EXERCISE TRAINING-INDUCED CHANGES IN INFLAMMATORY MEDIATORS AND HEAT SHOCK PROTEINS IN CANOEISTS

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Study Design; Data Collection; Statistical Analysis; Manuscript Preparation; Funds Collection

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Abstract. According to cytokine overtraining theory, skeletal muscle injuries are related to systemic inflammatory reaction. In response to inflammation, cells rapidly produce a series of proteins known as heat shock proteins (HSPs). These are considered to be molecular chaperones which play a universal role in maintaining cellular homeostasis. Among the subset of stress-responsive proteins, HSP27 and HSP70 are considered to be a new approach to monitoring exercise training and adaptive mechanisms. The study was designed to demonstrate the effect of sport training on changes in pro-inflammatory cytokines and HSPs, and their relation with muscle damage and body composition.

Six elite canoeists (19.8 ±2.9 yr) were observed during preparatory training period (March) at the 1st, the 4th and after 7 days of the conditioning camp, and then after 3 days of recovery. The canoeing training did not induce muscle damage, decreased in IL-1ß (r = 0.635; P < 0.001) and HSP70 (r = 0.651; P < 0.001). Any significant changes in body composition were not observed.

In conclusion, we could say that typical canoeing training improves cytokines and HSPs release, however, the changes are not related to muscle damage.

Key words: inflammation, cytokines, HSP27, HSP70, muscle damage

Introduction

The effectiveness of physical training depends on the training load, the individual toleration ability and the imbalance between the two may lead to under or over-training. One of the unique features of an exercise is that
it leads to a simultaneous increase of antagonistic mediators. On the one hand, exercise elevates catabolic pro-
inflammatory cytokines such as interleukin 1β (IL-1β) and tumour necrosis factor α (TNFα). On the other hand, exercise stimulates anabolic components such as heat shock proteins (HSPs) which protect against stressors. If the anabolic response is stronger, exercise will probably lead ultimately to an increased muscle mass and exercise adaptation (Noble et al. 2008; Pedersen 2011; Roubenoff 2007).

The role of pro-inflammatory cytokines in skeletal muscle growth still has not been fully explored. It has been observed that after IL-1β stimulation the total of protein synthesized does not increase, but rather the synthesis of the acute phase proteins is favoured (Weissman 1990). A study by Tayek (1996) has shown that TNFα has significant short- and long-term effects on protein synthesis. Earlier work demonstrated that TNFα can reduce weight gain and enhance muscle catabolism (Tracey et al. 1988). Nevertheless, the suppression of TNFα synthesis by using anti-inflammatory drug delays muscle restoration, but an excessive IL-1β and TNFα release may be responsible for the overtraining (Mackey et al. 2007; Main et al. 2009). The measurement of both pro-inflammatory cytokines IL-1β and TNFα within a population of athletes during training has not been widely reported (Borges et al. 2013; Main et al. 2009; Marin et al. 2011; Reinke et al. 2009; Zembron-Lacny et al. 2010). Nowadays, it is known that pro-inflammatory cytokines are elevated in sport activity and can be markers of overtraining syndrome, but we still have no information which level of inflammatory mediators is appropriate for athletes.

Heat shock proteins (HSPs) represent cell-protective system that may be induced by reactive oxygen species, cytokines, and hyperthermia. Under physiological conditions, constitutively expressed HSPs function as molecular chaperones, whereas under stress conditions, HSPs protect proteins against misfolding, aggregation and denaturation. In addition, HSPs may directly regulate specific stress-responsive signalling pathways and may antagonize signalling cascades that result in apoptosis (Madamanchi et al. 2001; Noble et al. 2008). Exercise-induced muscle damage is considered to be one of the stimulus which induce HSPs (Steinacker et al. 2004). Among the subset of stress-responsive proteins, HSP27 and HSP70 are considered as a new approach of monitoring exercise training and adaptive mechanisms (Banfi et al. 2006). The fibre type-specific expression of HSP70 is influenced by resistance and endurance training, whereas HSP27 is influenced only by endurance training, suggesting the existence of a training-modality-specific action on the adaptive processes including HSPs in human skeletal muscle (Folkesson et al. 2013). The function of HSP70 depends on ATP hydrolysis whereas that of HSP27 does not. Moreover, HSP70 is an early responsive protein whereas HSP27 is a late responsive one. Consequently, these proteins have been referred to as complementary protective proteins (Parcellier et al. 2003).

Basing on the gathered data on inflammatory response, the study was designed to evaluate the effect of 7-day training on HSP27 and HSP70 levels, and their relation to skeletal muscle damage and inflammation in elite canoeists.

Methods

Data collection and subjects. Six marathon canoeists, members of national team (Table 1), were observed during preparatory training period (pre-season, March) at the first, the fourth and after 7 days of the conditioning camp, and after 3 days of the recovery. The observations were performed at the Sport Centre in Gryfino (Poland). Details of the training program are presented in Table 2. All subjects occupied the same accommodations and followed the same training and diet schedules.
Table 1. Anthropometric and body composition data in canoeists; (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>1st day of camp</th>
<th>4th day of camp</th>
<th>After 7-day of camp</th>
<th>After 3-day recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
</tr>
<tr>
<td>Height cm</td>
<td>181.2 ±9.8</td>
<td>181.2 ±9.8</td>
<td>181.2 ±9.8</td>
<td>181.2 ±9.8</td>
</tr>
<tr>
<td>BM kg</td>
<td>79.7 ±6.6</td>
<td>80.1 ±6.1</td>
<td>80.1 ±5.9</td>
<td>80.0 ±6.2</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>24.3 ±2.1</td>
<td>24.5 ±2.2</td>
<td>24.5 ±2.1</td>
<td>24.4 ±2.1</td>
</tr>
<tr>
<td>FFM kg</td>
<td>68.9 ±6.0</td>
<td>70.8 ±5.0</td>
<td>70.1 ±5.2</td>
<td>69.9 ±5.4</td>
</tr>
<tr>
<td>FM kg</td>
<td>10.8 ±2.7</td>
<td>9.3 ±3.2</td>
<td>10.0 ±3.1</td>
<td>10.0 ±3.0</td>
</tr>
<tr>
<td>FM%</td>
<td>13.5 ±3.0</td>
<td>11.6 ±3.5</td>
<td>12.4 ±3.3</td>
<td>12.5 ±3.2</td>
</tr>
</tbody>
</table>


Table 2. The 7-day training program in canoe during conditioning camp (March)

<table>
<thead>
<tr>
<th></th>
<th>a.m. 10:00–12:00</th>
<th>TRAINING INTENSITY</th>
<th>p.m. 16:00–18:00</th>
<th>TRAINING INTENSITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day of camp</td>
<td>blood and body composition 7:00 and 8:00 a.m.</td>
<td>long distance paddling of 14 km (focus on technique)</td>
<td>moderate</td>
<td>repeat sets of 4 × 10'/3' break; distance of 14 km</td>
</tr>
<tr>
<td>2nd day of camp</td>
<td>repeat sets of 3 × (5 × 1'15'/5') break; distance of 20 km</td>
<td>moderate – high</td>
<td>repeat sets of 10 × 30'; distance of 12 km</td>
<td>high</td>
</tr>
<tr>
<td>3rd day of camp</td>
<td>repeat sets of 3 × 30'/5' break; distance of 20 km</td>
<td>moderate</td>
<td>rest; sauna</td>
<td>–</td>
</tr>
<tr>
<td>4th day of camp</td>
<td>repeat sets of 6 × 10'/2' break; distance of 16 km</td>
<td>high</td>
<td>long distance paddling of 12 km (focus on technique)</td>
<td>moderate</td>
</tr>
<tr>
<td>5th day of camp</td>
<td>repeat sets of 3 × (2'/1' + 4'/2' + 8'/3' + 4'/2' + 2'5') break; distance of 16 km</td>
<td>high</td>
<td>long distance paddling of 12 km (focus on technique)</td>
<td>moderate</td>
</tr>
<tr>
<td>6th day of camp</td>
<td>repeat sets of 4 × 15'/4' break; distance of 16 km</td>
<td>moderate</td>
<td>long distance paddling of 12 km (focus on technique)</td>
<td>moderate</td>
</tr>
<tr>
<td>7th day of camp</td>
<td>long distance paddling of 12 km (focus on technique)</td>
<td>moderate</td>
<td>strength training; 10 exercise; 6 × 10 reps (25 ton)</td>
<td>–</td>
</tr>
</tbody>
</table>

END OF CAMP
blood and body composition 7:00 and 8:00 a.m.
AFTER 3-DAY RECOVERY
blood and body composition 7:00 and 8:00 a.m.

All the subjects were informed of the aim of the study and were given their written consent for participation in the project. In two cases, the consent was obtained from parents. The protocol of the study was approved by the ethics committee at Medical University Poznan, in accordance with the Helsinki Declaration.

Body composition. Body mass (BM) and body composition (fat-free mass FFM and fat mass FM) were estimated using a bioelectrical impedance (BIA) by using Tanita Body Composition Analyser MC-980 (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 between 7.00 and 8.00 a.m. before blood sampling.

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Blood sampling. Blood samples were taken from the elbow vein between 7.00 and 8.00 a.m. after 15 minutes of body composition analysis. Within 20 min, blood samples were centrifuged at 1,000–2,000 x g and +4°C for 10 min. Aliquots of serum were stored at −80°C.

Skeletal muscle damage. Serum total creatine kinase (CK) activity was used as a marker of sarcolemma disruption and was evaluated by using commercially available reagents and Dr Lange analyser (Germany) at a temperature of 20–25°C. CK detection limit for the applied kit was 6 IU/L. The intra-assay coefficient of variation for the CK kit was <2%.

Inflammatory mediators. Serum interleukin-1β (IL-1β) and tumour necrosis factor α (TNFα) levels were evaluated by enzyme immunosassay methods using R&D Systems kits (USA). Detection limits for IL-1β and TNFα were 0.023 pg/mL and 0.038 pg/mL, respectively. The average intra-assay coefficient of variation was about 8.0%.

Heat shock proteins. Serum heat shock proteins HSP27 and HSP70 were evaluated by enzyme immunoassay methods using Calbiochem kit (USA) and Stressgen kit (USA). Detection limits for HSP27 and HSP70 were 0.02 ng/mL and 0.2 ng/mL, respectively. Intra-assay coefficients of variation (CV) were <5% for both HSPs.

Statistical analysis. For all tested parameters the arithmetic mean (×) and standard deviation (SD) were calculated. The conformity of distribution was tested with the Shapiro-Wilk test. The level of critical significance was set at p < 0.05. As the conditions of normal distribution or uniformity of variance were not met, non-parametric Friedman ANOVA test was used. The significance of differences between the dates of tests was assessed using a post-hoc Friedman ANOVA test. Statistical analysis was carried out using STATISTICA 10.0.

Results

Body composition. There were not significant changes in body composition but a tendency to high values in FFM. All subjects had normal BMI ranged from 21.5 to 27.7 kg/m².

Skeletal muscle damage (Table 3). The CK activity, as a marker of muscle damage, did not change significantly during the conditioning camp where canoe endurance training dominated.

Inflammatory mediators (Table 3). The concentrations of IL-1β and TNFα did not change at the same time. IL-1β level were significantly higher at the 1st day of camp whereas TNFα reached the highest level after 3 days of recovery. There was a negative correlation between IL-1β and TNFα (r = −0.490, P < 0.05).

Table 3. The serum levels of creatine kinase (CK), cytokines IL-1β and TNFα as well as heat shock proteins HSP27 and HSP 70 in canoeists; (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>1st day of camp n = 6</th>
<th>4th day of camp n = 6</th>
<th>After 7-day of camp n = 6</th>
<th>After 3-day recovery n = 6</th>
<th>Differences</th>
</tr>
</thead>
</table>
| CK IU/L        | 80.0 ±9.4              | 74.9 ±19.3            | 71.1 ±19.9                | 73.0 ±19.0                | Chi²ANNOVA = 3.8  
P = 0.283       |
| IL-1β pg/mL    | 1.24 ±0.09             | 1.19 ±0.16            | 0.98 ±0.09                | 1.04 ±0.10                | Chi²ANNOVA = 12.6  
P < 0.01      |
| TNFα pg/mL     | 3.14 ±0.13             | 3.36 ±0.12            | 3.68 ±0.11                | 4.13 ±0.17                | Chi²ANNOVA = 18  
P < 0.001     |
| HSP27 pg/mL    | 766 ±34                | 706 ±58               | 688 ±80                   | 650 ±88                   | Chi²ANNOVA = 9.8  
P < 0.05     |
| HSP70 ng/mL    | 1.24 ±0.07             | 1.10 ±0.09            | 1.28 ±0.09                | 1.42 ±0.06                | Chi²ANNOVA = 13.4  
P < 0.01     |
Heat shock protein (Table 3). Similarly to cytokines, HSP27 and HSP70 concentrations did not change at the same time. HSP27 level was significantly higher at the 1st day of camp whereas HSP70 reached the highest level after 3 days of recovery. Figure 1 show the significant negative relation between TNFα and HSP27 ($r = -0.562$, $P < 0.01$), and positive between TNFα and HSP70 ($r = 0.651$, $P < 0.01$). It shows that the release of HSP27 and HSP70 into the circulation may occur in response to exercise-induced inflammation.

![Figure 1](image-url)

**Figure 1.** The significant relations between TNFα and HSP27 (top; $r = -0.563$, $P < 0.01$) as well as between TNFα and HSP70 (bottom; $r = 0.651$, $P < 0.01$)
Discussion

In response to exercise-induced muscle damage (EIMD), 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 various molecules such as cytokines and HSPs that participate in muscle regeneration and hypertrophy (Shortreed et al. 2008).

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 (Brancaccio et al. 2010). Athletes, as a rule, have higher serum CK activity than non-athletes because of the regular strain imposed by training on their muscles. In the present study, CK did not reach the high activity after 7-day conditioning camp where the canoe endurance training dominated. It is commonly known that EIMD plays a role in promoting skeletal muscle hypertrophy (Flann et al. 2011; Sandri 2008; Shortreed et al. 2008). However, some researchers have questioned this hypothesis, noting that hypertrophy can occur in the relative absence of muscle damage. Although an eccentric exercise has greater hypertrophic effects compared with other types of actions, a cause-effect relationship directly linking these gains to EIMD is yet to be established. Moreover, if such a relationship does in fact exist, it is not clear to what extent of damage it is optimal for inducing maximum muscle growth (Schoenfeld 2012).

Although EIMD did not occur in marathon canoeists, both cytokines and HSPs concentrations changed following sport training. The serum concentrations of IL-1β and HSP27 were considerably higher at the first day while concentrations of TNFα and HSP70 were higher after 7-day conditioning camp. It is very interesting that TNFα and HSP70 reached the highest levels after 3-day recovery.

In our study, IL-1β and HSP27 constantly decreased after 7-day conditioning camp and after 3-day recovery. Data have shown that both molecules are expressed only in response to high-force eccentric exercise and are related to damaged myofibers (Fielding et al. 1993; Koh 2002; Paulsen et al. 2009). However, it does not seem likely that IL-1β and HSP27 have any direct involvement in muscle hypertrophy. However, HSP27 has been proposed to play a direct role in protecting skeletal muscle from contraction-induced damage via interactions with cytoskeletal elements and in regulation of the glutathione system (Koh 2002).

TNFα is considered to play a major role in the regulation of muscle mass. One of the possible actions of TNFα on skeletal muscle is generation of reactive oxygen species and modification of signalling pathways. On the other hand, TNFα can directly induce muscle catabolism by the inhibition of protein synthesis and myogenesis in myoblasts. Moreover, studies have indicated that TNFα is also responsible for triggering the death receptor mediated pathways of myonuclear apoptosis playing a significant role in muscle loss (Fernández-Celemin et al. 2002). The pro-catabolic action of TNFα was accompanied by increase in HSP70 (Figure 1). More recently, the presence of muscle-derived HSP70 in the circulation in response to aerobic and eccentric exercises has been demonstrated (Febbraio et al. 2002; Heck et al. 2012; Thompson et al. 2002). However, further studies have not confirmed that the muscle could be the major source of circulatory HSP70 precluded the ‘muscle hypothesis’, and suggested that other tissues should be responsible for the increase of HSP70 into the circulation (Heck et al. 2012). The serum and muscle HSP70 concentrations are dependent on both training intensity and volume (Liu et al. 2000). The elevated level of HSP70 was observed in rowers, soccer players, endurance runners and tennis players (Banfi et al. 2006; Fehrenbach et al. 2000; Liu et al. 2000; Ziemann et al. 2013).
Analysis of body composition in canoeists did not show significant changes in FM and FFM, and any relation with cytokines and HSPs. Our previous study demonstrated that FM and FFM in canoeists are similar to healthy non-athletes at the same age but significantly lower compared with judoists and wrestlers. In combat sports, the high FFM and CK activity were accompanied by high IL-1β and TNFα levels. This shows that type of sport discipline and training load markedly effect on relation of body composition with inflammatory response (Zembron-Lacny et al. 2013).

Conclusions
These results show that 7-day canoeing training modulates pro-inflammatory response which is related to HSPs release into the circulation, and reveal that skeletal muscle damage is not necessary to induce training-induced inflammation.

Acknowledgments
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References


