

Oxidative stress status in elite athletes engaged in different sport disciplines

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ABSTRACT

Exercise training may increase production of free radicals and reactive oxygen species in different ways. The training type and intensity may influence free radicals production, which leads to differences in oxidative stress status between athletes, but the results of the previous studies are inconsistent. The aim of our study was to estimate oxidative stress status in elite athletes engaged in different sport disciplines. The study included 39 male highly skilled professional competitors with international experience (2 Olympic players): 12 wrestlers, 14 soccer players and 13 basketball players in whom we determined the levels of advanced oxidation protein products (AOPP) and malondialdehyde (MDA), as markers of oxidative stress and the total antioxidative capacity (ImAnOX) using commercially available assay kits. The mean AOPP concentration was not significantly different between soccer players, wrestler and basketball players (60.0 ± 23.0 vs. 68.5 ± 30.8 and 80.72 ± 29.1 $\mu\text{mol/L}$ respectively). Mean ImAnOX concentration was not different between soccer players (344.8 ± 35.6 $\mu\text{mol/L}$), wrestlers (342.5 ± 36.2 $\mu\text{mol/L}$) and basketball players (347.95 ± 31.3 $\mu\text{mol/L}$). Mean MDA concentration was significantly higher in basketball players (1912.1 ± 667.7 ng/mL) compared to soccer players (1060.1 ± 391.0 ng/mL , $p=0.003$). In spite of this fact, oxidative stress markers levels were increased compared to referral values provided by the manufacturer. Type of sports (soccer, wrestler or basketball) have no impact on the levels of oxidative stress markers. Elite sports engagement is a potent stimulus of oxidative stress that leads to the large recruitment of antioxidative defense. Oxidative stress status monitoring followed by appropriate use of antioxidants is recommended as a part of training regime.

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KEY WORDS: oxidative stress, elite athletes, different sport disciplines

INTRODUCTION

The cells in our body continuously produce free radicals and reactive oxygen species (ROS) as part of metabolic processes. Free radicals are molecules or part of molecules which have one or more unpaired electrons in external electronic shell. Main characteristics of these molecules are very short life span and extremely high reactivity. Injurious effects of free radicals are induced by necessity to establish electronic stability and therefore they react with next stable molecule, taking its electron and creating new free radical. That way this molecules also becomes unstable and further interferes with other

molecules from its surrounding which leads to impairments of cellular components. Free radicals are created during the process of oxidative phosphorylation in mitochondria [1]. Oxidative stress occurs as a result of ROS activity and reduced protective mechanisms that lead to impairments in cells and tissues functions. It causes secondary damage through late cell death and inflammation [2]. Various studies have shown that oxidative stress represents pathogenetic foundation of many diseases [3]. ROS are normally neutralized by complex system of antioxidant defence [4]. The system of antioxidant defence can be divided into two groups: enzymes including superoxid dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX); and non-enzymes including vitamins C and E, retinol, bilirubin, uric acid, redox glutathione, thiols, coenzyme Q₁₀, stress proteins, albumins, as well as transport proteins and storage proteins for Fe²⁺ i Cu²⁺ which disable potentially harmful metal ions and their involvement in production of free radicals [5]. Nevertheless, low levels of ROS appear to be neces-

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sary for important physiological functions such as cell signaling, immune response, and apoptosis [6]. Many studies have shown that exercise induces oxidative stress and causes adaptations in antioxidant defences [7, 8]. Training can have positive or negative effects on oxidative stress depending on training load, training specificity and the basal level of training. Data suggest that regular long term training can induce antioxidant response to the oxidative stress. The results of a study which investigated the relationship between oxidative stress and exercise overtraining/overreaching support the possibility that the beneficial effect of physical exercise on oxidative stress might be associated with increased antioxidant defences [9]. It is also well known that active and non active skeletal muscles produce reactive oxygen and nitrogen species although it is not quite clear where oxidants originate during physical activity [10]. The degree of oxidative damage, as well as the time course for elevation in oxidative stress markers has varied across studies, and appears to be dependent, among all, on the type, intensity, volume and duration of exercise [11]. This leads to differences in oxidative status between athletes in different sport disciplines, but the results of the previous studies are inconsistent. Therefore the aim of our study was to estimate oxidative stress status in elite athletes engaged in different sports disciplines.

MATERIALS AND METHODS

Subjects

The study was performed on 39 young (22.1 ± 4.4 years old) male elite players. All the athletes were highly skilled professional competitors with international experience (two Olympic players) in three sport disciplines: 12 wrestlers, 14 soccer players and 13 basketball players. All participants underwent routine health checks and gave written informed consent to participate in the study. All participants completed a questionnaire assessing their daily and weekly training workload, duration of professional sports involvement. Any participant with suspect pathological findings during physical examination, recent history of disease or injuries, intake of medications that might have had influence on oxidative markers were excluded. All study procedures were in accordance with the Helsinki declaration. The study was approved by the Ethical committee of the Faculty of Medicine, University of Sarajevo.

Procedures

Two days prior to taking part in the study all participants refrained from strenuous physical training. One month prior to blood sampling, the athletes were instructed to abstain from any vitamin or antioxidant dietary supplementation. All par-

ticipants were nonsmokers. Before the beginning of the study, athletes passed standard sports-medicine examination that included a health questionnaire, electrocardiographic examination, blood pressure and anthropometrical measurement. BMI for each subject was calculated (weight in kilograms divided by height in meters squared). Height was measured with stadiometer and weight was measured with Toledo self-zeroing electronic digital scale (Mettler-Toledo, Inc., Worthington, OH.). Trained persons measured blood pressure using a mercury sphygmomanometer (MD10XX, MEDI, Shanghai, China) on the right arm after at least a 5-min rest.

Biochemical analysis

Blood samples were taken from athletes in order to determine the redox state. As the markers of oxidative stress we used advanced oxidation protein products (AOPP) and malondialdehyde (MDA) and ImAnOx as marker of total antioxidative capacity. Blood samples were taken from an antecubital vein into Vacutainer test EDTA tube and stored immediately.

AOPP Assay

Determination of AOPP was based on spectroscopic analysis of modified proteins using AOPP assay kit (Immunodiagnostic AG). Standards, controls and samples assayed for AOPP were placed in each well of a 96-well microtiter plate. The absorbance at 340 nm was measured at microplate reader (Statfax 2100, USA). Concentration of AOPP is expressed in chloramine units ($\mu\text{mol/l}$).

MDA Assay

Level of malondialdehyde in plasma was determined by using ELISA assay kit for MDA (Uscn Life Science Inc.). This assay employs the competitive enzyme immunoassay technique. A monoclonal antibody specific for MDA has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labeled MDA and unlabeled MDA (standards and samples) with the pre-coated antibody specific for MDA. After incubation the unbound conjugate is washed off. Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional concentration of MDA in the sample. After addition of the substrate solution, the intensity of color developed was reverse proportional to the concentration of MDA in sample. The absorbance was read at 450 nm.

ImAnOx (Total antioxidative capacity-TAC)

The determination of the total antioxidative capacity is performed by photometric test system ImAnOX (Immunodiagnostic AG, Bensheim). The antioxidants in the sample

reacted with the defined amount of exogenously provided hydrogen peroxide (H₂O₂) and eliminated a certain amount. The residual H₂O₂ is determined photometrically by an enzymatic reaction. The absorbance was measured at 450 nm.

Statistical analysis

Values are expressed as mean±SEM or median and interquartile range depending on data distribution. Normal distribution of continuous variables was tested using Shapiro-Wilk test. Differences in mean between groups were tested using ANOVA followed by post hoc Tuckey test and differences in median between the groups were tested by use of Kruskal-Wallis test followed by Mann-Whitney's test. Associations between continuous variables were tested with Spearman's rank or Pearson correlation analysis.

RESULTS

Baseline characteristics of the male elite athletes are given in Table 1. There was no significant difference in age and training habits between soccer players, wrestlers or basketball players. However, mean weight was found to be significantly higher in basketball compared

TABLE 1. Baseline characteristics of male elite athletes.

	Soccer players	Wrestlers	Basketball players	P value
Age (y)	22.1±4.4	21.7±6.0	20.2±2.3	NS
Weight (kg)	74.9±9.4	85.9±16.6	93.0±11.3	**p=0.004
BMI (kg/m ²)	22.6±1.8	26.3±4.4	23.6±1.5	*p=0.024
Duration of training (y)	13.2±4.1	12.3±5.6	10.5±3.1	NS
Training frequency (nr/week)	6.2±0.8	6.0±1.0	9.8±0.8	NS

* soccer players vs. wrestlers

** soccer players vs. basketball players

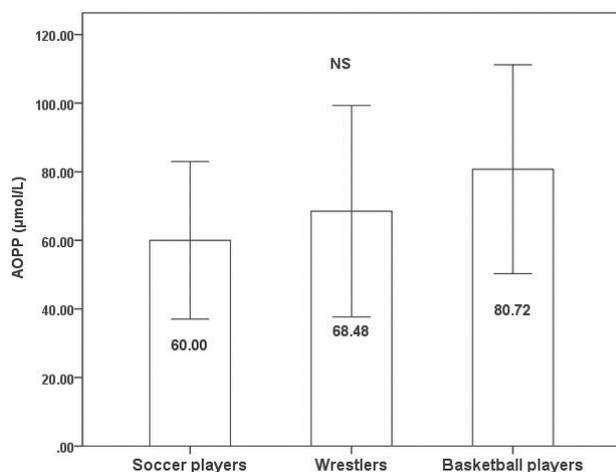


FIGURE 1. AOPP concentration in male elite athletes. Concentration of AOPP is expressed in chloramine units (µmol/l).

to soccer players, while mean BMI was significantly higher in wrestlers compared to soccer players (Table 1.). The mean AOPP concentration in soccer players was 60.0±23.0 µmol/L, in wrestlers 68.5±30.8 µmol/L and 80.72±29.1 µmol/L in basketball players, but the difference was not significant (p=0.424, NS)(Figure 1). The mean ImAnOX concentration was 344.8±35.6 µmol/L in soccer players, 342.5±36.2 µmol/L in wrestlers and 347.95±31.3

TABLE 2. Correlation coefficients between age, antropometric parameters, duration of training and oxidant/antioxidant markers in soccer players

	AOPP	ImAnOx	MDA
Age (y)	r=-0.19	r=0.33	r=-0.07
Weight (kg)	r=-0.2	r=0.11	r=-0.1
BMI (kg/m ²)	r=-0.35	r=0.18	r=-0.1
Duration of training (y)	r=-0.15	r=0.12	r=-0.17

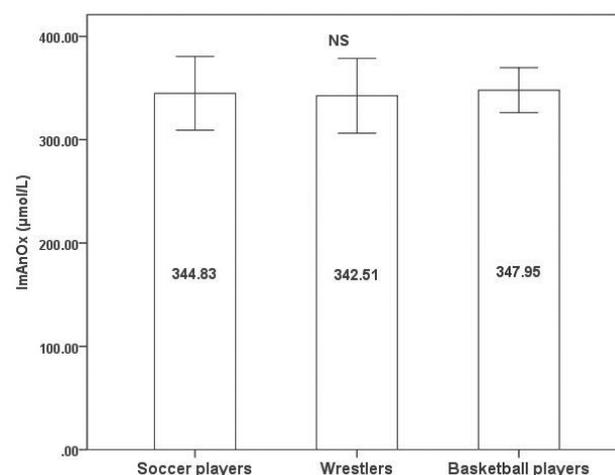


FIGURE 2. ImAnOx concentration in male elite athletes.

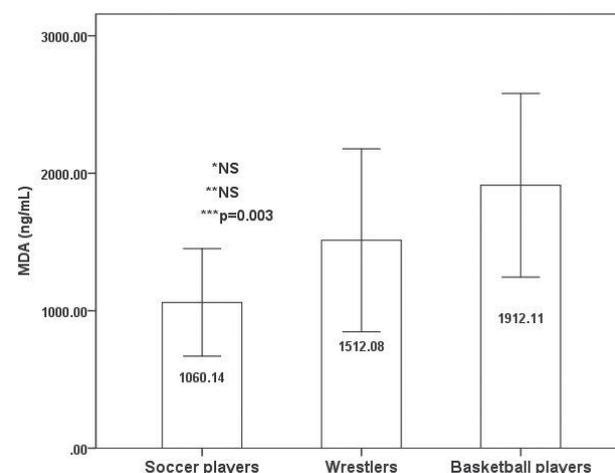


FIGURE 3. MDA concentration in male elite athletes.

* soccer players vs. wrestlers

**wrestlers vs. basketball players

*** soccer players vs. basketball players

TABLE 3. Correlation coefficients between age, antropometric parameters, duration of training and oxidant/antioxidant markers in wrestlers

	AOPP	ImAnOx	MDA
Age (y)	r=0.49	r=-0.1	r=-0.35
Weight (kg)	r=0.34	r=-0.04	r=-0.33
BMI (kg/m ²)	r=0.28	r=-0.19	r=-0.32
Duration of training (y)	r=0.33	r=0.0	r=-0.37

TABLE 4. Correlation coefficients between age, antropometric parameters, duration of training and oxidant/antioxidant markers in basketball players

	AOPP	ImAnOx	MDA
Age (y)	r=-0.19	r=-0.1	r=-0.03
Weight (kg)	r=-0.2	r=-0.3	r=-0.47
BMI (kg/m ²)	r=-0.35	r=-0.03	r=-0.1
Duration of training (y)	r=-0.15	r=0.4	r=0.58*

*p<0.05

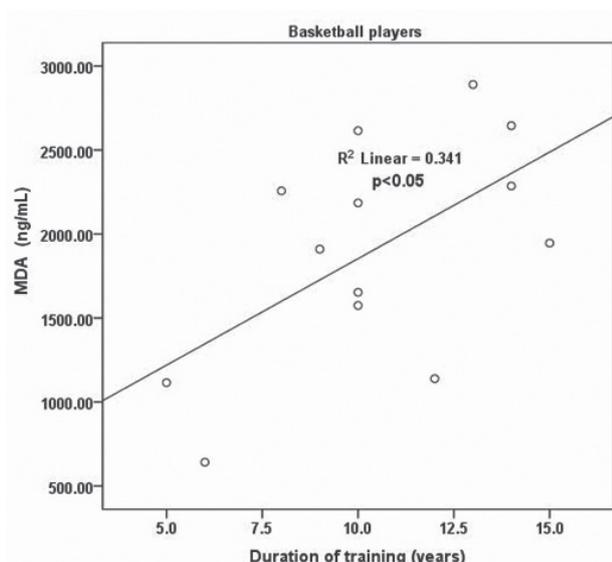


FIGURE 4. Relationship between duration of training and MDA concentration in basketball players.

TABLE 5. Correlation coefficients between AOPP, ImAnOx and MDA in male elite athletes.

	Soccer players	Wrestlers	Basketball players
AOPP			
ImAnOx	r=0.13	r=0.33	r=-0.12
MDA	r=0.04	r=0.18	r=-0.12

TABLE 6. AOPP, ImAnOx and MDA concentration in male athlete players with and without supplement

	AOPP	ImAnox	MDA	P value
Without supplement	74.6±29.1	348.0±29.3	1577.9±695.2	NS
With supplement	55.7±25.4	335.9±36.9	1292.2±591.1	NS

TABLE 7. Correlation coefficients between AOPP, ImAnOx and MDA in male elite athletes without supplement

	Soccer players	Wrestlers	Basketball players
AOPP			
ImAnOx	r=0.58	r=0.09	r=-0.22
MDA	r=0.12	r=0.39	r=-0.03

µmol/L in basketball players ($p=0.133$; NS)(Figure 2). Mean MDA concentration was significantly higher in basketball players (1912.1 ± 667.7 ng/mL) compared to soccer players (1060.1 ± 391.0 ng/mL)($p=0.003$). There was no significant difference in MDA concentration between wrestlers (1512.1 ± 666.1 ng/mL), soccer or basketball players (Figure 3). There was no significant correlation of AOPP, ImAnOx, MDA with age and anthropometric characteristic in soccer players, wrestlers and basketball players (Table 2, Table 3, Table 4). Duration of training was significantly positively associated with MDA levels in basketball players ($r=0.58$; $p<0.05$) (Table 4, Figure 4). There was no significant correlation between AOPP, ImAnOx and MDA levels in soccer players, wrestlers or basketball players (Table 5). There was no significant differences in AOPP, ImAnOx and MDA concentration in male athlete players with or without supplement (Table 6) and in male elite athletes without supplement in different sport disciplines (Table 7).

DISCUSSION

While ROS are constantly produced in small quantities within biological systems, their presence increases when exposed to both environmental and physical stressors [12]. Exercise is one such stressor. Simply stated, any situation in which the consumption of oxygen is increased, as during physical exercise, could result in an acute state of oxidative stress. In our study we evaluated oxidative stress status in elite athletes engaged in different sports disciplines including soccer, basketball and wrestling. We used AOPP (advanced oxidation protein products) and MDA (secondary product of lipid peroxidation) as markers of oxidative stress and ImAnOx as marker of total antioxidative capacity (TAC) which is defined as the sum of antioxidant activities of the nonspecific pool of antioxidants, consisting of antioxidant enzymes (GPX, catalase, superoxide dismutase), metal chelators, and nonspecific antioxidants (GSH, ascorbic acid, albumin, uric acid, tocopheroles, carotenoids, coenzyme-Q, bilirubin, and amino acids (cystein, methionine, tyrosine) [13]. The results of our study show that mean oxidative stress markers levels, both AOPP and MDA and total antioxidant capacity in elite athletes included in the study were increased compared to referral values provided by the manufacturer suggesting that physical activity leads to increased ROS fol-

lowed by increased antioxidant capacity in order to counter fight oxidative stress. Evidence for increased reactive oxygen and nitrogen species (RONS) production during and following exercise is provided by numerous investigations noting an increase in various oxidative stress biomarkers following both acute aerobic and anaerobic exercise [14]. In healthy males a period of intensified training elicited a biphasic TAC response, a significant increase after low- and high-volume training, and a decline after very-high-volume training [15]. The increase in TAC suggests that the body's antioxidant defense system is activated during exercise. Mobilization of antioxidant tissue stores may help to maintain the antioxidant status if needed [13]. The results in other researches [16, 17] where oxidative stress was assessed in elite soccer layers showed increased levels of oxidative stress but also an improved plasma antioxidant status together with more fluid erythrocyte membrane status, so they concluded that elevation in plasma activities of antioxidant enzymes and the higher levels of free radical scavengers of low molecular mass may compensate the oxidative stress caused by physical activity. Primary RONS generation in response to acute exercise can occur via several pathways. These include mitochondrial respiration (electron leakage from electron transport chain and subsequent production of the superoxide radical), prostanoic acid metabolism, the autooxidation of catecholamines, and oxidase enzymatic activity (NAD(P)H oxidase, xanthine oxidase) [18]. The initial increase in RONS during exercise, as well as following cessation of the work bout can lead to additional secondary generation of prooxidants via phagocytic respiratory burst, a loss of calcium homeostasis and/or the destruction of iron-containing proteins. Moreover, while the pathways listed above represent potential sources of RONS during exercise, specific RONS generation likely depends on the mode (aerobic, anaerobic), intensity, and duration of exercise, as varying types of exercise differ in their respective energy requirements, levels of oxygen consumption, and mechanical stresses imposed on the tissues [18]. Both acute aerobic and anaerobic exercise has the potential to result in increased free radical production, which may or may not result in acute oxidative stress [14]. In order for oxidative stress to occur, the ROS produced during exercise must exceed the antioxidant defense system present, thereby resulting in oxidative damage to specific biomolecules. Different exercise protocols may induce varying levels of RONS production, as oxidative damage has been shown to be both intensity and duration dependent [19, 20]. During low-intensity and duration protocols, antioxidant defenses appear sufficient to meet the RONS production, but as intensity and/or duration of exercise increases, these defenses are no longer adequate, potentially

resulting in oxidative damage to surrounding tissues [21]. Neubauer et al. [22] showed in their study that in well trained men, competitors in triathlon, increased levels of analyzed biomarkers of oxidative stress return to basal levels five days after competition and that there is also connection between training state, markers of oxidative stress and activity of antioxidant enzymes. Therefore, alternatives of antioxidant system defense in this trained population prevent appearance of long-term oxidative stress after intense exertion. The results of studies which determined oxidative stress biomarkers and antioxidant status in different sport disciplines showed mostly increased levels of oxidative stress markers but also more effective antioxidant protection. However, there are only few studies which compared the levels of oxidative stress markers among various sport disciplines. Our results showed no significant differences in the levels of oxidative stress status markers among various elite sports athletes. The difference in the mean concentrations of AOPP and ImAnOx between players of different sport disciplines was not significant. Mean MDA concentration was significantly higher in basketball players comparing to soccer players while there was no significant difference in MDA concentration between wrestlers, soccer or basketball players. These results are consistent with the results of Cubrilo et al. [23] who assessed oxidative stress and nitrite dynamics under maximal load in elite athletes in relation to sport type (aerobic, anaerobic and aerobic/anaerobic) measuring concentration of lactates, nitric oxide and thiobarbituric reactive substances (TBARS) as index of lipid peroxidation. Their results showed long term different training strategies establish different basal nitrites and lipid peroxidation levels in athletes which can be explained with different mechanisms of ROS induction by aerobic and anaerobic exercise. Nevertheless they found no statistically significant difference in oxidative stress parameters regardless of sport type although average concentrations (tests instructions proposed values) indicated high level of oxidative stress accompanied with increased antioxidative response in all groups. This can be explained with the fact that aero-anaerobic type of physical activity includes more mechanisms for production of oxidative stress. The results of a study by Stankovic et al. [24] suggested that increased production of RONS, as well as oxidative stress occurs in top-quality sportsmen under maximal physical exertion is independent of energetic requirement of sport type (aerobic, anaerobic, aero-anaerobic). The findings of Shi et al. [25] who in their study investigated the differences in oxidative stress caused by aerobic and anaerobic exercise due to different mechanisms suggest that similar workloads of anaerobic and aerobic exercise induce ROS differently: aerobic exercise seems to initially generate more ROS, whereas anaerobic exercise may in-

duce prolonged ROS generation. Although more oxygen was consumed during aerobic exercise, the generated ROS did not induce significant oxidative damage, so they concluded that oxygen consumption *per se* may not be the major cause of exercise-induced oxidative damage. The results of another study showed that aerobic training increased GPx activity in erythrocytes with a subsequent decrease in plasma TBARS (thiobarbituric reactive substances) levels but anaerobic training had no effect on this process [26]. Different impact on oxidative stress during acute and prolonged training was also shown by some studies. Pesic et al. [27] evaluated oxidative status in elite karate players during training session and their results showed that prolonged programmed physical exercise doesn't emphasize occurrence of oxidative stress unlike acute maximal physical exertion. Stankovic et al. [24] monitored changes of particular biomarkers of oxidative stress during taekwondo training and pilates training, where statistically significant increase of total antioxidant status was determined after taekwondo training, as well as catalase activity in plasma after pilates training. The authors suggested that sport athletes during longer period of exercise develop more effective antioxidant defence, respectively natural antioxidant defences of the body respond adequately to complex training program. When interpreting our results and the results from our study, it is important to consider other factors which impact the degree of antioxidant defences, including age, training status, and dietary intake [14]. If oxidative stress does occur, detection depends to a large degree on the tissue sampled, the timing of a given sample, as well as the sensitivity and specificity of the biomarker chosen [28]. In our study we chose two oxidative stress markers to analyse AOPP and MDA and found no difference in the average levels between different sport types. Oxidative stress has also been assessed by way of a variety of other miscellaneous markers. Assessment of lipid peroxidation, with MDA and TBARS are the most commonly used assays. The majority of authors have noted an increase in TBARS following a variety of exercise protocols, whereas null findings appear much more common when measuring MDA or isoprostanes specifically suggesting that TBARS lack of specificity of the assay which might explain possible discrepancies of the results [14]. From work over the past three decades, it is clear that exercise of sufficient volume, intensity, and duration can lead to an increase in RONS production, which may lead to the oxidation of several biological molecules (lipids, proteins, nucleic acids). Whether or not this condition is indicative of a harmful stimulus however, remains a topic of debate [29]. That is, due to the potential role of RONS in impairing exercise performance via altering contractile function and/or accelerating muscle damage/fatigue (secondary to the oxidation

of contractile and/or mitochondrial enzymes), coupled with their association with human disease [28], exercise-induced RONS have commonly been viewed as a detriment to physiological function. Hence, methods to reduce radical production and subsequent oxidative damage during and following physical exercise have been a priority of much research activity. While excessive prooxidant production, arising from any form of extreme aerobic or anaerobic exercise (i.e., marathon, aerobic/anaerobic overtraining) may have the potential to result in significant cellular disruption, there presently exist no "cause and effect" data to indicate that such an increase in RONS resulting from acute exercise actually causes ill-health and disease [14]. To the contrary, and in accordance with the principle of hormesis, a low grade oxidative stress appears necessary for various physiological adaptations [30]. Such a repeated exposure of the system to increased RONS production from chronic exercise training leads to an up-regulation in the body's antioxidant defense system and associated shift in redox balance in favor of a more reducing environment, thus providing adaptive protection from RONS during subsequent training sessions, as well as when exposed to non-exercise related conditions [14, 28]. Taken together, exercise-induced oxidative stress may operate in a similar fashion to all other principles of exercise science. That is, in order for an adaptation to occur (e.g., increased antioxidant defence, hypertrophy, strength), the physiological stimulus applied (in this case RONS production) must exceed a certain minimal threshold, effectively overloading the system. If overload is achieved, the physiological capacity of the body will expand or adapt; ultimately leading to improvements in health and/or human performance.

CONCLUSION

Type of sports (soccer, wrestler or basketball) have no impact on the levels of oxidative status markers. Elite sports engagement is a potent stimulus of oxidative stress that leads to the large recruitment of antioxidative defense implicating that oxidative stress status should be monitored. Consumption of antioxidants is recommended as a part of training regime.

DECLARATION OF INTEREST

The authors declare no conflict of interest for this study.

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