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Originalarbeiten

Studies on the tocopherol status in blood serum of premature babies and infants

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Summary: The level of naturally occurring tocopherols in blood serum of 88 preterm infants, aged from birth to 2 years, was determined by the high performance liquid chromatographic (HPLC) method using fluorescence detection.

In 11 cases, patients were assayed for their tocopherol status in longitudinal studies, receiving known amounts of vitamin E supplements orally and/or parenterally. No correlation was found between serum alpha-tocopherol level and gestational age nor birth weight. All preterm infants receiving the vitamin E preparation showed an average serum tocopherol content of more than 0.5 mg/100 ml. The highest alpha-tocopherol concentration registered during vitamin E therapy was 3.28 mg/100 ml. Infusion of Intralipid, a product derived from fractionated soybean oil, caused a significant increase of gamma- and delta-tocopherol in blood serum. The half-life of delta-tocopherol in serum was calculated to be about 24 hours.

Zusammenfassung: Bei 88 Frühgeborenen im Alter von 0 bis 2 Jahren wurde der Gehalt an natürlich vorkommendem Tocopherol im Blutserum durch Hochleistungsflüssigkeitschromatographie (HPLC) mit fluorimetrischer Detektion bestimmt.

In 11 Fällen wurde während eines längeren Zeitraums der Tocopherolstatus von Patienten, die bekannte Vitamin-E-Präparate oral und/oder parenteral aufnahmen, untersucht. Es wurde keine Korrelation zwischen alpha-Tocopherol-Spiegel und Gestationsalter bzw. Geburtsgewicht beobachtet. Alle Frühgeborenen, die Vitamin-E-Präparate erhielten, wiesen im Durchschnitt Serum-Tocopherolgehalte von mehr als 0,5 mg/100 ml auf. Die höchste alpha-Tocopherolkonzentration, die während einer Vitamin-E-Therapie gemessen wurde, war 3,28 mg/100 ml. Eine Infusion von Intralipid, einem Produkt, das aus fraktioniertem Sojaöl erhalten wird, verursachte einen signifikanten Anstieg der gamma- und delta-Tocopherolgehalte im Blutserum. Für die Halbwertszeit von delta-Tocopherol im Serum wurde ein Wert von etwa 24 Stunden berechnet.

Key words: Vitamin E, tocopherol, premature infant, HPLC

Introduction

Newborn infants have small body stores of vitamin E, particularly those born prematurely (1). In addition this lipid soluble vitamin is poorly absorbed from the gastrointestinal tract of preterm babies (2). As a natural antioxidant and free-radical scavenger vitamin E plays a role in the protec-

tion against the development of retrolental fibroplasia, bilirubinemia and other syndromes caused by peroxidative damage of cell membranes (3–5).

The purpose of our investigation was: 1) to establish a vitamin E profile, evaluating all naturally occurring tocopherols, and 2) to determine the influence of several vitamin E supplements on the amount and distribution of the four individual tocopherols in serum.

The development of high performance liquid chromatography (HPLC) systems in combination with fluorometric detection offers optimal instrumental conditions in separating and quantifing all tocopherols, being both very sensitive and specific. Compared with the other methods of vitamin E determination, the preparation of blood serum samples without saponification or derivation before chromatography has the advantage of a rapid and non-destructive procedure.

Materials and methods

Serum vitamin E levels of 88 patients were determined; the ages ranged from birth to 2 years. Serum samples were obtained from the Children's Hospital of the University of Kiel. In 11 preterm infants an attempt was made to establish the vitamin E consumption per day in relation to the registered serum tocopherol level. All children received as special vitamin E preparation E-Vicotrat (Heyl & Co.). In the case of parenteral nutrition, solutions of Intralipid and Multibionta (Merck) were given by infusion additionally. Orally applied food consisted of Pre-Aptamil

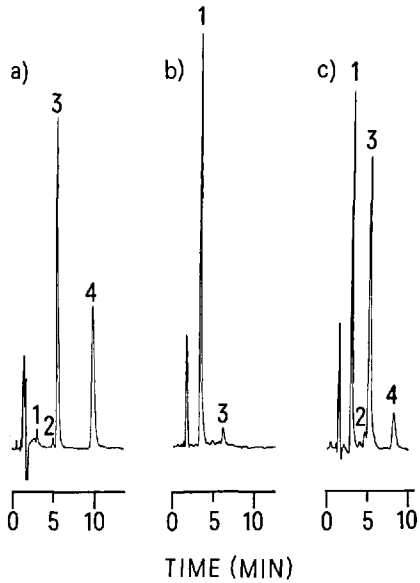


Fig. 1. Chromatograms obtained for separation of tocopherols in Intralipid a) and blood serum extracts of a 2 month aged infant before b) and after Intralipid infusion c).

1: alpha-tocopherol
2: beta-tocopherol
3: gamma-tocopherol
4: delta-tocopherol

Table 1. Alpha-tocopherol contents of several infant diets.

Preparation	Active substance	Content of D-alpha-tocopherol
E-Vicotrat	DL-α-TA	100 mg/ampoule
Multibionta, parent.	DL-α-TA	5 mg/10 ml
Multibionta, orally	DL-α-TA	4 mg/35 drops
Intralipid	DL-α-T	0.005 mg/ml
Pre-Aptamil	DL-α-TA	0.6 mg/100 ml
Humana I	DL-α-T	1.5 mg/100 ml
Alfaré	DL-α-TA	6 mg/100 g

T = tocopherol; TA = tocopherol acetate

(Milupa), Humana I (Humana), Alfaré (Nestlé) or mother's milk. Multibionta was also administered orally dropwise. The alpha-tocopherol contents of these preparations and infant foods are listed in Table 1. The infusional solution of Intralipid contains 10 g of fractionated soybean oil per 100 ml and shows the characteristic distribution of the four individual tocopherols (Fig. 1a). Beyond this the variation of serum tocopherol levels of preterm infants given known amounts of vitamin E with their food was studied during a longer period.

Blood serum was prepared for analysis immediately after separation from red blood cells; only in some cases the samples were 2 days old. Extraction of tocopherols was assayed in regard to the method of Jansson et al. (6). For the analysis 200 µl serum was pipetted into a 10 × 50 mm centrifuge glass tube and deproteinated with 100 µl abs. ethanol by mixing for 30 s on a Vortex mixer. 500 µl distilled n-hexane was then added to the tube, the contents mixed vigorously for 1 min and centrifuged at 4000 g for 5 min. The supernatant phase was removed and the extraction repeated twice. The combined unpolar phases were dried under nitrogen, reconstituted in 100 µl n-hexane and 20 µl was injected for HPLC analysis (Fig. 1b, c).

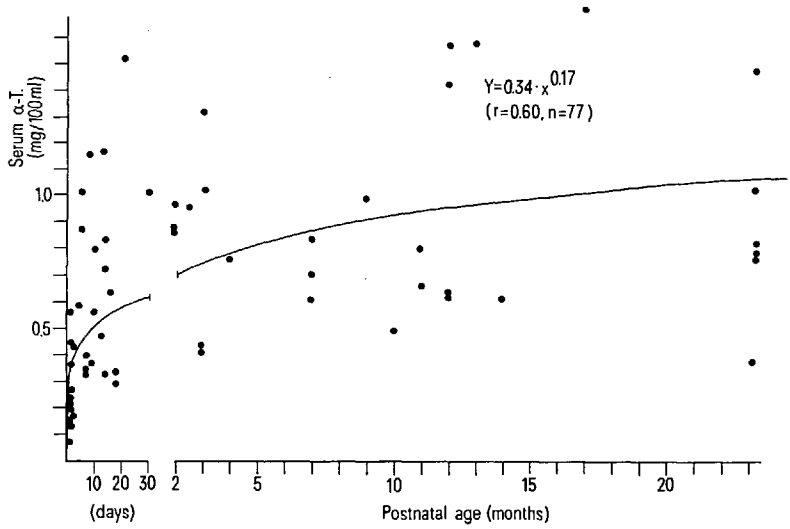


Fig. 2. Relationship between alpha-tocopherol (α-T.) level and postnatal age.

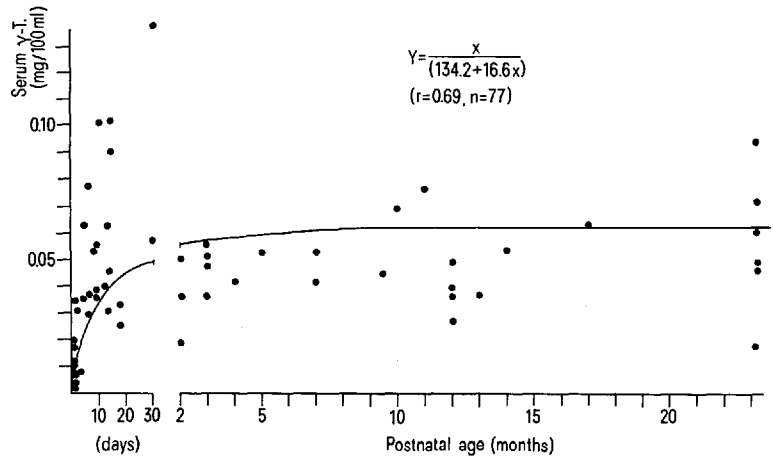


Fig. 3. Relationship between gamma-tocopherol (γ-T.) level and postnatal age.

Vitamin E content in serum samples was assayed by the following HPLC system: Waters Associates, Inc., consisting of a model 6000 A solvent delivery system, a model WISP 710 B automatic liquid chromatographic injector, a model RCM 100 radial compression separation system with Rad Pak cartridge 8 Si 5 μ and a model FS 970 fluorescence detector (Schoeffel).

The flow rate of the binary mobile phase used (isooctane: isopropanol, 99.5:0.5 v/v) was 2.2 ml/min. The column effluent was monitored at an excitation of 206 nm with an emission filter of 340 nm.

Registered peak areas were determined by an integrator C-R 1 B (Shimadzu). Serum tocopherol contents were calculated by the method of external standard with a two point calibration curve.

Results

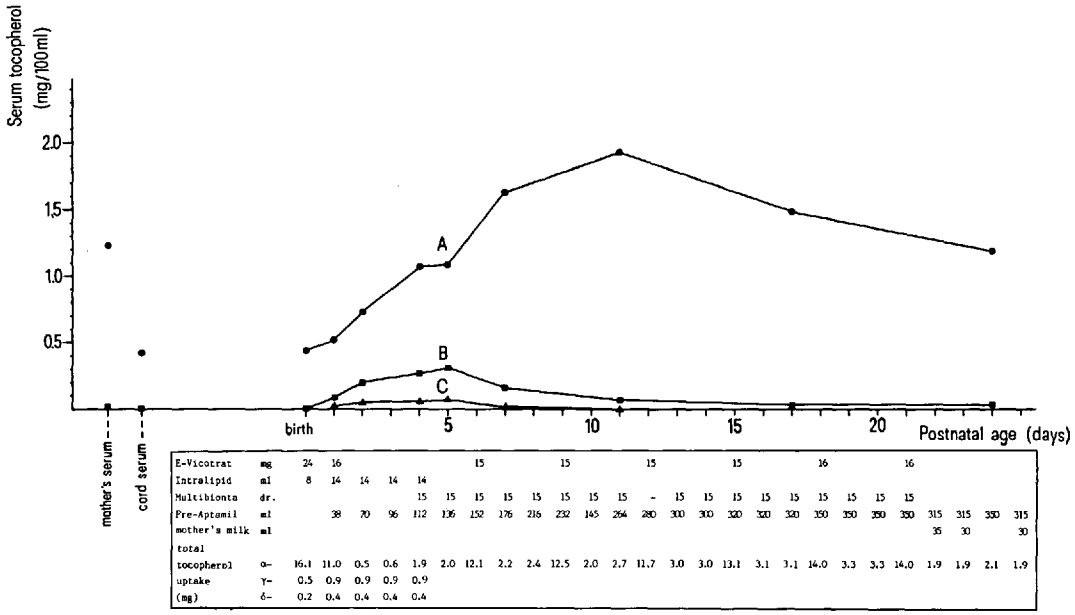
Profile study:

Figures 2 and 3 show the variation of alpha- and gamma-tocopherol levels of all 77 infants examined, in relation to time.

According to the highest correlation coefficients a potential and a hyperbolic function, respectively, were selected as regression curves.

Table 2. Geometric mean serum concentrations of alpha- (α-T.) and gamma-tocopherol (γ-T.) in neonate blood serum. (mg/100 ml).

Gestational age (month)	Birth weight (g)	Sex	α-T.	γ-T.
29	1010	♀	0.60	0.02
29	1230	♂	0.45	0.03
29	1300	♂	0.16	0.01
31	1650	♂	0.45	0.01
32	1500	♂	0.30	0.05
34	2200	♂	0.26	0.01
35/36	1740	♀	0.53	0.02
38	2900	♂	0.55	0.00



E-Vicotrat	mg	24	16						15			15			15			15			16			16						
Intralipid	ml	8	14	14	14	14																								
Multibionta	dr.					15	15	15	15	15	15	15	15	-	15	15	15	15	15	15	15	15	15							
Pre-Aptamil	ml		38	70	96	112	136	152	176	216	232	165	264	280	300	300	320	320	330	350	350	350	350	315	315	350	315			
mother's milk	ml																							35	30		30			
total tocopherol	α-	16.1	11.0	0.5	0.6	1.9	2.0	12.1	2.2	2.4	12.5	2.0	2.7	11.7	3.0	3.0	13.1	3.1	3.1	14.0	3.3	3.3	14.0	1.9	1.9	2.1	1.9			
uptake	γ-	0.5	0.9	0.9	0.9	0.9																								
(mg)	δ-	0.2	0.4	0.4	0.4	0.4																								

Fig. 4. Tocopherol serum levels of preterm infant (birth weight: 1650 g, gestational age at birth: 31 weeks) during vitamin E therapy. (A: alpha-tocopherol, B: gamma-tocopherol, C: delta tocopherol).

During the first months of life, the mean alpha-tocopherol levels increased from 0.527 ± 0.334 (0–1 month) to 0.914 ± 0.402 (1–6 months) and 1.027 ± 0.450 (6–24 months) mg/100 ml. In contrast to this finding mean gamma-tocopherol contents of these three age groups did not differ significantly. There was no correlation between these two tocopherols in serum.

Alpha- and gamma-tocopherol levels are demonstrated in Table 2 for 8 cases at the time of birth. No correlation was seen between the alpha-tocopherol content and gestational age nor birth weight. The mean values for alpha- and gamma-tocopherol were 0.41 ± 0.16 ($x \pm s$) and 0.02 ± 0.02 mg/100 ml serum, respectively.

Infants receiving Intralipid infusion show a significantly higher content of gamma-tocopherol in blood serum. Respectable amounts of delta-tocopherol, which has normally not been detected, were also registered (Fig. 1c). The level of gamma- and delta-tocopherol in sera depends on the dosage and duration of Intralipid infusion. A gamma-tocopherol content of more than 0.5 mg/100 ml was reached frequently.

Longitudinal study:

The exogenous vitamin E supplements of 11 premature babies were monitored in longitudinal studies. In Figure 4 the relation between tocopherol concentration in serum and age is presented from one of these premature babies in detail; the results of the other 10 cases show analog-

ous effects. The daily dosage of medicines and the corresponding calculated vitamin E content of supplements are listed below the figure. In all preterm babies alpha-tocopherol values increased after infusion with E-Vicotrat, Intralipid and Pre-Aptamil. Already at the first day after birth, serum has a tocopherol content of more than 0.5 mg/100 ml on average, which is recognized as a lower than normal limit. The highest serum alpha-tocopherol concentration obtained during vitamin E therapy was measured at 3.28 mg/100 ml, but the mean maximum value was 2.29 mg/100 ml.

After reaching the maximum, the alpha-tocopherol level fell back to a lower plateau between 1.0 and 1.5 mg/100 ml. Infusion of Intralipid caused a significant increase of gamma- and an appearance of delta-tocopherol in blood serum in detectable amounts. On stopping the supply of non-alpha-tocopherols, the concentrations decreased to the former levels.

Discussion

Most investigations of human vitamin E status have been carried out as single determinations of total tocopherol or of alpha-tocopherol using the photometrical Emmerie-Engel method (7). Many studies failed to distinguish the four individual tocopherols of differing biological activity (8). Because the non-alpha-tocopherols are known to be present in human breast milk, commercial formulas, and other nutritional products, it has become important to separate blood tocopherols by a new sensitive and selective technique such as HPLC.

Only a few studies on the relationship between infant formula diets or tocopherol supplements and blood serum tocopherol of premature babies have been published (9–11). Mino et al. (12) determined larger amounts of alpha-tocopherol in plasma of breast-fed infants in comparison to bottle-fed infants.

The vitamin E profile of our examined group of 77 infants leads to no significant correlation between serum tocopherol level and age. This is ascribed to the heterogeneous composition of patients in a children's hospital.

This finding was mentioned by other authors (12) recently. Some of the values from Figures 2 and 3, especially in the first month, are very high. This effect could be explained by an increased absorption of the vitamin E administered.

There is no correlation between alpha- and gamma-tocopherol levels. The intravenous supplementation of Intralipid or other artificial formulas significantly influences the level of gamma- and delta-tocopherol in blood serum. On the other hand, high dosage of alpha-tocopherol causes a rapid response in its serum concentration.

In order to reduce these influences of different nutritional and medical treatments, a group of 11 children given known amounts of vitamin E with the diets was investigated during a longer period. Since preterm infants absorb orally applied tocopherol poorly, the supply was administered parenterally. The resulting serum alpha-tocopherol concentrations rose to more than 1.2 mg/100 ml. This value is to be regarded as a concentration

limit, preventing risks like retrolental fibroplasia during short pregnancy, low birth weight or malabsorption syndromes (5). Cruz et al. (13) even suppose that a concentration of more than 2 mg/100 ml would be needed to be clinically efficient. An increased appearance of necrotizing enterocolitis caused by a tocopherol level of more than 3.5 mg/100 ml was registered by Sobel (14). In our study this high concentration level was never reached. The portion of absorbed food-tocopherols is very low, generally the alpha-tocopherol supply of daily applied infant formula diet (1.7 mg in the main) was found to be insufficient to stabilize the serum level. Colburn and Ehrenkranz (15) suppose that 4–5 mg per day during the first weeks in the nutrition of preterm infants is enough to maintain serum tocopherol levels. Dallmann (16) and Jansson et al. (17) even recommend 5–10 mg alpha-tocopherol per day to cover the needs.

All analyzed blood samples of children fed a synthetical infant milk contain no detectable amounts of delta-tocopherol. Only in the serum of children infused parenterally with Intralipid, do relatively high concentrations of gamma- and delta-tocopherol directly reach the blood. As demonstrated in Figure 4, the non-alpha-tocopherol level correlates significantly to the infused amount of Intralipid. The calculated turnover rate of delta-tocopherol, when the supply of Intralipid has been stopped, is in good agreement with the recently published half-life value (44 h) for alpha-tocopherol. The half-life of delta-tocopherol determined in our study is clearly lower than that of alpha-tocopherol, in accordance with a formerly postulated sequence (18).

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