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Research article

Exercise Training-Induced Changes in Inflammatory Mediators and Heat Shock Proteins in Young Tennis Players

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Abstract

Heat shock proteins (Hsp) represent proteins' groups, whose protective function, may be induced by heat, reactive oxygen species, cytokines etc. We evaluated blood levels of Hsp27 and Hsp70, and their relation to skeletal muscle damage and inflammation in young tennis players before and after the conditioning camp. Blood samples were collected directly after tournament season, 3-day rest and 14-day conditioning camp that followed. Hydrogen peroxide (H_2O_2) demonstrated the highest concentration directly after tournament season, which significantly decreased at camp's end. The pro-inflammatory cytokines IL-1β and TNFα decreased, whereas anti-inflammatory cytokines IL-6 and IL-10 increased after 3d rest and 14d camp. Hsp27 increased after 3d rest and remained so after 14d camp, while Hsp70 decreased from baseline to camp's completion. Hsp27 and Hsp70 correlated significantly with H_2O_2, IL-1β and TNFα. Muscle damage, observed as creatine kinase (CK) activity changes, increased after 14d camp similarly to Hsp27 and anti-inflammatory cytokines IL-6 and IL-10. Obtained data allows to conclude that decrease of Hsp27 and increase in pro-inflammatory cytokines could be a good indicator of overreaching. Reverse tendencies in these proteins may verify accuracy of conditioning camp. Finally, this training program caused an increase in the anti-inflammatory cytokines concentrations, improving individual status of recovery.

Key words: Hydrogen peroxide, cytokines, Hsp27, Hsp70, overreaching.

Introduction

The effectiveness of physical training depends on physiological parameters of participants, applied workload as well as individual susceptibility to tolerate fatigue. Imbalance between the last two may leads to under or overtraining. Depending on the applied workload, different immunological responses to training can be induced. A practice, imposing an excessive stress, result in an inflammatory response robust and likely, sufficiently powerful, to modify subsequent responses. The long term consequences of such impact may occur via mechanisms of immune tolerance and/or training-associated reduction in the innate immune response to brief exercise (Cooper et al., 2007). Thus, there is only a fine line between improved performance and deterioration (Smith, 2000).

Exercise triggers simultaneous increase of various antagonistic mediators, yet also, elevates catabolic pro-inflammatory cytokines such as interleukin 1β (IL-1β) and tumour necrosis factor alpha (TNFα). On the other hand, it also stimulates anabolic components such as interleukin 6 (IL-6), interleukin 10 (IL-10) and heat shock proteins (Hsps), which protect against stressors. If an anabolic response is stronger, training will probably, ultimately lead to an enhanced muscle mass and improved 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 was observed that after IL-1β stimulation the total of protein synthesized does not increase, but rather synthesis of the acute phase proteins is favoured (Weissman, 1990). A study by Tayek (1996) showed that TNFα has significant short- and long-term effects on protein synthesis. It was also demonstrated to be able to reduce weight gain and enhance muscle catabolism (Tracey et al., 1988), yet, the suppression of TNFα synthesis with anti-inflammatory drug delays muscle restoration. At the same time, 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- and anti-inflammatory cytokines IL-1β, TNFα, IL-6 and IL-10 within a population of athletes during training has not been yet widely reported (Main et al., 2009; Marin et al., 2011; Reinke et al., 2009; Zembron-Lacny et al., 2010). Nowadays, it is known that pro- and anti-inflammatory cytokines concentrations alter as a result of physical activity in a way dependent on a discipline; yet we still lack information on the levels of inflammatory mediators appropriate and most beneficial for athletes training a particular sport. Research in this area, particularly in tennis, is challenging due to numerous factors that require analysis, including number of matches played, their intensity and duration time. Their unpredictable occurrence makes running an investigation during a tournament season very demanding. Nevertheless, such research is vital to allow trainings to be planned in a way to stimulate and emphasize the anti-inflammatory response.

Heat shock proteins (Hsps) represent cell-protective system that may be induced by reactive oxygen species, cytokines, and hyperthermia. Under physiologically balanced conditions, constitutively expressed Hsps function as molecular chaperones, whereas under stress conditions, Hsps protect proteins against misfolding,

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aggregation and denaturation. Non adequate Hsps biosynthesis may be deleterious to cells and make them more sensitive to stress. HSPs may also directly regulate specific stress-responsive signalling pathways and may antagonize signalling cascades that result in apoptosis (Madamanchi et al., 2001; Noble et al., 2008). Hsps increase the stress tolerance and participate in the cellular repair processes. Moreover Hsp are involved in a number of remodeling processes associated with exercise training, such as facilitating mitochondrial biogenesis (Hood et al., 2000), regulators of apoptotic pathways (Samali and Orrenius, 1998), and inducing improvements in insulin sensitivity (Chung et al., 2008). No data are available about role of Hsp in overreaching syndrome.

Exercise-induced stress and muscle damage are considered two out of many stimuli, which induce Hsps synthesis (Steinacker et al., 2004). The sustaining high Hsps synthesis may indicate a state of inadequate regeneration even after a couple of weeks of recovery from exhaustive exercise (Lehmann et al., 1997). The elevated blood level of Hsp70 was observed in rowers, soccer players and endurance runners (Banfi et al., 2006; Fehrenbach et al., 2000; Liu et al., 2000). Among the subset of stress-responsive proteins, Hsp27 and Hsp70 are considered to be a new approach to monitoring exercise training and adaptive mechanisms (Banfi et al., 2006). The regulation of Hsp within intracellular environment is well understood, but extracellular Hsp can also exert important biological functions (Lancaster and Febbraio, 2005). For example Hsp27 seems to both directly scavenge the free radicals and protect against the toxicity of reactive oxygen species (ROS) (Wyttenbach et al., 2002).

One of the factors, which may induce synthesis of Hsp is hydrogen peroxide (H_2O_2). It is an important signalling molecule, generated during muscle contraction, involved in regeneration and adaptation of skeletal muscle to physical exercise. H_2O_2 is produced by the enzymes superoxide dismutase (isoforms CuZnSOD and MnSOD), which are localized in the muscle sarcolemma and mitochondria (Jackson et al., 2007). The studies in human isolated muscle and myotube culture demonstrated that H_2O_2 produced within contracting skeletal muscle is the key regulator of signalling pathways, leading to skeletal muscle adaptation (Powers et al., 2010).

Basing on the gathered data on immunological response indicators, the study was designed to evaluate the blood level of Hsp27 and Hsp70, as well as their relation to skeletal muscle damage and inflammation in tennis players. We hypothesized that our young tennis players experienced overreaching after a tournament season - a syndrome characterised by an increase in blood pro-inflammatory and lower anti-inflammatory cytokines. Consequently, we set our goal to verify the influence of Hsp70 and Hsp27 levels on restoring an immune balance.

Methods

Data collection and subjects

Our investigation was held during the sport camp (beginning of October 2011), organized annually by the Polish Tennis Association at the National Olympic Sport Centre in Cetniewo (Poland). All subjects occupied the same accommodations and followed the same training and diet schedules. Daily, energetic value of food offered in the menu did not exceed 4000 kcal. The proposed protein dose varied from 1.2-1.4 $g \cdot kg^{-1}$ of body mass.

The main purpose of the camp is to support development of the best young tennis players. Participants (n = 15, age 16 years old) are selected by the national coaches according to tennis players' annual achievements and rankings. The examination is officially approved by the Bioethical Committee of the Regional Medical Society in Gdansk NKEBN/39/2009 according to the Helsinki Declaration. Participation must be approved with written consents from the players' parents.

Blood was collected three times: directly after arrival at the camp (I), after a 3-day active rest (II) and at the end of the camp (III). The schedule of the training program was planed basing on our previous experiences, which had revealed that directly after arriving at the camp, low grade inflammation was noted. It suggested that players had been taking part in many different tournaments till the very end of the season to improve their rankings. In fact they did not experience sufficient recovery afterwards. Therefore, three days of an active rest, after arrival at the camp, were introduced, aimed to familiarize participants with stretching exercises and low-intensity training. After this period, body composition and aerobic assessment were held.

The presented training structure was applied in the first part of preparatory season, encountering for a half-year macrocycle. The main goal of the practice was to improve players' physical abilities via focusing on the main training components - strength, endurance and flexibility training. Consequently, 70% of training hours was assigned to strength, endurance and flexibility training, while the remaining 30% was used to develop other training components, vital in tennis: speed, agility, coordination as well as strokes timing. The strength practice based on developing local strength capability, as it is the first step to achieve a long-term strength level increase (dynamics of strokes and court movement). At the same time, endurance training implemented methods improving energy metabolism mechanisms, whereas flexibility was practiced through systematic exercises aimed to normalize muscle tension. Details of the training program are presented in Table 1, whereas it summary is included in Table 2.

Body composition assessment

Body mass (BM) and body composition were estimated using a multi-frequency impedance plethysmograph body composition analyser (InBody 720, Biospace Analyzer, Korea). Using a diverse range of frequencies from 1 kHz to 1 MHz, the InBody 720 accurately measured the amount of body water and body composition, including fat mass, free fat mass and skeletal muscle mass. The precision of the repeated measurements was expressed as the coefficient of variation, which was, on average, 0.6% for fat mass percentage (Lim et al., 2009; Volgyi et al., 2008). The measurements were taken one hour before breakfast. The participants emptied their bladders and

Table 1. The details and structure of the 2 weeks training program.

	Time (before lunch)	Training intensity	Time (after lunch)	Training intensity
Monday (1)	***Blood collection***		Training F (4:30-6:00 pm)	low intensity
Tuesday (2)	Training A (0:00-2:00 pm)	40% of 1 RM	Training D (8:30-9:30 pm)	low intensity
Wednesday (3)	Training D (7:15-8:00 am) Training E (11:00 am-1:00 pm)	low intensity moderate intensity	Training F (4:00-7:00 pm)	low intensity
Thursday (4)	***Blood collection, body composition*** Training D (7:15-8:00 am) Aerobic assessment	low intensity	Training G (4:00-5:30 pm) Training C (6:00-6:45 pm)	moderate intensity moderate intensity
Friday (5)	Training D (7:15-8:00 am) Training H (11:00 am-1:00 pm)	low intensity high intensity	Training E (4:00-7:00 pm)	high intensity
Saturday (6)	Training D (7:15-8:00 am) Training A (10:00-12:00 am) Training G (0:30-1:30 pm)	low intensity 60% of 1 RM moderate intensity	Training B (4:45-5:45 pm) Training C (6:00-6:45 pm) Training D (8:00-9:00 pm)	moderate intensity moderate intensity low intensity
Sunday (7)	Training F (10:30 am-1:00 pm)	moderate intensity	Training D (8:00-9:00 pm)	low intensity
Monday (8)	Training D (7:15-8:00 am) Training A (10:00-12:00 am)	low intensity 60% of 1 RM	Training I (4:00-6:00 pm) Training D (8:00-9:00 pm)	high intensity low intensity
Tuesday (9)	Training J (10:00-11:30 am)	high intensity	Training B (4:45-5:45 pm) Training C (6:00-6:45 pm)	moderate intensity moderate intensity
Wednesday (10)	Training D (7:15-8:00 am) Training A (10:00-11:30 am) Training E (0:00-1:00 pm)	low intensity 60% of 1 RM high intensity	Training F (4:00-7:00 pm)	low intensity
Thursday (11)	Training J (10:00-11:30 am)	high intensity	Training B (4:45-5:45 pm) Training C (6:00-6:45 pm)	moderate intensity moderate intensity
Friday (12)	Training D (7:15-8:00 am) Training A (11:00 am-1:00 pm)	low intensity 60% of 1 RM	Training H (4:00-6:00 pm)	high intensity
Saturday (13)	***Blood collection***	End of the camp		

HR –heart rate, AT-anaerobic threshold, RM-repetition maximum.
Training A: Strength training for local strength endurance (8 basic tennis exercises, each at 60% of 1 RM, involving arms and shoulders as follows: bench press, dumbbell pullovers, T-bar rows, reverse curls; legs as follows: squats, lunges; trunk as follows: crunches, dumbbell side bends).
Training B: line jumps in teams (agility, coordination, rhythm, sense of direction and adjustment abilities; alternately with the balance exercises on balls), Training C: swimming: 30-minute exercise, focusing mainly on upper limbs muscles; distance to cover- around 800m) , Training D: stretching exercise, "hold-relax" technique and basic yoga exercises.
Training E: conditioning exercise- team sports: soccer - regular match (2 times 45 minutes, 7x7 players), average heart rate at 60-95% HR_{max}.
Training F: regeneration (each player uses 2 hydrotherapy treatments for 40 minutes).
Training G: agility games with tennis balls on small (main stress on coordination, agility, accuracy).
Training H: interval training (2 series /5-10 second/in 6 repetitions, 80-95% $HR_{max,}$ work to rest ratio 1:3) Training I: endurance, continuous distance running for 60 minutes 70 -80% HR_{max}.
Training J: tennis training (developing tennis memory movement).
Training K: conditioning exercise team sports: soccer - short games with short periods (few seconds) with high intensity, average heart rate at 80-95% HR_{max}

Table 2. Summary of training program.

Motor ability	Training (hrs)	Relative Loads (%)	Type of training
Strength	12.7	27.5	A, C
Endurance	11.5	23	E, H, I
Flexibility	9.75	19,5	D, F
Coordination	6.5	13	B
Speed	5.5	11	K, G
Tactical and technical skills	3	6	J
TOTAL	50	100	

bowels prior to the assessment. During the measurement, the participants wore only briefs and remained barefoot.

Aerobic capacity

Aerobic capacity was determined during a VO_2 max test. Breath-by-breath pulmonary gas exchange was measured (MetaMax 3B, Cortex Biophysik GmbH, Germany) throughout the test. The participants performed a continuously graded multistage field tennis test according to the protocol suggested by Smekal et al. (2000). The series of 3-minute exercise stages, separated by 1-minute breaks for machine adjustments were based on typical tennis movements when reaching for a stroke. The participants alternated between forehand and backhand strokes with balls thrown by the HOT SHOT DXSR-1594 (Prince, USA) ball machine. They were allowed a 3-min warm up period before the test. Immediately following the warm up, the VO_2 max testing began and continued until a participant reached the point of volitional exhaustion (oxygen uptake did not increase any more or the frequency of the ball was so high that completing strokes became impossible). Before subsequent players began the test, the O_2 and CO_2 analysers were calibrated using standard gases at known concentrations in accordance with manufacturer guidelines. Additionally, during this test we determined the maximal heart rate, which was next used to monitor training intensity.

Personal evaluation

To determine individual mental state, players were asked to evaluate the undergone rest using perceived recovery status (PRS) scale. They were asked to estimate their perceived level of recovery, according to the provided and read, standardized instructions explaining how to interpret the PRS scale as well as the numerical and verbal anchors within it. The assessment was done twice, at the beginning and at the end of the conditioning camp (Laurent et al., 2011).

Biochemical measurement

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

Reactive oxygen species

Serum hydrogen peroxide (H_2O_2) was determined using Oxis Research kits (USA). H_2O_2 was measured immediately after serum collection. H_2O_2 detection limit was 6.25 µM. The intra-assay coefficient of variation for the H_2O_2 kit was <10%.

Pro- and anti-inflammatory cytokines

Serum interleukin-1β (IL-1β), tumour necrosis factor α (TNFα), interleukin-6 (IL-6) and interleukin-10 levels were determined by enzyme immunoassay methods using commercial kits R&D Systems (USA). Detection limits for IL-1β TNFα, IL-6 and IL-10 were 0.023, 0.038, 0.039 and 0.500 $pg \cdot mL^{-1}$, respectively. The average intra-assay CV was about 8.0% for all cytokines.

Heat shock proteins

Serum heat shock proteins Hsp27 and Hsp70 were evaluated by Elisa kit Calbiochem (USA) and Stressgen kit (USA). Detection limits were 0.2 $ng \cdot mL^{-1}$, and intra-assay coefficients of variation (CV) for the kits were <5%.

Muscle damage

Serum creatine kinase (CK) activity was used as a marker of muscle damage and was evaluated by Emapol kit (Poland) at a temperature of 20-25°C. CK detection limit for the applied kit was 6 $U \cdot L^{-1}$. The intra-assay coefficient of variation for the CK kit was 1.85%.

Statistical analysis

Statistical calculations were performed using STATISTICA 9.0. Statistical significance was assessed by repeated analysis of variance (ANOVA) and Tukey' 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 (x ±SD). Additionally, in order to assess the influence of this stimulus (the whole camp training program) the effect size (partial eta^2) by ANOVA, ranging between 0 and 1, was calculated.

Results

All participants completed the study with no adverse events being reported. The basic anthropometric characteristics of the subjects are summarized in Table 3. Repeated measurements indicated on diverse responses experienced directly after the tournament season and after the conditioning camp Table 4.

Reactive oxygen species

Hydrogen peroxide (H_2O_2) had demonstrated the highest concentration directly after the tournament season, yet it dropped significantly following the 12d conditioning camp. The effect size for these changes was 33%. The 3d active rest after the tournament season did not affect H_2O_2 concentration.

Table 4. The effect of the whole training program on blood hydrogen peroxide, cytokines and heat shock proteins concentrations . Values are means (± SD).

Variables	Directly after tournament season 1	3rd of the rest, before the camp 2	After the conditioning camp 3	*F*	*P* Value	Effect size	Test Power (α = .05)
H_2O_2 (µmol·mL^{-1})	10.79 (1.80) ^{3}#	9.75 (2.70)	8.25 (1.40) ^{1}	7.09	.003	.33	.90
IL-1β (pg·mL^{-1})	2.98 (2.50) ^{2}#^{3}#	1.13 (.80) ^{1}	.79 (.20) ^{1}	8.89	.001	.39	.95
TNFα (pg·mL^{-1})	4.05 (.50) ^{2}#	2.97 (.40) ^{3}*	3.69 (1.00) ^{2}	7.21	.002	.34	.90
IL-6 (pg·mL^{-1})	1.30 (.50)	1.36 (.40)	1.66 (1.20)	.79	.460	.05	.17
IL-10 (pg·mL^{-1})	9.33 (.90) ^{3}	9.27 (.90) ^{3}#	12.23 (3.30)	11.89	.0001	.46	.98
Hsp27 (pg·mL^{-1})	298 (54) ^{2,3}	983 (320) ^{1}	1029 (341) ^{1}	5.94	.007	.30	.84
Hsp70 (ng·mL^{-1})	4.74 (.90) ^{2,3}	3.62 (.67) ^{1}	3.45 (.71) ^{1}	6.06	.006	.30	.84
CK (IU·L^{-1})	307 (217) ^{3}*	149 (75) ^{3}	487 (197) ^{1,2}	12.71	.0001	.47	.99

ns - non significant differences, η^2_p - effect size expressed as partial eta^2, H_2O_2 - hydrogen peroxide; IL-1β - interleukin 1β; TNFα - tumour necrosis factor α; IL-6 interleukin 6; IL-10 - interleukin 10; Hsp27 and Hsp70 - heat shock proteins 27kDA and 70kDa.
Superscript*, superscript# and superscript denote p < 0.05, 0.01 and 0.001 respectively between the groups by Tukey' HSD.

Table 3. Anthropometric characteristics of young tennis players.

Variable	Means (±SD) [min-max]
Height, m	1.79 (.08) [1.65-1.93]
Weight, kg	67.8 (12.7) [44.9-90.6]
TBW, kg	45.0 (8.0) [31.0-61.9]
FFM, kg	61.4 (11.01) [42.0-84.2]
SLM, kg	58.0 (10.3) [39.8-79.5]
SMM, kg	34.8 (6.6) [23.1-48.0]
Fat, kg	6.5 (2.4) [2.9-11.5]
Fat, %	9.4 (2.5) [6.4-13.5]
BMI, kg·m^{-2}	21.1 (2.3) [16.5-24.3]

Values means (M), standard deviation (SD), minimal and maximal values (min-max),TBW –total body water, FFM - free fat mass SLM – Soft lean mass, SMM – skeletal muscle mass, Fat - fat mass, Fat% - percentage of body fat, BMI - body mass index

Pro- and anti-inflammatory cytokines

Similarly to H_2O_2, the cytokines IL-1β and TNFα had been at the highest levels after the tournament season, but later on, decreased by approx. 40% after the 3d rest and 14d camp. By contrast, the cytokines IL-6 and IL-10 reached the highest levels after the 3d rest and 14d camp; however, changes in concentration of IL-6 were not statistical significant. At the same time, the effect size for IL-10 was at 46%, which means that the applied program induced large changes in this anti-inflammatory cytokine.

Heat shock proteins

Hsp27 concentration increased 3-fold after the two weeks conditioning camp compared with level observed directly after tournament season. Also, the 3d rest resulted in a significant rise in Hsp27. By contrast, Hsp70 decreased after the 3d rest and at the end of camp. Hsp27 and Hsp70 correlated with H_2O_2, IL-1β and TNFα.

Table 5. Statistical relationships (correlation coefficients) between heat shock proteins Hsp27, Hsp70, hydrogen peroxide (H_2O_2), interleukins IL-1β and TNFα.

	H_2O_2 (µmol·mL^{-1})	IL-1β (pg·mL^{-1})	TNFα (pg·mL^{-1})
Hsp27 (pg·mL^{-1})	-.348 *	-.429 **	-.350 *
Hsp70 (ng·mL^{-1})	.397 **	.368 *	.313 *

* p < 0.05, ** p < 0.01.

Muscle damage

CK activity, as a marker of muscle damage, reached the highest value after the 14d conditioning camp, similarly to anti-inflammatory cytokines IL-6 and IL-10 as well as Hsp27.

Additionally, correlations between the pro-inflammatory cytokines and heat shock proteins were calculated (Table 5). Interestingly, directly proportional correlations were observed between Hsp70 and pro-inflammatory cytokines. On the other hand, correlations between Hsp27 and pro-inflammatory cytokines were indirectly proportional.

The assessment of PRS scale indicated on diversification of level of recovery status. It is striking that the average PRS before the camp had been equal 4.0 ± 3.0, yet by the end of the camp it increased to 7.5 ± 2.5.

Discussion

The original finding of this study demonstrates that after the tournament season, young tennis players experienced an overreaching syndrome, characterized by the low level of Hsp27 and the elevated concentration of pro-inflammatory cytokines IL-1β and TNFα. Moreover, we observed that 14 days of conditioning training program induced significantly the synthesis of heat shock protein Hsp27 and anti-inflammatory cytokine IL-10. These data confirm that properly adjusted training, supported with an appropriate diet, sleep and recovery might improve performance, simultaneously providing that the balance in inflammatory response is maintained. Interestingly, despite the moderate-intensity training program and even in some cases applied forced exercise, tennis players did not feel exhausted at the end of the camp. The PRS scale at camp's completion was higher than at its beginning. Although, the participating players were characterized by an elevated level of creatine kinase activity at the end of the camp, the synthesis of the Hsp27 increased 3.4fold compare to the baseline values, recorded before the camp.

These data suggest that low concentration of Hsp27 recorded directly after the tournament season may be a consequence of an overreaching syndrome. What is more, this low level of Hsp27 was accompanied by elevated concentrations of pro-inflammatory cytokines IL-1β and TNFα. Additionally, low assessment in perceived recovery status scale confirmed our biochemical data of this syndrome. It may be reasoned that the rise of IL-1β

and TNFα before the camp was caused by the tournament season impact. We have highlighted that arrival to the camp should have been proceeded by a short recovery period. However, in fact, tennis players competed in tournaments till the very end of the season, improving their rankings and leaving insufficient time to rest. These findings are in agreement with previous observations (Smith et al., 2000) as well as our results registered in high ranking professional tennis players (Ziemann et al., 2012).

Furthermore, the three days of active rest enhanced Hsp27 and applied training workloads caused this elevated concentration to sustain by the end of the camp. Blood Hsp27 has been proposed to play a direct role in protecting against oxidative stress induced by exercise and hypoxia (Brerro-Saby et al., 2010). Moreover, the elevated extracellular Hsp27 in vivo is anti-atherogenic (Rayner et al., 2008). Also, data of Miller-Graziano suggest that Hsp27 belongs to a new group of 'anti-danger signals' and macrophages might secrete this protein (Miller-Graziano et al., 2008). Although, we did not determine, which type of cells was a source for Hsp27, our data revealed that blood CK as an indicator of muscle damage, did not correlate with blood Hsp27. These observations suggest that Hsp27 was not released from damaged muscle. Additionally, after three days of active rest a drop in pro-inflammatory cytokines was recorded. These results might be explained by the anti-inflammatory effect of low-intensity exercise (Petersen and Pedersen, 2005). The training program, applied at that time, incorporated mainly aerobic work and low intensity.

Following the aim of the study, an attempt was made to determine the role of Hsps in an immunological response to exercise, in young tennis players. Collected data demonstrated discrepancies in blood concentration of Hsp. Due to comparable molecule size, Hsp72 and Hsp70 are treated as synonyms. Previous investigation indicated that skeletal muscle is capable of Hsp72 synthesis, yet intact skeletal muscle cells do not release it into the circulation (Febbraio et al., 2002). There are also suggestions that induction of Hsp72 is conditioned by an eccentric or mechanical stress, which leads to disruption patterns of the cellular homeostasis (Febbraio and Koukoulas, 2000; Puntschart et al., 1996). Furthermore, some investigators have also measured serum or plasma Hsp72 in response to exercise (Marshall et al., 2006; Walsh et al., 2001). Interestingly, investigation by Heck and co-workers, presented the role of Hsp70 as a novel fatigue signaling factor, sent from the immune system to the brain (Heck et al., 2011). They showed that increased levels of eHsp70 in plasma during an exercise and a considerable release of eHsp70 from lymphocytes during high-load exercise bouts may contribute to fatigue sensation, but also act as a danger signal from the immune system. This fact might provide an explanation for the observed elevation of Hsp70 at the beginning of the camp. Also, the long lasting mental stress, which appeared during the whole tournament season may have led to an increase in the concentration of this heat shock protein; however, already a 3-day recovery combined with a low-intensity training caused a decline in Hsp70 level. These lower values of Hsp70 were maintained by the end of the camp, most likely due to the fact that the forced physical workload applied during the camp had lacked mental stressors, connected with tournament competition.

Still, Liu et al. (2000) revealed that Hsp70 response to training seems to depend upon exercise intensity rather than its volume. Previous research indicated that close interactions exist between the activation of Hsp gene expression and IL-6 production. However, in our tennis players no statistically significant differences between IL-6 concentration from the beginning and the end of the camp occurred. Still, this lack of ascending or descending trends was accompanied by the changes in Hsps.

The main purpose of the training program, applied during the camp, was to prepare subjects for the upcoming tournament season, make them more resistant to stress, but also put a strong emphasis on the quality of undergone recovery processes. The observed overreaching syndrome at the beginning of the training camp is accompanied by an elevated H_2O_2 concentration, which progressively decreased, reaching the lowest value after 12 days of the camp. Interestingly, Hsp27 negatively correlated with H_2O_2 and pro-inflammatory cytokines. These data confirmed previous observations that Hsp27 may directly scavenge ROS (Wyttenbach et al., 2002). Moreover, it has been shown, that Hsp protect stress activated protein kinases (SAPK) form activation (Gabai et al., 1997). Recently our research group member revealed, basing on a cellular model, that activation of SAPK leads to iron-dependent ROS formation (Antosiewicz et al., 2007).

What is more, the applied training program resulted in an increase in the anti-inflammatory cytokine IL-10 concentration and decrease of the pro-inflammatory IL-1β and TNFα. It is striking that simultaneously with these changes and an increase of CK were observed in the group of tennis players. However, compare to the beginning of the camp, the assessment of perceived recovery status had grown significantly, up to 7.5 in scale proposed by Laurent et al. (2011), which meant that coaches might have expected improvement in athletes' performance.

Conclusion

To sum up, basing on the data collected and analysis conducted, we concluded that maintaining an immunological response balance is vital to achieve progress in tennis. The applied training program stimulated the anti-inflammatory response, which was supported by increase of Hsp27 and a drop in the pro-inflammatory cytokines and Hsp70.

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Key points

- The study demonstrating low grade inflammation-induced by the tournament season in young tennis player.
- Three days of active rest stimulated the anti-inflammatory response via rise of Hsp27 and anti-inflammatory cytokine IL-10.
- Observed decrease of blood Hsp70 may support mental recovery.
- Thirteen-day appropriate training program led to maintaining an immunological response balance.

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