

Time course of vitamin E repletion in the premature infant

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Plasma and erythrocyte (RBC) tocopherol-isomer concentrations were determined serially in forty-two premature infants (25–35 weeks gestation) from birth to 8 weeks of age. For comparison purposes vitamin E status was also determined in six term infants over the first 8 d following birth and in a group of thirteen adult volunteers. Vitamin E intakes in term and preterm infants were calculated from recorded food intakes and blood transfusions. In term infants plasma vitamin E concentration rose from 1.9 mg/l (day 1) to 8.2 mg/l by day 8. In comparison preterm plasma vitamin E concentration, 0.3 mg/l (day 1), did not change appreciably by day 8 (0.7 mg/l). Likewise RBC vitamin E concentration increased in term infants from 1.3 mg/l (day 1) to 2.7 mg/l (day 8), while in preterm infants it remained unchanged, 1.5 mg/l (day 1) v. 1.3 mg/l (day 8). Over the 3 weeks following birth, RBC vitamin E concentrations in the premature infants increased to adult values, while plasma vitamin E concentration did not reach the adult range until 8 weeks post-term. These slow changes in plasma vitamin E status occurred even though the vitamin E intake of these infants was similar to that proving adequate for term infants.

Erythrocyte vitamin E: Preterm infant: Vitamin E

Newborn infants have low circulating concentrations of vitamin E (Dju *et al.* 1958; Mino *et al.* 1977). It has been proposed that this is the result of placental impermeability (Wright *et al.* 1951; Dju & Mason, 1952; Cruz *et al.* 1983), and a transient lipoprotein deficiency at birth (Desai *et al.* 1984). In term newborn infants, vitamin E stores and hence circulating concentrations are rapidly replenished postnatally through either breast- or formula-feeding (Phelps & Dietz, 1981; Ostrea *et al.* 1986). Preterm infants, often due to their required management, may not have access to nutrients for several days, and may have restricted food intakes for many weeks. As a consequence the correction of low plasma vitamin E concentrations in preterm infants may take much longer (Bell *et al.* 1979; Gross & Gabriel, 1985; Huijbers *et al.* 1986).

The primary function of vitamin E is that of a lipid-soluble antioxidant, protecting cell membrane polyunsaturated fatty acids from free radical oxidative damage (Mason & Filer, 1947; McCay *et al.* 1972). Knowledge of membrane vitamin E concentration is, therefore, necessary in any study directed towards elucidating the actions of this vitamin. It is generally agreed that low plasma concentrations of vitamin E in preterm infants are associated with low tissue levels (Dju *et al.* 1958; Mino *et al.* 1977). Recently, however, Mino *et al.* (1985), utilizing erythrocyte (RBC) vitamin E concentrations as an indicator of tissue levels, suggested that the frequency of vitamin E deficiency in premature infants may be lower than previously assumed. Unfortunately these investigators did not include the gestational ages of the preterm infants utilized in their study. As only thirteen of the ninety infants considered had birth weights less than 2 kg we have assumed that these were not very premature infants relying on intensive clinical care.

In the present study we have assessed vitamin E status in a population of premature

Table 1. *Clinical details of term and preterm population*

	Term	Preterm
No. of infants studied	6	42
Male	3	25
Female	3	17
Gestational age (weeks)		
Median	40	30
Range	37-41	25-35
Birth weight (kg)		
Median	3.1	1.2
Range	2.2-3.9	0.6-2.4
No. with HMD	—	27
No. receiving oxygen	—	38
No. ventilated	—	37
No. with CLD	—	9
No. receiving blood transfusions	—	22

HMD, hyaline membrane disease; CLD, chronic lung disease.

infants (mean gestational age 30 weeks) by measuring both plasma and RBC tocopherol concentration serially over the first 8 weeks postnatally to determine whether the observations of Mino *et al.* (1985) also apply to infants who are born more prematurely.

METHODS

Study population

The study population consisted of forty-two premature infants admitted to our Unit between October 1986 and March 1988 (Table 1). Infants were excluded from the study if they had congenital malformation or inherited metabolic abnormalities. Consent from an informed parent was required for admission into the study. The study was approved by the Wessex Regional Authority Ethical Committee.

Preterm infants remained in the study for a variable period (17-56 d). Of the forty-two infants enrolled in the study ten were still available for analysis at 8 weeks of age. Of the thirty-two infants who did not complete the study, five died and twenty-seven were discharged before they were 8 weeks old. Six healthy full-term infants were also enrolled for comparative purposes (Table 1). A sample of umbilical venous blood was obtained at birth and a further venous sample was collected at 7-9 d from these infants. In addition, random blood samples were taken for plasma and RBC tocopherol measurement from thirteen healthy adult volunteers (age 21-43 years).

Vitamin E intake

Preterm infants were given expressed breast milk (either pasteurized pooled donor milk or their own mother's milk); artificial formulas such as SMA ordinary formula, SMA low birth weight formula (Wyeth Laboratories, Berkshire), Aptamil ordinary formula or Aptamil low birth weight formula (Milupa Ltd, Middlesex); or were fed by total parenteral nutrition (TPN) with a solution that contained the fat emulsion Intralipid® (Kabivitrum, Stockholm, Sweden).

All feeds were carefully recorded as the type of feed and the amount given daily. The vitamin E contents of SMA ordinary formula, SMA low-birth-weight, Aptamil ordinary

and Preaptamil formulas were (mg/l): 6.4, 10, 7 and 12.5 respectively (manufacturers' information). Our own analysis indicated that these values overestimated the vitamin E content of the formulas by less than 15% at the time of use. Four samples of pooled donor expressed breast milk were analysed and a mean vitamin E content of 4.7 mg/l was found to be present.

Whole blood and plasma transfusions were given as required and the volumes were carefully recorded. Samples of whole blood and plasma transfusions were routinely analysed for tocopherol content; mean values of 6.5 and 9 mg/l respectively were found. These values were also utilized in determining vitamin E intakes (Table 2, p. 635). None of the infants in the study received any vitamin E supplements.

Sample collection and analysis.

Whole blood (0.5 ml) was collected into heparinized tubes when blood was withdrawn for routine clinical purposes, twice weekly for the 1st week and once weekly thereafter. Samples were either stored at 4° for a short period (0–12 h) or centrifuged immediately and the plasma removed and frozen until analysis. The RBC were washed three times with normal saline (9 g sodium chloride/l) before resuspension in saline containing pyrogallol (5 g/l; 1:1, v/v). The presence of pyrogallol in the RBC preparation was essential to prevent oxidative loss of tocopherols during extraction. A sample of this suspension was removed for packed cell volume determination while the remainder was frozen. Batch analysis of plasma and RBC samples was carried out on a weekly basis. Plasma and RBC tocopherol isomers were separated using high-performance liquid chromatography (HPLC) as described by Bieri *et al.* (1979) with the following modifications.

Plasma samples

Portions (100 μ l) were mixed with 100 μ l anhydrous ethanol containing 5 μ g α -tocopheryl acetate (Sigma, Poole, Dorset) as an internal standard before extraction with 500 μ l hexane. Following vigorous mixing and centrifugation, the hexane layer was removed and evaporated under nitrogen. The extract was then redissolved in 400 μ l methanol.

RBC suspensions

A 0.25 ml sample was added to 5-ml stoppered test-tubes to which 1.5 ml cold methanol (cooled in a dry ice–acetone bath) were added drop-wise while the sample was mixed slowly on a vortex mixer. The samples were then processed in a similar manner to the plasma samples except 2 ml hexane were employed for extraction of the lipid-soluble components.

Plasma and RBC samples (100 μ l) were injected onto a 7 \times 100 mm stainless-steel column packed with 5 μ m C-18 packing (Jones Chromatography, Hengoed, Mid-Glamorgan) via an autosampler (SP 8780, Spectra Physics Ltd, St Albans, Herts). The mobile phase was methanol–water (39:1, v/v), and flow-rate 0.8 ml/min. Detection and quantification was made by monitoring absorbance at 292 nm. α -Tocopherol standards (2.2–12.1 mg/l) were run routinely for each weekly analysis. All samples (including standards) were assayed in duplicate. It was assumed that the recovery of α -tocopheryl acetate reflected the recovery of the other tocopherol isomers (Bieri *et al.* 1979; Gutcher *et al.* 1984). The recovery of the added α -tocopheryl acetate was 82 and 74% respectively for plasma and RBC suspensions.

Statistical analysis

Plasma and RBC vitamin E concentrations were compared using the Mann–Whitney *U* test for non-parametric data. Comparisons of plasma and RBC tocopherol concentrations from individual infants were made using Spearman's rank order correlation test.

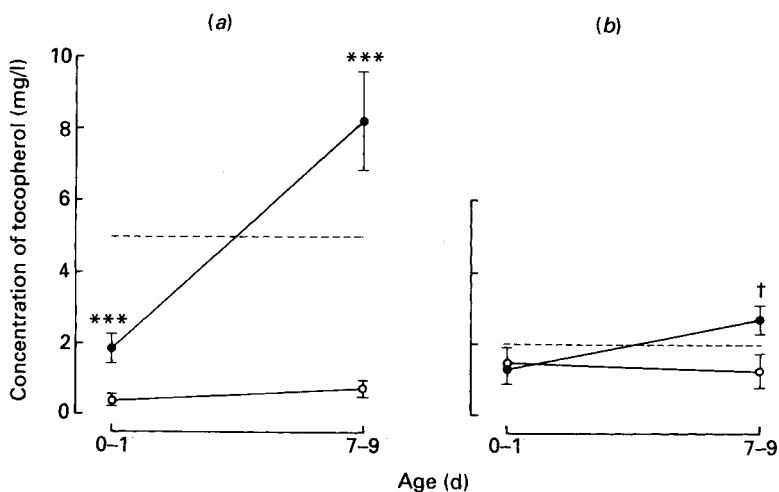


Fig. 1. Preterm and term (a) plasma and (b) erythrocyte (RBC) vitamin E concentrations during the 1st week following birth. Results are expressed as means, with their standard errors represented by vertical bars for eleven preterm infants (○) and six term infants (●). (-----), Lowest acceptable adult value, 5 mg/l and 2 mg/l for plasma and RBC respectively. Preterm, days 0-1 and days 7-9 plasma vitamin E concentrations were significantly lower than corresponding term values (** $P < 0.001$). Preterm RBC vitamin E concentrations were not initially different from term values, however by days 8-9 they were significantly lower († $P < 0.05$).

RESULTS

In the first 24 h following birth, plasma tocopherol concentration was extremely low in both term and preterm infants (Fig. 1). These plasma vitamin E concentrations (term, 1.9 mg/l, preterm 0.3 mg/l) were considerably lower than those determined in the adult population, 9.2 (SE 1.9; range 6.3-12.4) mg/l. During the 1st week of life, plasma vitamin E concentration of term infants increased significantly, while plasma vitamin E status of the preterm infants (0.7 mg/l) did not improve (Fig. 1). In contrast, the mean concentration of tocopherol in RBC membranes was similar in both term and preterm infants following birth, 1.3 and 1.5 mg/l respectively (Fig. 1). Although these values were less than those obtained in our adult population (mean RBC vitamin E was 2.8 mg/l, range 2.0-3.9 mg/l) the difference was relatively less than that found by comparing plasma values. By 7-9 d of age the tocopherol concentration in the RBC from the term infants had increased to 2.7 mg/l, while that of preterm RBC remained virtually unchanged (Fig. 1).

Over the next 7 weeks plasma tocopherol concentration increased slowly in the preterm infants, reaching a value of 7.1 mg/l by week 8 (Fig. 2). Statistical analysis revealed that preterm infants had a significantly lower plasma tocopherol concentration for the first 7 weeks when compared with the adult population. In comparison, RBC tocopherol concentration in these infants was significantly lower than that of the adult population for only 2 weeks following birth (Fig. 2).

The sustained low plasma vitamin E concentrations of preterm infants could not be explained by sustained low vitamin E intakes by these infants. Careful calculation of vitamin E intakes from daily feeding records and clinical management records revealed that vitamin E intakes of both term and preterm infants were approximately similar from the 2nd week following birth (Table 2).

As vitamin E consists of a number of individual isomers, α , β , γ and δ , the contribution of each of these to the plasma and RBC vitamin E concentrations was determined. At birth (or within the first 24 h) both plasma and RBC vitamin E totals consisted almost exclusively (> 95%) of α -tocopherol. Indeed this continued to be true for the majority of

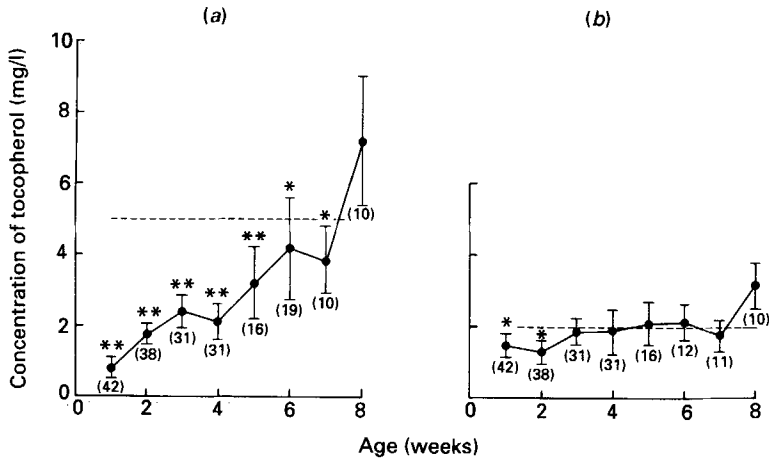


Fig. 2. (a) Plasma and (b) erythrocyte (RBC) vitamin E concentrations during the first 8 weeks of life following premature birth. Results are expressed as means, with their standard errors represented by vertical bars for no. of infants in parentheses. (-----), Lowest acceptable adult value, 5 mg/l (plasma) and 2 mg/l (RBC). Values at each time point were significantly different from those for adult vitamin E concentration (plasma 9.3 (SE 1.9) mg/l and RBC 2.8 (SE 0.6) mg/l): * $P < 0.05$, ** $P < 0.01$.

Table 2. Vitamin E intake (mg/kg per d) of term and preterm infants
(Mean values and standard deviations based on recorded intakes)

Age (weeks)...	1	2	3	4	5	6	7
Term*	0.5-0.9	0.7-1.4	0.7-1.4	0.7-1.4	0.7-1.4	0.7-1.4	0.7-1.4
Preterm							
Mean	0.07	1.12	1.19	1.27	1.39	1.31	1.27
SD	0.14	0.14	0.50	0.39	0.50	0.39	0.35

* Expressed as a calculated range, assuming a milk intake of 150-200 ml/kg per d (except week 1) and a tocopherol content of 4.7 mg/l for breast milk and 7 mg/l for cow's milk.

the infants during the period of the study. The correlation coefficients for plasma total tocopherol and α -tocopherol, and RBC total tocopherol were r 0.96 and r 0.99 respectively. The few infants who did not fit this pattern were identified as having received TPN for a period during the study. Our TPN regimen employed the lipid component Intralipid. Intralipid contains a high concentration of vitamin E, 146 mg/l; however, most of this vitamin is present as non- α -tocopherol isomers with α -tocopherol representing less than 10% of the total (Farrell, 1979; Gutcher *et al.* 1984; Kelly & Sutton, 1989). In those infants receiving TPN these non- α -tocopherol isomers appeared in the plasma and were rapidly incorporated into the RBC membrane (values not shown).

DISCUSSION

The majority of previous studies in this area have utilized plasma or serum concentrations alone or in relation to blood lipids in assessing preterm infant vitamin E status (Ostrea *et al.* 1986; Gross & Gabriel, 1985; Huijbers *et al.* 1986). In the present study we determined

plasma and RBC tocopherol concentrations as we hypothesized that the RBC membrane vitamin E concentration would be more informative as it represented the site of action of the vitamin. No difference was observed in the RBC vitamin E concentration of term and preterm infants during the first 24 h following birth (Fig. 1). RBC vitamin E concentration in both groups was, however, below the recorded adult range (2.0–3.9 mg/l). After 1 week, vitamin E concentrations of RBC from term infants were within this range (mean 2.7 mg/l), while those from the preterm infants were still considerably lower (1.3 mg/l). These results suggest that the feeding regimen over the first few days is extremely important in supplementing vitamin E pools to meet required levels. This occurs primarily in the case of breast-fed infants from the intake of colostrum. Colostrum contains a very high concentration of vitamin E (i.e. six to seven times that of mature breast milk) and it is this high vitamin E intake over the first few days that leads to the rapid correction of low vitamin E concentrations (Ostrea *et al.* 1986). In contrast, in the present study we observed that infants born prematurely, with low circulating concentrations of vitamin E, were unable to improve their vitamin E status. These infants, who received only fluids over the first few days, did not have the opportunity to accumulate vitamin E stores by the usual methods. Hence, by the end of the 1st week both their plasma and RBC vitamin E concentrations were still very low.

In the preterm infants, plasma vitamin E concentration increased very slowly, attaining the adult range only at 8 weeks post-partum. This deficient vitamin E state was maintained even though, as far as we could estimate, these infants were receiving comparable vitamin E intakes to term infants (Table 2). This would suggest that either the preterm infant's ability to absorb vitamin E is reduced, or alternatively the vitamin E requirement of these infants may be higher than that of term infants. At present there is no clear agreement regarding intestinal absorption of vitamin E in the premature infant, with studies suggesting both reduced (Melhorn & Gross, 1971; Farrell, 1979) and normal (Bell *et al.* 1979) absorption. A similar result was observed by Gross & Gabriel (1985) in a group of premature infants (< 1500 g) who received formula feeds with iron supplementation. By 6 weeks of age these infants still had depressed serum vitamin E levels (4.5 mg/l). In comparison, infants fed on formulas without the Fe supplementation had markedly higher serum E concentrations at week 6 (11.5 mg/l). A similar response was reported by Conway *et al.* (1986) in a study designed to consider the effect(s) of oral vitamin E supplementation. They observed that of those infants receiving formula alone without vitamin E or iron supplementation, 79% had vitamin E levels within the adult range by 6 weeks of age. Fe is known to affect vitamin E status in at least two ways. First, the presence of Fe in the intestine is known to decrease the absorption of vitamin E (Melhorn & Gross, 1971; Gross & Melhorn, 1972). Second, the oxidation of cellular lipids (normally prevented by vitamin E) is catalysed by Fe, hence the addition of Fe to the diet can further increase the vitamin E requirement. All the preterm infants in the present study received Fe supplementation at 21 d (2.75–5.5 mg). It is interesting to note that, in our study, the mean plasma vitamin E concentration actually fell between weeks 3 and 4 before recovering again by week 5. RBC vitamin E concentration did not change between weeks 3 and 4.

In comparison to the slow accumulation of vitamin E in the plasma of preterm infants, their RBC had attained adult concentrations of vitamin E by 3 weeks following birth (Fig. 2). This rise in RBC vitamin E concentration could not be accounted for by the provision of whole-blood transfusions to a number of these infants as removal of these babies from the analysis did not alter this result (values not shown). To infer from these results that preterm infants were vitamin E replete by week 3 depends however on two major assumptions. First, that the RBC vitamin E concentration reflects that found in other tissues, and second that the polyunsaturated fatty acid composition of the preterm infants'

RBC is similar to that of the adults' RBC. With respect to the first consideration, we are aware of only one study in which blood and tissue (lung and liver) vitamin E concentration have been determined over the 1st week of life. Butcher & Roberts (1981) reported that in rat pups allowed free access to the dam, whole blood and liver vitamin E levels peaked by day 1 while lung continued to accumulate vitamin E over the first 6 d. We are aware of no such study in which premature animals have been utilized. Second, there are now several studies in which marked differences have been found in the pattern of fatty acids in the total phospholipid of RBC from newborn infants, the proportion of polyunsaturated fatty acids being greater than those in the RBC from adults (Neerhout, 1968; Gercken *et al.* 1972). If this is also the case with RBC for preterm infants then these infants will in fact require greater concentrations of vitamin E in their RBC membranes to protect the increased amount of polyunsaturated fatty acids present.

In conclusion, the present study has shown that both term and preterm infants have low plasma and RBC tocopherol concentrations at birth when compared with an adult population. Whereas term infants rapidly replete vitamin E stores within the 1st week, preterm infants have low RBC vitamin E concentrations for about 3 weeks and low plasma vitamin levels for up to 8 weeks following birth. This situation arises even though the vitamin E intake of these infants is similar to that proving adequate for term infants. This apparent deficiency state may be in part influenced by the provision of an Fe supplement at 21 d to these infants.

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