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# Vitamin E status in Sudanese children with protein-energy malnutrition

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*Summary:* Total tocopherols and alpha-tocopherols were estimated in the plasma of children with severe malnutrition (14 marasmus; 11 marasmic kwashiorkor; five kwashiorkor) and related to the total plasma lipids and different plasma lipid classes. If the mere plasma concentrations were taken as an index of the vitamin E status, five children with marasmus, five children with marasmic kwashiorkor, and two children with kwashiorkor would have been regarded as deficient (< 500 µg/dl). However, if total tocopherols and alpha-tocopherols were related to the total plasma lipids, all malnourished children – except one – showed values within the limits found in healthy American children. The study shows that low tocopherol/lipid ratios are not a constant feature in severely malnourished children.

*Zusammenfassung:* Das Gesamttocopherol und alpha-Tocopherol wurden im Plasma von Kindern mit schwerer Mangelernährung (14 Maramus; 11 marantischer Kwashiorkor; 5 Kwashiorkor) bestimmt und auf die Gesamtlipide des Plasmas und auf die verschiedenen Plasmalipidklassen bezogen. Wenn die reinen Plasmakonzentrationen als ein Index des Vitamin-E-Status genommen würden, so wären 5 Kinder mit Marasmus, 5 Kinder mit marantischem Kwashiorkor und 2 Kinder mit Kwashiorkor als defizitär (< 500 µg/dl) anzusehen. Werden jedoch Gesamttocopherol und alpha-Tocopherol auf die Gesamtlipide bezogen, so zeigten alle mangelernährten Kinder – bis auf eines – Werte, die innerhalb der Grenzen lagen, die für gesunde amerikanische Kinder gefunden wurden. Die Studie zeigt, daß niedrige Tocopherol-Lipid-Verhältnisse kein konstantes Merkmal bei schwer mangelernährten Kindern sind.

*Key words:* protein-energy malnutrition, vitamin E status, children, Sudan, developing countries

*Schlüsselwörter:* Protein-Energie-Mangelernährung, Vitamin-E-Status, Kinder, Sudan, Entwicklungsländer

## Introduction

Several publications reported a reduced vitamin E status in children with protein-energy malnutrition starting with Scrimshaw et al. in 1957 (5,

7, 9, 12, 13). In these investigations (except those by Laditan and Ette (7)) plasma tocopherol concentrations were estimated. Because of the free radicals theory in the pathogenesis of kwashiorkor of Golden and Ramdath (5) it becomes necessary to know whether all or some of the chemical substances or processes which are involved in free radicals elimination are always or frequently deficient in kwashiorkor or other forms of protein-energy malnutrition (PEM).

Since the investigations of Horwitt et al. (6) it is known that plasma tocopherol levels have to be related to plasma lipid levels because they are transported within lipids and low lipid levels always include low tocopherol levels. According to extensive studies of Farrell et al. (4) a value of 0.6 mg total tocopherols/g of total lipids has been suggested as the lower limit of normal. However, discussion about the most practical references such as total lipids, cholesterol, triglycerides, and phospholipids continues. Therefore, we considered the ratios of total tocopherols/g total lipids, as well as the ratios of total tocopherols and alpha-tocopherol to the different lipid classes.

We estimated tocopherols in plasma of Sudanese children with different forms of malnutrition. This investigation was part of a broader investigation on the different nutritional factors which may cause or influence the clinical state of children with malnutrition in this part of Africa.

## Material and Methods

Thirty Sudanese children aged 5 to 48 months with severe PEM were admitted to Wad Medani Teaching Hospital during the period of December 1986 and April 1987. According to the Wellcome Working Party Classification (21) 14 patients had to be classified as marasmus, 11 as marasmic kwashiorkor, and five as kwashiorkor. Most of the children were formerly breast-fed adequately; however, they were supplemented late. The median age of supplementation with beikost (in months) being 7.1, 7.7, and 8.4 in children with marasmus, marasmic kwashiorkor, and kwashiorkor, respectively. Their mean age at investigation was 21 months (5 to 48 months), 24 months (13 to 48 months), and 24 months (14 to 36 months), respectively. Besides history, physical examination, and anthropometrical measurements, 5 ml of blood was routinely taken for estimation of essential hematological parameters, electrolytes, etc. An aliquot of EDTA plasma was immediately frozen and stored in darkness for the later estimation of tocopherols and lipids. This investigation had been acknowledged by the Research Committee of the Gezira University, Wad Medani, Sudan, and the Ethical Committee of the Faculty of Medicine of the University of Düsseldorf.

### *Tocopherols*

To each plasma sample (100 µl) 50 µg ethanol containing 0.09 % of 1,6-di-tert-butyl-4-methylphenol and 50 µg of ethanol containing 250 µg of retinyl acetate as internal standards were added. This mixture was stirred on a Vortex for 30 s. After addition of 600 µl of hexane the sample was stirred again for 2 min. The hexane layer was removed and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 100 µl of methanol of which 50 µl were used for high performance separation (HPLC) using a modification of a method of Bieri et al. (1). A Waters model 501 liquid chromatograph was used with a U6K injector, a Waters 30 cm column-C 18, particle size 4 µm, column diameter 4 mm, and the spectrophotometer model 481 connected to the Waters 740 data module. The solvent was methanol/water (97/3; V./V.), flow rate 1 ml/min., detection at 290 nm. The method separates

Table 1. Median and range of plasma alpha-tocopherol and total tocopherols in severe PEM (μg/dl)*.

| | Marasmus (n = 14) | Marasmic kwashiorkor (n = 11) | Kwashiorkor (n = 5) |
|---|---|---|---|
| Alpha-tocopherol | | | |
| median | 404 | 366 | 391 |
| range | 213– 763 | 68–425 | 209–1138 |
| Total tocopherols | | | |
| median | 583 | 529 | 551 |
| range | 318–1351 | 210–640 | 463–1447 |

* Differences between the various groups were tested by U-test; they were not significantly different.

alpha-tocopherol from a joint peak consisting of beta- and gamma-tocopherol. The efficiency of recovery of alpha- and gamma-tocopherol added to plasma was 98 ± 2 % for alpha- and 99 ± 3 % for gamma-tocopherol. The precision of the method for the same plasma determined on 10 consecutive days was equal or better than ± 2 %.

In a separate aliquot total cholesterol was measured by the method of Siedel et al. (14), phospholipids by the method of Zilversmit and Davis (22), and triglycerides by the method of Wahlefeld (20). Total plasma lipids were calculated by addition of the three major lipids triglycerides, phospholipids, and cholesterol. These lipids consist of about 97 % of the total lipids (2).

## Results

Table 1 shows the plasma concentrations of alpha- and total tocopherols. If one is considering concentrations of total tocopherols below 500 μg/dl as deficient, as proposed by Nitowsky et al. (11), then five children with marasmus (35.7 %), five children with marasmic kwashiorkor (45.5 %), and two children with kwashiorkor (20 %) have to be regarded as deficient. The lowest concentration observed was found in a child with marasmic kwashiorkor (210 μg/dl).

Table 2 outlines the concentrations of total cholesterol, triglycerides, phospholipids, as well as ratios of total tocopherols to total lipids, total cholesterol, phospholipids, and triglycerides, respectively. Additionally, it outlines the ratios of alpha-tocopherol to total lipids.

The medians of total tocopherols/g total lipids (1.60 mg, 1.41 mg and 1.48 mg for marasmus, marasmic kwashiorkor, and kwashiorkor, resp.) are far above the lower border of normal (0.6 mg) as defined by Farrell et al. (4). Only one child with marasmic kwashiorkor had a value of 0.57 mg.

## Discussion

Protein-calorie malnutrition is a common health problem in Sudan (17), and severe malnutrition accounted for a third of all hospital pediatric admissions (16). The ecological factors underlying protein-calorie malnu-

Table 2. Median and ranges of plasma lipids, tocopherols and various tocopherol/lipid ratios in 30 children with PEM*.

| | Marasmus (n = 14) | Marasmic kwashiorkor (n = 11) | Kwashiorkor (n = 5) |
|---|---|---|---|
| Cholesterol (mg/dl) | | | |
| median | 92 | 122 | 125 |
| range | 68–197 | 53–254 | 59–199 |
| Triglycerides (mg/dl) | | | |
| median | 148 | 126 | 198 |
| range | 52–291 | 84–230 | 118–264 |
| Phospholipids (mg/dl) | | | |
| median | 129 | 96 | 130 |
| range | 77–161 | 77–143 | 87–142 |
| Total lipids (mg/dl) | | | |
| median | 370 | 348 | 434 |
| range | 270–514 | 272–496 | 312–502 |
| Total tocopherols/ Total lipids (mg/g) | | | |
| median | 1.60 | 1.41 | 1.48 |
| range | 0.74–2.63 | 0.56–2.24 | 0.94–3.76 |
| Total tocopherols/ Cholesterol (mg/g) | | | |
| median | 5.92 | 4.33 | 7.78 |
| range | 2.94–9.80 | 1.58–10.53 | 2.37–11.55 |
| Total tocopherols/ Triglycerides (mg/g) | | | |
| median | 4.83 | 4.24 | 3.78 |
| range | 1.44–7.38 | 1.43–6.61 | 2.09–12.35 |
| Total tocopherols/ Phospholipids (mg/g) | | | |
| median | 4.60 | 4.61 | 6.34 |
| range | 2.70–10.49 | 2.33–7.88 | 3.56–10.16 |
| Alpha-tocopherol/ Total lipids (mg/g) | | | |
| median | 1.00 | 0.86 | 0.87 |
| range | 0.46–1.67 | 0.18–1.31 | 0.42–2.95 |

* Differences between the groups were testet by U-test; they were not significantly different.

trition in Sudan and particularly in the irrigated area of the Gezira have been reported by Taha previously (18). It is characteristic for this part of the Sudan that the children are weaned relatively late, that weaning is frequently performed abruptly which often induces problems leading to malnutrition, that infants and toddlers are usually then fed an adult diet that contains cotton seed oil as the main staple fat.

Based on the values of total tocopherols/g of total lipids and probably of alpha-tocopherol/g of total lipids only one malnourished child was found to be deficient and most of the other children were in the range of well supplied healthy American probands (4). The vitamin E status of normally nourished breast-feeding Sudanese women is comparable to that found in Europe and America (3). The results of the malnourished children investigated in this study are also in the range of these Sudanese women (3). Because normal Sudanese toddlers receive nearly the same food as these women they will probably also show comparable values.

It has to be emphasized that all children with kwashiorkor elicit values of tocopherols/g of total lipids were within the normal range of healthy American probands. However, if only tocopherol concentrations are considered nearly half of these patients could be regarded wrongly as deficient.

There is only one study from Nigeria (7) which related alpha-tocopherol values to total lipids in PEM and considered 0.8 mg alpha-tocopherol/g of total lipids as the lower limit of normal. However, if one is considering the relation of total tocopherols/g of total lipids with 0.6 mg/g as lower limit of normal (4) then a lower limit of 0.8 mg alpha-tocopherol/g total lipids, as proposed by Laditan and Ette, is far too high.

If the ratio of total tocopherols/g of cholesterol, another frequently used lipid reference, is considered, the ranges of 2.94–9.80 for marasmus, 1.58–10.53 for marasmic kwashiorkor, and 2.37–11.55 for kwashiorkor scatter as much as the values of total tocopherols/g of total lipids. But they are also within or above the values of American children of an inner city (mean 2.97 ± 0.23) who show no signs of vitamin E deficiency (4). In summary, our data and the data of Laditan and Ette (7) show that in Sudan and Nigeria, low plasma vitamin E values do not frequently occur together with severe malnutrition. Whether this is also true for other areas has to be investigated as age and dietary fat supply may influence the tocopherol status, at least in animals (10).

As expected, alpha-tocopherol is quantitatively the most important tocopherol; as is well known, beta- and gamma-tocopherols show a lower vitamin E efficacy. Therefore, the ratio of alpha-tocopherol/g of total lipids is probably a better approach to total vitamin E efficacy than is total tocopherols/g of total lipids. However, because most clinically important investigations relate to total tocopherols/g of total lipids this term cannot be abandoned presently. The differences between the terms of alpha-tocopherol/g of total lipids, of total tocopherol/g of total lipids, and highly probably, of total vitamin E efficacy/g of total lipids are not so different that they change the importance of this investigation.

Do the results of this paper mean that free radicals do not participate essentially in the pathogenesis of kwashiorkor? In selenium-deficient animals the production of alkanes as an indicator of peroxidation of polyunsaturated fatty acids cannot be normalized by vitamin E alone (19). Certainly, production, action, and elimination of free radicals are very complex processes, and prevention of damage cannot be related to tocopherols only (for review see (15)). Additionally, our knowledge on production and elimination of free radicals in malnourished humans is too limited to enable a definitive answer on the role of free radicals in the

pathogenesis of certain symptoms of malnutrition presently.

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