

Does the plasma level of vitamins A and E affect acne condition?

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Summary

Background. Vitamin A and E are lipid soluble antioxidants that are necessary for our health. Deficiency in these vitamins can cause serious diseases. Administration of vitamin A and E to patients with acne was shown to improve their acne condition.

Aims. To test the relationship between plasma vitamin A and E levels and acne.

Methods. Plasma vitamin A and E concentrations were determined by high performance liquid chromatography in 100 newly diagnosed untreated patients with acne and were compared with those of 100 age-matched healthy controls. Patients were carefully graded using the Global Acne Grading System.

Results. We found that plasma vitamin A concentrations in patients with acne were significantly lower than those of the control group (336.5 vs. 418.1 µg/L, respectively) $P = 0.007$. We also found that plasma vitamin E concentrations in patients with acne were significantly lower than those of controls (5.4 vs. 5.9 mg/L) $P = 0.05$. In addition, we found that there is a strong relationship between decrease in plasma vitamin A levels and increase in the severity of acne condition. Patients with severe acne had significantly lower plasma concentrations of vitamins A and E than did those with lower acne grade and the age-matched healthy controls.

Discussion. Based on our results, we conclude that low vitamin A and E plasma levels have an important role in the pathogenesis of acne and in the aggravation of this condition.

Introduction

Vitamins A and E, the main lipid-soluble antioxidants, are a group of organic compounds occurring naturally in food and are necessary for good health. Lack of these vitamins can lead to a number of diseases.^{1,2}

Vitamin A is required for vision, reproduction, and for the maintenance of differentiated epithelia and mucus secretion.^{1,3} It is also necessary for correcting defects in keratinization in a number of skin diseases, especially in acne vulgaris.^{4,5} Oral and topical vitamin A was reported to improve and significantly modify acne

condition.^{4,6} 13-*cis*-retinoic acid, a form of vitamin A, has a highly effective inhibitory effect on sebaceous gland activity and sebum production, which leads to a significant decrease in the population of *Propionibacterium acnes*, a bacterium known to be involved in the inflammation process of acne.⁷⁻⁹ The comedogenesis process was usually suppressed by retinoids, as they influence the differentiation of epidermal cells and keratin production, and stop the development of the comedones into more severe inflammatory lesions.⁸⁻¹²

Vitamin E is widely distributed in foods, the richest sources being vegetable oils and their products. The D- α -tocopherol form of vitamin E in plasma is an effective antioxidant in stabilizing unsaturated lipids against auto-oxidation. It accumulates in circulating lipoproteins and in cellular membranes, where it reacts rapidly with molecular oxygen and free radicals, acting as a scavenger for these compounds, protecting membranes, lipoproteins and fatty acids from peroxidation reactions,

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providing a high level of protection to skin against ultraviolet irradiation, and enhancing the immune response.^{13–16}

Oral synergism of vitamins A and E in the treatment of acne was reported to significantly improve acne condition.^{11,17} To our knowledge, there are no previous studies investigating the relationship between the status of plasma vitamin A and E concentrations and acne condition. Thus, in this work we measured plasma retinol and D- α -tocopherol levels in patients with acne to test the relationship between these levels and the appearance and severity of acne.

Materials and methods

In total, 200 participants were enrolled in this study. Of these, 100 were patients with untreated acne, of both genders, either attending the Dermatology Clinic at Princess Basma Teaching Hospital or students at Jordan University of Science and Technology in Irbid. The other 100 were age-matched healthy volunteers, who formed the control group. They were not taking any medication and had no personal or family history of acne. Both groups were interviewed with full informed consent. Acne grading for each patient was based on Global Acne Grading System (GAGS).¹⁸

Venous blood samples were collected in EDTA tubes after 12–14 h of fasting and immediately spun in a low-speed refrigerated centrifuge (Hettich Zentifugen D.78532; Tuttlingen, Germany) at 1500 *g* for 30 min at 4 °C to separate the plasma. Plasma was divided into aliquots and kept frozen at –30 °C until used for analysis.

For the measurement of plasma vitamin A and E levels, the following chemicals, all high-performance liquid chromatography (HPLC) grade, were used; *n*-hexane (95% purity, Laboratory Chemicals, Germany), methanol (99.9% purity, Riedel-de Haen, Germany), ethanol (99.8% purity, Hayman Limited, UK), anhydrous diethylether (Scharlau Chemies, Spain) and water (Merck, Germany). Vitamin A standards (all-*trans*-retinol, all-*trans*-retinyl acetate) with 99.0% purity for HPLC analysis were purchased from Fluka Chemie GmbH (USA). Vitamin E standards (D- α -tocopherol and D- α -tocopheryl acetate) with 98.0% purity for HPLC analysis were obtained from Applichem Biochemica Chemica Synthesis Services (Germany).

Plasma retinol (vitamin A) and α -tocopherol (vitamin E) were measured by reverse-phase HPLC as described by George *et al.*¹⁹ Briefly, plasma was deproteinized with ethanol containing the internal standards for vitamin A and E (all-*trans*-retinyl acetate and D- α -tocopheryl

acetate, respectively). Vitamins A and E were extracted using hexane and then evaporated to dryness under a stream of nitrogen. The residues were dissolved in diethyl ether and gently mixed before dilution with methanol solvent. A sample (100 μ L) of this solution was injected into the reverse-phase C18 HPLC column (250 mm in length \times 4.6 mm internal diameter). The mobile phase consisted of 950 mL of methanol solvent diluted to 1000 mL with deionized water. This solution was filtered and de-gassed before being used. The flow rate of the mobile phase was set at 2.5 mL/min. The absorbance of vitamins A and E and their internal standards were measured at 280 nm. The peak heights were recorded and used to quantify vitamin concentrations. Sample handling and measurements were carried out in the dark or with light protection to prevent photodestruction of vitamins. Stock solutions of the alcohol and acetate forms of vitamins A and E were prepared and used to construct the standard curve for each vitamin (Figs 1, 2).

Statistical analysis

Statistical analysis of the data was performed using SPSS software (SPSS Inc., Chicago, IL, USA). Student's *t*-test was performed to investigate the significance of the mean differences between two groups. ANOVA was used to compare between more than two mean groups. Differences were considered significant at $P = 0.05$.

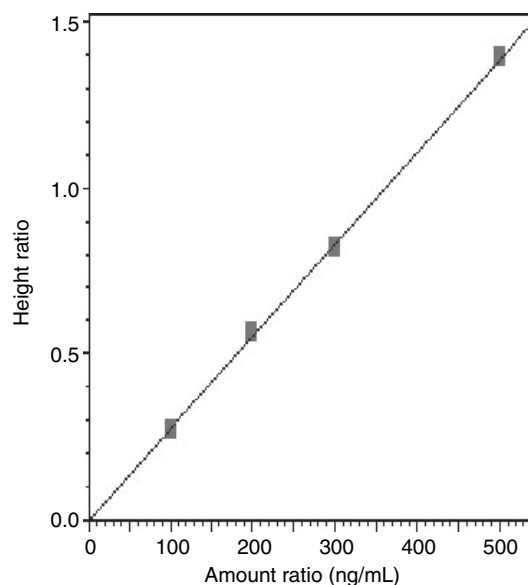


Figure 1 Standard curve of vitamin A. Peak height ratio (*y*-axis) vs. amount ratio (*x*-axis) for (vitamin A) retinol/retinyl acetate (internal standard), slope = 0.99946.

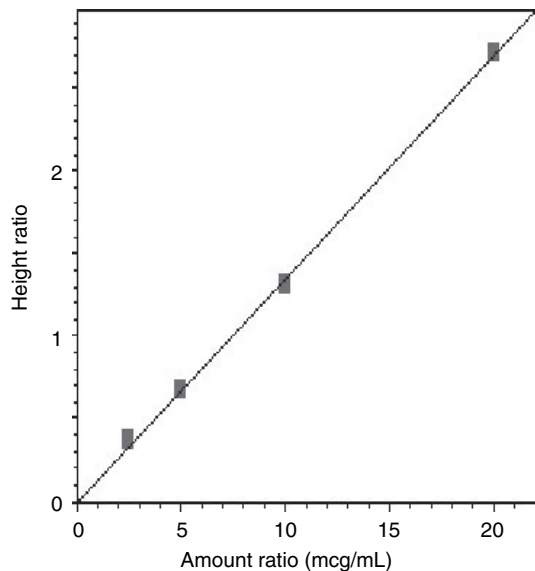


Figure 2 Standard curve of vitamin E. Peak height ratio (*y*-axis) vs. amount ratio (*x*-axis) for (vitamin E) *D*- α -tocopherol/*D*- α -tocopheryl acetate (internal standard), slope = 0.999127.

Results

There were 100 untreated patients with acne and 100 age-matched controls included in this study. The average age of patients with acne and controls was 21.0 ± 5.4 and 21.3 ± 5.3 years, respectively. The mean weight of patients with acne was 65.7 kg, and

that of controls 64.3 kg. Patients with acne were divided into three groups according to the severity of their acne condition using GAGS. Mild acne scored 6–18, while the moderate and severe grades scored 20–30 and 31–36, respectively. Overall, 30 patients had mild, 45 had moderate and 25 had severe acne. Standard curves of vitamin A and E were linear through all recommended concentrations (as shown in Figs 1 and 2, respectively). The slope for the standard curves of vitamin A and vitamin E were 0.99946 and 0.999127, respectively.

Figure 3 demonstrates the chromatogram of normal plasma vitamin A (all-*trans*-retinol) and vitamin E (*D*- α -tocopherol) peaks with the internal standard for each vitamin. The retention times for all-*trans*-retinol, all-*trans*-retinyl acetate, *D*- α -tocopherol and *D*- α -tocopheryl acetate were 3.2 min, 4.68 min, 13.47 min and 19.03 min, respectively. Peaks of vitamin A and vitamin E in plasma samples were identified by comparing their retention times with those of the standards and quantified from the standard curves.

We found that plasma vitamin A (retinol) and vitamin E (α -tocopherol) concentrations were significantly lower in patients with acne ($336.5 \pm 119.7 \mu\text{g/L}$, $P = 0.007$; and $5.4 \pm 2.1 \text{ mg/L}$, $P = 0.05$, respectively), than in control subjects ($418.1 \pm 100.4 \mu\text{g/L}$ and $5.9 \pm 1.4 \text{ mg/L}$) (Table 1).

In this study, we found that there is a strong relationship between plasma vitamin A and E concen-

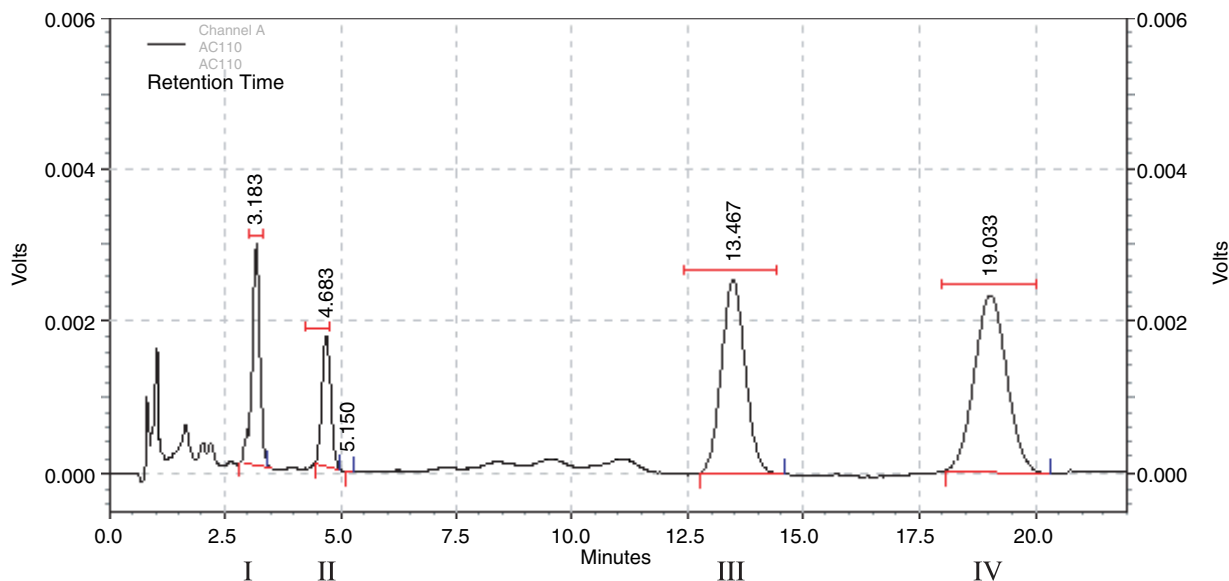


Figure 3 Chromatogram of normal plasma sample with internal standards for each vitamin (A and E). Peaks: I, retinol (vitamin A); II, all-*trans*-retinyl acetate (vitamin A internal standard); III, *D*- α -tocopherol (vitamin E); and IV, *D*- α -tocopheryl acetate (vitamin E internal standard). X-axis is the retention time (minutes).

Table 1 Vitamin A and vitamin E plasma concentrations in patients with acne and controls.

Parameters	Patients with acne (n = 100)	Controls (n = 100)	P-value
Vitamin A*	336.5 ± 119.7	418.1 ± 100.4	< 0.05
Vitamin E	5.4 ± 2.1	5.9 ± 1.4	< 0.05

*Plasma vitamin A values expressed in mean ± SD (µg/L); plasma vitamin E values expressed in mean ± SD (mg/L); significant differences, $P = 0.05$.

trations and the severity of acne. Significant differences were noticed in plasma vitamin A concentrations between controls and patients with moderate acne, controls and patients with severe acne, patients with mild and moderate acne, patients with mild and severe acne, and patients with moderate and severe acne. Vitamin A concentration was lowest in patients with severe acne with a trend towards decreasing vitamin A concentration from mild to moderate to severe grades. Vitamin E plasma levels were significantly lower in patients with severe grades of acne compared with those of controls, and with patients with mild and moderate acne (Table 2).

Discussion

Based on our knowledge about the anti-inflammatory effect of vitamin A, we suggest that insufficient plasma vitamin A concentrations might aggravate acne in patients, as low plasma vitamin A concentrations can influence the acne process through increasing the size and the activity of the sebaceous glands that produce much of the sebum, which leads to a significant increase in the growth of *P. acnes*. This enhances inflammation by increasing the levels of toxic oxygen metabolites and neutrophils accumulation in the affected tissue. Low concentrations of vitamin A also increase cell shedding, keratin production and the 'stickiness' of cells in the follicles, which helps in the development from comedones to more severe inflammatory lesions and thus increases the disease duration.^{4,7-12} This suggestion is in agreement with our results, which showed that acne

is more severe among patients with low concentrations of plasma vitamin A compared with controls. There was an obvious and significant correlation between plasma vitamin A concentrations and acne severity. The decrease in plasma vitamin A concentrations was associated with an increase in the severity of acne. These findings demonstrated the importance of considering low concentrations of plasma vitamin A as a risk factor for the occurrence of severe acne conditions. Our results also confirm the observations made by many other researchers, who have reported that administration of oral vitamin A to patients with severe acne significantly improved their acne condition.^{4,6-11,20}

Concerning vitamin E levels in patients with acne, which were found to be significantly low in severe cases compared with controls, we have to note that severity of acne condition is not directly related to plasma vitamin E concentrations. The decrease in plasma vitamin A concentrations observed in our patients with acne could be due to the increased demands by the infective organism or to the nutritional and metabolic status of the body. The decrease in plasma vitamin A concentrations that is directly proportional to the severity of acne, which has been clearly demonstrated in our work, might be due to the increased consumption of vitamin A as an anti-inflammatory agent in acne. David Shoseyov and coworkers demonstrated that vitamin A utilization is increased during repeated allergen challenge and allergic bronchitis in rats.²¹ On the other hand, restricted intake of foods rich in vitamin A (retinol) or its provitamin β-carotene, may cause insufficient plasma concentrations of this vitamin. Patients with acne in our study mentioned that their diet was not high in vegetables, fruits and meats, and that they concentrated on eating carbohydrates and fast foods. Absorption of vitamin A and its transport, associated with plasma lipoproteins or via retinol-binding proteins, can also influence its plasma concentration.

The expected reason for low plasma vitamin E concentrations in patients with acne, besides low intake, might be the consumption of vitamin E as an antioxidant agent in the maintenance of normal immune function in patients with acne. It protects the immune

Table 2 Vitamin A and E plasma concentrations in controls and patients with acne with different grades of acne.

Parameters	Controls (n = 100)	Mild acne (n = 30)	Moderate acne (n = 45)	Severe acne (n = 25)	P-value
Vitamin A*	418.1 ± 100.4	398.8 ± 100.9	355.2 ± 81.8	202.8 ± 53.5	< 0.05
Vitamin E	5.9 ± 1.4	5.9 ± 1.7	5.7 ± 2.3	4.1 ± 1.4	< 0.05§

*Plasma vitamin A values expressed in mean ± SD (µg/L); plasma vitamin E values expressed in mean ± SD (mg/L). Significant differences at $P = 0.05$ between pairs of groups: control and moderate, control and severe, mild and moderate, mild and severe, and moderate and severe; §significant difference at $P = 0.05$ between pairs of groups: control and severe, mild and severe, and moderate and severe.

cells from reactive oxygen species produced during the inflammatory process and prevents the oxidation of polyunsaturated fatty acids in the skin of those patients. In general, exogenous factors such as smoking have been reported to decrease plasma concentrations of vitamins A and E.^{2,22} This was also observed in our study, comparing patients with acne who were smokers with nonsmoking patients, but the difference was not statistically significant. Thus, the effect of smoking on acne in our patients was negligible and these results were not included due to the small number of the smokers in this study.

In conclusion, the observation of low plasma concentrations of vitamin A and vitamin E in Jordanian patients with acne is consistent with our hypothesis that low plasma concentrations of vitamins A and E might play a big role in the development of acne.

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