Effect of vitamin A administration on free radicals and lactate levels in individuals exercised to exhaustion

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Abstract: This study was performed to explore the effect of vitamin A administration on Free Radicals production and antioxidant system activity and lactate levels in individuals exercised to exhaustion The study registered 10 healthy sedentary males their mean age was 22,85±0,26 years. The subjects were orally administrated with 300 mg vitamin A (retinol) for 4 weeks and engaged in strenuous exercise (using the Bruce protocol) once a week. Blood samples were collected from the subjects at four different times, before and after the supplementation and before and after exercise to analyze Malondialdehyde (MDA), Nitric oxide (NO), Glutathione (GSH), Glutathione peroxidase (GSH-Px), Catalase (CAT), Superoxide dismutase (SOD) levels using colorimetric ELISA test kits and plasma lactate levels using an autoanalyzer. Exhaustion exercise leaded to an increase in both MDA, NO, and lactate, and GSH, GSH-Px, CAT and SOD levels compared to resting levels both before and after supplementation ($p<0.05$). Increased NO levels found in pre-supplementation exhaustion showed a significant decrease after the supplementation of vitamin A ($p<0.05$), but the other parameters were not changed after vitamin A administration. The results of our study demonstrate that the increase caused by 4-week strenuous exercise in the levels of the free radical NO was offset by vitamin A supplementation. It can be suggested that supplementation of vitamin A at physiological doses has a limited effect on lipid peroxidation caused by strenuous exercise.

Keywords: Vitamin A, exercise, free radicals, antioxidant activity, lactate.

INTRODUCTION

Physical performance is enhanced by exercise and regular nutrition. Athletes need mineral and vitamin support for their health and athletic performance (Rodriguez *et al.,* 2009a; Rodriguez *et al.,* 2009b). Vitamins are regulators of metabolic functions. The theory underlying vitamin use is based upon the specific metabolic function of each vitamin in relation to sports (Williams, 1989). Research shows that vitamin deficiency lowers performance (Williams, 1989; Kujala *et al.,* 2003). It has been mentioned water-soluble vitamins are important for maintaining performance and protecting weight in athletes engaged in weight-loss sports (Rodriguez *et al.,* 2009a; Rodriguez *et al.,* 2009b). The importance of vitamin and mineral supplementation is emphasized to achieve better performance by delaying fatigue in athletes (Sobal and Marquart, 1994). Therefore, there has been a growing interest in research about the relation between exercise and vitamins supplementation. One of the most commonly supplemented vitamins is vitamin A (Briefel *et al.,* 2006). The muscle injury-preventing effect of vitamin A may be critical in eliminating the free radical products that arise particularly in exercise (Stacewicz-Sapuntzakis, 1997). Actually, Ross (1999) noted that vitamin A supplementation might be effective in preventing lipid peroxidation which caused muscle injury. However, there is no solid evidence showing that vitamin A has a role in enhancing performance or preventing lipid peroxidation. The present study performed to investigate the effects of vitamin A administration on free radical production and antioxidant defence system and lactate levels in individuals who performed strenuous exercise.

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MATERIALS AND METHODS

Total 10 healthy male students participated to this study from at the School of Physical Education and Sports of Selcuk University, but not actively engaged in any specific branch of sports. The mean age of the subjects was 22,85±0,26 years and their mean weight was 69,74±2,09 kg.

The subjects were supplemented with 300 mg vitamin A (retinol) daily for 4 weeks and subjected to exhaustion exercise once a week. The study protocol was approved by local ethic committee.

Vitamin A supplementation

The subjects were given 300 mg vitamin A (retinol) in the form of a tablet through the oral route at 10.00 after the breakfast for 4 weeks.

Exhaustion exercise (Bruce protocol)

Exercise procedures were carried out once a week for 4 weeks in the form of exhaustion exercise. Bruce protocol which is the most commonly used clinical exercise test where the incline and speed are increased in 3-minute intervals (on Cosmed T150 trademark treadmill) was applied up to the point where the subjects could no longer continue.

Collection of blood samples from the subjects

Blood samples were collected from the forearm vena of the subjects at 09.00 a.m. (on an empty stomach) for a total of four times before and after the supplementation and during rest and exhaustion. The samples collected as such were centrifuged at 3000 rpm for 10 minutes to separate the serum, which was then stored at -80^{o}C until the time of analysis.

Biochemical analyses

Serum MDA analysis

MDA analyses were conducted using Cayman brand (catalogue no: 705002) commercial kits. The results were presented as nmol/ml.

Serum GSH analysis

GSH analyses were carried out using Cayman brand (catalogue no: 7003002) commercial kits. The results were expressed as mg/dl.

Serum Glutathione Peroxidase (GPx) analysis

GPx was analyzed using Cayman brand (catalogue no: 703102) commercial kits following. The results were established as nmol/ml.

Serum Superoxide Dismutase (SOD) analysis

SOD analysis was performed using Cayman brand (catalogue no: 706002) commercial kits. The results were presented as U/ml.

Serum Nitric Oxide (NO) analysis

Nitric oxide was analyzed using Cayman brand (catalogue no: 780001) commercial assay kits. The results were expressed as μM.

Serum Catalase analysis

Catalase analysis was carried out using Cayman brand (catalogue no: 707002) commercial. The results were indicated as nmol/ml.

Plasma Lactate analysis

Plasma lactate was analyzed in blood samples taken from the earlobes into heparinized capillary tubes using a lactate analyzer (VARIO Photometer, Germany). The results were established as mmol/l.

Statistical evaluations

SPSS 16.0 computer software package was used in the statistical evaluation of results. Repeated Measures Variance Analysis was employed to identify the differences between measurement times. Differences for which $p<0.05$ was accepted significant.

RESULTS

MDA, NO, GSH, GSH-Px, CAT, SOD, and lactate levels measured during exhaustion both in the pre- and post-supplementation periods were found higher than the levels measured during rest ($p<0.05$, table 1, fig. 1 and fig. 2). NO levels which were elevated in exhaustion before supplementation of vitamin A were observed to fall significantly after supplementation ($p<0.05$), while other parameters remained unaffected by vitamin A supplementation (table 1, fig. 1 and fig. 2).

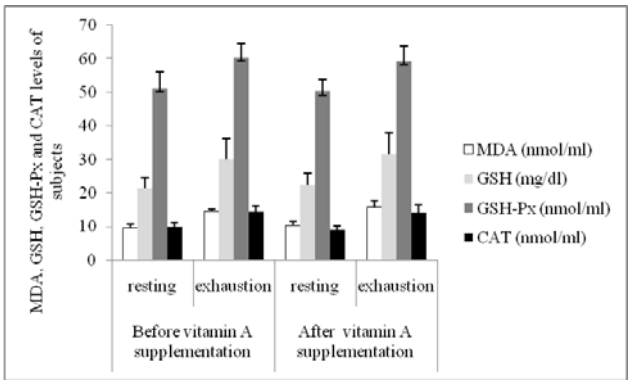

Fig. 1: MDA, GSH, GSH-Px and CAT levels of subjects

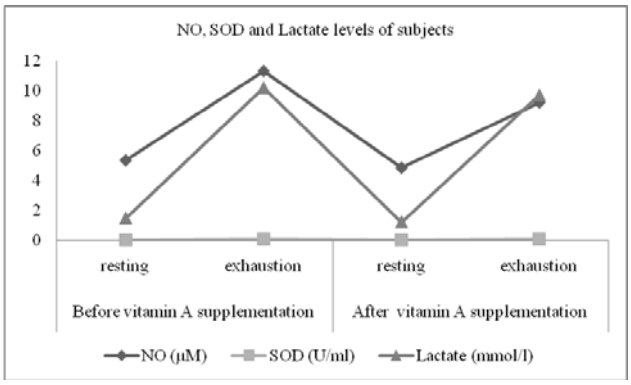

Fig. 2: NO, SOD and Lactate levels of subjects

DISCUSSION

In the present study, exhaustion MDA values before and after vitamin supplementation were found significantly higher than the resting MDA values. Physical activity causes an increase in free radical products and leads to tissue damage. Oxidative stress that develops parallel to increased oxygen consumption during exercise is considered the main reason for increased free radical production (Aslan, 1997). Elevated MDA values found in exhaustion result from acute exercise and are consistent with the relevant literature reports (Aslan, 1997). Especially athletes who compete in weight classes and perform weight-loss exercises need water- and fat-soluble vitamin supplementation (Rodriguez *et al.,* 2009a; Rodriguez *et al.,* 2009b). In the same vein, it is claimed that vitamin A may be critical in not only athlete health and performance, but also in the prevention of oxidative stress (Lane *et al.,* 2008). The results of our study related to MDA parameter indicate that physical activity caused an increase in MDA, but vitamin A supplementation failed to reduce the elevated MDA levels.

Table 1: Biochemical parameters of study subjects

Antioxidant Parameters	Before vitamin A supplementation		After vitamin A supplementation	
	resting	exhaustion	resting	exhaustion
MDA (nmol/ml)	9.70 ± 0.96^{B}	14.64 ± 0.66^{A}	10.28 ± 1.19^{B}	15.80 ± 1.78^{A}
NO (µM)	5.34 ± 1.95^{C}	11.30 ± 3.76^{A}	4.84 ± 1.33^{C}	9.20 ± 2.57^{B}
GSH (mg/dl)	21.28 ± 3.29^{B}	29.98 ± 6.18^{A}	22.42 ± 3.43^{B}	31.58 ± 6.47^{A}
GSH-Px (nmol/ml)	51.20 ± 4.88^{B}	60.22 ± 4.22^{A}	50.28 ± 3.60^{B}	59.28 ± 4.47^{A}
SOD (U/ml)	0.04 ± 0.01^{B}	0.07 ± 0.01^{A}	0.04 ± 0.01^{B}	0.08 ± 0.01^{A}
CAT(nmol/ml)	9.84 ± 1.33^{B}	14.30 ± 1.92^{A}	8.94 ± 1.39^{B}	14.24 ± 2.17^{A}
Lactate (mmol/l)	1.48 ± 0.40^{B}	10.20 ± 1.23^{A}	1.22 ± 0.29^{B}	9.71 ± 1.08^{A}

A,B,C: Differences between means with different superscripted letters in the same line are statistically significant ($p<0.05$). A>B>C

Nitric oxide values measured during exhaustion both before and after vitamin A supplementation were significantly higher than the resting nitric oxide values. NO is a lipophilic free radical gas and a hetrodiatomic molecule with a low molecular weight. It can react rapidly with the oxygen in the medium and be converted to toxic nitrogen dioxide (NO_2) (Tschakovsky and Joyner, 2008). When NO reacts with superoxide, peroxynitrite ($ONOO^-$) is formed and the reaction between peroxynitrite and tyrosine results in the formation of 3-nitrotyrosine (Drew and Leeuwenburgh, 2002). In the present study, we could not find any study to compare our results regarding the relation between nitric oxide and exercise. However, that nitric oxide (NO) was shown to regulate vessel tonus and to cause vasodilation after exercise (Gilligan *et al.,* 1994) can explain the increased nitric oxide levels we found after exercise. Besides, the report that exercise produces a significant stimulator effect for nitric oxide production by increasing blood flow (Shen *et al.,* 1995) is again an important piece of information that supports our NO findings.

Four-week vitamin A supplementation in the present study significantly reduced exhaustion NO values in comparison to the pre-supplementation exhaustion values. This result is remarkable, as although low concentrations of nitric oxide can contribute to physiological processes such as controlling the vascular tonus, neurotransmission, learning and memory (Sezer *et al.,* 2004), high NO concentrations can rapidly react with the oxygen in the medium and be converted to toxic nitrogen dioxide (NO_2) (Tschakovsky and Joyner, 2008). It is interesting that vitamin A supplementation significantly reduced exhaustion NO levels relative to pre-supplementation values. This result may highlight the importance of supplementation of the concerned vitamin in the nutrition and performance of athletes.

In this study, exhaustion GSH, GPx, SOD, and CAT values before and after vitamin supplementation were found significantly higher than resting GSH, GPx, SOD, and CAT values. Antioxidant levels are known to vary depending on the intensity, type and duration of exercise (Fıçıcılar *et al.,* 2003). However, it was reported that SOD and catalase increased following acute exercise (Banerjee *et al.,* 2003). In general, exercise can enhance the antioxidant defense. Previous studies established elevated erythrocyte GSH, catalase and glutathione reductase activities after exercise (Clarkson and Thompson, 2000). An experimental animal study demonstrated that the increase in free radical formation as a result of exercise was too high and the increase in antioxidant system was not able to offset the free radical production (Fıçıcılar *et al.,* 2003). The increase we found in the antioxidant parameters during exhaustion before supplementation is consistent with the reports cited above. This elevation in antioxidant parameters during exhaustion exercise is probably a compensation mechanism intended to hinder the increased free radical production. Our study shows that regular physical activity activates the antioxidant system. As it was reported, regular exercise and training stimulates the biosynthesis of antioxidant enzymes (Viña *et al.,* 2000). Therefore, regular exercise is accepted to be a significant adaptation mechanism for reducing the oxidative stress that is enhanced after exercise (Brites *et al.,* 1999). This condition can be explained by the successful adaptation of the athletes' antioxidant defense systems. It was found that plasma ascorbic acid, alpha-tocopherol, uric acid, and total GSH levels of female elite footballers who trained regularly increased after a 40-minute football match. This increase in the antioxidant defense can be explained by the endogen antioxidants' and/or antioxidants that have accumulated in the tissues going into circulation as a result of exercise (Andersson *et al.,* 2010). All these reports lend support to the elevated antioxidant parameters we found after exercise.

Exhaustion lactate levels we found before and after vitamin A supplementation in this study were significantly higher than resting lactate levels. As before, four-week vitamin A supplementation did not alter the resting lactate values.

CONCLUSION

An overall evaluation of the study results reveals that exhaustion exercise causes an increase in free radical production in healthy individuals. Enhanced antioxidant activity in individuals engaged in an exhaustion exercise fails to prevent free radical production. In the same vein,

the study results demonstrate that the increase in the free radical NO levels caused by 4-week exhaustion exercise can be prevented by vitamin A supplementation. It can be concluded that vitamin A supplementation at physiological doses can have a limited effect on lipid peroxidation resulting from exhaustion exercise.

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