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ORIGINAL

Changes in leukocyte *HSPA1A*, *HSPB1* mRNA in basketball players after plyometric training

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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abstract

Background Exercise-induced stressors activate leukocyte *HSPA1A* and *HSPB1* gene transcripts. However it is not clear how plyometric training affects the expression of these genes in basketball players under plyometric exercise. Therefore, the aim of this study was to investigate the changes in leukocyte *HSPA1A* and *HSPB1* mRNA, in male basketball players after plyometric training.

Material/Methods Twelve male college basketball players (age 22.1 ±2.96 years) took part in this study. Peripheral blood (2.0 ml) was collected from the ulnar vein of each participant before and after a plyometric exercise to assess *HSPA1A* and *HSPB1* mRNA relative expression of leukocyte via quantitative reverse transcription polymerase chain reaction.

Results A significant increment of leukocyte *HSPA1A* mRNA expression (Qt from 1.67 ±0.93 to 3.17 ±0.97, p = 0.003) after plyometric exercise was found. However, there was no significant change in leukocyte *HSPB1* mRNA expression, indicating the high stability of this gene during exercises.

Conclusions *HSPA1A* mRNA was found to be a very sensitive indicator and could be used to assess physiological adaptation to a physical load and time requirements for complete recovery in basketball players.

Key words gene expression, heat shock proteins, basketball, plyometric training

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INTRODUCTION

Basketball is considered as a physically demanding team sport that combines repeated bouts of short intensive intermitted exercises and bodily collisions between the players. Intense physical training and too frequent competitions result in catabolic/anabolic imbalance, muscle damage and protein breakdown [1, 2]. Plyometric training is often used in basketball by combining it with specific basketball drills by alternating intensity and duration. This type of training causes muscle damage and increased production of reactive oxygen species and oxidative stress [3, 4, 5, 6, 7]. This is the main reason for increased transcriptional activity associated with stress response in those muscle damages [8].

In cells, the protective role of HSP during physical effort is mediated by inhibition of apoptosis [9]. Therefore, overexpression of genes encoding HSP, especially *HSPA1A* mRNA, may indicate the greater impact of stress factors. Buttner et al. [10] suggested that the level of *HSPA1A* mRNA is associated with the training load and therefore may be useful as a target gene for monitoring training. In the last years, Radom-Azik et al. [9] have conducted a study on the expression of genes encoding HSP and interleukin in leukocytes after 30-min physical effort. These authors suggested that high-intensity exercises result in overexpression of genes associated with apoptosis like those of *HSPA1A*. Maltseva et al. [11] determined the expression of *HSPA1A* and *HSPB1* mRNA in peripheral blood leukocytes of young men after 30-min running with a moderate load. They found overexpression of *HSPA1A* mRNA but a constant level of *HSPB1* mRNA, whereas Ryan et al. [12] reported only minor changes in the expression of *HSPA1A* mRNA in five healthy men after a 2-h effort on a treadmill under heat stress conditions. Surprisingly, Lui et al. [13] observed overexpression of *HSPA1A* mRNA even four weeks after the training period was completed. Diversified gene expression in athletes may result from both varying effort intensity and different sport levels. Furthermore, some authors reported a lower expression of *HSPA1A* mRNA after physical effort in trained subjects in relation to the untrainedones [10, 14]. Moreover, Ferenbrach et al. [15] reported that well-trained individuals have a decreased basal *HSPA1A* mRNA expression as adaptation to a repeated training load.

In the present study we investigated the expression of genes encoding heat shock proteins *HSPA1A* and *HSPB1* mRNA in peripheral blood leukocytes of basketball players after plyometric training. According to Zeibig et al. [16], determination of gene expression in peripheral blood leukocytes is justified since it indicates the global body response to the training load. Additionally, peripheral blood is a highly accessible source of these cells [11, 16]. According to Radom-Azik et al. [9], the association of neutrophil gene expression with exercise is more than just a stereotypical response to stress. Probably, it represents an integrated or summed response to various physiological changes associated with physical activity. According to Buttner [17] and Kregel [18], physical effort is one of the factors leading to increased level of HSP, especially those of the 70- or 90-kDa families.

When these observations are taken together, there is no unambiguous answer to the following question: How does gene expression vary as a function of muscle work intensity, time of effort, physiological characteristics of individual players, and muscle damage? Moreover, the biochemical factors that stimulate gene expression in leukocytes have not been fully identified yet.

Therefore, the aim of this study was to determine the changes in *HSPA1A* and *HSPB1* mRNA in basketball players' leucocytes after plyometric training.

MATERIALS AND METHODS

PARTICIPANTS AND STUDY DESIGN

12 college level male basketball players, with the following characteristics (mean ±SD): age 22.1 ±2.96 years, weight of 87.3 ±9.36 kg, height of 195.7 ±5.46 cm, maximal oxygen consumption (VO^2max) of 50.0 ±6.06 ml · kg^{-1} · min^{-1}, and average levels of Hb 14.5 ±5.78 g/dL at the beginning of the study, were involved in the current study. All players had engaged in basketball training for 5–8 years and trained five times per week for 2 hours. This study was performed at the end of the preseason period. In order to determine expression of HSPA1 and *HSPB1* mRNA, blood samples were collected before and after plyometric exercise (Table 1). Players did not have any intensive exercises for 48 hours before testing procedures. A training session involved: warm-up (15 min), plyometric exercises (30 min), individual technical exercises (40 min) and stretching (5 min) (Table 1). The warm-up exercises were focused on muscles preparation for plyometric exercises. The main part involved plyometric exercises composed of hurdle jump with regard to the body height of the players (height >187 cm – a hurdle was 80 cm; height <187 cm – a hurdle was 75 cm) and jumps over the bench (30 cm). Depth jumps on 40 cm and 80 cm height of box were performed. Players also performed technical exercises with low intensity physical activity.

According to the guidelines of Helsinki Declaration, players participating in the study were informed about the test procedures and provided written consent of participation in the project. The study protocols were revised and received ethical approval from the Ethical Committee of the Regional Medical Chamber.

ANTHROPOMETRY

Height (cm) was measured with a Martin metal anthropometer to the nearest 0.1 cm according to the standard technique. Body mass (kg) was measured using medical electronic scales (A&D Instruments, Abingdon, UK) and recorded with 0.05 kg precision with the subject wearing light clothes. The body mass index (BMI; kg/m^2) was calculated.

BLOOD ANALYSIS

Peripheral blood (2.0 ml) was collected from the ulnar vein of each participant before and after plyometric training. Prior to RNA extraction, erythrocytes were lysed and discarded using RBCL buffer (A&A Biotechnology, Poland) for 20 min in ice. Then, blood was centrifuged (300 rpm for 10 min) and the obtained leukocytes were lysed using Fenozol (A&A Biotechnology, Poland) and the RNA was subsequently precipitated using the method described by Chomczynski and Sacchi [19]. After extraction, RNA was treated with DNaseI (Invitrogen) to digest any remaining DNA. Complementary DNA (c-DNA) was synthesized using the Transcript Me system (Blirt, Gdańsk, Poland) following the manufacturer's instructions. A mixture of master mix (10 µL), enzyme (2 µL) and RNA (2 µg), and water (sufficient to a final volume of 20 µL) was added to each tube. Samples were then incubated (25°C, 10 min; 55°C, 30 min; and 85°C, 5 min). Quantitative RT-PCR analyses for *HSPA1A* and *HSPB1* were

performed using the Step One real-time PCR system (Applied Biosystems). Each qRT-PCR reaction mix consisted of Semi Fast Sybr Green qPCR master mix (10 µl; Biolone, UK), cDNA (2 µl), each primer (0.8 µL; 10 pM) (final volume: 20 µL). Thermal cycling conditions included an initial hold (95°C for 2 min) followed by 40 cycles (95°C for 15 s; 60°C for 10 s; and 72°C for 20 s. All samples were assayed in triplicate. Target gene expression was normalized to the expression of the reference gene Tata Box Protein (TBP).

STATISTICAL ANALYSIS

Statistical analysis was performed with GraphPad software (GraphPad Prism 6.0). Means and standard deviations were calculated (±SD).

Relative gene expression levels were calculated using the delta-delta CT method [20]. The normality check was done using Shapiro-Wilks test. The differences of variables before and after plyometric training were tested using parametric analysis t-test for independent samples was utilized. For non-parametric analyses, the Wilcoxon test was used. Pearson correlation coefficients were used to determine the relationships between the variables. The significance level was set at $p < 0.05$.

RESULTS

Physical characteristics of the subjects are presented in Table 1. In Figure 1 relative expressions of *HSPA1A* and *HSPB1* are presented (Figure 1).

The number of the copy of transcript *HSPA1A* mRNA was significantly greater ($p = 0.04$). After training a significant increase in *HSPA1A* mRNA (from 1.67 ±0.93 to 3.17 ±0.97, $p = 0.003$) was marked. According to literature, authors consider that changes higher than 2-fold show up-regulation of expression. Hence, the plyometric training caused overexpression of *HSPA1A*. The changes in *HSPB1* mRNA were little and insignificant (from 0.24 ±0.1 to 0.29 ±0.16).

Table 1. Plyometric training

Plyometric exercises
Front obstacle jumps over hurdles 4S/8R, break between the sets – 45 s
Depth Jumps (box height 40 cm – ground – 80 cm box – ground –40 cm box – 1 repetition). Focusing on speed. 4S/2R, break between the sets – 60 s
Lateral obstacle jumps (75 or 80 cm), jumping over hurdles 4S/6R, break between the sets – 45 s
Depth Jumps (box 40cm height)+ Jump straight (as quickly as possible, jump back up straight into the air. Focusing on airtime). 4S/5R, break between the sets – 60 s
Lateral obstacle jumps (30 cm) (without pause between jumps) 4S/10R, break between the sets – 60 s

Explanation: S– sets, R – repetitions

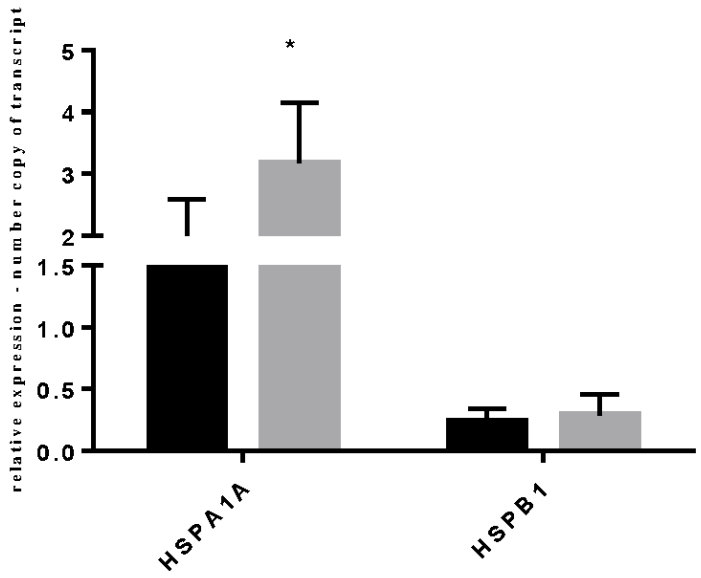

Fig. 1. Relative expression of *HSPA1A* mRNA and *HSPB1* mRNA (mean ±SD) before (dark bars) and after (grey bars) plyometric training

DISCUSSION

Acute exercises are followed by a significant disruption in body temperature, blood pH, O_2 saturation, and circulating cytokines. Each of them is known to affect the activity of neutrophils [9, 17, 21].

This study reveals changes in gene expression *HSPA1A* mRNA in peripheral blood leukocytes after plyometric training applied to basketball players. The highest and significant expression of *HSPA1A* mRNA and a stable number of copy of *HSPB1* transcript was observed in players. The remaining data are compatible with those of Buttner et al. [10], who suggested that *HSPA1A* mRNA levels may indicate a training load. Therefore, we also suggest that a *HSPA1A* mRNA level may be useful for monitoring training [10] and, in our opinion, it is very sensitive to intensive physical exercises.

It is known that *HSPA1A* mRNA is sensitive to physical activity and its over-expression is associated with cell protection via anti-apoptotic functions [3, 22]. Some studies focused on the impact of the nature of muscle work on both genes expression [12, 23, 24] and the type of muscle fibres involved in the training, including their relative proportions [14]. Donnikov et al. [23] and Sakharov et al. [25] observed an increase in *HSPA1A* mRNA expression after brief efforts. However, another group of investigators found only minor changes in the gene expression in peripheral blood leukocytes of healthy young men after a 2-h effort on a treadmill in heat stress conditions [12]. In this study, plyometric training resulted in a significantly increased *HSPA1A* mRNA level after complete training. Radom-Azik et al. [9] also reported that 30-min acute exercises affected gene expression only in untrained men. This result may be associated with too short time as well as with too lower a load for athletes.

Overexpression of *HSPB1* mRNA was also associated with physical performance and was induced by heat stress [26, 27, 28]. A study on *HSPA1A* and *HSPB1*

mRNA expression and physical performance was conducted by Maltseva et al. [11]. These authors reported a constant level of *HSPB1* mRNA during 30 min of medium-intensity physical activity. In our study, the low changes in *HSPB1* mRNA expression were determined immediately after the training had been completed. The authors of this article suggest that activation *HSPB1* mRNA occurs later (unpublished data), according to its function, i.e. the necessity of degradation of proteins caused by the stressor effect. These results point to the differences in activation of both genes.

Regarding changes in the expression of genes encoding HSP proteins, it is likely that activation of *HSPA1A* mRNA is immediate whereas *HSPB1* mRNA expression is delayed and occurs after the muscle work has been completed. It can be considered that *HSPB1* plays a very important role in degradation of damaged proteins during physical effort [26, 29], and an increase in expression occurs later.

Transcriptional activity can be stimulated by muscle damage [28]. In our opinion for this reason *HSPA1A* mRNA is sensitive because its activation occurs as a result of acidosis [3], oxidative stress [18, 30], and increased temperature [22], which are known to influence the expression of genes considered in our study.

The cellular adaptation to stress could also be mediated by a signalling effect of Hsp70 on metabolic rates, which requires adequate levels of ATP [21].

CONCLUSIONS

Thus, *HSPA1A* mRNA seems to be a very sensitive indicator for plyometric training and shows that this type of training is severe and causes increased protection to apoptosis.

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