Pro-Oxidative Processes and Cytokine Response to Training in Professional Basketball Players

by

Agnieszka Zembroń-Łacny1, Małgorzata Slowińska-Lisowska2, Edward Superlak3

In the present study, we evaluated the plasma concentration of inflammatory mediators including cytokines and their relation with oxidative damage markers in training cycles of basketball players. Sixteen professional players of the Polish Basketball Extraleague participated in the study. The basketball players were observed during the preparatory period and the play-off round. Twenty healthy and untrained males composed of the reference group. The comparative study has shown significantly higher levels of lipid peroxidation (TBARS) and protein carbonylation (PC) in nonathletes than in basketball players during the observed training periods. Tumour necrosis factor α (TNFα), similarly to TBARS and PC, was significantly higher in nonathletes than athletes, except at the end of the play-off round. Interleukin-6 (IL-6) was lower in nonathletes than athletes in the preparatory period but it was higher in athletes in play-off round. In basketball players, the high level of IL-6 directly correlated with TBARS (r = 0.763, p<0.001) and PC (r = 0.636, p<0.001) during the preparatory period, whereas the high level of TNFα inversely correlated with TBARS (r = -0.601, p<0.001) and PC (r = -0.650, p<0.001) in the play-off round. The activity of creatine kinase (CK) was significantly increased during the training mesocycles in basketball players compared with nonathletes, and reached the highest activity at the end of the play-off round. CK activity did not correlate with oxidative damage markers and cytokines in both untrained and trained subjects. Our results have shown the reduction in oxidative damage and improvement in cytokine response following professional training, as well as the relationship between inflammatory and pro-oxidative processes in basketball players.

Keywords: cytokines, lipid peroxidation, protein carbonylation, athletes

Introduction

Physical exercise is an important and well-known factor, both in animals and human studies, for inducing reactive oxygen and nitrogen species (RONS) generation and improving antioxidant defence. RONS are physiological products of aerobic metabolism and are used by cells for variety of metabolic tasks such as gene expression, protein turnover, inflammatory reaction, arachidonic acid immobilisation,
erythropoiesis etc. RONS are released from muscle, endothelial and immunological cells to stimulate an adaptation to intense physical exercise, which include the immune system and energetic status related to cytokine synthesis, such as tumour necrosis factor α (TNFα) and interleukine-6 (IL-6) (Radak et al. 2008; Valko et al. 2007).

Previously, it was hypothesized that the exercise-induced increase in these cytokines is a consequence of an immune response due to local damage in the working muscles (Nieman et al.; 1998). However, Plomgaard et al. (2005) demonstrated that the immune cells are not the source of the plasma TNFα and IL-6 during intense exercise. TNFα and IL-6 are found to be expressed in human skeletal muscle in a strict fiber type specific fashion. TNFα is expressed by fast-twitch fibers; whereas the expression of IL-6 is more prominent in slow-twitch fibers and dependent on RONS level (Kosmidou et al. 2002; Plomgaard et al., 2005). TNFα increases mainly after eccentric exercises, whereas IL-6 dramatically increases after long-term endurance exercises. The high level of TNFα impairs glucose uptake by skeletal muscle; whereas IL-6 ensures glucose homeostasis and intramuscular glycogen stores (Petersen & Pedersen 2005; Steensberg et al. 2002). Nevertheless, both cytokines play an important role in muscle reconstruction after strenuous exercise, and also in tolerance to oxidative damage (Steensberg et al. 2002). TNFα initiates the breakdown of damaged muscle tissue, whereas IL-6 stimulates the proliferation and differentiation of satellite cells (Peake et al., 2005).

The ability of exercise to modulate immune response through cytokine production has prompted some researchers to explore the effects of training on immune status. However, few studies have looked at cytokine response and its relation with oxidative stress in professional training activity (Gokhale et al., 2007; Huffman et al., 2008; Rämson et al., 2008; Rong et al., 2008). The aim of the present study was to investigate the plasma concentration of inflammatory mediators including IL-6 and TNFα, and their relation to oxidative damage markers in training activity of basketball players.

**Material & Methods**

Sixteen professional players of the Polish Basketball Extraleague were enrolled for the study (age 26.1 ± 3.2 yr, height 198.3 ± 10.2 cm, body mass 98.2 ± 4.1). They engaged in sports professionally for an average of 12.5 ± 2.8 years. The concentrations of oxidative damage markers and cytokines, as well as creatine kinase activity, were measured two times during the preparatory period before the final play-off round of the season (November, December), and two times during the play-off round (April, May).
All subjects underwent the same standardized training program. All details of the training mezoycles reported by the coach are shown in table 1. Twenty healthy, non-smoking and untrained males (age 21.8 ± 1.1 yr, height 180.3 ± 7.8 cm, body mass 82.2 ± 8.7 kg) made the reference group. At the time of our investigation the studied subjects avoided drugs or nutrition supplement that could interfere with evaluating pro-antioxidant capacity.

All subjects were informed of the aim of the study and voluntarily gave their written consent for participation in the project. The protocol of the study was approved by the local ethics committee in accordance with the Helsinki Declaration.

Each of the studied subjects was asked to avoid physical effort for 24 h before laboratory measurements. Blood samples were taken from the elbow (antecubical) vein at 8 a.m., after 15 minutes of rest (and an overnight sleep). Within 10 min, the blood samples were centrifuged at 2500 g and 4°C for 10 min. Aliquots of plasma were stored at -20°C. All samples were analysed within 7 days.

Creatine kinase (CK) activity was immediately evaluated after plasma collection using the diagnostic assays for the kinetic enzyme analyser Konelap 60 BioMerieux (France). CK detection limit for the applied kit was 6 U · l⁻¹. The

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Untrained Subjects</th>
<th>BASKETBALL PLAYERS</th>
<th>PREPARATORY PERIOD</th>
<th>PLAY-OFF ROUND</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>November</td>
<td>December</td>
<td>Nov vs. Dec</td>
<td>April</td>
</tr>
<tr>
<td>CK U · l⁻¹</td>
<td>106 ± 90 a, c, d</td>
<td>238 ± 74</td>
<td>186 ± 45 b, d</td>
<td>ns</td>
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<tr>
<td>TBARS μmol · l⁻¹</td>
<td>2.150 ± 0.279 a, b, c, d</td>
<td>1.884 ± 0.387 ac</td>
<td>0.888 ± 0.136 bc</td>
<td>P &lt; 0.001</td>
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<tr>
<td>PC nmol · mg⁻¹ d⁻¹</td>
<td>1.408 ± 0.231 a, b, c, d</td>
<td>1.072 ± 0.082</td>
<td>0.798 ± 0.119 bc</td>
<td>P &lt; 0.001</td>
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<tr>
<td>IL-6 ng · l⁻¹</td>
<td>0.795 ± 0.251 a, b, c</td>
<td>1.311 ± 0.315 ac</td>
<td>0.821 ± 0.201 bc</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>TNFα ng · l⁻¹</td>
<td>1.417 ± 0.229 a, b, c</td>
<td>0.868 ± 0.261</td>
<td>0.854 ± 0.190 bd</td>
<td>ns</td>
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The single letters indicate statistically significant differences (P < 0.01) between untrained subjects (NT) and basketball players (T): a NT vs. T (Nov), b NT vs. T (Dec), c NT vs. T (April), d NT vs. T (May). The double letters indicate statistically significant differences (P < 0.01) between mezoycles of the preparatory period and play-off round: a NT vs. T (Nov), b NT vs. T (Dec), c NT vs. T (April), d NT vs. T (May). The double letters indicate statistically significant differences (P < 0.01) between mezoycles of the preparatory period and play-off round: a NT vs. T (Nov), b NT vs. T (Dec), c NT vs. T (April), d NT vs. T (May).
Plasma lipid peroxidation products were estimated using the measurement of thiobarbituric acid – reactive substance (TBARS) level according to the method of Buege and Aust (1991). To avoid further peroxidation, plasma samples were deproteinized with 15% trichloroacetic acid (TCA), containing 0.25M HCl, immediately after separation of plasma. TBARS level was expressed as nmol of malondialdehyde, using 1,1,3,3-tetraethoxypropane as a standard. TBARS detection limit was 0.13 nmol·ml⁻¹.

Plasma protein carbonyls (PC) were measured by the method of Levine et al. (1990), using 2,4-dinitrophenyl hydrazine. The carbonyl content was calculated using an extinction coefficient of 22000 M⁻¹·l⁻¹·cm⁻¹ and expressed as nmol PC per mg of plasma protein. Protein concentration was determined by the Bradford method (1976). The intra-assay coefficient of variation (CV) for PC and TBARS procedures were <10%.

Plasma tumour necrosis factor (TNFα) and interleukin-6 (IL-6) levels were determined by enzyme immunoassay methods using commercial kits (R&D Systems, USA). Detection limits for the TNFα and IL-6 were 0.038 pg·ml⁻¹ and 0.04 pg·ml⁻¹, respectively. The intra-assay coefficient of variation (CV) was <8.0% for both cytokines.

The results are expressed as mean and standard deviation (x ± SD). Statistical significance was tested using one-way ANOVA and post-hoc Tukey’s test. Correlation between variables was tested with Pearson’s correlation analysis. The statistics were done using STATISTICA 8.0.

**Results**

**Nonathletes vs. Basketball players.** The comparison of the muscle damage markers and cytokines have shown significant differences between untrained and well-trained subjects (Table 2). CK activity was almost two-fold higher in basketball players than in nonathletes. Contrary to CK, TBARS and PC were more than 30% lower in athletes than in nonathletes. The low levels of TBARS and PC in basketball players were presumably related to the enhancement of antioxidant capacity by systemic physical exercise and the fast elimination of lipid peroxidation and protein carbonylation products from blood.

Cytokine TNFα changed similarly to pro-oxidative parameters (i.e., it was lower by over 30% in athletes than in nonathletes), except at the end of play-off round (May). It was very interesting that cytokine IL-6 behaved remarkably differently compared with TBARS, PC and TNFα. In basketball players, IL-6 was significantly higher in the preparatory period (November) and lower in the play-off round (April), compared with nonathletes.

**Preparatory period vs. Play-off round.** CK activity reached the highest value in basketball players at the end of the play-off round, in which the high lactate-anaerobic efforts were observed. The increase in CK activity did not correlate with oxidative damage and cytokine response. The analysis of oxidative damage markers, as well as cytokines, has shown the regularity which was dependent on exercise load in particular training mezocycles. In the preparatory period, in which high aerobic efforts were observed, the TBARS concentration...
was significantly elevated, compared with values observed in the play-off round. The highest level of TBARS was found at the beginning of the preparatory period (November). PC concentration was similar in both training periods. However, the measurement of oxidative damage markers demonstrated the regularity (i.e., TBARS and PC were always elevated at the beginning of the analysed periods (November, April), and then the values dropped at the end of training periods (December, May). The changes in TBARS and PC were directly and positively correlated. The Person’s coefficient for TBARS vs. PC was $r = 0.630$ ($p < 0.001$) in the preparatory period, and $r = 0.458$ ($p < 0.01$) in the play-off round (Table 1-2).

IL-6 was significantly higher in the preparatory period than in the play-off round. It reached the highest level at the beginning of the preparatory period (November), similarly to TBARS. TNF$\alpha$ behaved in contrast to interleukine-6. TNF$\alpha$ was high in the play-off round, where the anaerobic-alactate efforts prevailed. TNF$\alpha$ reached the highest level at the end of the season (May), similarly to CK activity (Table 1-2). In the preparatory period, the high level of IL-6 positively correlated with TBARS ($r = 0.763$, $p<0.001$) and PC ($r = 0.636$, $p<0.001$) (Figure 1). In the play-off round, the high level of TNF$\alpha$ inversely correlated with TBARS ($r = -0.601$, $p<0.001$) and PC ($r = -0.650$, $p<0.001$) (Figure 2).

**Discussion**

Intense physical exercise causes the disruption of homeostasis in skeletal muscle cells, which is associated with production of RONS, damage of muscle proteins and enzymes, release of inflammatory mediators and growth factors, ultrastructural changes in muscle architecture, etc.

The plasma CK activity is the common biochemical marker of muscle fiber damage. It rises slowly after exercise and usually peaks after one or two days; then it declines even more slowly towards baseline. Athletes, as a rule, have higher plasma CK activity than nonathletes because of the regular strain imposed by training on their muscles. In our study, CK activity was almost two-fold higher in basketball players than nonathletes, and it reached the highest value in athletes at the end of play-off round, similarly to pro-inflammatory cytokine TNF$\alpha$. Nevertheless, an increase in CK did not correlate with either cytokines or oxidative damage markers, contrary to others studies (Bruunsgaard et al., 1997; Drewa et al., 1999; Malm et al., 2004).

The detection of lipid peroxidation and protein carbonylation products using thiobarbituric acid and dinitrophenyl hydrazine have been the most widely used markers of RONS generation and oxidative damage. Although the plasma TBARS and PC have been non-specific techniques, using them can offer an empirical view on the complex process of peroxidation and carbonylation followed by single exercise or training (Allesio et al., 2000; Knez et al., 2007; Mena et al., 1997; Metin et al., 2003). The present study has shown significant lower level of lipid peroxidation in basketball players than in nonathletes. The similar results were previously obtained by Mena et al. (1997) and Metin et al. (2003) in professional cyclists and soccer players compared to nonathletes. Furthermore, Sentürk et al. (2001) established that oxidative damage takes place in untrained, but not in trained animals, and Oztasan et al. (2004) confirmed that endurance training is useful to prevent acute exhaustive exercise-induced oxidative stress by upregulating of the antioxidant system.

In basketball players, TBARS level directly correlated with PC in both training periods, indicating an integration of the peroxidation with the carbonylation process. It has been known that lipid radicals induce protein damage, and oppositely, thiyl radicals can cause the peroxidation of lipids, such as arachidonic acid (Sagrista et al., 2002).

The cytokine response to physical exercise has frequently been investigated within the past few years (Fischer et al., 2004; Plomgaard et al., 2005; Steensberg et al., 2002; Steinberg et al., 2007; Suzuki et al., 2002). It was observed that plasma IL-6 and TNF$\alpha$ increase in an exponential fashion with exercise and is related to exercise intensity, duration, the mass of muscle recruited, and one’s endurance capacity (Petersen & Pedersen, 2005). It was also demonstrated that mRNA for IL-6 and TNF$\alpha$ are upregulated in contracting skeletal muscle, and that the transcriptional rate of the IL-6 and TNF$\alpha$ genes are markedly enhanced by a single exercise (Fischer et al., 2004).
et al., 2004; Plomgaard et al., 2005). Our results have shown that systemic exercise significantly affects cytokine response. The differences are especially visible in TNFα, which was lower in basketball players than nonathletes. TNFα is a pro-inflammatory cytokine, therefore its decreased level in athletes is interpreted as an anti-inflammatory effect of systemic physical exercise (Petersen & Pedersen, 2005). In basketball players, TNFα increased only at the end of the play-off round when muscle damage was markedly enhanced; the high activity of CK. The muscle damage attract neutrophils and macrophages to the site of injury, which contribute to the degradation of damaged tissue by releasing the pro-inflammatory cytokines, such as TNFα (Peake et al., 2005). Therefore, an increase in plasma TNFα can be an index of phagocytosis and reconstruction of muscle after intense exercise.

The main findings of our study were the relationships between changes in cytokine levels dependent on training periods, as well as integration of ROS activity with cytokine response. IL-6 reached the highest level in the preparatory period where the aerobic efforts were performed. TNFα significantly increased in games during the regular season, where the anaerobic-alactate efforts prevailed.

According to Petersen and Pedersen (2005), IL-6 acts in a hormone-like manner to maintain glucose homeostasis, induce lipolysis and fat oxidation, exert inhibitory effect on TNFα production, and also stimulate the proliferation of satellite cells and differentiation of myoblasts. Taking into account the increase in IL-6, which was observed during the preparatory period, it is essential to reach an optimal level of adaptation to intense physical exercise. The decline in IL-6, observed in the play-off round, may be related to the muscle lactate accumulation during competition (McInnes et al., 1995). The high lactate production during intense exercise probably impairs the ability of muscle cells to produce IL-6 (Suzuki et al., 2002).

Both cytokines correlated with markers of oxidative damage, however, IL-6 positively correlated in the preparatory period, whereas TNFα was inversely correlated in the play-off round. The first relationship confirms the participation of RONS in IL-6 synthesis and release from muscle (Kosmidou et al., 2002). RONS is also involved in TNFα release from muscle and immune cells. In addition, TNFα induces the RONS synthesis in the reaction of NADPH oxidase (Dröge, 2001). The high level of this cytokine is essential for the regeneration of injured muscle fibers (Peake et al., 2005). Nevertheless, we observed the negative correlation between TNFα and oxidative damage markers, which excludes complicity of RONS in TNFα synthesis during the game season. The main reason for an increase in plasma TNFα was muscle damage, regardless of RONS generation.

In conclusion, our results have shown 1) professional sport training reduces oxidative damage and improve IL-6 and TNFα release from muscle, 2) cytokine IL-6 dominates during the preparatory period, whereas TNFα prevails in the play-off round, and 3) cytokine response is markedly related to pro-oxidative processes in basketball players.

References


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