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The effect of vitamin A and E supplements on the plasma melatonin levels of smoking sportspeople

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Abstract. The aim of this study is to determine the effect of smoking on the melatonin levels in sportspeople and the regulatory role of vitamin A and E supplements on this effect. 14 voluntary people who do sports actively participated in the study. Methods, sportspeople were separated into two groups: sportspeople who had been smoking at least 10 cigarettes a day for the last year and non-smoking sportspeople. Two groups were given a two-hour exercise program which would be implemented three times a week for two weeks. An hour before the exercises, 200 mg of oral vitamin A and E supplements were given. Melatonin level measurements were done before, right after and two hours after the exercises, and measurements of serum vitamin A and E levels were done at the beginning and end of the exercise program. Inter-group variables and independent variables were compared via t test, and intra-group variables were compared via paired t test. Results.The melatonin and vitamin A and E levels of the smoking sportspeople prior to the exercises were found to be less than non-smoking sportspeople; however, no statistically significant difference was observed between these levels ($p>0.05$). Nevertheless, melatonin levels which increased right after the exercises were found to decrease to the pre-exercise levels two hours later. Moreover, post-exercise melatonin levels of the smoking sportspeople were determined to significantly increase compared to the non-smoking sportspeople, especially after the second week, not the first week ($p<0.01$). Inter-group comparison after the exercise program and the comparison of the first and final vitamin A and E levels were observed to be significantly different ($p<0,05$; $p<0.01$). As a result, While smoking was determined to have a decreasing effect on the melatonin and vitamin A and E levels, the antioxidant agent supplements such as vitamin A and E were found to be protective regarding the oxidative effects of smoking and to increase the melatonin levels in sportspeople.

Key Words: *smoking, exercise, melatonin, vitamin A and E.*

Introduction

Human beings repeat their physiological and bio-chemical behaviors 24 hours a day. Day-night cycle is the primary environmental factor affecting the circadian rhythm in humans. The suprachiasmatic core in the hypothalamus works as a circadian clock. Melatonin receptors in the suprachiasmatic core indicate to the importance of melatonin in the control of circadian rhythm (1,2).
Melatonin lowers the blood pressure, vascular tonus and norepinephrine levels. This lowering effect of melatonin on blood pressure is thought to be related to sympatic nerve system inhibition and postsynaptic $\alpha_1$adrenergic receptor blockage (3). Although there are different experimental results, differences may be observed between exercise and melatonin level depending on the duration of exposure to light and the time of the exercises during the day (4). Moreover, vitamin A, E and C and non-enzymatic antioxidants such as Selenium (Se) take the high-energy electrons in the free oxygen radicals (FOR) into their structures, and help decreasing the oxidative damage caused by FOR, while antioxidant compounds destroy the oxygen radicals.
Antioxidant compounds melatonin, vitamin A, E and C and Se have quite an important role in this defense (5,6).
Melatonin is also one of the most powerful free radical receivers. It is a very effective hydroxyl (OH) radical receiver. This antioxidant effect enables melatonin to react with toxic OH radicals, and to protect the biomolecules in all the cell compartments against oxidative damage with its high lipophilic characteristic.
Hazardous radicals in the smoke disrupt the oxidant-antioxidant balance and increase oxidative damage to the cell and tissues; and destruct the cell membranes and cause inefficient physiological functions, thus affecting the plasma lipid profile and antxoidant exyme levels (7).

On the other hand, maintaining the balance between the formation and destruction of the oxidants in biologic systems is important in maintaining the biological integrity of the cell and tissue. There are several antioxidants in cells against the hazardous effects of free radicals resulting from smoking. Many antioxidants in the body such as vitamin A and E work as defense systems against the damages caused by free radicals (8).

As a result, smoking may increase lipid peroxidation reactions by causing oxidative damage in the lung tissue in sportspeople. Therefore, this study examines the effect of smoking on the melatonin levels in sportspeople and the regulatory role of Vitamin A and E supplements on this effect.

Material and Method

This study was conducted in accordance with the Declaration of Helsinki. The risks and aim of the study were explained to the participants, and their written consents were sought. Study group consisted of 14 voluntary male amateur basketball players at the age of 19–24 who had been playing basketball for 4 years and whose weights and heights varied between 65–85 kilograms and 1.75–1.92 meters, respectively. Sportspeople were separated into two groups: sportspeople who had been smoking at least 10 cigarettes a day for the last year and non-smoking sportspeople.

Both groups were given a two-hour exercise program which would be implemented three times a week for two weeks. The exercise program was prepared on a systematic basis specifically for the groups in order to obtain the most appropriate physiologic effects. The program included the type, degree and frequency of the exercises. Exercise program consisted of three parts: warm-up, exercise and cooling-down.

The sportspeople were informed of the possible side effects of vitamin A and E. Each sportspeople was given a vitamin A and E praparates of 200 mg one hour before the exercise explaining him how s/he should take it considering the daily dosage. Melatonin level measurements were done before, right after and two hours after the exercises, and blood samples were taken twice into EDTA tubes in order to determine the vitamin A and E levels at the beginning and end of the two-week exercise program. The blood samples were centrifuged with 3000 spin/minute for 10 minutes in cooled centrifuge, and kept at -20°C in the deep freeze until the results were examined. Melatonin Hormone Levels were measured as stated in ELISA DRG KIT.

Statistical analysis. Inter-group variables and independent variables were compared via t test, and intra-group variables were compared via paired t test. An analysis of variance (ANOVA) was conducted in order to compare the melatonin levels of the smoking and non-smoking groups right after the exercises of the 1st and 2nd weeks. Significance level was considered to be $p<0.05$.

Results and Conclusion

14 sportspeople performed the exercises determined for the study. They participated in all the activities and took the vitamin A and E at the determined dosage and time. The ages, weights and lengths of the male participants were compared in groups: smoking and non-smoking groups, and the results were given in Table I. No significant difference was observed in the ages, weights and lengths of the two groups ($p>0.05$).

Melatonin and vitamin A and E levels were measured from the blood samples prior to the exercise program, and the values were compared in terms of smoking and non-smoking sportspeople.

These measurements and the results of the comparison were given in Table II. No significant difference was observed between the melatonin and vitamin A and E levels of the smoking and non-smoking sportspeople ($p>0.05$).

The pre-exercise melatonin levels of the non-smoking sportspeople were found to be higher than smoking sportspeople; however, no statistically significant difference was observed between these levels ($p>0.05$). Moreover, melatonin levels of the smoking and non-smoking sportspeople were determined to increase right after the two-week exercise program and to decrease to the pre-exercise levels two hours later. These increases and decreases were considered to be statistically significant ($p<0.01$) (Graph 1).

The increase in the melatonin levels of the smoking and non-smoking sportspeople right after the second exercise, not the first exercise, was found to be higher in non-smoking sportspeople. These increases were considered to be statistically significant ($p<0.01$) (Graph 2).

The melatonin and vitamin A and E levels were measured from the blood samples at the end of the two-week exercise program, and then compared in terms of smoking and non-smoking sportspeople. The measurements and the comparison results were given in Table III.

Melatonin and vitamin A and E levels were found to be significantly higher in non-smoking sportspeople compared to the smoking ones (p<0,01). Comparison of the differences in the intra-group, pre-exercise and post-exercise variables indicated that the melatonin and vitamin A and E levels of both smoking and non-smoking sportspeople significantly increased (p<0.05, p<0.01). The comparison of the intra-group, pre-exercise and post-exercise variables of the smoking and non-smoking groups was given in Table IV.

Table I**.** Comparison of the ages, weights and lengths of the smoking and non-smoking sportspeople. (means ± SD; n=7 per group).

Characteristic	Smoking	Non-smoking
Age	21.14±1.57	21.85±1.57*
Weight	73.14±4.74	77.14±4.67*
Lenght	1.85±0.05	1.84±0.04*

p>0.05, Independent variables were compared via t test.

Table II. Comparison of the pre-exercise vitamin A and E and melatonin levels of the smoking and non-smoking sportspeople. (means ± SD; n=7 per group).

Characteristic	Smoking	Non-smoking
Vitamin A (gr/dL)	0.04±0.00	0.04±0.00*
Vitamin E (gr/ml)	0.08±0.01	0.08±0.01*
M. D. (pg/ml)	6.80±0.258	6.82±0.327*

*p>0.05, M.D. (Melatonin level)

Table III**.** Comparison of the vitamin A and E and melatonin levels of the smoking and non-smoking sportspeople at the end of the two-week exercise program. (means±SD; n=7 per group).

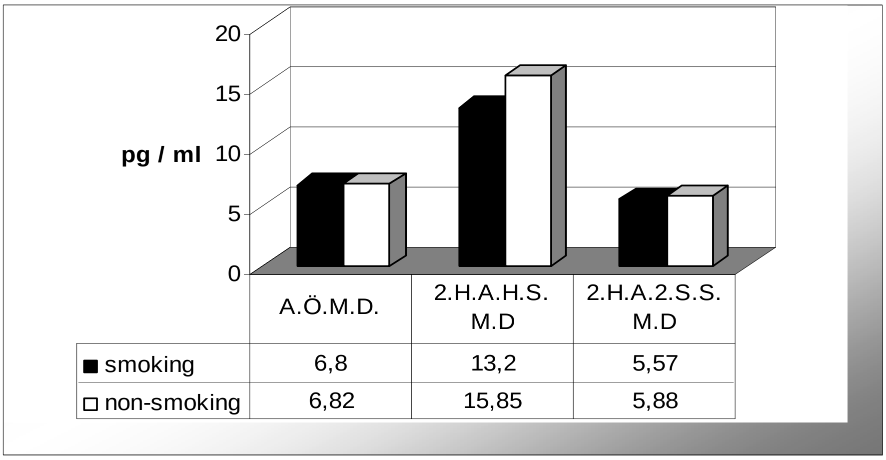
Characteristic	Smoking	Non-smoking
Vitamin A (gr/dL)	0.10±0.00	0.24±0.03**
Vitamin E (gr/ml)	0.25±0.45	0.49±0.53**
M.L.R.A.E. (pg/ml)	13.22±0.508	15.85±2.038**
M.L.2.H.A.E (pg/ml)	5.57±0.340	5.88±0.254**

**P < 0.01, M.L.R.A.E. (melatonin level right after the exercise) M.L.2.H.A.E (melatonin level 2 hours after the exercise)

Table IV. Changes in the vitamin A and E and melatonin levels of the smoking and non-smoking sportspeople after the two-week exercise program and the vitamin A and E supplements. (means ± sd; n = 7 per group).

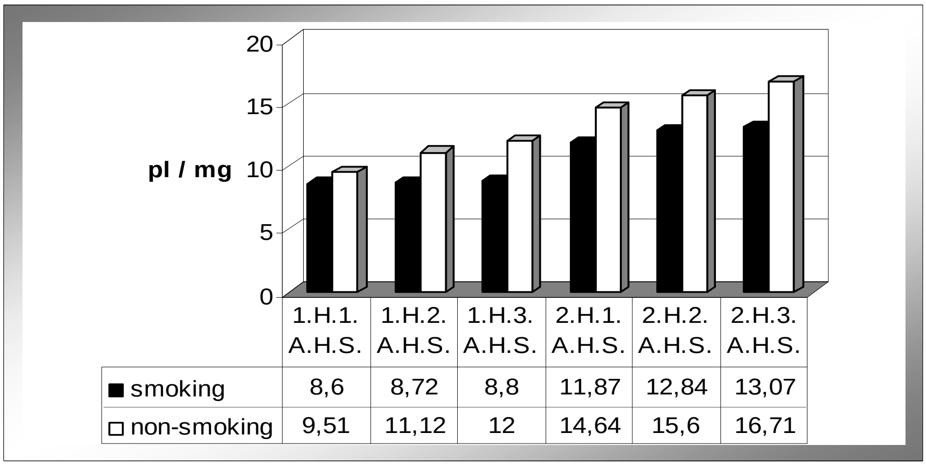
Variables	**Groups**	**Pre-exercise**	**Post-exercise**
Vitamin A (gr/dL)	Smoking	0.04±0.00	0.10±0.00*
	Non-smoking	0.04±0.00	0.24±0.03**
Vitamin E (gr/ml)	Smoking	0.08±0.01	0.25±0.45*
	Non-smoking	0.08±0.01	0.49±0.53**
P.E and M.L.A.2.W.E.(pg/ml)	Smoking	6.80±0.258	4.57±0.340*
	Non-smoking	6.82±0.327	4.88±0.254*

**P < 0.01, *p>0,05. P.E. and M.L.A.2.W.E. (Pre-exercise and melatonin level after the two-week exercise)



	A.Ö.M.D.	2.H.A.H.S. M.D	2.H.A.2.S.S. M.D
■ smoking	6,8	13,2	5,57
□ non-smoking	6,82	15,85	5,88

Graphic 1. Melatonin Levels of the smoking and non-smoking sports people
****:** p<0.01; A.Ö.M.D.:Melatonin level before the exercise; .2.H.A.H.S.M.D.:melatonin level right after the two-week exercise; 2.H.A.2.S.S.M.D.: melatonin level 2 hours after the two-week exercise



	1.H.1. A.H.S.	1.H.2. A.H.S.	1.H.3. A.H.S.	2.H.1. A.H.S.	2.H.2. A.H.S.	2.H.3. A.H.S.
■ smoking	8,6	8,72	8,8	11,87	12,84	13,07
□ non-smoking	9,51	11,12	12	14,64	15,6	16,71

Graphic 2. Melatonin levels of the smoking and non-smoking sportspeople right after the first and second weeks
****:** p<0.01; 1.H. (1.2,3.) A.H.S.M.D.: First week (1.,2. and 3.) Melatonin levels right after the exercises 2.H (1.2.3.).A.H.S..M.D.: Second week (1.,2.and 3.) Melatonin levels right after the exercises

Discussion

Today, smoking is the most important health problem. Approximately 1.1 billion people are smoking, 800 million of which live in developing countries. 100 million people died out of smoking in the twentieth century, and this number is expected to increase to 1 billion in twenty first century if nothing is done in this regard (9).

The negative effect of smoking is known to be related to the oxidative stress in tissues. Oxidative stress is a process in which free radicals cause physical damage in tissues. This damage is to be recovered with antioxidant agents (3). Smoking causes oxidative stress by not only resulting in the production of reactive oxygen types but also damaging the antioxidant defense mechanisms (7).

A similar study (10) examined the plasma melatonin levels of 21 smoking and non-smoking female students, and suggested that the melatonin levels of the smoking students were lower than the non-smoking students. Another study conducted by Ursing et al. (11) upon seven males and one female who smoked at least 20 cigarettes a day determined that smoking decreased the serum melatonin level.

Again, the study of Pilaczynska et al. (12) compared the melatonin levels of people who performed low and high physical activity, and found a negative correlation between plasma melatonin levels and low physical activity and a positive correlation between plasma melatonin levels and high physical activity. These data indicated that regular physical activity increases melatonin levels, while smoking decreases melatonin levels. These findings supported the findings of the present study (Graph 1 and 2).

Moreover, vitamin A and E may have a protective effect against other oxygen radicals because of their oxygen cleaning characteristics. Vitamin E deficiency is observed to increase hydroperoxide, aldehydes and other oxidation types caused by metabolic activity, while vitamin E supplement is observed to decrease the formation of free radicals causing lipid peroxidation (13). On the other hand, maintaining the balance between the formation and destruction of the oxidants in biologic systems is important in maintaining the biological integrity of the cell and tissue. There are several antioxidants in cells against the hazardous effects of free radicals resulting from smoking. Many antioxidants in the body such as vitamin A and E work as defense systems against the damages caused by free radicals (14).

The present study (Graph 1) determined the pre-exercise melatonin levels of the non-smoking sportspeople to be higher than smoking sportspeople; however, no statistically significant difference was observed between these levels ($p>0.05$).

Moreover, melatonin levels of the smoking and non-smoking sportspeople were determined to increase right after the two-week exercise program and to decrease to the pre-exercise levels two hours later. These increases and decreases were considered to be statistically significant ($p<0.01$). Melatonin levels of the smoking and non-smoking sportspeople were determined to significantly increase right after the second week, not the first week ($p<0.01$) (Graph 2).

Moreover, the vitamin A and E levels of the smoking and non-smoking sportspeople before and after the two-week exercise were compared. Pre-exercise vitamin A and E levels were found to significantly increase in both groups. Although no significant difference was observed between the inter-group vitamin A and E levels before the exercise, a significant increase was observed in the vitamin A and E levels of the non-smoking sportspeople after the exercise ($p<0.05$, $p<0.01$).

Smoke includes many compounds of oxidants and radicals starting or maintaining oxidative stress; however, the organism develops an antioxidant defense system against the hazardous effects of free radicals after smoking. The most important antioxidants of these systems are considered to be vitamin A and E, which are scavenging, quencher, repairing and chain breaking (15).

On the other hand, exercise may disrupt the balance between free radicals, namely the oxidative stress, and the antioxidants. During the exercise, O_2 consumption may be 10-15 times more than the resting condition, thus temporarily increasing the free radical production capacities of mitochondria. In their study, Buxton et al. (16) determined minimum O_2 consumption in 3 hours to be 40-60% and maximum O_2 consumption in 1 hour to be 75% in gentle exercise, and significant increases were found in the melatonin levels. These findings indicated that physical activity increased plasma melatonin level.

The production of oxidants is known to increase during the exercise. Especially heavy physical activities induce the formation of reactive oxygen species (ROS) in the organism.

The amount of O_2 used in relation to the increasing metabolic activity increases, and ROS are formed. In case that these oxidants cannot be removed during the exercise, significant oxidative damage can occur in cellular biomolecules.

However, regular exercise protects myocytes against the hazardous effects of oxidants, and enables adaptation in the antioxidant capacity of skeletal muscles so as to prevent cellular damage (17). Smoking can be said to decrease the exercise tolerance level and restrict physical activity, while regular exercise can be said to increase the maximal respiration capacity.

The effect of exercise on the melatonin level depends on certain factors such as the level, duration and hour of the exercises; and melatonin reduces the free radical damage caused by smoking in the respiratory system and has a strong antioxidant effect against oxidative damage in the lungs. As a consequence, smoking was determined to have a decreasing effect on the melatonin levels of sportspeople.

However, as for the oxidative damage caused by smoking in cells, antioxidants such as vitamin A and E supplements were found to protect against the toxic effects of smoking and increase the melatonin levels.

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