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Effect of Zinc Supplementation on Antioxidant Activity in Young Wrestlers

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Abstract This study aims to examine the effect of zinc supplementation on free-radical formation and antioxidant system in individuals who are actively engaged in wrestling as a sport. The study registered a total of 40 male subjects, of whom 20 were wrestlers and 20 were sedentary individuals. The subjects were equally allocated to four groups: group 1, zinc-supplemented sportsmen group; group 2, sportsmen group without supplementation; group 3, zinc-supplemented sedentary group; group 4, sedentary group without supplementation. Blood samples were collected from all subjects twice, once at the beginning of the study and once again at the end of 8-week procedures. The blood samples collected were analyzed to determine the levels of malondialdehyde (MDA), serum glutathione (GSH), serum glutathione peroxidase (GPx) activity, serum superoxide dismutase (SOD) activity (ELISA colorimetric method) and zinc (colorimetric method). No difference was found between MDA levels of the study groups in the beginning of the study. The highest MDA value at the end of the study was obtained in group 4 ($p<0.01$). MDA levels in group 2 were established to be significantly higher than those in groups 1 and 3 ($p<0.01$). GSH level, GPx, and SOD activities and zinc level measured in the beginning of the study were not different between groups. Measurements performed at the end of the study showed that groups 1

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and 3 (zinc-supplemented groups) had the highest GSH level, GPx, and SOD activities and zinc level ($p<0.01$). These parameters were not different in the groups without supplementation (groups 2 and 4). Results obtained at the end of the study indicate that zinc supplementation prevents production of free radicals by activating the antioxidant system. In conclusion, physiologic doses of zinc supplementation to athletes may beneficially contribute to their health and performance.

Keywords Zinc · Exercise · Free radicals · Antioxidant activity

Introduction

Free radicals are highly reactive molecules which destroy cell membranes and damage DNA. They are formed during processes of the cellular metabolism and are generally detoxified by the body's antioxidant defense system. Consequently, oxidative stress is defined as an imbalance between free-radical production and antioxidant defense, which results in cellular injury [1]. When compared to resting conditions, intense physical activity can bring about a ten to 15 times increase in the body's need for oxygen. Increased mitochondrial oxygen consumption and electron transport induces oxidative stress, which causes reactive oxygen species (ROS) formation and lipid peroxidation [2]. Free-radical production in physical activity can increase through ways other than the increase in the need for oxygen: Severe exercise may make active muscles hypoxic; concentration of catecholamine rises during exercise, which in turn may result in the auto-oxidation of ROS. Hyperthermia caused by exercise may also cause oxidative damage; and lastly, exercise may increase the auto-oxidation of oxyhemoglobin to methemoglobin, which may culminate in superoxide production [3]. Additionally, there is a growing body of evidence suggesting that cytotoxic reactive oxygen species may lead to muscle disorders associated with exercise, like muscle exhaustion or muscle injury [4].

Discovery of the protective role of zinc against free-radical formation and oxidative stress [5] has triggered studies into the antioxidant effect of zinc and its involvement in the antioxidant defense system [6]. Zinc is found in the structure of superoxide dismutase, an enzyme effective in the antioxidant system, and metallothioneins, which protect tissues against the harmful effects of free radicals [7]. These effects of zinc on the antioxidant system, as revealed in studies, have led researchers to question the relations between athlete health and performance, and zinc. The importance of zinc intake through diet in athletes has been emphasized, and it has been argued that zinc deficiency in the diet may unfavorably influence not only performance, but also cellular immune system, enhancing the predisposition to infections [8, 9]. It was shown that zinc deficiency increases free-radical formation and lipid peroxidation during exercise, negatively affecting antioxidant activity [10]. Thus, the report to the effect that zinc supplementation inhibited the formation of reactive oxygen radicals in exercise [11] is not only very important in terms of the relationship between zinc and the antioxidant system, but also demonstrates that zinc is closely related with athlete's health and performance.

The present study aimed to examine how zinc supplementation to individuals actively engaged in wrestling as a sport affects free-radical formation and antioxidant system.

Materials and Methods

Study Groups

The study registered a total of 40 subjects in the same age group, of whom 20 were young male wrestlers (who have been engaged in sports for about 6 to 7 years) and 20 sedentary males. The study protocol was approved by the ethics committee of Selcuk University School of Physical Education and Sports.

Subjects were equally allocated to four groups:

Group 1. Zinc-supplemented sportsmen group ($n=10$): subjects in group 1 were involved in an exercise program for 2 months (4 to 6 days/week) and were also supplemented with oral zinc sulfate (5 mg/kg/day) for 8 weeks.

Group 2. Sportsmen group without supplementation ($n=10$): subjects in group 2 were involved in an exercise program for 2 months (4 to 6 days/week)

Group 3. Zinc-supplemented sedentary group ($n=10$): The subjects in group 3 were supplemented with oral zinc sulfate (5 mg/kg/day) for 2 months (8 weeks).

Group 4. Sedentary group without supplementation ($n=10$): the subjects in this control group were not subjected to either exercise or zinc supplementation.

Preparation of Zinc Sulfate ($ZnSO_4$) Preparations

Zinc sulfate preparations were concocted as 100, 150, 200, 250, 300, and 350 mg capsules in the Tekno-Med laboratory (Konya).

Measurement of Weight and Height

Weight was measured using scales with 0.1 kg precision and a metal rod on the scales, while height was measured using a height measurer with 0.01 cm?

Collection of Blood Samples from Subjects

Blood samples were collected from the forearm veins of the subjects using a 5-cc injector twice, once in the beginning of the study and once at the end of the 8-week procedures (at 9 a.m. after fasting). The samples were centrifuged at 3,000 rpm for 10 min to separate sera and then they were stored at −80°C until analysis.

Biochemical Analyses

Serum MDA Analysis

Malondialdehyde (MDA) analyses were conducted using Cayman trademark (catalog no: 705002) commercial kits according to enzyme-linked immunosorbent assay (ELISA) colorimetric method. The results were determined as nanomole per milliliter.

Serum Glutathione Analysis

Serum glutathione (GSH) analyses were carried out using Cayman trademark (catalog no: 7003002) commercial kits in compliance with ELISA colorimetric method. The results were established as micromole per milliliter.

Serum Glutathione Peroxidase Analyses

Serum glutathione peroxidase (GPx) was analyzed using a Cayman trademark (catalog no: 703102) commercial kit in accordance with ELISA colorimetric method. The results were expressed as nanomole per milliliter per minute

Serum Superoxide Dismutase Analyses

Serum superoxide dismutase (SOD) analyses were conducted using a Cayman trademark (catalog no: 706002) commercial kit according to ELISA colorimetric method. The results were established as units per milliliter.

Serum Zinc Measurements

Serum zinc was measured using a 5×10 ml packaged Spinreact trademark kit in accordance with colorimetric method. Zinc analyses were carried out in BPC trademark Prime model spectrophotometer. The results were presented as micrograms per deciliter.

Exercise Program

The sportsman group (n=20) regularly trained in their sports club for 2 months. All 20 athletes followed the same exercise program. The training sessions were conducted under the supervision of trainers. Wrestling and technical training were of moderate (ranging between 60 and 80%) intensity, while strength training was of high (ranging between 70 and 100%) intensity.

Statistical Evaluations

Statistical analysis of data was performed using SPSS 10.3 package software programmer. In the statistical analysis of findings, paired t test was employed in the dual comparison of samples. In addition, the same software was used to calculated mean age, height and weight, and standard deviations for all groups. Variance analysis was utilized in multivariate comparisons. Duncan test was applied to determine the degree of importance of data, which were found to differ in the variance analysis. The results were presented as mean±SD, and $p<0.05$ was accepted as the level of statistical significance.

Results

Subjects constituting study groups were not different in terms of age, height, and body weight (Table 1).

Table 1 Physical Features of Study Groups

Groups (n=10)	Age	Height (cm)	Body weight (kg)
1 Zinc-supplemented sportsmen group	16.20±0.13	174,00±0,01	69.50±3.95
2 Sportsmen group without supplementation	15.80±0.24	169.50±0,02	65.50±4.66
3 Zinc-supplemented sedentary group	17.00±0.25	173.90±0.02	64.80±5.98
4 Sedentary group without supplementation	17.00±0.25	172.50±0.02	64.80±5.98

There was no significant difference between serum MDA levels of study groups in the beginning of the study. At the end of the study, the highest MDA value was found in the sedentary group (group 4; $p<0.01$). Serum MDA level in the sportsman group without supplementation (group 2) was significantly higher than those in zinc-supplemented sportsmen (group 1) and zinc-supplemented sedentary group (group 3; $p<0.01$). MDA levels obtained in the groups which were not supplemented with zinc (groups 1 and 3) did not differ at the end of the study (Table 2).

Although serum GSH level, GPx, and SOD activity levels measured in the groups in the beginning of the study were not different, these levels in zinc-supplemented groups (groups 1 and 3) were found to be significantly higher than those in non-supplemented groups (groups 2 and 4) at the end of the study ($p<0.01$, Tables 3, 4, and 5).

Serum zinc levels of the groups are presented in Table 6. Accordingly, there was no significant difference between serum zinc levels of groups in the beginning of the study. However, at the end of the study, groups which were supplemented with zinc for 8 weeks (groups 1 and 3) had higher serum zinc levels than the groups which were not supplemented with zinc (groups 2 and 4; $p<0.01$).

Discussion

No significant difference was found between age, height, and weight of groups in the study. The similarity between physical features of the subjects is important in that it enables a well-grounded discussion of the results obtained in the study.

Serum MDA levels measured in the zinc-supplemented sportsmen group (group 1) after 8 weeks of supplementation were found significantly lower, in comparison to the levels of before supplementation ($p<0.05$). Demonstration of the protective role of zinc, which plays in the protection against free-radical formation and oxidative stress [5], has triggered studies exploring the antioxidant effect of zinc and its involvement in the antioxidant defense system [6]. Zinc is found in the structure of superoxide dismutase, which is an enzyme

Table 2 Serum MDA Levels of Study Groups (nmol/ml)

Groups (n=10)	MDA (before study)	MDA (after study)
1 Zinc-supplemented sportsmen group	0.60±0.05	$0.25±0.01^{c}$
2 Sportsmen group without supplementation	0.55±0.05	$0.35±0.06^{b}$
3 Zinc-supplemented sedentary group	0.58±0.06	$0.27±0.03^{c}$
4 Sedentary group without supplementation	0.58±0.07	$0.55±0.07^{a}$

Means with different superscripted letters in the same column indicate statistical significance ($p<0.01$)

Table 3 Serum GSH Levels of Study Groups (μmol/ml)

Groups (n=10)	GSH (before study)	GSH (after study)
1 Zinc-supplemented sportsmen group	16.56±1.44	33.64±3.19[a]
2 Sportsmen group without supplementation	17.50±3.19	21.00±3.81[b]
3 Zinc-supplemented sedentary group	17.37±1.79	39.62±2.83[a]
4 Sedentary group without supplementation	17.18±5.98	21.35±3.30[b]

Means with different superscripted letters in the same column indicate statistical significance (p<0.01)

effective in the antioxidant system, and metallothioneins, which protect tissues against the harmful effects of free radicals [7]. Zinc deficiency was shown to increase free-radical formation and lipid peroxidation during physical activity, thus negatively affecting the antioxidant activity [10]. Additionally, the report indicating that zinc supplementation in exercise prevented the formation of reactive oxygen radicals [11] not only points to the relation of zinc with the antioxidant system, but also shows that zinc is closely associated with athlete health and performance. In our study, oral zinc supplementation to athletes for 8 weeks significantly reduced serum MDA levels. Oxidative stress induced by cadmium in rat testes was shown to increase in zinc deficiency [12]. In another study, it was reported that only dietary zinc deficiency, per se, elevated the oxidative stress in rat testes [13]. MDA production in the plasma, and liver and pancreas tissues significantly increased in rats fed on a zinc-deficient diet, while zinc supplementation to the same rats significantly suppressed the increased MDA levels [14]. Another study demonstrated that acute exercise increased plasma MDA levels in rats subjected to acute swimming exercise, and that MDA levels displayed a further increase in zinc deficiency, whereas zinc supplementation suppressed MDA levels [10]. This result is in harmony with the lower MDA levels we found in the zinc-supplemented sportsman group (group 1) in this study.

Serum GSH level, GPx, and SOD activity levels, as measured at the end of the 8-week supplementation period in the zinc-supplemented sportsman group, were found significantly elevated compared with the levels before supplementation. This indicates that 8-week zinc supplementation activates antioxidant system in athletes. It was reported that zinc deficiency in rats lessened the SOD activity in the blood and liver, while 3-week zinc supplementation to the same rats significantly stepped up the SOD activity which was previously curtailed [15]. Another study showed that subsided GSH levels in the brain tissues of rats exposed to an electromagnetic field increased with zinc supplementation, which is an impressive example of the effect of zinc on the antioxidant system [6]. The glutathione-dependent antioxidant system, consisting of reduced glutathione (GSH) and an array of functionally related enzymes, plays a fundamental role in cellular defense against

Table 4 Serum GPx Levels of Study Groups (nmol/ml/minutes)

Groups (n=10)	GPx (before study)	GPx (after study)
1 Zinc-supplemented sportsmen group	1136±37.61	1648±78.74[a]
2 Sportsmen group without supplementation	1174±47.23	1250±82.83[b]
3 Zinc-supplemented sedentary group	1088±53.41	1516±75.27[a]
4 Sedentary group without supplementation	1161±87.31	1245±87.55[b]

Means with different superscripted letters in the same column indicate statistical significance (p<0.01)

Table 5 Serum SOD Levels of Study Groups (U/ml)

Groups (n=10)	SOD (before study)	SOD (after study)
1 Zinc-supplemented sportsmen group	0.19±0.01	0.47±0.03[a]
2 Sportsmen group without supplementation	0.21±0.03	0.24±0.07[b]
3 Zinc-supplemented sedentary group	0.22±0.03	0.46±0.05[a]
4 Sedentary group without supplementation	0.22±0.03	0.23±0.03[b]

Means with different superscripted letters in the same column indicate statistical significance (p<0.01)

reactive free radicals and other oxidant species [16], of these enzymes, glutathione peroxidase (GPx) is a selenoprotein, which reduces lipidic or nonlipidic hydroperoxides as well as H_2O_2 while oxidizing glutathione. Reports indicating that GSH levels that decreased in rats subjected to acute swimming exercise showed a further decline in zinc deficiency, while zinc supplementation to the same rats significantly elevated GSH levels [10] lend substantial support to elevated GSH, GPx, and SOD values we obtained with zinc supplementation in humans.

When compared to the values measured at the beginning of the study, 8-week zinc supplementation culminated in an increase in serum zinc levels of the sportsman group (group 1). Increased zinc levels we found in group 1 can be considered a natural consequence of the 8-week supplementation.

Serum MDA levels measured in the zinc-supplemented sedentary group (group 3) at the end of the 8-week supplementation period were significantly lower than those measured before supplementation (p<0.01). Serum GSH level, GPx, and SOD activity levels and zinc levels measured after supplementation, however, were found significantly elevated, relative to the levels measured before supplementation (p<0.01). These values we obtained in the sedentary group are similar to those we found in the zinc-supplemented sportsman group (group 1) and discussed above. Decreased MDA and increased GSH, GPx, and SOD values we found in the zinc-supplemented sedentary group have dual significance: First, these values obtained not only in the sportsman, but also sedentary group, indicate that zinc prevents free-radical formation by activating the antioxidant system. Secondly, these values found after zinc supplementation are independent of exercise, since the same values were obtained in both the sportsman and sedentary groups.

Pre- and post-supplementation serum MDA, GSH, GPx, SOD, and zinc levels in the non-supplemented sportsmen group were not different. Regular muscle exercises, which are known to have various beneficial effects, lead to an increase in the production of radicals and other reactive oxygen species. There is evidence suggesting that reactive oxygen species are the underlying cause of muscle homeostasis disorders which are associated with exercise and which may result in muscle tiredness or injury [17]. Increased MDA and

Table 6 Serum zinc Levels of Study Groups (μg/dl)

Groups (n=10)	Zinc (before study)	Zinc (after study)
1 Zinc-supplemented sportsmen group	88.75±12.55	155.00±21.00[a]
2 Sportsmen group without supplementation	91.50±11.20	95.00±19.00[b]
3 Zinc-supplemented sedentary group	89.60±13.45	160.00±20.00[a]
4 Sedentary group without supplementation	87.55±15.30	90.00±22.00[b]

Means with different superscripted letters in the same column indicate statistical significance (p<0.01)

creatine kinase findings were obtained in half-marathon runners after exercise. This is a finding indicating incompetence of the antioxidant defense mechanism in half-marathon runners [18]. Likewise, another study demonstrated that lipid peroxidation increased and GSH levels showed a parallel increase in exercised rats [19]. This result suggests that exercise causes an increase in free-radical production on the one hand, and activates the antioxidant system and counteracts this negative effect on the other. As a matter of fact, some researchers showed that antioxidant system was activated in children who were engaged in regular swimming exercises [20]. It was also demonstrated that 8 weeks of treadmill run attenuated the exhaustive exercise induced increase in MDA level in erythrocytes and liver tissue [21, 22].

In our study, no difference could be found between pre- and post-supplementation MDA, GSH, GPx, and SOD values in the non-supplemented sportsman group. This lack of difference may have two reasons: first, collection of blood samples during rest may have caused this lack. Secondly, subjects included in the study have been engaged in wrestling sport for about 6 years, which may have produced an adaptation of the antioxidant system, and consequently, duration of engagement in sports may be another factor.

Pre- and post-supplementation serum MDA, GSH, GPx, SOD, and zinc levels in the non-supplemented sedentary group (group 4) were not different. This lack of difference in the sedentary group before and after the study is an indicator that the study was conducted properly and is important for a sound discussion of data obtained from other study groups.

In conclusion, supplementation of physiological doses of zinc during exercise activates the antioxidant system by increasing SOD and GPx activities and also GSH levels. Supplementation of zinc to athletes, particularly in periods of intense exercise, may uphold the antioxidant system and contribute to their performance. In the light of the results obtained from the present study, it is suggested that zinc supplementation to athletes in physiologic doses may be beneficial to their health and performance.

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