as well as on *HSPA1A* expression (P = 0.0129) in rowers were detected. The 6-week low-intensity aerobic training significantly affected the physiological parameters and *HSPA1A* expression in the rowers. Therefore, we suggest that the response of leukocytes by activating *HSPA1A* was dependent on the type of training. The 6-week period proved sufficiently long to of adapting leukocytes in athletes to high intensity exercises.

Key words: Genes expression; Aerobic training; Till exhaustion test; Rowers

INTRODUCTION

Low-intensity training is equally important in the process of preparing rowers for competition as is high intensity training. It is typically performed at the beginning of the preparation, after a detraining period. Its duration is dependent on the current level of functional capacity of the athletes, as well as on the adopted training objectives. It is known that low-intensity training may improve the exercise ability of athletes. Therefore, it can also affect gene expression associated with the adaptation of the body to physical effort. There are numerous references indicating that the expression of genes encoding heat shock proteins (HSPs) is dependent on various factors such as heat stress (Morimoto, 1998), physical effort (Kregel, 1985), the increase in production of reactive oxygen species-oxidative stress (Radák et al., 2002; Łaszczyńska and Seweryn, 2007), and other exogenous and endogenous factors (Arya et al., 2007; Kaźmierczuk and Kiliańska, 2007). However, little is known about the impact of exercises of different intensity on expression of these genes in leukocytes. The determination of their expression can be a valuable source of information about metabolic changes and purposeful limitation of applied training loads, especially in high level athletes, among whom the variability of physiological indicators is not very dynamic (Zeibig et al., 2005; Maltseva et al., 2012). The anti-apoptotic effects of proteins that are encoded by *HSPA1A* and *HSPB1* constitute a protective mechanism of the organism during intensive physical exertion, which is accompanied by severe disturbances in homeostasis (Arya et al., 2007). However, reports concerning gene expression alterations after training are not uniform, because of the specificity of muscular activity (Ecochard et al., 2000), the different character of physical effort involving slow twitch or fast twitch fibers (Sakharov et al., 2009), or the variety of their ratio in subjects (Kaźmierczuk and Kiliańska, 2007). Furthermore, Łaszczyńska and Seweryn (2007) reported individual differences in expression of the *HSP* genes. Additionally, Ryan et al. (1991) found that the expression of *HSPA1A* increased only modestly in the white cells of venous blood during a 2-h run on a treadmill in conditions of heat stress in a healthy man. However, other authors, for example Liu et al. (2000), observed an increased expression of *HSPA1A* in rowers, even at four weeks after the end of the training period. These data confirm the divergent results obtained by researchers. These results might be affected by the high antioxidative capacity of leukocytes (Nielsen et al., 2008). Changes in gene expression in these cells suggest a systemic response, although this might not always be the same in each athlete, despite the application of similar training loads (Maltseva et al., 2005). According to Maltseva et

al. (2012), the expression of *HSPB1* compared to *HSPA1A* during physical exertion has rarely been studied, and no data have been identified for *LDHb* expression in human leukocytes. *LDHb* is a marker gene for muscle cells (Yoshioka et al., 2003), and its overexpression hinders oxidative phosphorylation and improves the efficiency of lactate glycolysis. Therefore, the analysis of *LDHb* expression in light of the previously discussed excessive expression of genes that encode HSPs seems relevant (Zeibig et al., 2005). In order to demonstrate accurate impact, the effects of selected training loads on the expression of genes encoding HSPs should be considered, including the level of advancement in the discipline of the subjects, the use of a unified training program, as well as a selection of specific testing efforts (Morimoto, 1998).

The aim of this study was to determine the impact of a 6-week low-intensity training regime on changes in indicators of aerobic capacity and on genes encoding HSP proteins (HSP 70, HSP 27) or lactate dehydrogenase in leukocytes of peripheral blood in top-level Polish rowers. We hypothesized that the applied training would have a significant impact not only on the adaptation of athletes to aerobic effort determined by maximal oxygen consumption (VO_{2max}), anaerobic threshold (AnT), or concentration of lactate, but also on expression of the queried genes in peripheral blood leukocytes.

MATERIAL AND METHODS

Ethic statement

According to the guidelines of the Helsinki Declaration (2008), rowers participating in the experiment were informed in detail about the test procedure and provided written consent of participation in the project. The study protocols received ethical approval from the Ethical Committee of the Regional Medical Chamber (KB-3/12). The athletes tested could withdraw consent at any time for any reason.

Participants

The research was conducted on nine lightweight male rowers (mean age 21.7 ± 3.77 years, mean body mass 74.2 ± 1.76 kg, mean height 184.8 ± 4.58 cm). Ten subjects were members of the National Team, one participated in the Olympic Games in London, and eight were the finalists of the Championships of Poland in the last two years. On average, they had eight years of training experience. During the experiment (conducted during the preparation period, just before the competition) all subjects were subjected to the same training program created by the coach of the Polish National Team and did not participate in any top tournaments.

Approach to the problem

Rowers were subjected to a 6-week low-intensity training regime and nine training units were completed in each microcycle. According to the test results obtained at the beginning of the experiment, the load on the ergometer and running as well as swimming were selected individually for each subject. For low-intensity training performed by athletes the accepted load is less than AnT (<75% VO_{2max}) or 50% of maximum strength (F_{max}) (Table 1).

The highest effective exercise times rowers have achieved in anaerobic and anaerobic-aerobic energy zones are shown in Table 2.

Table 1. Typical training microcycle during 6-week low-intensity training.

Day	Training	Exercise	Duration (effectiveness time)	$\%VO_{2max}$	$\%F_{max}$
Monday	10:00-11:30 a.m.	Ergometer for rowers	20 s	60	
		Strength	30 s	-	40
	Afternoon		Free		
Tuesday	10:30-12:00 a.m.	Running	75 s	50	-
	5:30-7:00 p.m.	Ergometer for rowers	45 s	60	-
		Strength	20 s	-	45
Wednesday	10:00-11:30 a.m.	Ergometer for rowers	20 s	60	-
		Strength	30 s	-	40
	Afternoon		Free		
Thursday	10:30-12:00 a.m.	Running	75 s	50	-
	5:30-7:00 p.m.	Ergometer for rowers	45 s		-
		Strength	20 s	-	40
Friday	10:00-11:30 a.m.	Running	60 s	50	-
	5:00-6:30 p.m.	Swimming	60 s	60	-
Saturday	10:00-11:30 a.m.	Team sports (recreation)	90 s	70	-
	Afternoon		Free		
Sunday			Free day		

VO_{2max} = peak oxygen uptake; F_{max} = maximal strength.

Table 2. Training loads performed by the subjects during 6-week low-intensity training.

	AP (min)	MAAP (min)	ALP (min)	ANLP (min)
Monday	570	-	-	-
Tuesday	710	140	30	20
Wednesday	265	-	20	-
Thursday	645	110	-	10
Friday	430	140	30	-
Saturday	-	530	-	10
Sunday	Free day	Free day	Free day	Free day
Total load (min)	2620	920	80	40
			3660	

AP = aerobic performance; MAAP = mixed aerobic-anaerobic performance; ALP = anaerobic lactate performance; ANLP = anaerobic nonlactate performance.

Procedure for conducting special progressive resistance tests "until refusal" and the designation of lactate in blood

The athletes performed the "until refusal" test twice, before and after the 6-week experiment. The test was carried out in a room with ambient air temperature of 20°C, atmospheric pressure of 991 hPa and humidity at 56%. The subjects were assigned to a certain time between 10:00 a.m. and 2:00 p.m. They performed the test "till exhaustion" on a Concept II indoor rower (model-C, Vermont, USA, 2009). An Oxycon-Mobile gas analyzer (Erich JAEGER GmbH, Hoechberg Germany, 2012) was used to determine the maximal oxygen uptake. The initial load was 170 W during the first 3-min effort. During each consecutive 3-min interval, the load was increased by 30 W until exhaustion of the participant and termination of performance. The load was determined electronically. The highest value of oxygen uptake that was sustained for 15 s obtained during the maximal effort was assumed as VO_{2max} (Hahn et

al., 2000). AnT was determined as a value of load (W) at which lactate concentration reached 4 mM in the blood (Beaver et al.,1986). During the test between the change in load was followed by 1-min break. The concentration of lactate in the blood was determined with an enzymatic reagent kit (Randox). The absorbance reading was performed on an EPOLL 20 spectrophotometer (Serw-med s.c., Brand, Poland, 2006).

Genetic research methodology

RNA extraction and reverse transcription

Blood for gene expression research was collected twice, just before and immediately after completing the exercise test, both before and after the 6-week low-intensity training. Total RNA was extracted from 2 mL venous blood. In the first step, erythrocytes were lysed by RBCL buffer (A&A biotechnology, Gdynia, Poland). Next, isolated leukocytes were lysed by Fenozol (A&A Biotechnology) and RNA was extracted according to the method published by Chomczyński and Sachi (1987). The quantity and quality of the isolated RNA was determined by spectrophotometry (Eppendorf Biophotometer Plus, Hamburg, Germany). To degrade any contaminating DNA, RNase-free DNase I (Life Technology, Warsaw, Poland) was added to the samples. Total RNA (2 µg) was reverse transcribed using the TranscripMe Kit (Blirt, Gdańsk, Poland) in 20 µL total volume.

Quantitative polymerase chain reaction (qPCR) assay to determine the HSPA1A, HSPB1, and LDHb mRNA levels

The quantification of *HSPA1A*, *HSPB1*, and *LDHb* gene expression was carried out using an Applied Biosystems Step One Cycler (Applied Biosystem) with Sybr®Green I as the fluorophore. Expression of genes analyzed was determined with Livak's comparative method $2^{-\Delta\Delta Ct}$ relative to the geometric mean of the expression levels of the *TBP* housekeeping gene. The reaction mixture (10 µL) included 0.2 µL cDNA, 0.4 µM of each forward and reverse primers, and 5 µL real-time PCR Sensi Fast Sybr (Bioline, UK). The cycling conditions were those recommended by Bioline. All reactions were repeated three times. Dynamic melting curve analysis was performed for all reactions. Primers used in the reaction were: for *TBP* reverse primer: 5'-TCT GTC GGC TCC GCT CTG AGA T-3' and forward 5'-ACT CCC GTT CCC AAG GCT TC-3'; for *HSPA1A* reverse primer: 5'-TTC GGA GAG TTC TGG GAT TGT A-3' and forward 5'-TGG ACT GTT CTT CAC TCT TGG C-3'; for *HSPB1* reverse primer 5'-GAG GAA ACT TGG GTG GGG TCC A-3' and forward 5'-AAG GAT GGC GTG GTG GAG ATC A-3'; and for *LDHb* reverse primer: 5'-ACC TGC CAC ATT CAC ACC ACT CC-3' and forward primer: 5'-GAA ACT AAG TGG ATT ACC CAA ACA CCG C-3'.

The data were collected and relative expression was analyzed in Excel 2005 (Microsoft Corp., Redmond, WA, USA). In order to calculate the level of gene expression, the Schmigtten and Livak (2008) method was used. To assess the statistical significance the following tests were used: the normality of the distribution was checked with the Shapiro-Wilk test. The non-parametric Wilcoxon test (comparing results before and after the test) and the Mann-Whitney U test (comparing results between groups) were applied to dependent samples. Person's correlation was calculated between genes and physiological parameters. All calculations were performed in Statistica 10 (StatSoft, Inc. Tulsa, OK, USA) and graphics were

performed in GraphPad Prism 6.0 (www.graphpad.com). Statistically significant differences were considered as $P \leq 0.05$.

RESULTS

The applied training load in the low-intensity range had a statistically significant effect on increased VO_{2max}, $[La]_{max}$, and anaerobic threshold (W/AT) in the rowers tested (Tables 1 and 2; Figure 1).

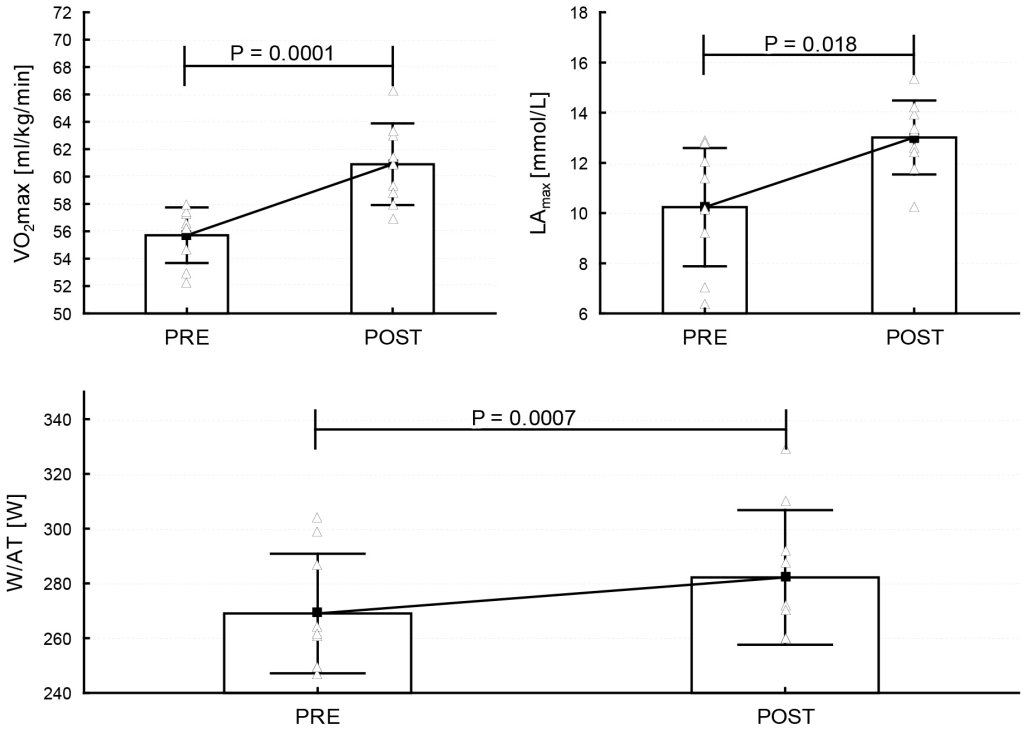

Figure 1. Mean value of VO_{2max} $[LA]_{max}$ and anaerobic threshold pre and post 6 weeks of low-intensity training.

Gene expression

The resting levels of gene expression before performing the "until refusal" test between subjects did not differ significantly. Figures 2-4 show the average change in post-exercise expression compared to the resting value.

In Figure 2, the mean changes in expression of *HSPA1A* are presented as mean changes pre-exercise compared to post-exercise. There was a statistically significant change in *HSPA1A* expression after the 6-week low-intensity aerobic training.

A decrease in the expression of *HSPB1* was also observed after the 6-week low-intensity aerobic training, but it was not statistically significant.

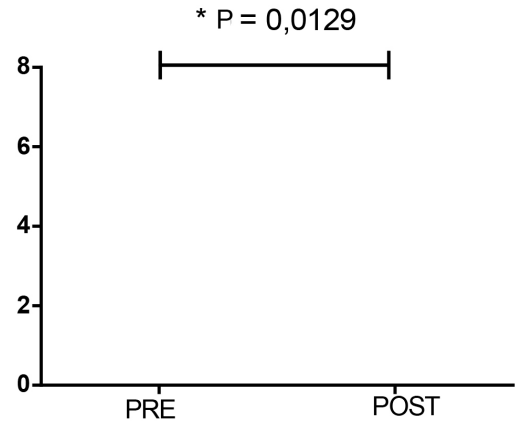

Figure 2. Mean changes in gene expression of *HSPA1A* measured before and after tests to exhaustion pre and post 6-week low-intensity training.

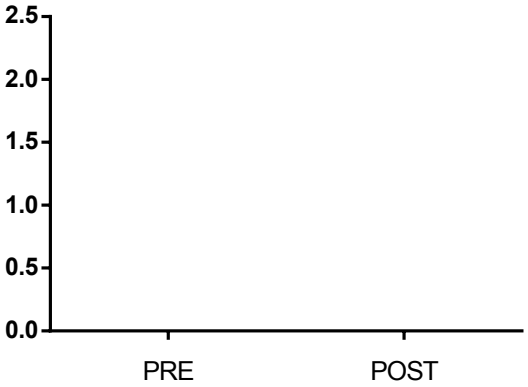

Figure 3. Mean changes in gene expression of *HSPB1* measured before and after tests to exhaustion of work pre and post 6-week low-intensity training.

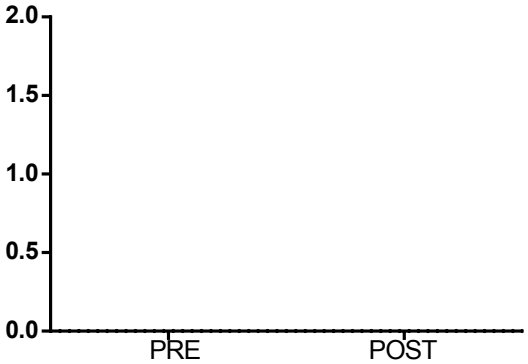

Figure 4. Mean changes in gene expression of *LDHb* measured before and after tests to exhaustion of work pre and post 6-week low-intensity training.

For *LDHb* an increase in expression in peripheral blood leukocytes was observed during the second "until refusal" test, performed after the 6-week low-intensity training. However, this change was not correlated with the increase in lactate concentration in the blood in athletes tested at the same time.

DISCUSSION

In terms of the physiological parameters measured significant changes were identified after the 6-week low-intensity training in the lightweight rowers studied. However, changes in the expression of the *HSPA1A*, *HSPB1*, and *LDHb* genes at this time were varied. For *HSPA1A*, significantly lower mean expression was detected after the 6-week training; however changes in expression of the two other genes were not statistically significant. There are few studies in the literature associated with *HSPB1* and *LDHb* expression during the physical efforts of professional athletes, especially in "until refusal" tests. In this study it was assumed that the energy exhaustion accompanying the "until refusal" effort and the developing oxidative stress would significantly affect the expression of genes encoding HSPs in rowers (Arya et al., 2007; Kaźmierczuk and Kalińska, 2007). Significant differences in *HSPA1A* activity between first (pre) and the second (post) laboratory tests suggested that the type of training affected gene expression during the "until refusal" tests. Liu et al. (2000) studied rowers during the training period and after its termination and found increased expression of *HSPA1A* during training and even four weeks after its termination. An increase in expression of *HSPA1A* after a 4-week training period was observed. In our study, 6 weeks of low-intensity training caused a significant decrease in *HSPA1A* expression in the rowers tested, compared to the results obtained before this period. The overexpression of genes encoding HSPs during physical effort conditions is a factor that keeps human cells alive and protects them from apoptosis. Morton et al. (2009) suggested that the overexpression of genes encoding HSPs occurs after crossing a critical threshold. This threshold is associated with the individual characteristics of a person, the level of training, and the antioxidant status. This might suggest an association between the level of expression of genes encoding HSPs and the body stress load. The changes in expression of genes associated with HSPs are hard to interpret. On the one hand, their overexpression suggests the level of stress load (Morton et al., 2009), whereas on the other hand, a decrease in their expression, as seen in this study, might be associated with the absence of adequate protection against factors disrupting metabolic functions within the body. The authors of this study are inclined to conclude that excessively long periods of low-intensity training increases the adaptation to the exhaustive efforts so that the same load induces relatively less stress. The varying activity of *HSPA1A* depending on the intensity of muscle effort and its duration has been reported by such authors as Ryan et al. (1991), Donnikov et al. (2008), and Sakharov et al. (2009). Our studies indicate that different training loads also determine the quantity of transcripts of genes tested. Unquestionably, little is known about changes in *HSPB1* expression in conditions of physical effort. There were no changes in *HSPB1* expression during 30-min moderate intensity exercises among young men as studied by Maltseva et al. (2012). In contrast, in our experiment the mean changes in *HSPB1* expression in the testing exercise performed before the low-intensity training period were considerable (1.4 fold more than the resting value). However, after the 6-week low-intensity training period the change in expression of this gene was not statistically significant. Previous studies have reported that the increased expression of *HSPA1A* hinders oxidative phosphorylation and simultaneously

intensifies generation of energy through anaerobic glycolysis pathways (Wang and Subject, 2013). We observed that the increase in *HSPA1A* was associated with an increase in *LDHb* expression, but only during the first "until refusal' test. In the second test, the direction of change was the opposite (an increase in *LDHb* and decrease in *HSPA1A* expression). Therefore, our results are not consistent with the suggestions of Wang and Subject (2013), and might indicate other, more significant factors that affect the activity of *LDHb*. Despite the lack of statistical significance of the differences observed in our study, we made the observation of an increase in the acidity of plasma that coincided with the increase of the expression of *LDHb*. However, at this stage of research it is difficult to draw far-reaching conclusions regarding how acidification might affect blood *LDHb* expression in leukocytes. The correlated expression of *LDHb* and *HSPA1A* with [La] after exercise in peripheral blood leukocytes requires further study as it might provide a more accurate response measure to stimulating factors related to the expression of these genes.

Overall, our study demonstrated that a 6-week low-intensity aerobic training resulted in a statistically significant effect on VO_{2max}, W/AT, and $[La]_{max}$ as well as *HSPA1A* expression in lightweight class rowers. On the basis of these data, the authors suggest that the response of leukocytes (treated as systemic) in the form of activation of genes tested is dependent upon the type of training. Furthermore, low-intensity training appeared to increase the tolerance of leukocytes to the "until refusal" effort. A 6-week low-intensity training was therefore sufficient to increase the tolerance of the athlete's body to high intensity effort.

Conflicts of intererest

The authors declare no conflict of interest.

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