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Original Research

Plasma Concentrations of Vitamin E, Vitamin A and β-Carotene in Healthy Men

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Abstract

The aim of the study was to adapt the method for the determination of vitamins E, A, and β-carotene and to assay them quantitatively in plasma of municipal transport drivers. The study embraced 147 municipal transport male drivers, aged 23–58 years. The Waters 2695 integrated analytical system of high-pressure liquid chromatography (HPLC), equipped with a UV-VIS detector was used to determine the studied compounds.Our analysis of the quantitative data by age and seniority did not show significant differences in the concentrations of the analyzed compounds between the study groups, except for the concentration of β-carotene, which was significantly lower in drivers aged over 46 years with the longest employment (over 16 years) compared to the younger groups.

Keywords: β-carotene, vitamin E, vitamin A, plasma, humans

Introduction

Vitamin E (α-tocopherol), vitamin A (retinol), and β-carotene (provitamin A) are low molecular compounds that exhibit high biological activity against oxidants and free radicals [1, 2]. They are found in fats and fatty food components of both vegetable and animal origin. These substances, absorbed by blood via the lymphatic system, reach the liver and fatty tissues where they are stored. Vitamin E is a mixture of compounds containing four tocopherols (α, β, γ, and δ) and four tocotrienoles, including the most active α-tocopherol. Vitamin E prevents oxidative damage to cellular structures and tissues through breaking reactions of free radicals, participates in the maintenance of an appropriate structure of cellular membranes, inhibits the generation of microclots, and blocks the production of nitrosamines. Epidemiological studies evidence the association between tocopherol concentration in blood and/or its dietary intake, and mortality from cardiovascular and neoplastic diseases [3–6]. Vitamin E is not a toxic substance and its overdosage may result merely in headache, fatigue, drowsiness, muscular weakness and dyspepsia, abdominal pain, diarrhea, and/or nausea. A group of plant carotenoids, diversified in terms of their chemical structure, forms provitamin A, of which vitamin A is enzymitically synthesized in the walls of small intestine and liver. β-carotene, the most active among over 50 carotenoids, is responsible for the biological activity of vitamin A, directly or after it is transformation into two molecules of this vitamin. Vitamin A and β-carotene are essential for normal vision, building up epithelium and skin, tissue growth, stabilization of epithelial cells, synthesis of adrenal cortex hormones, thyroid thyroxine secretion, maintenance of normal neural shields, immune reactions, production of erythrocytes and protection against cancer development [7]. Vitamin A deficiency can induce severe health effects, but its abundance can also be dangerous. These are manifested by heaviness, muscular weakness, growth inhibition, hemorrhages, idiopathic bone fractures, dysfunctions of the heart, kid-

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neys and central nervous system. Therefore, adequate bodily concentrations of vitamin A are essential for the maintenance of good health. High body concentrations of β-carotene can induce yellow pigmentation, but this is not harmful. There are well documented studies which indicate that adequate plasma concentrations of vitamin E and β-carotene inhibit oxidation of low density lipoproteins, and thus decrease the risk for the development of atherosclerosis, cardiovascular diseases, cancers and age-related macular degeneration [8-11]. Vitamin concentrations in cells and extracellular fluids diminish with increasing age, which is linked for example with constant exposure to environmental oxidants, dietary habits (low consumption of fruits and vegetables), diseases and related medications. A statistically significant relationship between plasma vitamin E concentration and psychomotor functions has been revealed in persons aged over 60 years [12]. Environmental exposure to ozone is responsible for a statistically significant decrease in plasma β-carotene concentration [13]. Oxidative stress induced by exposure to chemical compounds in the work environment can also exert its effect on plasma concentrations of anti-oxidative vitamins [14]. A significantly lower concentration of vitamin E in erythrocytes was found in workers occupationally exposed to heavy metals, organic solvents and nitric oxides, compared with non-exposed persons living in the same region [15 and our own studies]. Lower blood plasma concentrations of vitamin E and β-carotene have also been reported in heavy smokers [16]. The aim of the study was to adapt the method for the determination of vitamins E, A and β-carotene and to assay quantitatively their concentration in the plasma of healthy men employed as municipal transport drivers.

Materials and Methods

Chemical Reagents

D1- α-Tocopherol (Sigma), Retinol (Sigma), β-Carotene (Sigma), 95% n-hexane and ethyl acetate (J.T. Baker) for high-pressure liquid chromatography (HPLC), 99.8% ethyl alcohol (POCH S.A.) were used in the study.

Material

A group of 147 healthy men aged 23–58 years who were employed as municipal transport drivers was eligible for the study. The Local Ethics Committee for Scientific Research at the Nofer Institute of Occupational Medicine, Łódź, approved the study protocol.

Sample Preparation

Venous blood was collected during routine control examinations using Venoject vacutainers containing lithium heparin as an anticoagulant. Blood was centrifuged (4000 g, 10 min, 4°C) to separate the plasma. The collected plasma was stored at –70°C. 200 µl of ethyl alcohol was added to 200 µl of plasma (1:1; v/v), mixed on a vortex and then 2 ml of hexane was added. Samples were shaken for 5 min and centrifuged for 2 min (4000 g). The hexane layer was transferred to other test-tubes. Extraction was repeated, hexane layers were mixed and evaporated to dryness under the argon atmosphere. The dry residue was dissolved in 200 µl of mobile phase; 20 µl of the sample was collected for chromatographic analysis. The Alpha Diagnostics test was used to determine cholesterol concentration in plasma.

Apparatus and Determination Conditions

An integrated analytical system of high-pressure liquid chromatography (HPLC), Waters 2695 Integrity System equipped with a UV-VIS detector (Waters 996) with the range of 190–800 nm was used to determine the study compounds. Chromatographic separations were performed on a LC-NH2-NP column (25 cm · 4.6 mm, 5 µm) with the Supelguard ™ LC-NH2-NP precolumn (SUPELCO). Determination conditions: mobile phase – hexane: ethyl octane (7:3; v/v), mobile phase flow – 0.8 ml/min, injection volume – 20 µl, length of analytical wave: 454 nm – β-carotene, 325 nm – retinol (vitamin A), 292 nm – D1- α-tocopherol (vitamin E).

Linearity, Operating Range, Limits of Determinability and Detection

To assay the range of linearity, limits of determinability and detection, a series of 10 standardized samples with known concentrations was analyzed. Regression parameters were calculated using the least square method. The limits of detection were defined as the concentration value of the studied compound for which the signal (S) to noise (N) ratio was higher than 3 (S/N > 3).

Extraction Capacity

Extraction capacity was defined by comparing nominal concentrations with concentrations of samples extracted following the addition of the known amount of standards. Three samples were analyzed for each point of standard curve.

Correctness

To check the correctness of the applied analytical methods, two human plasma concentrations of each compound, vitamin A, vitamin B and β-carotene, were determined in the certified reference material (Fast Soluble Vitamins, Carotenoids in Human Serum, NIST 968, USA).

Precision and Repeatability

Precision and repeatability were determined by a four-fold analysis of the same sample of known concentrations for vitamin A, vitamin E and β-carotene.

Results

Chromatographic Analyses

The assayed vitamins were identified by comparing the retention time of the compounds with the retention time of the standards. The following values were obtained: 4.06 min for β-carotene, 5 min for vitamin E (D1- α-tocopherol), and 8.3 min for vitamin A (retinol).

Based on the analysis of 10 standard samples of known concentrations, the limit of detection, determinability, operating ranges, and linearity were assayed (Table 1).

Extraction Capacity

Extraction capacity was 85.3 (13.3)% for vitamin A, 98.2 (11.0)% for vitamin E, and 85.5 (5.9)% for β-carotene (mean ±SD).

Correctness of the Method

Concentrations of the compounds and their nominal values along with the correctness of the method are given in Table 2.

Precision and Repeatability

Precision and repeatability were assayed by a four-fold analysis of samples of the nominal concentration. The values were: 1.5 μg/ml for vitamin A, 15 μg/ml for vitamin E, and 1.5 μg/ml for β-carotene. Coefficients of variation (CV%) amounted to 5.9, 2.3, and 1.5 respectively.

Samples

Concentrations of β-carotene, vitamin A, and vitamin E were determined by age and duration of employment in blood serum of 147 drivers under study (Table 3).

Discussion

The applied HPLC method allows for precise determinations of the studied compounds: β-carotene, vitamin A, and vitamin E. Our analysis of the quantitative data on

Table 1. Characteristics of the method used to determine vitamin A, vitamin E, and β-carotene.

Compound	Detection limit	Determinability	Operating range	Linearity r
β-Carotene	0.056 μmol/l	0.14 μmol/l	0.14 – 0.37 μmol/l	0.999
			0.23 – 4.66 μmol/l	0.999
Vitamin A	0.14 μmol/l	0.44 μmol/l	0.44 – 17.45 μmol/l	0.999
Vitamin E	0.696 μmol/l	2.32 μmol/l	2.32 – 46.45 μmol/l	0.999

r – correlation coefficient

Table 2. Comparisons between the certified reference material and nominal values of that material.

Compound	Reference material x_{ref} (μmol/l)	Determined concentration x_i (μmol/l)	Correctness $(x_i - x_{ref})\cdot 100/x_{ref}$ (%)
β-Carotene	0.318 ± 0.032	0.307 ± 0.014	-3.5
	0.812 ± 0.064	0.766 ± 0.016	-5.7
Vitamin E	17.3 ± 1.1	19.40 ± 0.83	12.1
	39.0 ± 1.8	34.85 ± 0.81	-10.6
Vitamin A	1.69 ± 0.041	1.84 ± 0.045	8.8
	2.93 ± 0.094	3.16 ± 0.119	7.8

Table 3. Concentrations of β-carotene, vitamin A, and vitamin E.

Group		No. of subjects	Plasma concentration of study compounds (μmol/l)		
			β-Carotene	Vitamin A	Vitamin E
Total		147	1.01 ± 074	4.1 ± 0.9	22.8 ± 7.3
Smokers		94	0.95 ± 0.63	4.1 ± 1.0	23.4 ± 8.1
Non-smokers		53	1.12 ± 0.91	4.0 ± 0.8	21.8 ± 5.5
Age (years)	< 35	51	1.09 ± 0.86	4.1 ± 0.9	22.9 ± 7.3
	36 – 45	53	1.09 ± 0.84	4.1 ± 1.0	21.9 ± 6.2
	> 46	43	0.80 ± 0.35*	4.0 ± 08	23.9 ± 9.2
Duration of employment (years)	1 – 5	35	1.06 ± 0.61	42 ± 1.1	23.2 ± 7.2
	6 – 10	39	1.23 ± 1.15	4.2 ± 1.1	23.4 ± 9.9
	11 – 15	35	0.95 ± 0.59	4.1 ± 0.9	22.1 ± 5.2
	>16	38	0.80 ± 0.26#	3.8 ± 0.9	22.6 ± 5.8

*- $p < 0.05$ compared to the age groups, # – $p < 0.05$ compared to the groups employed for 1 to 5 years

Table 4. Comparison of plasma β-carotene, vitamin A and vitamin E concentrations in people living in different regions of the world.

Study group [reference]	β-Carotene (μmol/l)	Vitamin A (μmol/l)	Vitamin E (μmol/l)
Healthy men living in Łódź, Poland	1.01 ± 0.74	4.1 ± 0.9	22.8 ± 7.3
Japanese living in the USA [18]	0.75 ± 0.58	2.52 ± 0.60	30.39 ± 13.73
Japanese living in Japan [18]	0.49 ± 0.47	2.55 ± 0.66	20.17 ± 7.84
People of European origin living in the USA [18]	0.63 ± 0.65	2.77 ± 0.53	29.19 ± 11.54

Table 5. Plasma β-carotene, vitamin A and vitamin E concentrations (converted into) in terms of mg cholesterol, depending on age and duration of employment in the examined drivers.

Group		No. of subjects	Plasma concentration of study compounds μmol/mg cholesterol[A]		
			β-carotene	Vitamin A	Vitamin E
Total		147	0.25 ± 0.17	0.56 ± 0.21	4.78 ± 2.41
Smokers		94	0.24 ± 0.14	0.58 ± 0.23	4.98 ± 2.62
Non-smokers		53	0.27 ± 0.20	0.54 ± 0.8	4.44 ± 1.98
Age (years)	< 35	51	0.29 ± 0.19	0.62 ± 0.27	5.27 ± 2.71
	36 – 45	53	0.26 ± 0.18	0.53 ± 0.16	4.3 ± 11.46
	> 46	43	0.19 ± 0.09	0.54 ± 0.17	4.78 ± 2.88
Duration of employment (years)	1 – 5	35	0.29 ± 0.16	0.64 ± 0.31	5.34 ± 2.67
	6 – 10	39	0.29 ± 0.23	0.57 ± 0.15	4.93 ± 3.07
	11 – 15	35	0.23 ± 0.14	0.54 ± 0.18	4.34 ± 2.17
	>16	38	0.20 ± 0.07	0.52 ± 0.15	4.53 ± 1.38

[A] – arithmetic mean (± SD)

these compounds did not reveal significant differences in their concentrations between the study groups, except for the concentration of β-carotene in the drivers aged over 46 years, which was significantly lower than in the younger groups. A significantly lower concentration of β-carotene was also found in the group of drivers with the longest duration of employment (16 years and more) compared to the drivers with a shorter duration of employment (1–5 years). It is likely that low β-carotene concentrations in the oldest group of drivers is associated with low consumption of β-carotene-rich products [17]. After having compared the results of our studies with those of other authors, we can conclude that the concentrations of the studied compounds do not differ from those observed in healthy people living in other regions of the world (Table 4).

The amounts and sources of vitamin A differ depending on the region. In developing countries, carotenoids are the major source of vitamin A, except for Africa, where it comes from vegetable oils, whereas in Asian countries vitamin A comes almost entirely from fruits and vegetables or vegetables and eggs. In the majority of studies, β-carotene, vitamin A, and vitamin E concentrations are expressed in μmoles, but bearing in mind that the fat-soluble vitamins are transported in the body along with lipoprotein fractions, some of the authors are of the opinion that the conversion of their concentrations into mg cholesterol produces more precise results [19, 20]. Table 5 gives concentrations of the study compounds in μmol/mg cholesterol. Even with this approach, no significant differences between study groups were observed.

Conclusions

Concentrations of β-carotene, vitamin A and vitamin E assayed in the serum of healthy people working in road traffic do not significantly differ from those reported by other authors. Lower plasma β-carotene concentrations in the group of drivers aged over 46 years may result from their dietary habits, i.e. low intake of products rich in β-carotene.

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