Z. Ernährungswiss. **18**, 81–87 (1979)

ISSN 0044-264 X

Universitätskinderklinik Erlangen (Direktor: Prof. Dr. K. Stehr) und Institut für experimentelle Ernährung (Direktor: Dr. W. Fekl) e.V.; Erlangen

Deficiencies of essential fatty acids and vitamin E in cystic fibrosis

H. Böhles, H. Heid, K. Stehr, and *W. Fekl*

With 5 figures

(Received August 10, 1978)

Introduction

Problems of lipid assimilation are well known in cystic fibrosis of the pancreas (7, 10, 19, 21). Deficient activity of pancreatic lipase has been fully described (19). In consequence, dietary substitution with pancreatic enzymes has been established as standard therapeutic approach in cystic fibrosis (7, 10). The effect of a disturbed assimilation on serum and tissue levels of lipids continue to be the subject of further studies (3, 8). In particular, polyunsaturated fatty acids and vitamin E have become a center of interest during the last years (1, 2, 3, 5, 8, 18, 20, 22, 23, 24, 25).

Materials and methods

Study population

The study was done on 25 patients (13 female; 12 male) with cystic fibrosis. The diagnosis was confirmed by sodium sweat analysis. The patients are under regular control of the outpatient department of the University Children's Hospital of Erlangen. The age of the patients ranged from 6 months up to 16 years.

24 control children were matched for age and sex. These were patients of the hospital with mild upper respiratory tract infections or orthopedic problems. Their history showed no intestinal problems. The parents of all our patients were informed about the purpose of our study and gave their consent.

Growth velocity

The growth velocity for height of the cystic fibrosis patients was calculated for the year before the beginning of the study. Data were taken from the charts. Data were available for 12 patients with cystic fibrosis and 11 control children.

Analysis

About 3 cc of blood were taken from patients and controls in the fasting state during the morning hours. Patients were selected randomly as they presented at the outpatient department for their routine check up. Total serum vitamin E was determined within half an hour after the withdrawl of blood. The remaining serum was kept frozen under nitrogen for the later fatty acid analysis. The fatty acid analysis was done within a month after the sampling of blood.

Vitamin E

Total serum vitamin E was determined using the method of *Quaife* et al. (17).

Fatty acids

Lipids were extracted from serum according to *Folch* et al. (4). The total lipid was chromatographed on silica gel G in hexane/diethyl ether/acetic acid (80:20:1; v:v:v). Fatty acid methyl esters of the separated serum triglyceride, of the cholesterol ester and of the phospholipid fractions were prepared (5% methanolic H_2SO_4; 3 hr; 70 °C) and separated by gas liquid chromatography (Hewlett-Packard 5830 A equipped with hydrogen flame ionization) using glass columns packed with 10% DEGS on 100/120 mesh chromosorb W-HP. Analysis started at 160 °C followed by temperature programming at 2°/min to 200 °C, from then, isothermally. Commercially obtained standards were used for reference.

Results

Serum tocopherol

Serum tocopherol was decreased considerably in the patients with cystic fibrosis (0.30 ± 0.26 mg/dl) compared to the control group (1.02 ± 0.24 mg/dl) ($p = 0.01$) Fig. 1). The results in the cystic fibrosis group show a wide scatter, ranging from 1.07 mg/dl to immeasurable values (≙ 0.00 mg/dl).

Fatty acids

The fatty acid distribution in the cystic fibrosis group showed no significant difference in any of the studied ester fractions, compared to the control group (fig. 2, 3, 4). However, there is a wide scatter in all three ester fractions and trends towards differences are demonstrable. The concentration differences observed can be seen best in the cholesterol ester fraction

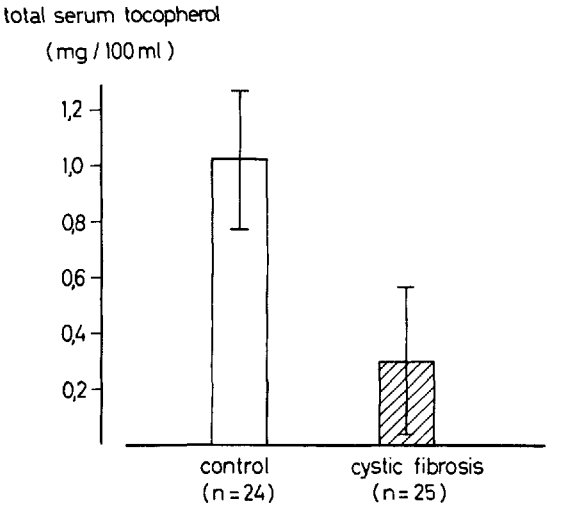

Fig. 1. Total serum tocopherol levels in patients with cystic fibrosis and in controls (mean ± 1 SD).

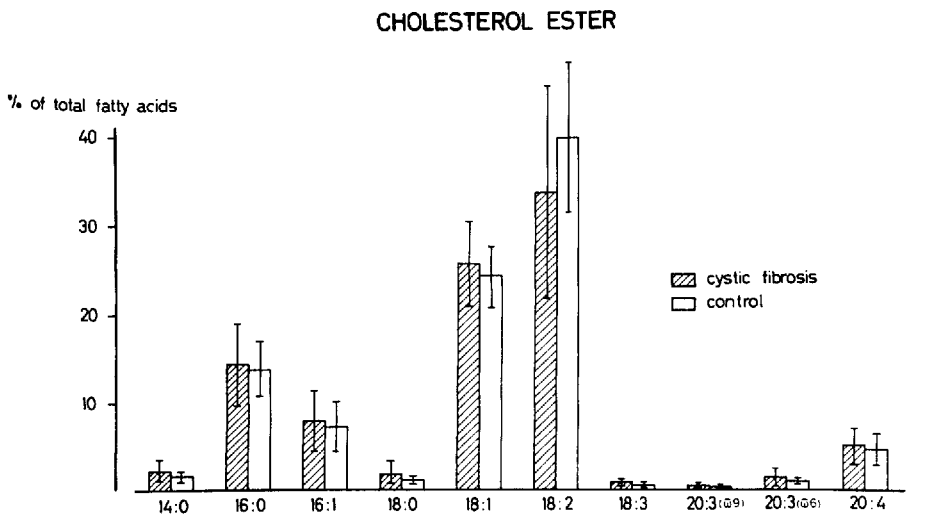

Fig. 2. Fatty acid pattern of the cholesterol ester fraction from patients with cystic fibrosis (n = 25) and controls (n = 24) (mean ± 1 SD). 14:0 myristic acid; 16:0 palmitic acid; 16:1 palmitoleic acid; 18:0 stearic acid; 18:1 oleic acid; 18:2 linoleic acid; 18:3 linolenic acid; 20:3 (n-9) eicosatrienoic acid; 20:3 (n-6) eicosatrienoic acid (natural); 20:4 arachidonic acid.

(fig. 2). Among the polyunsaturated fatty acids, linoleic acid (18:2) shows a distinct tendency to be decreased, compared to controls. In all three ester fractions, 5,8,11-eicosatrienoic acid (20:3; n-9) is slightly increased – compared to controls.

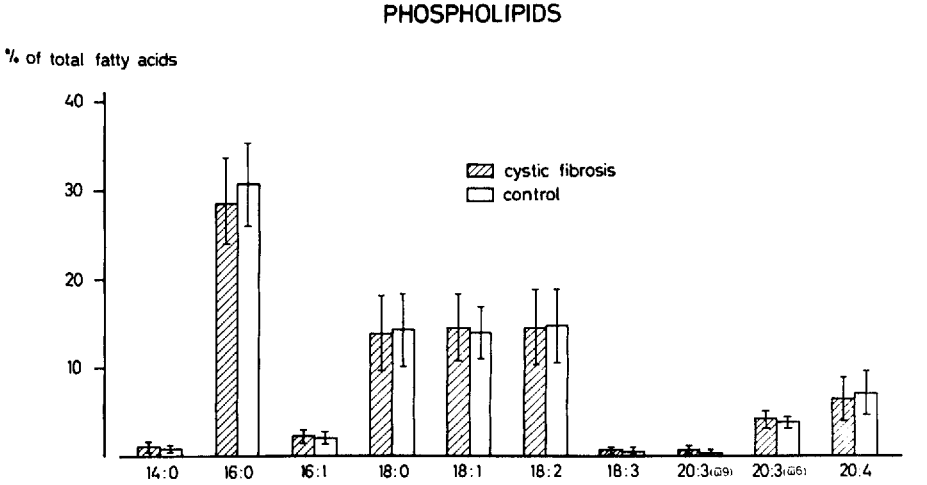

Fig. 3. Fatty acid pattern of the phospholipid fraction from patients with cystic fibrosis (n = 25) and controls (n =22) – (mean ± 1 SD).

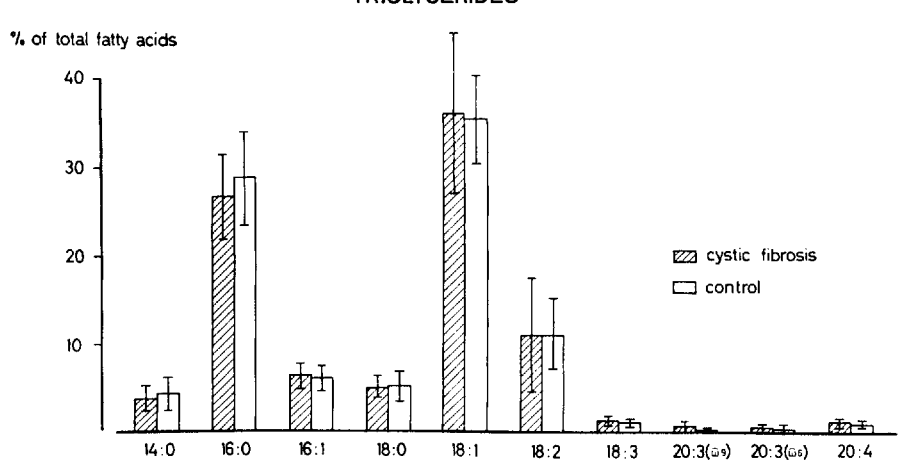

Fig. 4. Fatty acid pattern of the triglyceride fraction from patients with cystic fibrosis (n = 25) and controls (n = 24) – (mean ± 1 SD).

Discussion

Low serum tocopherol levels have been extensively described in patients with malabsorption especially in patients with cystic fibrosis (6, 8, 11, 13, 14, 15). We can confirm these results. The formation of chylomicrons and a sufficient intraluminal bile acid concentration has been understood as the most important factors with respect to vitamin absorption (12). Vitamin E deficiency in cystic fibrosis was thought to occur possibly on the basis of a decreased micelle formation and the ensuing decreased solubility of apolar lipids, like tocopherol (12). *Underwood* et al. (22) describe decreased plasma and erythrocyte tocopherol levels in patients with cystic fibrosis. The serum tocopherol levels of our cystic fibrosis population (0.30 ± 0.24 mg/dl) are in accordance to those found by *Underwood* et al. (0.24 ± 0.20 mg/dl). The decreased serum tocopherol levels are interpreted by these authors only as a consequence of chronic lipid malabsorption in cystic fibrosis. All our patients are substituted with pancreatic enzyme preparations. The average daily amount of pancreatic enzymes taken by our cystic fibrosis population corresponds to 6900 mg pancreatin[1]). This amount is about half of 15,000 mg pancreatin that *Harris* et al. (7) recommend as highest daily substitute. Beyond this amount these authors could not see any further effect.

The pattern of changes in fatty acid concentrations is in accordance to the changes found by *Underwood* et al. (22) in total serum fatty acids. The biggest changes were observed in the cholesterol ester fraction. This is confirming the results of *Zöllner* et al. (26). According to these authors, very small changes in fatty acid concentration are reflected in the choleste-

[1]) Pankreon forte® (Kali Chemie Pharma GmbH, Hannover, West Germany) 6900 mg pancreatin contain: 591 lipase units, 493 amylase units, 1479 protease units, 739 mg bile concentrate (60% bound bile acids) (units = Willstätter units).

rol esters. The decreased concentration of linoleic acid (18:2) and the compensatorily increased concentrations of the monoenoic acids-palmitoleic acid (16:1) and oleic acid (18:1) characterize a deficiency in essential fatty acids, as does the appearance of 5,8,11-eicosatrienoic acid of the n-9 family. Despite of the wide scatter there is a trend towards an increased concentration in the cystic fibrosis group (0.33 ± 0.30%) compared to controls (0.17 ± 0.15%). *Holman* (17) defined the increment of the 5,8,11-eicosatrienoic acid/arachidonic acid – ratio beyond the value 0.4 as a diagnostic criterion for essential fatty acid deficiency. Only in two patients we found a value higher than 0.4 in the cholesterol ester fraction. Both patients showed an extremely low linoleic acid level at the same time.

Several authors reviewed recently the problem of essential fatty acid dificiency in cystic fibrosis (3, 18, 20, 24). We cannot confirm the results supported by *Rivers* and *Hassam* (20) that 5,8,11-eicosatrienoic acid is not present in the fatty acid pattern of cystic fibrosis. The highest 5,8,11-eicosatrienoic acid concentrations were found to be in the cholesterol esters up to 1.01%, in the phospholipids up to 1.76% and in the triglyceride fraction in one case up to 2.63% of total measurable fatty acids. In almost all patients this acid was found in some concentration. Our results are in accordance with those of *Robinson* (18) who demonstrated 5,8,11-eicosatrienoic acid concentrations in serum and in erythrocyte membranes up to 2.5% of total fatty acids.

5,8,11-eicosatrienoic acid (n-9) was found also in the control population in 63.5% of the total fatty acid analysis. The highest concentration of 1.03% was found in the phospholipid fraction of a 6-month-old control patient. These results confirm those by *Watts* et al. (24) and *Paulsrud* et al. (16), who could demonstrate 5,8,11-eicosatrienoic acid also in apparently healthy children, especially if very young. Out of 22 phospholipid fraction analysis

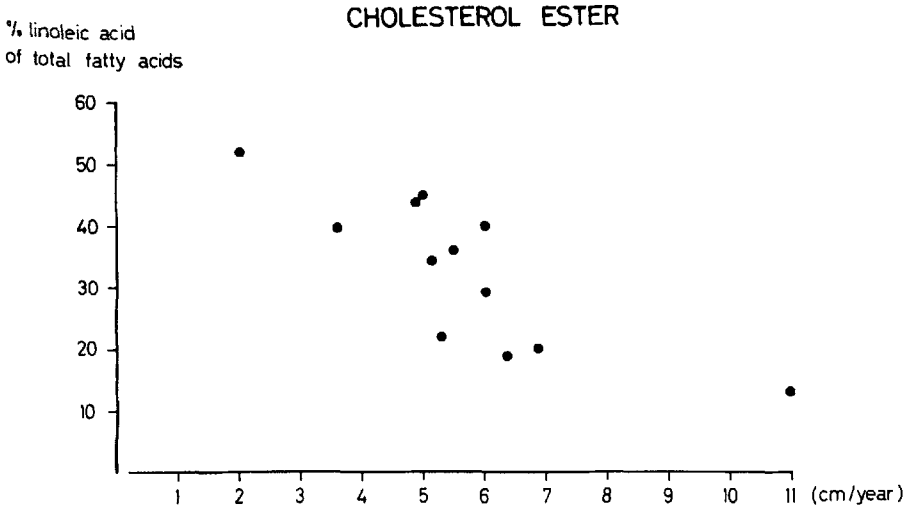

Fig. 5. Relationship between percent linoleic acid of total fatty acids in the cholesterol ester fraction and height velocity (cm/year) in children with cystic fibrosis. $r = -0.76$, $y = -4.26 \times + 57.61$.

we could demonstrate 5,8,11-eicosatrienoic acid in 19. The amount in percentage of total fatty acids related to the age of control patients shows the tendency towards a negative correlation ($r = -0.58$). This confirms the results by *Watts* et al. (24) and *Paulsrud* et al. (16) who found a decreasing 5,8,11-eicosatrienoic acid content with increasing age.

Both tocopherol and essential fatty acid levels in serum of patients with cystic fibrosis may theoretically reflect:

1. Decreased absorption from the gastrointestinal tract.
2. Increased utilization of these nutrients by tissues.
3. Decreased absorption accompanied by increased utilization by the appropriate tissue.

As a point of discussion we want to stress an inverse correlation ($r = -0.76$) in patients with cystic fibrosis between linoleic acid level of cholesterol esters and height velocity, which is supposed to reflect utilization of essential fatty acids (fig. 5). There is no such correlation in the control group ($r = -0.03$). Lowest linoleic acid levels in those patients with best growth rates may reflect increased utilization for tissue synthesis in obviously decreased compensatory absorption of lipids despite of pancreatic enzyme substitution. Further investigation is needed to clarify this observation.

Summary

In 25 children (13 male; 12 female) with cystic fibrosis aged 6 months to 16 years and 24 matched controls total serum vitamin E levels and fatty acid patterns of serum cholesterol esters, phospholipids and triglycerides are demonstrated.

Compared to controls (1.02 ± 0.24 mg/dl) the total serum vitamin E levels are significantly decreased in patients with cystic fibrosis (0.30 ± 0.26 mg/dl) ($p < 0.01$). There is no significant difference comparing the fatty acid patterns of the serum ester fractions of both groups. Differences can be seen best in the cholesterol ester fraction. In this fraction linoleic acid shows a trend to be decreased in the cystic fibrosis patients compared to the control group.

A possible influence of height velocity on the levels of essential fatty acids is discussed.

Zusammenfassung

Bei 25 Patienten mit Mukoviszidose (13 männlich, 12 weiblich) und entsprechenden Kontrollen wurden der Serum-Vitamin-E-Spiegel und das Fettsäuremuster von Serumcholesterinestern, Serumphospholipiden und Serumtriglyzeriden untersucht. Im Vergleich zu Kontrollpatienten ($1{,}02 \pm 0{,}24$ mg/100 ml) war der Serum-Vitamin-E-Spiegel bei den Patienten mit Mukoviszidose signifikant erniedrigt ($0{,}30 \pm 0{,}26$ mg/100 ml) ($p < 0{,}01$). Zwischen beiden Gruppen besteht kein signifikanter Unterschied des Fettsäuremusters. Unterschiede sind jedoch aus der Serumcholesterinesterfraktion am besten ersichtlich. Hier ist die Linolsäurekonzentration im Mittelwert bei den Patienten mit Mukoviszidose im Vergleich zu den Kontrollen erniedrigt.

Ein möglicher Einfluß der Wachstumsgeschwindigkeit wird diskutiert.

References

1. Anonymous, Vitamin E and cystic fibrosis of the pancreas. Nutr. Rev. **17**, 13 (1959). – 2. *Blanc, W. A., J. D. Reid, D. H. Andersen,* Avitaminosis E in cystic fibrosis of the pancreas. Pediatrics **22**, 494 (1958). – 3. *Elliott, R. B., P. G. Robinson,* Unusual clinical course in a child with cystic fibrosis treated with fat emulsions. Arch. Dis.

Child. **50,** 76 (1975). – 4. *Folch, J., M. Lees, G. H. Sloane-Stanley,* A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. **226,** 497 (1957). – 5. *Gordon, H. H., H. M. Nitowsky, J. T. Tildon,* Tocopherol deficiency in infants and children with steatorrhea. Trans. Amer. Clin. Climat. Ass. **68,** 155 (1956). – 6. *Gordon, H. H., H. M. Nitowsky,* Some studies of tocopherol in infants and children. Amer. J. Clin. Nutrition **4,** 391 (1956). – 7. *Harris, R., A. P. Norman, W. W. Payne,* The effect of pancreatin therapy on fat absorption and nitrogen retention in children with fibrocystic disease of the pancreas. Arch. Dis. Child. **30,** 424 (1955). – 8. *Harries, J. T., D. P. R. Muller,* Absorption of different doses of fat soluble and water miscible preparations of vitamin E in children with cystic fibrosis. Arch. Dis. Child. **46,** 341 (1971). – 9. *Holman, R. T.,* The ratio of trienoic-tetraenoic acids in tissue lipids as a measure of EFA requirement. J. Nutr. **70,** 405 (1960). – 10. *Jensen, S. B., M. Schneidelbach,* On the effectiveness of our pancreatic enzyme preparations. A study of fat and protein absorption and of the total amount of faeces. Acta paediatrica **45,** 70 (1956). – 11. *McWhirter, W. R.,* Plasma tocopherol in infants and children. Acta paed. Scand. **64,** 446 (1975). – 12. *Muller, D. P. R., J. T. Harries, J. K. Lloyd,* The relative importance of the factors involved in the absorption of vitamin E in children. Gut **15,** 966 (1974). – 13. *Nitowsky, H. M., M. Cornblath, H. H. Gordon,* Studies of tocopherol deficiency in infants and children. II. Plasma tocopherol and erythrocyte hemolysis in hydrogen peroxide. Amer. J. Dis. Child. **92,** 164 (1956). – 14. *Nitowsky, H. M., J. T. Tilden,* Some studies of tocopherol deficiency in infants and children. III. Relation of blood catalase activity and other factors to hemolysis of erythrocytes in hydrogen peroxide. Amer. J. Clin. Nutr. **4,** 397 (1956). – 15. *Nitowsky, H. M., H. H. Gordon, J. T. Tilden,* Studies of tocopherol deficiency in infants and children. IV. The effect of alpha-tocopherol on creatinuria in patients with cystic fibrosis of the pancreas and biliary atresia. Bull. Johns Hopkins Hosp. **98,** 361 (1956). – 16. *Paulsrud, R. J., L. Pensler, C. F. Whitten, S. Stewart, R. T. Holman,* Essential fatty acid deficiency in infants induced by fat-free intravenous feeding. Amer. J. Clin. Nutr. **25,** 897 (1972). – 17. *Quaife, M. L., N. S. Scrimshaw, O. H. Lowry,* A micromethod for assay of total tocopherols in blood serum. J. Biol. Chem. **180,** 1229 (1949). – 18. *Robinson, G. R.,* Essential fatty acids in cystic fibrosis. Lancet **1975/II,** 919. – 19. *Ross, C. A. A.,* Fat absorption studies in the diagnosis and treatment of pancreatic fibrosis. Arch. Dis. Child. **30,** 316 (1955). – 20. *Rivers, J. P. W., A. G. Hassam,* Defective essential fatty acid metabolism in cystic fibrosis. Lancet **1975/II,** 642. – 21. *Shmerling, D. H., J. C. W. Forrer, A. Prader,* Fecal fat and nitrogen in healthy children and in children with malabsorption or maldigestion. Pediatrics **46,** 690 (1970). – 22. *Underwood, B. A., C. R. Denning, M. Navab,* Polyunsaturated fatty acids and tocopherol levels in patients with cystic fibrosis. Ann. New York Acad. Sci. **203,** 237 (1972). – 23. *Underwood, B. A., C. R. Denning,* Blood and liver concentrations of vitamin A and E in children with cystic fibrosis of the pancreas. Pediatr. Res. **6,** 26 (1972). – 24. *Watts, R., S. Taylor, R. Postuma, C. A. Smalley,* Essential fatty acid metabolism in cystic fibrosis. Lancet **1975/II,** 983. – 25. *Witting, L. A.,* Vitamin E-polyunsaturated lipid relationship in diet and tissues. Amer. J. Clin. Nutr. **27,** 952 (1974). – 26. *Zöllner, N., G. Wolfram,* Cholesterinester im Plasma als Parameter der Linolsäureversorgung des Menschen. Z. Ges. Exptl. Med. **146,** 89 (1968).

Für die Verfasser:

Dr. *Hansjosef Böhles,* Universitätskinderklinik, Loschgestraße 15, 8520 Erlangen