ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effect of combined antioxidant treatment on oxidative stress, muscle damage and sport performance in female basketball players

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SUMMARY

Introduction/Objective We determined the impact of antioxidant supplementation by GE132® on sports performance, oxidative stress markers, and muscle enzymes activities in professional female basketball players.

Methods Repetitive strength, explosive power, anaerobic endurance, and agility performance were measured before/after the 45-day supplementation period. The FORT (Free Oxygen Radicals Test) and FORD analysis (Free Oxygen Radical Defense) were assessed before/after basketball specific exercise bout, at the beginning/end of observational period. The grade of muscle damage was evaluated by aspartate aminotransferase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH).

Results After supplementation period, significant difference was not recorded regarding the basic motor skills tests. Basketball specific exercise bout induced significant increase in FORT ($p < 0.05$) only at the beginning of supplementation period. Both FORT and FORD significantly decreased over the observational period ($p < 0.001$, $p < 0.01$, respectively). CK and LDH were remarkably lower at the end of observational period ($p < 0.05$), compared to the baseline.

Conclusion Exogenous supplementation with protective nutraceuticals such as those found in GE132®, could reduce acute/chronic oxidative stress and muscle damage, but had no effect on sport performance in basketball players.

Keywords: antioxidants, oxidative stress; FORT; FORD; basketball; muscle damage

INTRODUCTION

Moderate physical activity, associated with a balanced diet, provides numerous health benefits. However, exhaustive and/or intense training sessions are associated with increased production of reactive oxygen species (ROS) and might lead to oxidative stress (OS) in skeletal muscles, blood, and perhaps other tissues [1, 2]. An increasing body of evidence implicates OS in the pathogenesis of numerous diseases, including diabetes, certain cancers, and cardiovascular disease. Importantly, exercise-induced OS might be associated with fatigue, muscle damage, and increased recovery time, which can all affect exercise performance [3–6].

The body contains an antioxidant defense system that depends on dietary intake of antioxidant vitamins and minerals and the endogenous production of antioxidant compounds such as antioxidant enzymes and numerous non-enzymatic antioxidants, involved in the quenching or removal of free radicals [7]. Physical training may enhance the antioxidant defense system to offset the barrage of ROS generated during exercise [8, 9]. However, the body's natural antioxidant defense system might not be sufficient to counteract the increase in ROS production during high-intensity or prolonged intermittent aerobic or anaerobic exercise [10, 11]. Additionally, a large number of athletes failed to ingest sufficient quantities of fruits and vegetables, which also suggest suboptimal intake of various antioxidants.

Previous research has demonstrated that antioxidants obtained through antioxidant rich diet or supplementation can reduce lipid peroxidation [12–15] and muscle damage [13, 16–19], indicating reduced OS. Although isolated studies reported potential ergogenic properties of antioxidants [20, 21, 22], overall conclusion is that there is limited evidence that dietary supplementation with antioxidants improves human performance.

We investigated the effect of proprietary nutraceutical blend GE132® on OS, muscle damage and sport performance in female basketball players. This blend contains several components with various biological effects including antioxidant properties. Each capsule contains 100 mg of *Ganoderma lucidum* extract (20%), 130 mg of royal jelly (26%), 80 mg of resveratrol (16%),

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30 mg of shark protein complex (6%), 80 mg of green tea extract (16%), and 80 mg of rosehip extract (16%). It was shown that these components have many therapeutic effects including anticarcinogenic and antihypertensive effects, immunomodulatory effect, rheumatism alleviation, and hepatic disease prevention, health benefits related to gastrointestinal disorders, metabolic diseases, and allergies [23–26].

The efficacy of this blend has never been tested before. Because of the great interest in using antioxidant nutrients as a preventive and therapeutic tool in clinical medicine and in physical activity, the aim of the present study was to determine the effects of this dietary supplement on OS, muscle damage and sport performance in female basketball players during competitive half season. It was hypothesized that athletes would demonstrate lower OS and muscle damage in response to exercise and training after supplementation period.

METHODS

Subjects

Fourteen senior female basketball players, who play in the first league club Red Star, Belgrade, Serbia, participated in this study. Athletes gave written consent after explanation of the purpose, demands, and possible risks associated with the study. The protocol was in accordance with the Declaration of Helsinki for Research on Human Subjects and it was approved by the Ethical Committee of Sport Medicine Association of Serbia. All participants passed sports medical examination and were eligible for participation in competitive sport. None of the subjects reported any serious injury or disease six months prior to or during the study. All subjects were non-smokers and did not use oral contraceptives, anti-inflammatory drugs, or dietary supplements (i.e. antioxidants) one month before and during the study. Subjects were instructed to restrain themselves from making any drastic changes in the diet. All of them had regular menstrual cycles and none of them were in the menstrual phase at the time of blood sampling.

Study design and supplementation

The study was conducted during a competitive half season, over the 45-day period. During this period, athletes were engaged in a controlled training program, and participation in the study did not have any effect on previously determined training and competition schedule. Subjects completed two basketball specific exercise bouts, at the beginning and at the end of the observational period. Supplementation started after the first exercise bout and continued for 45 days. Before and after each exercise bout, capillary blood samples were collected for OS measurement. Venous blood samples were collected at the beginning and at the end of observational period for muscle enzyme analysis. In addition, all players performed basic motor skills tests at the beginning and at the end of the study, in order to evaluate strength, endurance, and agility as the most commonly used motor skills in basketball.

All subjects received antioxidant complex supplement, GE132®, during 45 days. Athletes were told to comply with supplementation protocol and to take two capsules daily, one before lunch and the other before dinner. Capsules were counted upon return of the capsule bottles to assess compliance.

Procedures and measurements

Baseline measurements: Prior to enrolling in the study, all subjects completed a body composition assessment, standard blood chemistry screening, medical history, and physical activity questionnaire. Anthropometric and body composition characteristics were determined by using Seca height measuring instrument (Seca GmbH, Hamburg, Germany) and Tanita scale BC-418MA (Tanita Corp., Tokyo, Japan).

Basketball-specific exercise bout: Each exercise bout consisted of a general warm up and stretching (approx.10 min), technical-tactical training (approx. 30 min), heavy training, including training of counterattacks and simulated full- or half-court basketball games (approx. 40 min), and finally a cool-down phase (approx. 10 min). Each subject served as self-control to eliminate any biological variability in the response to antioxidant supplementation. The exercises were carried out under the same conditions, in the same place and time of the day to avoid circadian variations.

Basic motor skills tests

Strength: In order to evaluate repetitive strength, players performed push-ups and sit-ups to failure. Push-ups were done by placing hands just wider than shoulders. Subjects were told to keep their elbows fairly close to body and point them back and not to flare them out to the sides. They lowered until their chests were just above the floor, paused for a split second, and then pressed themselves back up. In order to do sit-ups, subjects were told to raise the torso from a supine to a sitting position and then lie back down again without moving the legs. Knees were bent at an angle of 90°and arms were held crossed behind the neck during the test. Only correct repetitions were taken into account.

Explosive strength was assessed by Globus Ergo Tester Platform. Squat jump (SJ), countermovement jump (CMJ), vertical jump (VJ), left leg (LL) and right leg (RL) jumps values were obtained. The subjects stood on the contact platform connected to a digital timer that recorded the flight time and height of all jumps. The timer was triggered by the release of the player's feet from the platform, and stopped at the moment of touchdown. SJ was performed from a starting position of 90° knee angle without allowing any counter movement. The subjects were told to jump as high as they can without performing a countermovement. The hands were held on the hips during the jump, thus avoiding any arm swing. During the CMJ, subjects were in the position with knees slightly bent and moved into a semi-squat position before jumping. Subject's hands also remained on the

hips throughout CMJ. One leg jumps (RL and LL) were performed in the same way as the CMJ. The only difference was that subjects were jumping from and landing on the same leg. Considering the VJ, the similar technique was used like in the CMJ, but subjects were able to perform the arm swing during the jump. Each player performed three jumps and the highest values achieved were recorded.

Endurance: Anaerobic endurance, as an important aspect of basketball, was evaluated by 300-yard shuttle test. Marker cones and lines were placed 25 yards apart to indicate the sprint distance. Stopwatch was used to record results of the test. Athletes were told to run as fast as they can to the opposite 25-yard line, touch it with their foot, turn and run back to the start. This was repeated six times without stopping (covering 300 yards total). After a 5-minute rest, the test was repeated. The average values of the two 300-yard shuttles were recorded.

Agility: Agility performance of basketball players was assessed by agility t-test. Four cones were set in the court (5 yards = 4.57 m, 10 yards = 9.14 m), and the subjects started at cone A. When the times sounded off, the subjects sprinted to cone B and touched the base of the cone with their right hand. Then, they turned left and shuffled sideways to cone C, also touching its base, this time with their left hand. The subjects were then shuffling sideways to the right to cone D, touching the base with the right hand. At last they shuffled back to cone B, touching it with the left hand, and ran backwards to cone A. The stopwatch was stopped as they passed the cone A. The test was performed three times and average value of all three attempts was taken into account.

Oxidative stress and biochemical measurements: In order to evaluate OS status, approximately 15 minutes before and 15 minutes after each basketball specific exercise bout, capillary blood samples were collected for FORT (free oxygen radicals test) and FORD (free oxygen radical defense) measurements. The free radical analysis system FORM PLUS 3000 (Callegari S.P.A., Parma, Italy), incorporating a spectrophotometric device reader, was used to measure these parameters. Test kits used with this instrument are highly reliable, rapid, and user-friendly for the global evaluation of the oxidative status (radical-induced damage index and the total antioxidant capacity) in the body from capillary blood.

FORT assay provides an indirect measurement of hydroperoxide, which are intermediate oxidative products of lipids, amino acids and peptides and therefore useful measure of OS. It is a colorimetric test based on the ability of transition metals, such as iron, to catalyze the breakdown of hydroperoxide into derivative radicals. These derivative radicals are then preferentially trapped by a suitably buffered chromogen: 4-Amino-N-ethyl-N-isopropylaniline hydrochloride and develop, in a linear kinetic based reaction at 37°C, a colored fairly long-lived radical cation spectrophotometrically detectable at 505 nm. The intensity of the color correlates directly with the quantity of radical compounds, which is related to the oxidative status of the sample [27].

FORD test provides an estimation of the overall antioxidant capacity of blood plasma. This test is based on the ability of antioxidants present in plasma to reduce a preformed radical cation. A stable colored cation (photometrically detectable at 505 nm) is formed in the presence of an acidic buffer (pH = 5.2) and an oxidant ($FeCl_3$). Antioxidant compounds present in the analyzed sample, reduce the radical cation of the chromogen, quenching the color and producing a discoloration of the solution, which is proportional to their amount in the sample [27].

To examine the extent of muscle damage, venous blood samples were collected from the antecubital vein of athletes in serum separator tube using vacutainer system (Greiner Bio-One, Kremsmünster, Austria). The serum separator tubes were placed on ice and left to stand for 30 minutes to facilitate clotting before being centrifuged at 3500 g for 15 minutes to obtain serum. Creatine kinase (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) were determined in a clinical laboratory using current bioassays based on methods by Johnson et al. [28]. LDH activity determination is based on the conversion of pyruvate to L-lactate by monitoring the nicotinamide adenine dinucleotide (NADH) oxidation. AST is assayed in a coupled reaction with malate dehydrogenase in the presence of NADH. In the determination of CK activity, the enzyme reacts with creatine phosphate and adenosine diphosphate to form adenosine triphosphate, which is coupled to the hexokinase/guanosine diphosphate reaction generating NADPH.

Statistical analysis

Statistical analyses were performed with the software IBM SPSS Statistic version 20.0 (IBM Corp., Armonk, NY). All data were assessed for normality (one-sample Kolmogorov–Smirnov test). FORT and FORD were analyzed using two-way analysis of variance (ANOVA) with repeated measures. Significant changes in muscle enzyme activities at rest, as well as values of basic motor skills test obtained at the beginning and at the end of the study were analyzed using paired sample t-tests. Data were expressed as mean ± SD. A p-value < 0.05 was considered statistically significant.

RESULTS

Descriptive characteristics of the basketball players are shown in Table 1. All subjects consumed the appropriate amount of product throughout the study period. None of the subjects reported adverse effects related to the dietary supplement.

Statistically significant difference was not recorded regarding the results of basic motor skills tests after 45-day supplementation period. The obtained results are shown in Table 2.

Basketball specific exercise bout induced significant increase in FORT at the beginning of observational period ($p < 0.05$), (Figure 1). However, these changes were not recorded after 45 days of supplementation. In addition, the FORT significantly decreased after 45-day supplementation period ($p < 0.001$). ANOVA repeated measures re-

vealed significant decrease in FORD over the observational period ($p < 0.01$).

We established the overall OS status of the athletes based on both the FORT and FORD results, according to the manufacturers' direction. Five major profiles: normal status NS, latent OS, compensated OS, at risk of OS and OS in progress – have been depicted [27]. The number of athletes with OS in progress was reduced from 71% (10/14 athletes) to 0% (0/14 athletes) and the number of athletes with NS (2/14 athletes) was increased from 14% to 86% (12/14 athletes) as a result of antioxidant supplementation.

The CK and LDH levels at rest, as indicators of muscle damage, significantly decreased after 45 days of supplementation ($p < 0.05$); while no changes were detected, regarding the AST levels (Table 4).

DISCUSSION

In the present study, female basketball players were supplemented with complex antioxidant supplement GE132® during 45 days. The study was performed just before the start of regular basketball season and after completion of basic conditioning training period. This period was chosen since preseason training is highly demanding for athletes because they are engaged in both frequent and high intensity workouts with little or no time to recover. This training program allows neuromuscular and endocrine systems to adapt after the loads placed to them and potential redox status adaptations occur [7, 9, 29, 30].

The major findings of this study indicate that:

1. single basketball training session can increase OS in trained females;
2. the antioxidant combination treatment with GE132® used in this study can significantly attenuate the rise of blood OS markers and muscle damage after basketball exercise and training;
3. GE132® supplementation does not provide benefit for enhancing motor skills of female basketball players.

Table 1. Characteristics of female basketball players

Variables	Mean ± SD
Age (years)	20.6 ± 2.7
Height (cm)	178.1 ± 7.8
Weight (kg)	72.9 ± 10.9
Body Mass Index (kg/m^2)	22.7 ± 2.2
Percent body fat (%)	20.7 ± 4.5
Years of training (years)	8.2 ± 2.7
Hours per week of training (h)	14.2 ± 4.8

Values are presented as mean ± SD

Table 2. Performance changes before and after supplementation period in female basketball players

Motor skills	Before supplementation	After supplementation	p
Push-ups (N)	31.5 ± 10.2	38.3 ± 10.9	< 0.05
Sit-ups (N)	95.5 ± 50.1	121.3 ± 50.4	n.s.
Squat jump (cm)	22.4 ± 2.9	23.4 ± 2.8	n.s.
Countermovement jump (cm)	27.5 ± 5.6	28.1 ± 4.3	n.s.
Vertical jump (cm)	36.1 ± 7.91	36.77 ± 6.55	n.s.
Right leg jump (cm)	14.7 ± 3.7	15.8 ± 2.8	n.s.
Left leg jump (cm)	15.2 ± 2.9	15.9 ± 2	n.s.
Anaerobic endurance (sec)	75.8 ± 5.7	74.1 ± 4.9	< 0.05
Agility (sec)	11.1 ± 0.5	10.9 ± 0.7	n.s.

Values are presented as mean ± standard deviation; N – number of repetitions; cm – centimeters; sec – seconds

Table 3. Muscle enzyme activities at rest before and after supplementation period in female basketball players

Enzyme levels	Before supplementation	After supplementation	p
AST (IU/L)	21.7 ± 3.6	19.5 ± 2.3	n.s.
CK (IU/L)	143.5 ± 35.6	108.7 ± 32.3	< 0.05
LDH (IU/L)	179.4 ± 14.4	164 ± 25.6	< 0.05

Values are presented as mean ± standard deviation; AST – aminotransferase; CK – creatine kinase; LDH – lactate dehydrogenase

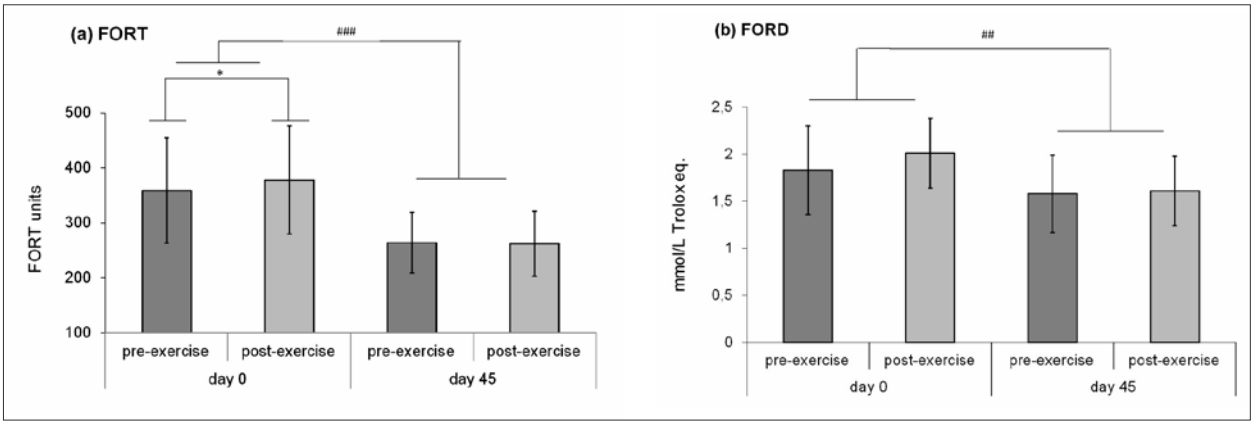

Figure 1. a) The levels of reactive oxygen species measured by the Free Oxygen Radicals test (FORT) before and after supplementation, in pre-exercise and post-exercise conditions; b) the capacity of plasmatic antioxidants Free Oxygen Radical Defense test (FORD), measured by FORD test before and after supplementation, in pre-exercise and post-exercise conditions; values are presented as mean ± standard deviation;

*p < 0.05;
##p < 0.01;
p < 0.001

Based on FORT assay measurement, we found that OS was significantly increased in response to basketball specific exercise bout before supplementation. Basketball is one of the mixed sports that include aerobic phases (intermittent running at different intensity) and anaerobic phases (jumps, sprints). Therefore, the increased free radical generation in basketball can occur via several pathways: mitochondrial respiration, oxidase enzymatic activity (NADPH oxidase, xanthine oxidase), via phagocytic respiratory burst, a loss of calcium homeostasis and/or the destruction of iron containing proteins [2, 31]. The increase in ROS production resulting from any of the above sources, could lead to oxidative changes of different biomolecules, and increased levels of OS. The antioxidant supplementation attenuated this increase after exercise bout at the end of study period, as evidenced by the non-significant changes in FORT levels. Additionally, the overall decreased FORT levels indicate less oxidative damage after 45 days of supplementation. The attenuated OS response is consistent with other studies using antioxidant supplementation [12, 15, 18, 32–36]. On the other hand, similar changes might occur as a result of adaptive response to chronic exercise [7, 9]. However, since present study was conducted after the conditioning preseason training, which allowed redox status adaptations, the reduced OS observed in female basketball players might be the result of antioxidant supplementation alone.

The antioxidant system capacity of plasma, measured by FORD test, depends on individual and synergic effects of different molecules, such as proteins, glutathione, vitamin E, ascorbate, carotenoids, and phenolic compounds. We detected no changes of FORD in response to exercise at the beginning of neither the observational period or at the end. Mobilization of tissue antioxidant stores into the plasma is an accepted phenomenon that would help maintain antioxidant status in plasma at certain level and protect body against ROS [37]. In addition, soluble plasma antioxidants work synergistically to defend against oxidant production, meaning that when one antioxidant nutrient is lacking at a particular period in time, another could substitute or it may be regenerated by another that is in abundance [38]. These rapid, dynamic responses in order to maintain redox homeostasis could be the reason for non-significant changes observed in FORD after the exercise. However, plasma levels of antioxidants, measured by FORD, decreased over the entire observational period in response to supplementation. This may not increase athlete's susceptibility to OS, since supplementation decreased oxidative modifications of various biomolecules, as indicated by FORT test. In addition, OS status of the female basketball players was improved after supplementation, judging by the increased number of athletes with NS and decreased number of athletes with OS in progress. Therefore, this antioxidant supplement may provide protection against the negative health consequences of free radicals produced during training.

Although the mechanisms behind exercise-induced muscle damage are not precisely known, it is believed that along with initial mechanically induced disruption, secondary damage is caused by the free radical production and subsequent OS [39]. Some markers, such as AST, CK, and LDH, have been used as a way to indicate the grade of muscle cell damage, especially after playing a sport, since microfiber breakdown releases cell content [5, 40]. The supplementation with antioxidants significantly reduced plasma muscle enzyme activities (CK and LDH), suggesting the involvement of oxidant mechanisms on tissue injury induced by the exercise. This finding can be explained by protective effect of antioxidants against lipid peroxidation, resulting in less muscle membrane damage. Our results are in accordance with several studies, which reported beneficial effects of antioxidants in terms of minimizing the rise in muscle enzyme activity in response to exercise [16–19, 41, 42].

There has been a general inconsistency of outcomes when investigating the role of antioxidant supplementation in exercise performance, with the majority of the studies reporting no benefits. In accordance, in the present study no statistically significant difference was observed regarding repetitive or explosive strength, endurance, or agility performance after supplementation period in comparison to baseline.

The limitations of the study include the small number of subjects and the short duration of supplementation. However, particular strength of this study is the fact it was conducted during a regular competitive half season, reflecting habitual conditions of nutrition and training program. In addition, this is the first study examining the effects of nutraceutical blend GE132®.

CONCLUSION

Previous studies showed that professional athletes are exposed to increased OS over the periods of intensive training. Exercise-induced OS might be associated with fatigue, muscle damage, and increased recovery time that can all affect exercise performance. For that reason, reliable and quick tests for OS status measurements, such as FORD and FORT assays, might be very useful for training and supplementation planning. Exogenous supplementation with protective nutraceuticals such as those found in GE132®, could reduce acute and chronic OS during high intensity efforts, and provide beneficial effect on muscle function recovery. The results of the present study suggest that GE132® supplementation does not enhance performance of female basketball players, but rather provide protection against detrimental health consequences of ROS produced during training.

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Утицај комбиноване суплементације антиоксидансима на оксидативни стрес, оштећење мишића и спортску способност код кошаркашица

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САЖЕТАК

Увод Утврдили смо утицај комбинације антиоксиданата *GE132*® на спортску способност, маркере оксидативног стреса и активности мишићних ензима код професионалних кошаркашица.

Циљ истраживања је да се испита поузданост и валидност *OHIP*-14 код црногорског становништва старости 65 и више година и да утврди утицај оралног здравља на квалитет њиховог живота.

Методе Поновљена снага, експлозивна снага, анаеробна издржљивост и агилност су мерени пре/после 45-дневног периода суплементације. *FORT* (тест слободних кисеоничних радикала) и *FORD* (тест антиоксидативне заштите) процењени су пре/после специфичног кошаркашког тренинга на почетку/крају периода суплементације. Степен оштећења мишића је процењен мерењем активности аспартат-аминотрансферазе, креатин-киназе и лактат-дехидрогеназе у серуму.

Резултати После суплементације није забележена значајна разлика у резултатима тестова моторичких способности у односу на период пре суплементације. Кошаркашки специфични тренинг је изазвао значајно повећање *FORT*-а ($p < 0,05$) само на почетку периода суплементације. И *FORT* и *FORD* су значајно опали током посматраног периода ($p < 0,001$, $p < 0,01$). Креатин-киназа и лактат-дехидрогеназа су биле значајно ниже на крају периода посматрања ($p < 0,05$) у поређењу са вредностима пре суплементације.

Закључак Суплементација са заштитним нутритивним препаратима, као што су они у *GE132*®, може смањити акутни/хронични оксидативни стрес и оштећење мишића, али није показан утицај на спортске перформансе кошаркашица.

Кључне речи: антиоксиданси, оксидативни стрес; *FORT*; *FORD*; кошарка; оштећење мишића