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The level of pro-inflammatory cytokines and reactive oxygen species in wrestlers compared to non-athletes

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Summary

Introduction. 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 pro-inflammatory cytokines response and its relation with reactive oxygen species (ROS) generation in professional training activity. The aim of the present study was to investigate the plasma concentration of cytokines including IL-1β and TNFα, and their relation with muscle damage, hydrogen peroxides (H_2O_2) and markers of ROS activity (lipid peroxides LPO and protein carbonyls PC) in wrestlers compared to nonathletes.

Material and methods. Nineteen wrestlers, members of national team, participated in the study. The athletes were observed during the preparatory period (January). Twenty healthy and untrained males made a reference group.

Results. The comparative study has shown the significantly higher level of creatine kinase (CK) and H_2O_2 as well as cytokines TNFα and IL-1β in wrestlers then non-athletes. LPO and PC concentrations tended to lower values in wrestlers but the differences between groups were not significant. There also was observed the high correlation between CK activity and TNFα concentration ($r=0.912$, $P<0.001$).

Conclusions. The study has demonstrated the significant effect of sport training on plasma level of hydrogen peroxide and pro-inflammatory cytokines as well as the relationships between muscle damage and TNFα.

Introduction

The recent study has shown that reactive oxygen species (ROS), such as hydroperoxide (H_2O_2), are important signalling molecules generated during muscle contraction and are involved in the regeneration and adaptation of skeletal muscle to physical work. H_2O_2 is produced by enzyme superoxide dismutase (SOD) which is localized to the muscle sarcolemma (isoenzymes CuZnSOD) and mitochondria (isoenzymes MnSOD) [1]. The studies in human isolated muscle and myotube culture demonstrated that H_2O_2 produced within contracting skeletal muscle is key regulators of pretranslational signalling events leading to cytokines expression [2].

The cytokine response to physical exercise has been studied by many authors in recent years [3-7]. The plasma cytokines increase exponentially during or after physical effort in relation to exercise intensity, duration, mass of working muscles and type of contractions (concentric vs. eccentric). In spite of the large number of studies investigating changes in cytokines, only a few other studies have compared the effects of sport training on the pro-inflammatory cytokines [8-12].

In our previous study, we reported that TNFα reached the highest values during the start period in basketball players when the anaerobic-alactate efforts and muscle damage were occurring [12]. Based on these findings, the aim of the present study was to compare the plasma concentration of inflammatory mediators including IL-1β and TNFα in wrestlers and non-athletes, and to examine the relationships between the levels of muscle damage, cytokines and ROS.

Material and methods

Subjects. Nineteen senior wrestlers participated in the study. All of them were members of national team during the

preparatory period (January). Twenty physically active untrained males made a reference group (Tab. 1).

Each of the studied subjects was asked to avoid drugs or nutrition supplement that could interfere with immunological and pro-antioxidant evaluation. All the subjects were informed of the aim of the study and 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.

Blood samples were taken from the elbow vein at 8 a.m. Within 10 min, the blood samples were centrifuged at 2500 g and 4°C for 10 min. Aliquots of plasma were stored at -80°C.

Anthropometric measurements. Body mass (BM) and body composition were estimated using a bioelectrical impedance floor scale (Akern, Poland) calibrated in accordance with manufacturer guidelines prior to each test session.

Muscle damage. Plasma creatine kinase (CK) activity was used as a marker of muscle damage and was evaluated by Emapol kit (Poland). CK detection limit for the applied kit was 6 U · 1^{-1}. The intra-assay coefficient of variation (CV) for the CK kit was 1.85%.

Markers of ROS activity. The markers of ROS activity i.e. plasma hydroperoxide (H_2O_2), lipid peroxide (LPO) and protein carbonyls (PC) concentrations were determined by using Oxis Research kit (USA) and Alexis Biochemicals (USA). H_2O_2 were measured immediately after plasma collection, at day of exercise study. The limit detection for H_2O_2 was 6.25 nmol · ml^{-1}, and for LPO and PC were 0.1 nmol · ml^{-1}. The intra-assay coefficient of variation (CV) for the kits of H_2O_2, LPO and PC were <10%.

Pro-inflammatory cytokines. Plasma interleukin-1β (IL-1β) and tumour necrosis factor (TNFα) levels were determined by enzyme immunoassay methods using commercial kits (R&D Systems, USA). Detection limits for IL-1β and TNFα were 0.023 and 0.038 pg · ml^{-1}, respectively. The average intra-assay coefficient of variation (CV) was <8% for both cytokines.

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. 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).

Results

The comparison of the muscle damage markers and cytokines has shown the significant differences between untrained and well trained subjects (Tab. 2). CK activity was five-fold higher in wrestlers than in nonathletes, similarly to TNFa. CK significantly correlated with TNFα (r=0.912, Fig. 1). H_2O_2 and IL-1β also were higher in athletes than nonathletes.

This is very interesting that markers of ROS activity (LPO and PC) tended to lower values in wrestlers compared to nonathletes. The low levels of LPO and PC in athletes were presumably related with the enhancement of antioxidant capacity by systemic physical exercise and/or the fast elimination of lipid peroxidation and protein carbonylation products from blood.

Discussion

The strength exercise has been used widely as a means of inducing muscle damage and may be a valuable tool in the study of inflammation. The muscle tissue damage promotes

Tab. 1. Anthropometric characteristics of untrained and trained subjects; BMI body mass index, FM fat mass, FFM free fat mass, MM muscle mass

	Age [years]	Body mass [kg]	High [cm]	BMI	FM [kg]	FFM [kg]	MM [kg]
NONATHLETES N=20	21.2±1.3	77.6±7.5	182.0±7.6	23.5±1.9	14.0±2.6	60.0±4.7	41.8±2.9
WRESTLERS N=19	21.5±3.6	76.6±13.3	175±8	24.9±2.2	10.7±3.9	65.9±9.8	42.5±4.1

Tab. 2. Levels of plasma creatine kinase (CK), lipid peroxides (LPO), interleukin 1β (IL-1β) and tumour necrosis factor α (TNFα) in untrained and trained subjects

	NONATHLETES	WRESTLERS	Significant level non-athletes vs. wrestlers
H_2O_2 μM	5.51±1.06	7.22±0.96	$P<0.01$
LPO nmol · ml^{-1}	2.90±0.50	2.65±0.55	*ns*
PC nmol · ml^{-1}	5.86±0,81	5.21±0.76	*ns*
IL-1β pg · ml^{-1}	0.58±0.17	1.00±0.13	$P<0.001$
TNFα pg · ml^{-1}	0.85±0.22	5.90±0.49	$P<0.001$
CK U · l^{-1}	163±75	746±158	$P<0.001$

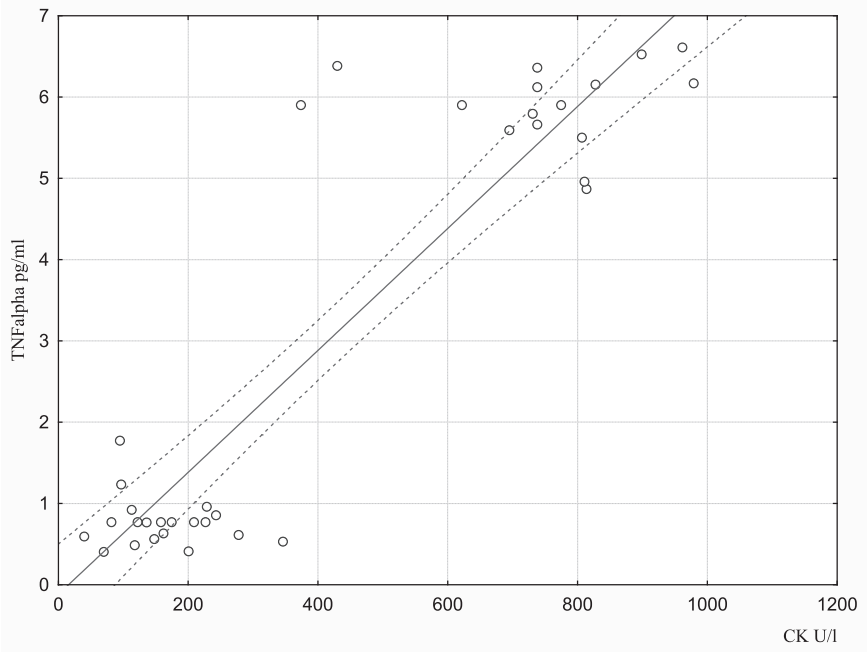

Fig. 2. The relationship between creatine kinase (CK) activity and tumour necrosis factor α concentration (TNFalpha)

infiltration by inflammatory cells that, in conjunction with local muscle, endothelial, and satellite cells, produce an array of molecules to regulate the regeneration process, including TNFα, IL-1β and H_2O_2. Typical responses to muscle injury induced by eccentric and isometric contractions include loss of force-generating capacity, decreased range of motion, delayed-onset muscle soreness, swelling, and delayed increases in circulating intramuscular proteins [13-14].

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 considerably higher in wrestlers than nonathletes. The high activity CK not correlated with oxidative damage markers (LPO and PC), contrary to others studies [15-16].

The detection of LPO and PC has been the most widely used markers of ROS activity and oxidative damage. The measure of plasma LPO and PC can offer an empirical view on the complex process of peroxidation and carbonylation followed by single exercise or sport training [17-19]. The present study has shown the tendency of LPO and PC to lower values in wrestlers compared to nonathletes. Earlier, Sentürk et al. [20] and Oztasan et al. [21] demonstrated that physical training is useful to prevent acute exhaustive exercise-induced oxidative stress by upregulating of the antioxidant system.

The cytokines IL-1β and TNFα were significantly higher in wrestlers then nonathletes. Moreover, TNFa highly correlated with muscle damage (CK). The long-term presence of pro-inflammatory cytokines is related to muscle restoration [22-23]. It should be stressed that the suppression of inflammatory response by using anti-inflammatory drugs attenuates the exercise-induced increase in satellite cell number after eccentric exercise [24].

Conclusions

In conclusion, our results have shown: 1) the professional sport training reduces an oxidative damage and improve in TNFa release from muscle and immune cells, 2) the cytokine TNFa could be a marker of a normal reconstruction of muscle after intense exercise and adaptation to physical exercise.

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