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Research Paper

Combined and Separated Supplementation of Zinc and Selenium Change Lipid Peroxidation but Not Hematological Parameters and Performance of Road Cyclists

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Abstract

Introduction: Many of athletes look for supplementation of trace element in order to improve their performance. we aimed to compare the effect of combined and separated supplementation of zinc and selenium on the serum level of Malondialdehyde, several hematological parameters, and also the performance of the road cyclists after exhausting exercise. Materials and methods: Thirty-two road cyclists from Tehran Traffic Cycling Team were assigned into four groups of zinc supplement (Zn), selenium supplement (Se), zinc-selenium supplement (Zn-Se), and placebo (P). In order to assess the basic values of the participants' zinc, selenium, hematocrit (Hct), hemoglobin (Hb), and red blood cell count (RBC), blood samples were retrieved from each participant before supplementation. In addition, blood samples were collected before and after exhausting and their exhaustion time was recorded. After four weeks of supplementation, blood samples were collected again from the participants before and after performing exhausting exercise. Results: No significant difference was observed between groups in terms of exhausting exercise and supplementation on time to exhaustion and hematological parameters ($P > 0.05$). Resting MDA level was lower in Zn-Se group compared to placebo ($P < 0.05$). Conclusion: Four weeks of combined and separated supplementation of zinc and selenium did not lead to a significant change in the hematological parameters (Hb, Hct, and RBC) after exhausting exercise. It seems that when athletes do not show any deficiencies in Zinc and Selenium, supplementation will not be beneficial in order to improve their time to exhaustion.

Keywords: Zinc, Selenium, Hematological, Hemoglobin, Bike riding, Exhausting exercise, Hematocrit

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Introduction

Minerals and trace elements are inorganic micronutrients found in a variety of plant and animal foods [1]. Insufficient trace element intake has been linked to a number of health conditions, such as diabetes, cardiovascular and kidney disease, aging and fracture risk [1-3]. These micronutrients play a role in myriads of biological processes relevant to exercise and athletic performance, including energy storage/utilization, protein metabolism, inflammation, oxygen transport, cardiac rhythms, bone metabolism and immune function [4, 5]. Zinc and selenium microelements play important role in more than 300 metabolic reactions of the body [6, 7]. It has been shown that both zinc and selenium microelements have potential to increase antioxidant defense system and decrease byproducts of oxidative stress such as Malondialdehyde (MDA) [8-10].

The critical role of sustained energy production for the athletes in order to continue activity and postpone the time to exhaustion and paying attention to the athletes' health at the meantime, would be a potential challenge. Many studies have indicated that blood composition changes as a result of exercise. Vigorous exercise causes many metabolic changes in the body, including hematological disorders, iron deficiency, anemia, and development of oxidative stress and lipid peroxidation which particularly occur in endurance athletes [11, 12]. Hemolysis and iron released from transferrin due to increased oxidative stress decline athletes' performance [13]. Moreover, the exercise-induced anemia may occurr as a result of oxidative damage to red blood cells. The positive and significant relationship between hemoglobin concentration, RBCs, hematocrit, and maximum oxygen consumption ($Vo2_{Max}$) has been well demonstrated, which can determine the aerobic capacity of athletes and their performance [14]. Kelkar et al. (2008) examined the combined effect of antioxidants on oxidative stress, hematological parameters, and performance among Indian athletes, and concluded that supplementation with antioxidants led to a decrease in oxidative stress and improvement of the runners' hematological status and performance [15]. Furthermore, some studies have shown that zinc and selenium supplementation can be helpful to the antioxidant defense system [16, 17]. One of the possible paths through which zinc and selenium supplementation can affect hematological profile is their antioxidant properties.

In situations of high metabolic demand, such as exercise or athletic training, insufficient circulating and cellular trace elements may impair optimal physiological performance and may require supplementation [18]. The exact impact of these deficiencies or supplementations on athletic performance remains generally unclear [19]. However, there may be ergogenic properties of trace elements in achieving or possibly surpassing the Recommended Dietary Allowance (RDA), that are specifically designed for the general population health. Due to side effects of increasing consumption amounts of zinc and selenium, the amount of zinc and selenium supplements used in this study was according to RDA for supplementation.

In this study, we first revealed no deficiency in serum level of zinc and selenium among elite road cyclists. Then, we aimed to see the effect of supplementation of zinc and selenium on several hematological parameters, time to exhaustion, and MDA level in the absence of deficiency. By using RDA for supplementation of these trace element, then we tested if there is any synergic effect by combining zinc and selenium on above-mentioned parameters.

Materials and Methods

Participants

Forty-seven cyclists from Tehran Cycling Club provided their written consent, and completed health worksheets indicating their health and lack of specific diseases. After the worksheets were examined, 32 male cyclists were selected and assigned into four groups (n=8) including zinc supplement, selenium supplement, zinc-selenium supplement, and placebo. All participants were asked not to consume any vitamin supplements, minerals, or amino acid supplements containing vitamins and minerals during study as well as two weeks prior the study. Body composition and aerobic power of each participant were measured one week before the study. Afterwards, fasting blood samples were collected from the participants 24 hours after their last exercise session in order to measure the basic values of zinc and selenium before the supplementation period. It is also worthwhile to mention that participants were members of same club, hence, they had been training under same exercise plan over period of experiment. All procedures were approved by Ethics Committee of Faculty of Medicine at university of Tehran.

Supplementation

Participants in the zinc supplement group consumed one 30-mg zinc capsule (zinc gluconate) on a daily basis. The participants in the selenium supplement group took a 200-μg selenium capsule (selenium selenite) every day, and those in the zinc-selenium supplement group a 30-mg and a 200-μg selenium capsule on a daily basis. In addition, the placebo group a similar capsule of the same shape and size (250 mg) as the other groups every day. Four weeks after supplementation and before conduction of the exhausting program, blood samples were obtained from the participants in order to assess the factors of interest.

Food Analysis

In order to assess the participants' diet and level of their daily zinc and selenium intake, their food frequency and 24-hour recall were taken. The collected data were analyzed using ESHA Food Processor (Food Processor Version 11.x), and the daily amounts of each nutrient including zinc and selenium were determined in the participants' diet. Afterwards, those amounts were compared with the RDA related to the mentioned elements, and no deficiency was revealed (information is provided in table ٣ in the appendix).

Exercise capacity Test

Each participant carried out two types of exercise program: 1) The program related to measure aerobic power [20] and 2) The program of exhausting exercise [21]. In order to measure the aerobic power, Monark bicycle (made in Sweden) confirmed by Physical Fitness Assessment Center, was used by the National Olympic Academy of Iran. The program was carried through the following procedure: First, participants warmed up themselves with 110 watts for 10 minutes. Afterwards, 35 watts were added to the resistance every four minutes, and one minute of break was considered between each extra weight stage and the previous one. This process continued until the participants could not tolerate the last extra resistance for four minutes and the speed of pedaling dropped below 75 rpm. Afterwards, the data recorded during the test were used to calculate the aerobic power based on following equation:

$$Wmax = wf + (T/240) \times 35$$

Where wf is the resistance that was preserved for complete four minutes before the test, T is the time of tolerating the last resistance in seconds, and 35 is the difference between the resistances.

To perform exhausting protocol, 50%, 70%, 80%, and 90% of WMax of each participant were calculated and the protocol was carried out as following: First, the participants were required to warm themselves up using 50% of their maximum aerobic power. Afterwards, they were asked to pedal using 90% and 50% of their aerobic power in 2-minute alternates until they could not keep the 90% of their aerobic power for completing the two minutes. After this step, participants were asked to pedal using 80% and 50% of their aerobic power in 2-minute alternates until they could not preserve 80% of their aerobic power for complete two minutes and at this point, they continued pedaling using 70% of their aerobic power, which continued until they could not preserve 70% of their aerobic power for complete two minutes.

Data Collection

Six ml of blood were taken from the participants' brachiocephalic vein. Serum and plasma of each participant were separated using centrifuge and kept in -20oc for further analysis. Sysmex cell counter (model mxs800i) was used in order to measure Hb, Hct, and RBC. Serum level of MDA was measured using ELISA kit (Zellbio, Germany).

Statistical Analysis

One-way ANOVA was used to determine the difference between the participants' individual characteristics and time to exhaustion. Two-way ANOVA was used to analyze the study's variables, and whenever the main effects of the group and the interactions (time x group) were significant, Bonferroni post-hoc test was used for pair comparisons. Due to heterogeneity of the variances, nonparametric Kruskal-Wallis test was used to analyze the level of hemoglobin (Hb). The level of statistical significance was set at $p < 0.05$. SPSS 21.0 software was used to analyze the results.

Results

Table 1 presents the participants' individual characteristics. There was no significant difference between the supplements and placebo groups in terms of their individual characteristics.

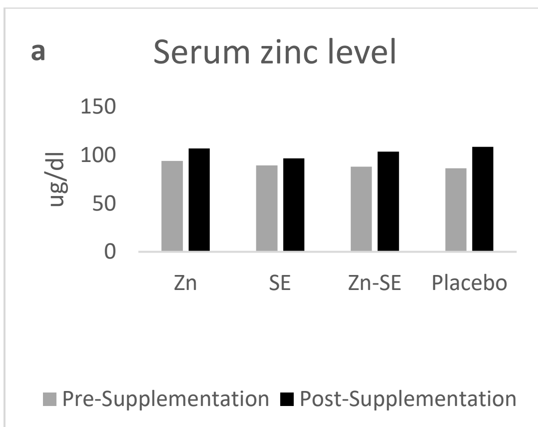
Table 1- The Participants' Individual Characteristics (The Values are Based on Mean and Standard Deviation)

Indices	Zn group	Se group	Zn-Se group	Placebo	P value
Height (CM)	176.87±8.2	177.75±4.23	174.81±0.75	176.87±8.02	0.824
Weight (Kg)	68.11±8.66	61.66±4.75	63.17±8.45	66.15±6.4	0.299
BMI (kg/m^2)	21.75±2.5	20.5±1.26	21.57±0.5	21.12±0.98	0.528
Fat Percentage (%)	13.54±3.09	11.56±1.63	15.42±2.98	11.49±1.76	0.1
Vo2max (ml/kg*min)	57.28±8.49	59.93±7.41	56.27±5.57	56.61±4.87	0.696
Aerobic power (watt)	299.88±30.17	323.06±31.99	296.12±41.54	291.5±44.56	0.359

Before supplementation, no deficiency was revealed in terms of zinc and selenium level after comparison to borderlines (table2). As for supplementation and exhausting exercise, no significant changes were observed in serum level of zinc and selenium after exhausting exercise (figure1 a,b).

Table 2- Study variables before supplementation. Values are based on mean and standard deviation.

Variable	Zn group	Se group	Zn-Se group	Placebo	Borderline
Serum Zinc (μg/dl)	80.73±1.14	81.83±2.14	79.5±5.9	87.41±8.89	75-125
Serum Selenium (μg/dl)	89.55±4.21	87.62±7.07	90.88±7.09	100.37±6.71	46-143
RBC	5.49±0.08	5.33±0.27	5.49±0.23	5.46±0.40	4.5-6.2
Hb (mg/dl)	16.28±0.30	15.96±0.53	16.58±0.96	16.03±0.45	13-16.5
Hematocrit (%)	47.32±0.87	47.32±1.13	48.38±0.96	48.10±0.96	38-50



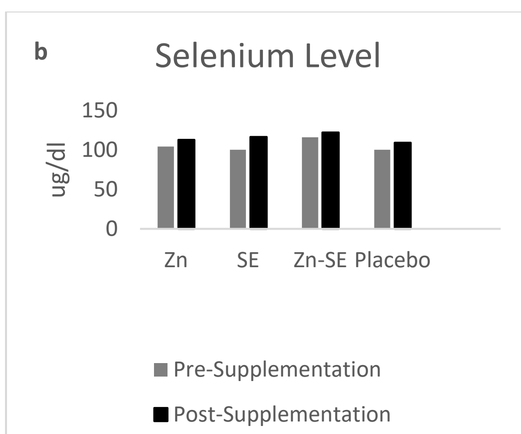


Figure 1- Zinc and selenium level in serum of participants, before and after supplementation.

Hematological Parameters

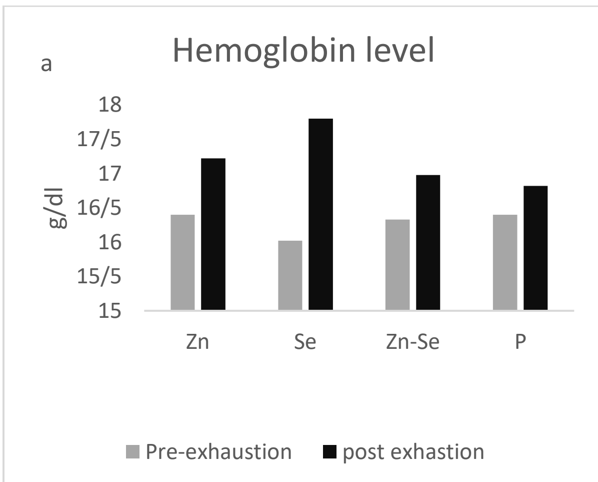
Analysis of Hb, Hct, and RBC showed an increase in all groups under the effect of exhausting exercise with no significant difference between groups. Moreover, supplementation in all groups did not lead to any significant effect on rest values of hematological parameters (Hb, Hct, and RBC) (Figure2.a-c).

Malondialdehyde

MDA level in serum increased after exhausting exercise and no significant difference was observed between groups. As for supplementation, significant difference ($p = 0.023$) was observed in resting value (prior to exhausting exercise) between Zn-SE and placebo group (figure3).

Time to Exhaustion

Results showed that there was no significant difference ($p=0.589$) between groups before and after supplementation (figure4).



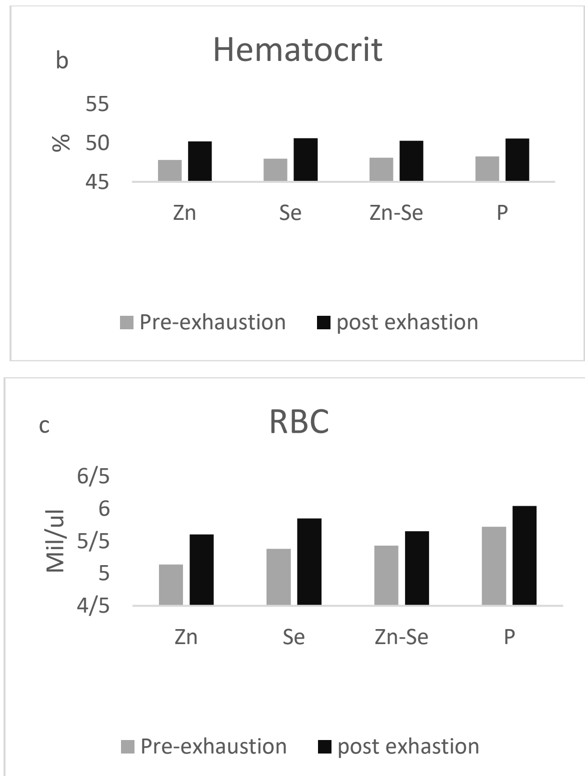


Figure 2- four weeks supplementation did not lead to significant changes in hematological parameters.

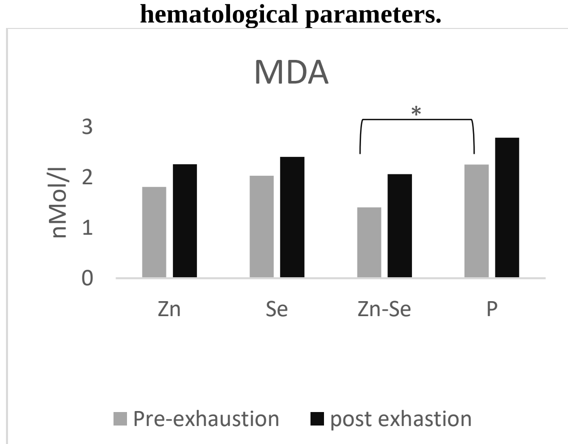


Figure 3- Resting MDA level changed after combined supplementation of zinc and selenium.

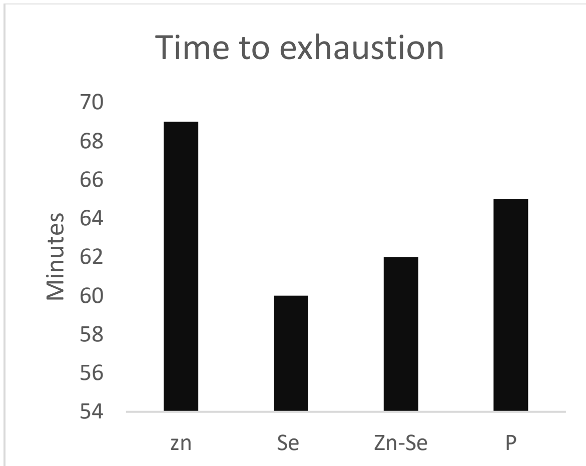


Figure 4-time to exhaustion did not lead to significant changes after supplementation.

Discussion

The results of the present study showed that Hb, Hct, and RBC increased as a result of exhausting exercise; however, four weeks of combined and separate supplementation of zinc and selenium did not cause a significant difference in study's variables between groups, except for resting value of MDA in zn-se group. Moreover, the results showed that four weeks of combined and separate supplementation of zinc and selenium did not lead to a significant difference in the time to exhaustion in each of the supplementation groups.

Exhausting exercise led to increase hematocrit and hemoglobin in this study. There is no general agreement on exercise-induced increase in number of erythrocytes. It is argued that increase in number of erythrocytes and other blood factors during exercise efforts can be the result of mechanisms of increased blood concentration. For example, after exercise, a decline in plasma level increases the concentration of red blood cells, which enhances blood concentration and the ability to transfer oxygen to the tissues.

The study conducted by Baltace et al. (2003) [22], demonstrated an improvement in hematological profile. The contradiction between our results and the one reported by Baltace can be attributed to the difference in the type and level of the participants and the dosage of the supplements; since the amount of zinc consumed in the study of Baltaci was much higher than the allowed level. Moreover, we used supplementation of zinc and selenium in situation which there was no sign of zinc and selenium deficiency as well as iron deficiency and anemia.

Hence, supplementation in case there is no deficiency in the athletes, could not improve hematological parameters related to cycling performance.

There are some studies that have shown long-term endurance training results to decrease in zinc and selenium level [23, 24]. However, we found no deficiency in road cyclists participating in our study. In the light of our findings, Baltaci et al. stated that zinc levels of child sportsmen were not affected by training [25]; Mogulkoc et al. reported that training did not affect zinc levels in sportsmen [26]. Since many endurance athletes keep trying to increase their hemoglobin and hematocrit level in order to increase oxygen delivery and eventually performance, it is not surprising that time to exhaustion as an index of endurance performance did not change in the absence of hematological improvement. Exhaustion/fatigue has very complicated mechanisms, and it cannot be attributed to a certain factor. Some studies have reported the positive effect of antioxidant supplements on time to exhaustion [27, 28], and since antioxidant properties of zinc and selenium have been shown in many studies [16, 17, 29, 30], it is not irrational to expect that these supplements affect the cyclists' time exhaustion. However, it should be noticed that in those studies examined the positive effects of such supplements on time to exhaustion, they used participants who were not at a high level of performance. Among these participants, changes in time to exhaustion and performance occur much faster than trained individual.

Conclusion

Our findings demonstrated that four weeks combined and separated supplementation of zinc and selenium did not affect performance and hematological parameters; however, a significant reduction in resting MDA level was observed between Zn-Se and placebo group. In terms of lack of deficiency, supplementation of Zinc and selenium by RDA dosage could not lead to remarkable improvement in performance and hematological parameters as well as oxidative stress. Hence, athletes in this situation should not consider these elements with RDA dosage as an ergogenic aid in order to improve their performance. So, for better understanding of these trace elements on athletic performance in lack of deficiency, it is required to measure more parameters regarding performance and oxidative stress. Also, it remains unclear if athletes can take an advantage of supplementation by using amounts of supplement beyond RDA.

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Appendix

Table 3- Dietary analysis of participants by Dorosty food processor. Data are provided by mean and standard deviation.

Nutrients	Consumed	RDA (mg)	Consumed/RDA percentage
Energy (Kcal)	3466 ± 405.27	3018.52	115.41 ± 12.28
Protein (mg)	83.16 ± 24.09	54.94	152.86 ± 44.02
Carbohydrate (mg)	440.05 ± 106.5	420.5	104.67 ± 25.3
Dietary Fiber (mg)	0.47 ± 0.238	290	0.24 ± 0.015
MUFA (mg)	20.05 ± 11.91	72.5	27.65 ± 16.43
PUFA (mg)	48.15 ± 17.9	72.5	66.42 ± 24.69
Total Fat (mg)	121.83 ± 28.95	217.5	56.02 ± 13.31
Cholesterol	54.11 ± 31.5	165	14.93 ± 8.96
Vitamin A (mcg)	1228.87 ± 368.76	1000	123.11 ± 3701
Vitamin B1 (mg)	1.79 ± 0.234	1.2	119.59 ± 15.6
Vitamin B6 (mg)	1.56 ± 3.03	1.3	79 ± 151.4
Folate (mcg)	94.98 ± 25.32	200	197.49 ± 12.16
Vitamin B12 (mcg)	1.75 ± 0.98	2.4	87.67 ± 49.21
Vitamin C (mg)	60.86 ± 17.79	60	101.43 ± 29.66
Vitamin E (mg)	14.95 ± 12.97	10	329.47 ± 129.7
Iron (mg)	19.4 ± 3.78	12	161.97 ± 37.8
Copper (mg)	870 ± 0.305	900	96.67 ± 20.34
Sodium (mg)	1419.81 ± 436.28	701	202.32 ± 62.16
Potassium (mg)	1950.68 ± 426.74	1200	99.39 ± 21.6
Magnesium (mg)	368.62 ± 39.08	400	92.61 ± 11.17
Calcium (mg)	1788.62 ± 143.02	1200	149.05 ± 11.72
Phosphorus (mg)	1122.25 ± 211.81	1200	93.5 ± 17.65
Selenium (mcg)	64.45 ± 19.03	50	128.9 ± 27.19
Zinc (mg)	16.34 ± 1.23	15	108.93 ± 8.24