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Serum Levels of Vitamin E and β-carotene in Relation to Sex and Medical Status in a Population from North Jordan

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aim: The aim of this study is to assess whether serum vitamin E (α-tocopherol) and β-carotene are affected (or not) by different medical conditions such as hypertension, diabetes, smoking, and pregnancy.
Methodology: Total serum vitamin E and β-carotene concentrations were measured in a group of 946 volunteers (males and females with age range between 18 and 60 years); 432 were healthy and non-smokers (161 men and 271 women), and 514 were having a medical condition (90 men and 75 women with high blood pressure, 40 men and 85 women were diabetes, 98 men and 50 women were smokers, and 76 pregnant women). Total serum vitamin E and β-carotene were measured by HPLC. Sera were extracted from blood samples collected from a population from the north of Jordan.
Results: The total vitamin E serum concentrations in male healthy individuals was found to be 32.3 ± 0.9 µmol/l compared to 17 ± 0.4 µmol/l for smokers, 19.2 ± 0.8 µmol/l with diabetes, and 20.1 ± 0.9 µmol/l with high blood pressure; and 26.6 ± 0.1 µmol/l in healthy women compared to 21.1 ± 0.5 µmol/l for smokers, 20.5 ± 0.6 µmol/l with diabetes, and 21.7 ± 0.4 µmol/l with high blood pressure.
The total β-carotene serum concentrations in male healthy individuals was found to be 1.26 ± 0.03 µmol/l compared to 0.83 ± 0.04 µmol/l for smokers, 0.85 ± 0.06 µmol/l with diabetes, and

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0.81 ± 0.053 µmol/l with high blood pressure; and 2.4 ± 0.3 µmol/l in healthy women compared to 1.4 ± 0.2 µmol/l for smokers, 0.97 ± 0.03 µmol/l with diabetes, and 0.78 ± 0.06 µmol/l with high blood pressure. The levels of serum concentrations of both vitamins were significantly lower ($p < 0.05$) in the tested subjects compared with the values registered in the healthy subjects. Pregnancy was also found to affect the levels of serum concentrations of both vitamins, our results showed a decrease in the serum concentration levels of the pregnant women who participated in this study versus the healthy subjects.

Conclusion: Vitamin E and β-carotene absorption by the body was found to be influenced by the health status of the individual. This was clearly shown by the differences between healthy individuals from the test group and those in the healthy counter parts.

Keywords: vitamin E (α-tocopherol); β-carotene; serum concentrations; HPLC.

1. INTRODUCTION

Vitamins are trace-amount organic compounds that regulate the physiological functions of an organism. Vitamins are classified into two main groups, water-soluble (vitamins B and C) and fat soluble (vitamins A, D, E and K). Water soluble vitamins are not stored by the body and therefore should be supplied daily in our diet, on the other hand, fat soluble vitamins are dissolved in fat before they are absorbed in the bloodstream to carry out their functions; excess of these vitamins is stored in the liver, and is not needed every day in the diet. Vitamin E exists in eight chemical forms (alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol) that have varying levels of biological activity [1]. Alpha- (or α-) tocopherol is the only form that is recognized to meet human requirements. Vitamin A and its analogs are considered to be important regulators of cell differentiation, proliferation, and cell apoptosis, and are involved in immune functions. Retinoic acid, a metabolite of vitamin A, is involved in embryonic kidney development by controlling branching morphogenesis [2,3]. β-carotene is converted into vitamin A (retinol), an important vitamin for good vision and eye health, for a strong immune system, and for healthy skin and mucous membranes.

Fat-soluble vitamins such as vitamin E (α-tocopherol) (Fig. 1A) and β-carotene (Fig. 1B) are considered to be important antioxidants protecting body tissue from damage caused by free radicals [4-6]. Reduced levels of these antioxidants in plasma were associated with different diseases, such as, cancer, atherosclerosis, and cardiovascular diseases [4,7-9]. Beside their antioxidant properties, carotenoids (of which, β-carotene) have other important functions such as enhancement of gap junctional communication, immunomodulation, protection of DNA against peroxidation, and tumor-suppressive activity [10]. Women were found to have elevated levels of fat-soluble antioxidant vitamins, especially carotenoids [10]. Several factors such as: age, sex, dietary intake, drinking and smoking habits and seasonality were found to influence plasma concentrations of these compounds [11-13].

In this study we investigated the concentrations of β-carotene and vitamin E (α-tocopherol) in a population of individuals with different health conditions (high blood pressure, diabetes, smokers, and pregnancy) and compare them with healthy individuals (people with no medical condition and non-smokers) from the northern parts of Jordan in order to assess whether or not they should worry about their vitamins intake because of their medical condition.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

β-carotene > 97.0%, and retinyl acetate, α-tocopherol acetate > 96% and γ-tocopheryl acetate were obtained from Sigma-Aldrich and used without further purification. All solvents were Merck grade and used as received.

2.2 Population Description

946 volunteers were selected for this study, 389 men and 557 women, aged 18 to 60 years old;432 were healthy non-smokers and with no medical condition (161 men and 271 women),and 514 (228 men and 286 women) were having different health conditions including diabetes, high blood pressure, pregnancy and smokers.

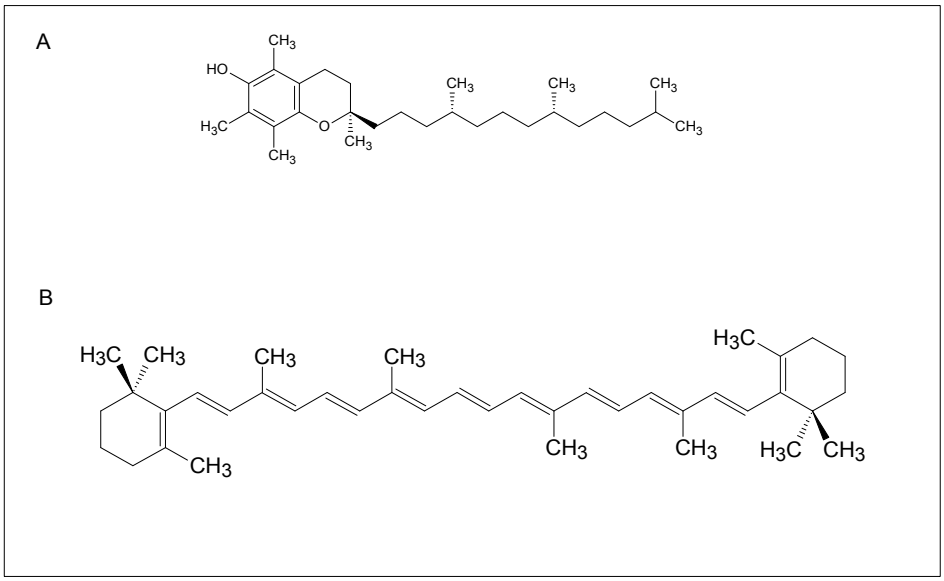

Fig. 1. Chemical structure of vitamin E (α-tocopherol) (A), and β-carotene (B)

2.3 Blood Sample Collection

Blood was collected from each subject and stored in glass tubes at the medical laboratories in Al Mafraq hospital (Al Mafraq, Jordan), samples were then centrifuged to separate the serum form other blood constituents. Sera were stored in vials at -20°C until use. The study protocol was conducted according to the Jordanian legislation regarding research ethics (Jordan Food and Drug Administration (JFDA), 2001 clinical research law).

2.4 Sample Preparation

For the analysis of α-tocopherol and β-carotene, 100 µl of serum was deprotenized with 100 µl of absolute ethanol and then extracted with 600 µl of chloroform. The extracts were shaken for 5 min before centrifugation. The organic layer was extracted from the samples and dried by evaporation under nitrogen. The dried extracts were dissolved in 100 µl absolute methanol and stored at -20°C until analysis. The total concentrations of α-tocopherol and β-carotene present in serum were measured by High Performance Liquid Chromatography (HPLC). Retinyl acetate and α-tocopheryl acetate were used as internal standards for β-carotene and α-tocopherol respectively. Both standards and serum samples were spiked with known amount of internal standards (20 µmol/l) and then extracted using the described procedure.

2.5 Chromatographic System

A water mode 600 solvent delivery system was used together with a Supelcosil LC-18 (250 x 4.6 mm) column packed with 5µm particle size. Samples of α-tocopherol and β-carotene were injected using a rheodyne injector with a 20 µl sample loop. Detection was done with a UV/Vis diode array detector (Water PD486), the absorbance detector was operating at 254 nm forα-tocopherol and 450 nm for β-carotene. Peak evaluations and quantitizations were made using water millennium software. The mobile phase consisted of 90% (v/v) methanol: water and flow rate of 1.5 ml/min. The concentration of α-tocopherol was quantified from the peak area ratio of α-tocopherol /γ-tocopherol acetate (internal standard), and the concentration of β-carotene was obtained from the peak area ratio of β-carotene/retinyl acetate (internal standard).

2.6 Statistical Analysis

Data was reported as mean ± Standard Deviation (SD). Differences between studied groups were analyzed using the two way ANOVA test (software Origin Pro 8). *P*-value < 0.01 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Validation of the Analytical Method

Different concentrations of the standard solutions containingα-tocopherol and β-carotene, in the

presence of internal standards, were made for the construction of the calibration curve. The calibration curve was used to determine the linear range and limit of detection of both α-tocopherol and β-carotene. Spiking of sample sera with internal standards was also used to validate the extraction and the analytical method. The concentration of α-tocopherol was quantified from the peak area ratio of α-tocopherol/γ-tocopheryl acetate (internal standard) and the concentration of β-carotene was obtained from the peak area ratio of β-carotene/retinyl acetate (internal standard).

Fig. 2 shows the typical chromatogram of α-tocopherol and β-carotene in serum samples as well as the peaks of the internal standards; retinyl acetate (internal standard used for β-carotene) appears at 5.0 min retention time (RT), γ-tocopheryl acetate (internal standard used for α-tocopherol) appears at 7.5 min RT, α-tocopherol appears at 9.8 min RT, and β-carotene appears at 12.8 min RT. The concentration of α-tocopherol was quantified from the peak area ratio of α-tocopherol/γ-tocopheryl acetate, and the concentration of β-carotene was obtained from the peak area ratio of β-carotene/retinyl acetate.

Fig. 3 shows the calibration curves for α-tocopherol and β-carotene.. The calibration curves follow a linear relation in the range between 5± 1.0 – 90±0.9 µmol/land 0.5 ± 0.09 – 50 ± 0.3 µmol/l for α-tocopherol and β-carotene, respectively. The calibration curves for both β-carotene and α-tocopherol were found to follow a linear relationship as represented in equations 1 and 2 for β-carotene and α-tocopherol, respectively.

$$y = 1.056 + 0.171\ x \text{ with RSD is } 0.997 \quad (1)$$

$$y = 0.00379 + 0.0817\ x \text{ with RD is } 0.995 \quad (2)$$

Where y in equation 1 represents the peak area ratio of β-carotene/retinyl acetate, and y in equation 2 represents the peak area ratio ofα-tocopherol/γ-tocopheryl acetate. The calibration results show that 1.8 ± 0.09 µmol/l and 0.15 ± 0.06 µmol/l were the smallest concentrations of α-tocopherol and β-carotene, respectively (LOD). On the other hand, the limits of the quantification of both compounds were also determined as 3.6 ± 0.8 µmol/l for α-tocopherol and 0.3 ± 0.03 µmol/l for β-carotene (LOQ).

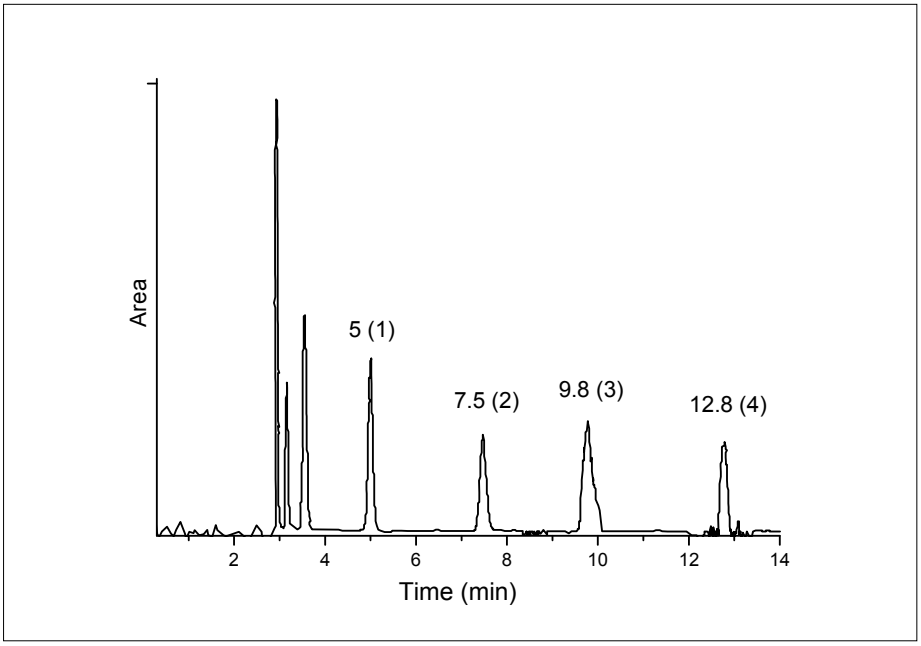

Fig. 2. HPLC chromatogram of (1) Retinyl acetate (2) γ-tocopheryl (3) Vitamin E (α- tocopherol) and (4) β-carotene in serum samples. HPLC conditions: Isocratic elution (mobile phase: methanol-water 90:10 v/v). Column: Supelcosil LC-18 (250 x 4.6 mm; I.D 5 µm; Flow rate 1.5 ml/min)

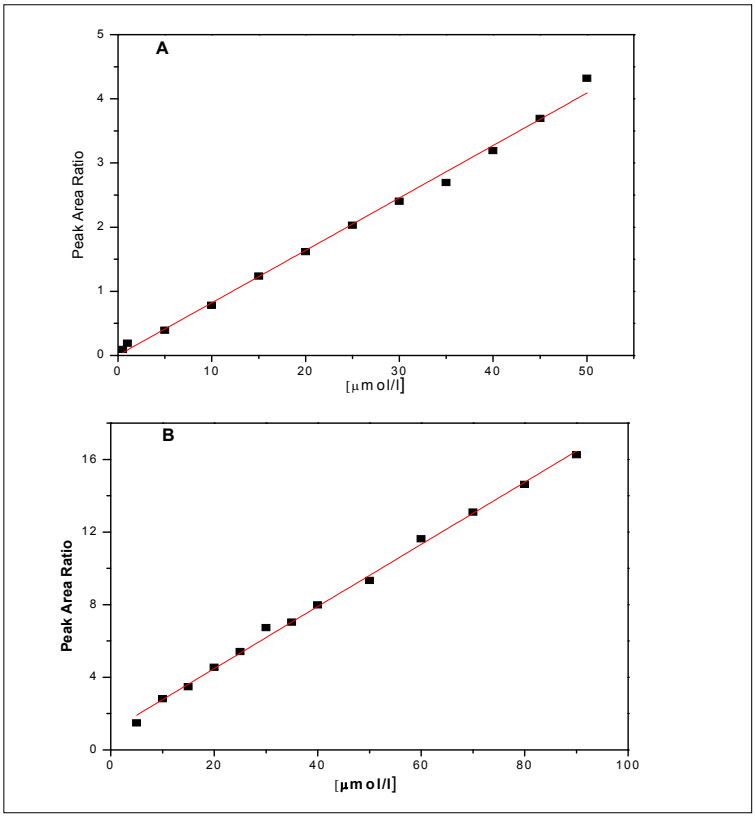

Fig. 3. Calibration curve for the analysis of α-tocopherol and β-carotene standard samples in the range between 5 ± 1.0 – 90 ± 0.9 µmol/l for α-tocopherol (A), and 0.5 ± 0.09 – 50 ± 0.3 µmol/l for β-carotene (B)

3.2 Levels of α-tocopherol and β-carotene Detected in the Participants

Vitamin E (α-tocopherol) and β-carotene serum concentrations were found to be associated with different conditions; Lower levels of β-carotene were detected in patient with stenosis [10], vitamins A, E, C, α- and β-carotene were also found to be lower in patients with chronic obstructive pulmonary disease [14], other studies showed elevated levels of fat-soluble antioxidant vitamins in plasma was associated with atherosclerotic progression in arteries [7,15,16].

In this study, we evaluated the levels of vitamin E and β-carotene in sera of 946 individuals; the subjects were classified depending on their sex (men vs women), their health condition (healthy non-smoker individuals vs individuals smokers and with a medical history of diabetes, high blood pressure or pregnancy), and depending on the number of smoked cigarettes per day (< 10 to > 20 cigarettes per day) for smokers. The results were illustrated in tables representing the above categories. All participants were from a northern region of Jordan, they were aged between 18 and 60 years, 389 were males and 557 were females. In the male category, 161 were healthy and non-smokers and 228 had a medical condition and were distributed as fellow: 90 had high blood pressure, 40 were diabetics, and 98 were smokers. Of the 557 females participating in this study, 271 were healthy non-smokers, 75 had high blood pressure, 85 were diabetics, 50 were smokers, and 76 were pregnant. Table 1 summarizes the number of men and women investigated in this study as well as their distribution depending on their sex and medical status.

Table 2 shows the mean serum concentrations of α-tocopherol and β-carotene in the tested population with different health conditions from 18 to 60 years; the mean values of serum α-tocopherol concentrations among healthy individuals were 32.3 µmol/l in males and 26.6 µmol/l in females, males seem to have higher serum concentrations of this vitamin than

women. For β-carotene, the mean serum concentration values in healthy males and females were 1.26 µmol/l and 2.4 µmol/l respectively, although females seem to have higher serum concentrations than males (Table 2). These results were in accordance with those found by other researchers [10,17,18].

The tested population with a medical condition were divided into 4 groups depending on their medical status (high blood pressure, diabetes, smokers, and pregnancy); when comparing the male and female subjects with high blood pressure, we found that both sexes had similar mean values regarding their serum concentrations of α-tocopherol (20.1 µmol/l in males and 21.7 µmol/l in females) and β-carotene (0.81 µmol/l in males and 0.78 µmol/l in females). But when comparing these values with the healthy subjects from the tested population, we found that for α-tocopherol, there was a 1.6 fold decrease in the mean serum concentrations in men and 1.2 decrease in women, although this difference was not statistically significant in women compared with the healthy subjects. For β-carotene, our results showed a similar decrease in the mean serum concentrations of this vitamin in both sexes with 1.5 fold decrease in men and 3 fold decrease in women compared with the healthy subjects (Table 2). In a recent study on different vitamin intake conducted by Llopis-González and his group on patients with hypertension, they found that there was no difference in their levels of vitamin E compared with non-hypertension participants and high levels of vitamin A (both men and women) compared with non-hypertension subjects [19]. In our study, both sexes showed lower levels of serum concentrations of both α-tocopherol and β-carotene.

Table 1. Distribution of the tested population depending on their sex, age and medical status

Participants description	Sex	Sample number	Age range / years
Total number		946	18-60
Sex	Male	389	18-60
	Female	557	18-60
Healthy individuals (non-smokers, neither high blood pressure nor diabetic problems)	Male	161	18-60
	Female	271	18-60
High blood pressure	Male	90	30-60
	Female	75	30-60
Diabetics	Male	40	30-60
	Female	85	30-60
Smokers	Male	98	18-60
	Female	50	18-60
Pregnant women		76	18-40

**P< 0.01, statistically significant different from the control (Healthy individuals)*

Table 2. Mean concentrations of serum vitamin E and β-carotene in the collected samples from 946 individual, males and females, healthy and with different medical conditions

Sample description	Sex	Sample number	Mean concentration of vitamin E (µmol/l)	Mean concentration of β-carotene (µmol/l)
Healthy individuals	Male	161	32.3 ± 0.9	1.26 ± 0.03
	Female	271	26.6 ± 0.1	2.4 ± 0.3
High blood pressure	Male	90	20.1* ± 0.9	0.81* ± 0.053
	Female	75	21.7 ± 0.4	0.78* ± 0.06
Diabetes	Male	40	19.2* ± 0.8	0.85* ± 0.06
	Female	85	20.5* ± 0.6	0.97* ± 0.03
Smokers	Male	98	17.0* ± 0.4	0.83* ± 0.04
	Female	50	21.1 ± 0.5	1.4* ± 0.2
Pregnant females		76	25.3 ± 0.7	1.1* ± 0.6

**P< 0.01, statistically significant difference from the control (healthy individuals)*

For the tested individuals with diabetes, a similar pattern was observed for both vitamins, with the mean serum concentration values for α-tocopherol in the range of 19.2 µmol/l for men and 26.6 µmol/l for women, these values were statistically significant ($P < 0.05$) when compared with the healthy subjects with a 1.68 fold decrease in males and 1.29 fold decrease in females. For β-carotene, the mean serum concentration values found were 1.26µmol/l in males and 2.4 µmol/l in females with a statistically significant decrease in both sexes in comparison with the healthy subjects with 1.5 fold decrease in these values in males and 2.47 fold decrease in females (Table 2). Again, these findings were in accordance with studies showing that individuals with a medical status had lower serum levels of vitamins compared to healthy individuals [19-22]. Pregnant women who took part in this study were aged between 18 and 40 years, they had their mean serum levels of α-tocopherol and β-carotene of 25.3 µmol/l and 1.1 µmol/l respectively; these values were similar to those registered in healthy women (26.6 µmol/l) forα-tocopherol and significantly low for β-carotene (2.18 fold lower compared with healthy women) (Table 2). It was found that the administration of β-carotene among other vitamins in the second trimester of pregnancy decreases the incidence of preterm premature rupture of membranes before the onset of contractions [23], also high intake of β-carotene during late pregnancy was found to be associated with offspring bone mineralization [24]. Other studies demonstrated that low levels of β-carotene and α-tocopherol were associated with early-onset preeclampsia, suggesting that oxidative stress may play a greater role in the pathophysiology of early-onset preeclampsia [25].

The tested subjects with smoking habits had lower mean serum concentration values for both vitamins with 17.0 µmol/l in males and 21.1 µmol/l in females for α-tocopherol and 0.83µmol/l in males and 1.4 µmol/l in females for β-carotene; these values were 1.9 fold lower in males and 1.26 fold lower in females for α-tocopherol, and 1.51 fold lower in males and 1.71 fold lower in females for β-carotene when compared with the healthy group from the tested population (Table 2). Smoking was found to cause many damage to health including oxidative stress due to the abundance of free radicals in, or produced by, cigarette smoke [26,27]. Our study showed that smoking affects men more than women when it comes to α-tocopherol serum levels but for β-carotene serum levels, women smokers were found to be more affected than men smokers. Table 3 shows the distribution of smokers depending on the number of smoked cigarettes per day (less than 10 cigarettes/day to more than 20 cigarettes/day). The mean serum concentration levels for α-tocopherol in men were decreasing with increased number of smoked cigarettes / day (19.8 µmol/l, 16.7 µmol/l, and 14.6 µmol/l, respectively); in the female tested population there were also a decrease in the mean serum concentration values with increasing numbers of cigarettes / day with 23.5 µmol/l, 20.3 µmol/l, and 19.3 µmol/l, respectively. The mean serum concentrations of β-carotene showed the same pattern shown for α-tocopherol for both sexes with decreasing levels of this vitamin with increasing numbers of smoked cigarettes / day (1.01 µmol/l, 0.82 µmol/l, and 0.65 µmol/l, respectively in men and 1.7 µmol/l, 1.4 µmol/l, and 1.1 µmol/l, respectively in women). These values showed that men smokers had lower levels of serumα-tocopherol than women smokers and women smokers had lower levels of β-carotene than men smokers. These values were significantly higher when compared with those found in the healthy group involved in this study, although, there was no statistically significant difference in α-tocopherol serum levels between women smokers of less than 10 cigarettes / day and the healthy subjects (1.13 times decrease only) (Table 3). Other studies showed that smoking was affecting peoples' health as well as their vitamin serum levels which were found to drop with elevated number of cigarettes smoked daily in both men and women [18,20,28,29]; our data were in accordance with these finding.

The results of the present study showed that sex, smoking habits, and the medical condition of the subjects who participated in the study can affect their serum levels of antioxidants; these results were similar to those found by many other research groups around the wold [21-23,30,31]. Our study showed that men had high levels of α-tocopherol serum concentrations than women and low levels of β-carotene serum concentrations than in men. Different studies done by other research groups [18,19] showed that women had higher levels of β-carotene than men and this was probably related to the nutrition diets rich in this vitamin usually consumed by women more than men. Research related to nutritional regimens has shown that the consumption of large amounts of fruit and

Table 3. Mean serum vitamin E and β-carotene concentrations of smokers (males and females) aged 18-60 years and stratified by number of cigarettes smoked/day

		Males (n = 98)			Females (n = 50)		
		Sample number	Mean concentration of vitamin E (μmol/l)	Mean concentration of β-carotene (μmol/l)	Sample number	Mean concentration of vitamin E (μmol/l)	Mean concentration of β-carotene (μmol/l)
Healthy individuals		161	32.3 ± 0.9	1.26 ± 0.03	271	26.6 ± 0.1	2.4 ± 0.3
Smokers (No of cigarettes/ day)	< 10	13	19.8* ± 0.32	1.01 ± 0.02	25	23.5 ± 0.5	1.7* ± 0.9
	10- 20	40	16.7* ± 2.3	0.82* ± 0.06	10	20.3* ± 0.9	1.4* ± 0.1
	> 20	45	14.6* ± 1.4	0.65* ± 0.08	15	19.3* ± 0.3	1.1* ± 0.6

**P< 0.01, statistically significant different from the control (Healthy individuals)*

vegetables have lower incidences of many diseases including tumors, cardiovascular diseases, and strokes; although the precise mechanisms for this protective effect are not fully understood. Also, vitamin supplements were shown to reduce the risk of many diseases such as high blood pressure [20,23], and the protection against some types of cancers [32].

4. CONCLUSION

As a conclusion to this study, conducted on a population from the northern parts of Jordan, we can state that the sex and health status of an individual can affect the serum levels of vitamins. The study of α-tocopherol and β-carotene as antioxidants and common players in maintaining a healthy body as well as preventing the occurrence of some diseases or lowering the incidence of others; we observed that people with high blood pressure, diabetes, smokers and pregnant women, all have lower levels of both vitamins when compared with healthy people, and these people should be aware of the health risks resulting from the inadequate intake of these vitamins; taking into consideration the Recommended Dietary Allowances (RDA) of both α-tocopherol and β-carotene according to the Institute of Medicine-Food and Nutrition Board which are: 15 mg (34.8 μmol) for individuals aged 14+ (both sexes and pregnant women) for α-tocopherol, and 900 μg (3.14 μmol) of vitamin A, of which β-carotene, for men aged 14 +, 700 μg (2.44 μmol) for women aged 14 +, and 750 μg (2.61 μmol) for pregnant women (14 + years) [31].

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this paper and accompanying images'.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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