Expression of serum HSP27 under exercise-induced muscle damage in elite greco-roman wrestlers

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Key words: heat shock proteins, muscle damage, sport training

Summary

Introduction. In response to stress, cells rapidly produce proteins known as heat shock proteins (HSPs) that are considered to be molecular chaperones, playing a universal role in maintaining cellular homeostasis. Among the subset of stress-responsive proteins, evaluation of HSP27 level is considered to be a new approach to monitoring exercise training and adaptive mechanisms. The aim of the study was to assess whether the wrestling training increases production of HSP27 which can stimulate the regeneration of damaged skeletal muscle in athletes.

Material and methods. Blood samples were collected from nine elite Greco-Roman wrestlers during the conditioning camp at in-season period (June) including efforts of a comprehensive impact such as running, kayaks, games, strength (50.8%), efforts of the impact of targeted force (15.4%), and special/wrestling training (30.8%). Nine healthy and untrained males made a reference group.

Results. It was found that HSP27 concentration was approx. 40% higher in wrestlers than non-athletes and correlated with total plasma creatine kinase (CK) activity (r = -0.734, P<0.05). Wrestlers showed a body mass index (BMI), fat mass (FM) and free fat mass (FFM) on higher level than in non-athletes. BMI, FM and FFM significantly correlated with CK activity. Moreover, CK activity inversely correlated with nitro-oxidative index NO/H₂O₂ (r = -0.798, P<0.05).

Conclusions. In conclusion, muscle damage induced by wrestling training is important factor modulating changes in nitro-oxidative ratio and HSP27 expression as well as body composition. High HSP27 may be implicated in the regenerative process in Greco-Roman wrestlers.
and H₂O₂ are produced during muscle work by the enzymes nitric oxide synthase (isoforms nNOS, eNOS and iNOS) and superoxide dismutase (isoforms CuZnSOD and MnSOD) which are localized to the muscle sarclemma and mitochondria [7,8]. The studies in human isolated muscle and myotube culture demonstrated that NO and H₂O₂ produced within contracting skeletal muscle are key regulators of pre- and post-translational signalling events leading to HSPs expression [7,9].

The aim of the study was to assess whether the wrestling training increases production of NO, H₂O₂ and HSP27 which can stimulate the regeneration of damaged skeletal muscle in Greco-Roman wrestlers.

Material and methods

Subjects. Nine elite Greco-Roman wrestlers, members of the national team (Tab.1), were observed during competition period (in-season, June). The training loads were demonstrated using program Trening 1.2. prepared by Department of Sport Theory at University School of Physical Education Warsaw (tab.2). During the study, athletes participated in tournaments of Bundesliga Wrestling (Germany) and prepared for World Wrestling Championships (Moscow, September 2010). Nine healthy and untrained males made a reference group (Tab.1).

All the subjects were informed of the aim of the study and were given their written consent for participation in the project. The protocol of the study was approved by the ethics committee at Medical University Poznan, in accordance with the Helsinki Declaration (nr 184/10; 02/11).

Body composition. Body mass (BM) and body composition were estimated using a bioelectrical impedance (Tanita Body Composition Analyser BC-418MA, Japan) calibrated prior to each test session in accordance to the manufacturer’s guidelines. Duplicate measures were taken with the participant in a standing position; the average value was used for the final analysis. The recurrence of measurement was 98%. The measurements were taken one hour before breakfast.

During the measurement, the participants wore only briefs and remained barefoot. BM and body composition have been measured immediately after serum collection for the first six consecutive days of the conditioning camp.

Blood sampling. Blood samples were taken from the elbow vein between 7.00 and 7.30 a.m. after 15 minutes of rest (and an overnight sleep). Within 20 min, they were centrifuged at 3000 g and +4°C for 10 min. Aliquots of serum were stored at -80°C.

Muscle damage. Serum total creatine kinase (CK) activity was used as a marker of sarclemma disruption and was evaluated by using commercially available reagents and Dr Lange analyser (Germany) at a temperature of 20-25°C. The CK activity has been measured immediately after serum collection for the first six consecutive days of the conditioning camp. At 24 h after peak serum CK activity, the levels of NO, H₂O₂ and HSP27 were evaluated.

Reactive oxygen/nitrogen species. Serum nitric oxide (NO) and hydroperoxide (H₂O₂) concentrations were determined using Oxis Research kit (USA). NO and H₂O₂ detection limits were 0.5 µM and 6.25 µM, respectively. The intra-assay coefficient of variation (CV) for the NO kit and the H₂O₂ kit was <10%.

Heat shock protein 27. Serum heat shock protein HSP27 was evaluated by ELISA kit supplied by Calbiochem (USA). Detection limit was 0.2 ng/mL, and intra-assay coefficients of variation (CV) for the kit was <5%.

Statistical analysis. Statistical calculations were performed using STATISTICA 9.0. Statistical significance was assessed by one-way analysis of variance (ANOVA) and Tukey’s post-hoc test (Tukey’ HSD). Associations among measured parameters were analyzed using Pearson’s linear regression (coefficient, r). Statistical significance was set at \( P < 0.05 \). Results are expressed as mean and standard deviation (\( \pm \) SD).

Results

Body composition. Wrestlers have shown BMI, FM and FFM on higher level than non-athletes (Tab.1). Body composition did not change significantly during the conditioning camp. However, FM reached the lowest level and FFM the highest level at fourth day of conditioning camp after training including efforts a comprehensive impact (strength) and special-wrestling training.

Skeletal muscle damage. The rest CK activity was two-fold higher in wrestlers than in non-athletes. CK activity reached >2000 IU/L at the second day of condition camp and then permanently increased to >4000 IU/L (Tab. 3). The highest CK activity was at the third and sixth day. The high CK activity was preceded an intensive special-wrestling training.

Table 1. Anthropometric characteristic of subjects

<table>
<thead>
<tr>
<th>Age</th>
<th>Height</th>
<th>BM</th>
<th>BMI</th>
<th>FAT</th>
<th>FM</th>
<th>FFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>yrs</td>
<td>cm</td>
<td>kg</td>
<td>%</td>
<td>kg</td>
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</tr>
<tr>
<td>NON-ATHLETES N=9</td>
<td>21.8 ± 1.6</td>
<td>180.4 ± 7.7</td>
<td>76.2 ± 8.3</td>
<td>23.4 ± 2.3</td>
<td>19.7 ± 3.4</td>
<td>14.5 ± 3.4</td>
</tr>
<tr>
<td>WRESTLERS N=9</td>
<td>23.0 ± 3.2</td>
<td>177.0 ± 7.8</td>
<td>88.2 ± 22.7</td>
<td>27.8 ± 5.1</td>
<td>16.1 ± 4.9</td>
<td>15.1 ± 8.7</td>
</tr>
<tr>
<td>1st day of conditioning camp</td>
<td>23.0 ± 3.2</td>
<td>177.0 ± 7.8</td>
<td>88.2 ± 22.8</td>
<td>28.1 ± 5.0</td>
<td>17.2 ± 5.9</td>
<td>16.5 ± 10.0</td>
</tr>
<tr>
<td>2nd day of conditioning camp</td>
<td>23.0 ± 3.2</td>
<td>177.0 ± 7.8</td>
<td>88.2 ± 22.5</td>
<td>27.8 ± 5.0</td>
<td>16.1 ± 4.9</td>
<td>15.1 ± 8.7</td>
</tr>
<tr>
<td>3rd day of conditioning camp</td>
<td>23.0 ± 3.2</td>
<td>177.0 ± 7.8</td>
<td>88.5 ± 23.2</td>
<td>28.2 ± 5.3</td>
<td>15.4 ± 4.2</td>
<td>14.6 ± 8.0</td>
</tr>
<tr>
<td>4th day of conditioning camp</td>
<td>23.0 ± 3.2</td>
<td>177.0 ± 7.8</td>
<td>86.8 ± 22.6</td>
<td>28.1 ± 5.0</td>
<td>16.0 ± 4.9</td>
<td>15.1 ± 8.5</td>
</tr>
<tr>
<td>5th day of conditioning camp</td>
<td>23.0 ± 3.2</td>
<td>177.0 ± 7.8</td>
<td>86.8 ± 22.1</td>
<td>28.0 ± 4.9</td>
<td>16.2 ± 5.1</td>
<td>15.3 ± 8.9</td>
</tr>
</tbody>
</table>

BM body mass; BMI Body Mass Index; FM Fat Mass; FFM Free Fat Mass
Table 2. Training protocol

<table>
<thead>
<tr>
<th>Training period</th>
<th>Type of training</th>
<th>Training load %</th>
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<tr>
<td>COMPETITION in season, June</td>
<td>COMPREHENSIVE: running, kayaks, games, strength, intervals, toss from knees, back</td>
<td>50.8</td>
</tr>
<tr>
<td></td>
<td>TARGETED - special force: intervals, toss from knees, back suples, reverse waist, turns, gym</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td>SPECIAL - wrestling training: elevation from the low position, keys, trolleys, throws with different amplitude of movement, combat task</td>
<td>30.8</td>
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</tbody>
</table>

Table 3. Creatine kinase (CK) activity as well as hydrogen peroxide (H₂O₂), nitric oxide (NO) and heat shock protein 27 (HSP27) concentrations in non-athletes and wrestlers

<table>
<thead>
<tr>
<th></th>
<th>CK IU/L</th>
<th>NO μM</th>
<th>H₂O₂ μM</th>
<th>NO/H₂O₂</th>
<th>HSP27 pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NON-ATHLETES N=9</td>
<td>178 ± 59</td>
<td>13.29 ± 0.85</td>
<td>26.20 ± 5.28</td>
<td>0.53 ± 0.11</td>
<td>531 ± 52</td>
</tr>
<tr>
<td>WRESTLERS N=9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st day of conditioning camp</td>
<td>404 ± 399</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2nd day of conditioning camp</td>
<td>2480 ± 1083</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3rd day of conditioning camp</td>
<td>3233 ± 1669</td>
<td>16.12 ± 3.84</td>
<td>13.60 ± 4.83</td>
<td>1.36 ± 0.53</td>
<td>734 ± 358</td>
</tr>
<tr>
<td>4th day of conditioning camp</td>
<td>2211 ± 1794</td>
<td></td>
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<td>5th day of conditioning camp</td>
<td>2911 ± 1942</td>
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<tr>
<td>6th day of conditioning camp</td>
<td>4477 ± 1765</td>
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</tbody>
</table>

Figure 1. The relationship between creatine kinase (CK) and body mass index (BMI) in wrestlers; $r = 0.532, P<0.05$

Figure 2. The relationship between creatine kinase (CK) and fat mass (FM) in wrestlers; $r = 0.550, P<0.05$
Figure 3. The relationship between creatine kinase (CK) and free fat mass (FM) in wrestlers; $r = 0.555$, $P<0.05$

Figure 4. The relationship between creatine kinase (CK) and nitro-oxidative ratio (NO/H$_2$O$_2$) in wrestlers; $r = -0.798$, $P<0.05$

Figure 5. The relationship between creatine kinase (CK) and heat shock protein 27 (HSP27) in wrestlers; $r = -0.733$, $P<0.05$
activity significantly correlated with BMI, FM and FFM (Fig. 1-3).

Reactive oxygen/nitrogen species. NO concentration was higher, whereas H$_2$O$_2$ concentration was lower in wrestlers than non-athletes. The NO/H$_2$O$_2$ ratio significantly correlated with CK activity in athletes (Fig. 4).

Heat shock proteins. HSP27 concentration was higher by 38% in wrestler than non-athletes and significantly correlated with CK activity (Fig. 5). This indicates that HSP27 may be implicated in the regenerative process.

Discussion

In response to exercise-induced muscle damage, myogenic satellite cells become activated, proliferate, and repopulate the myofiber population by fusing together or fusing with existing myofibers. This process is mediated largely through ROI/NS, cytokines, growth factors and HSPs that participate in muscle regeneration [10].

Disruption of the myofiber integrity is reflected by increased serum or plasma CK activity. In human and animal models, increased CK activity is observed after mechanical stress (e.g. extensive physical exercise) and in the course of muscle degenerative diseases [11]. Athletes, as a rule, have higher serum CK activity than non-athletes because of the regular strain imposed by training on their muscles. Brancaccio et al. [11] reported that significant increase of CK was observed after fourth day of football training and then was dropping till tenth day of training, what is likely an adaptation to exercise. In the present study, CK reached the high activity >3000 IU/L after training session where special and wrestling training elements dominated. A well-designed study by Barbas et al. [12] on Greco-Roman wrestlers performing five matches in one-day tournament has shown that values of serum CK reached the highest level in the last of the five matches.

Exercise-induced muscle damage plays role in promoting skeletal muscle hypertrophy [13,14]. However, other researchers have questioned this hypothesis, noting that hypertrophy can occur in the relative absence of muscle damage [15,16]. We observed the positive correlations between CK activity, FM and FFM which indicate that muscle damage may be implicated in hypertrophy.

There is a large body of evidence demonstrating exercise-induced muscle damage is associated with factors involved in the accretion of muscle proteins such as ROI/NS. Hypertrophic effects associated with ROI/NS may be carried out through increased mitogen-activated protein-kinases (MAPKs) signalling. In vitro analysis has shown that treatment of myoblasts with ROI/NS increases the various MAPKs. Eccentric actions have been shown to increase MAPKs activation to a greater extent than concentric or isometric actions [17]. ROI/NS also may modulate muscle protein synthesis via enhanced growth factors such as IGF-I [18]. Our study has demonstrated that wrestling training increases NO generation which is related to muscle damage; NO/H$_2$O$_2$ ratio correlated with CK activity in wrestlers.

In high-force eccentric exercise, the cytoprotective role of HSP27 has been indicated by translocation of the small HSPs from the cytoplasm to myofibrillar structures during exercise [19]. It stabilizes and prevents damage to these structures during the mechanical strain of high-force eccentric exercise [20]. We observed a HSP27 response to exercise-induced muscle damage. The high HSP27 level induced a decrease in CK activity in wrestlers.

Conclusions

1. Muscle damage induced by wrestling training is important factor modulating changes in nitro-oxidative ratio and HSP27 expression as well as body composition.
2. Moreover, high HSP27 level may be implicated in the regenerative process in Greco-Roman wrestlers.

Acknowledgments

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References

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