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Cystic fibrosis
of the pancreas
liver
transport
vitamin A
vitamin E

Blood and Liver Concentrations of Vitamins A and E in Children with Cystic Fibrosis of the Pancreas

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Extract

The median vitamin A level in plasma of patients with cystic fibrosis of the pancreas (CFP) (18 μg/100 ml) was less than half that of a control group (39 μg/100 ml), whereas the median vitamin A level in liver (57 μg/g) was nearly 3.5 times higher (Table II.). Concentrations of vitamin E in both the plasma and the liver of the patients were less than one-third those of the control group. The data suggest that vitamin A-supplemented children with CFP are unable to maintain normal circulating levels of vitamin A because of a defect in mobilization or transport from storage tissue, rather than because of tissue depletion. Low blood levels of vitamin E, however, reflect tissue depletion and not defective transport.

Speculation

The cause of the apparent defect in ability to mobilize or transport vitamin A from the liver in children with CFP may be due to a limited ability to synthesize transport proteins or to a reduced activity of hepatic retinyl ester hydrolase enzymes. These alternatives are under investigation.

Introduction

Lipid absorption is impaired in most children with cystic fibrosis of the pancreas (CFP). Enzyme replacement therapy partially, but inconsistently, corrects the problem [1, 8, 19]. Clinical, anatomic, and biochemical evidences for deficiencies of the fat-soluble vitamins A, D, E, and K have been described in children with CFP who received no vitamin supplementation [5, 12, 22, 23, 24, 27, 28]. Some evidences of deficiency may disappear after feeding the responsible vitamin, but the fasting concentration of vitamin A in plasma often remains low, and the level of vitamin E responds very slowly, in spite of oral administration of high doses of these vitamins [3, 10, 16].

The present study was planned to determine whether the low plasma concentrations of vitamins A and E usually seen in patients with CFP are a reflection of tissue depletion caused by malabsorption or whether there is a defect in the transport of these vitamins from storage tissues. Levels of cholesterol, vitamins A and E, and carotene in plasma were determined on blood from 48 randomly selected patients with CFP after a 12–18 hr fast. Of these 48, 12 patients died and, at autopsy, plasma and tissue were obtained and assayed for vitamins A and E.

Materials and Methods

All patients were diagnosed as having CFP on the basis of the clinical picture, elevated sweat electrolytes, and deficiency of trypsin in the duodenal contents. At the time of diagnosis, all patients were routinely placed on daily multivitamin supplements (including at least

enzymes, and a high protein, high caloric diet containing as much fat as could be tolerated. Supplementation with vitamin E was not routine.

Blood samples were taken from 48 informed and consenting patients randomly selected from a group of 300 who were currently attending the CFP clinic after a fast of 12–18 hr. The subjects studied ranged in age from 6 months to 22 years. Blood was drawn into Vacutainers containing ethylenediaminetetraacetate monohydrate (EDTA). Most of the 48 patients had two or more specimens analyzed at intervals of 6–12 months.

Autopsies were performed within 24 hr on the 12 patients who died. Liver tissue was removed from the central portion of the right lobe and immediately frozen in an atmosphere of nitrogen. Skeletal muscle was excised and treated similarly in some patients. Analyses for vitamins A and E (α-tocopherol) were completed within 1 week of death.

Plasma concentrations of vitamin A and carotene were determined by the micromethod of Neeld and Pearson [21], using trifluoroacetic acid (TFA). Vitamin A levels in liver were measured on the nonsaponifiable extract from 1 g finely homogenized frozen tissue by the TFA method. Pyrogallol was added during saponification to minimize oxidative losses. Recoveries of variable quantities of vitamin A added to liver tissue were consistently above 93%.

Vitamin E levels in plasma were measured by the method of Hashim and Schuttringer [13]. The concentration of α-tocopherol in liver was determined spectrophotometrically after isolation from the nonsaponifiable lipids by two-dimensional thin layer chromatography as described by Bieri [4]. Recoveries of 5–20 μg α-tocopherol added to liver tissue were consistently above 90%.

Cholesterol was measured by standard procedures in an AutoAnalyzer. Total protein in plasma was determined by the biuret procedure; albumin was determined by electrophoresis on silica gel-coated cellulose-acetate strips. Lipid content of liver was determined gravimetrically on a chloroform-methanol extract [9].

Results

The plasma levels of vitamins A and E and carotene from 48 patients with CFP, and cholesterol values for 31 of those patients, are shown in Table I. The age range of this group was 6 months–22 years, with a mean of 11 years. For comparison, data are given for a group of 47 normal children 6–14 years of age admitted to the hospital for elective surgical procedures, and for a group of 642 normal New York City school children 10–13 years of age as reported by Baker *et al.* [2]. All patients with CFP received daily multivitamin supplements including at least 10,000 IU vitamin A, but no vitamin E. It was not known whether the hospital control group received vitamin supplementation; 29% of the New York City school control group were reported to receive supplementary vitamins, but no mention was made of the nature of the supplement.

Table I. Plasma levels of vitamin A, carotene, vitamin E, and cholesterol in children with cystic fibrosis of the pancreas and in control subjects

	Cystic Fibrosis			Normal	
	$\bar{X} \pm$ SD	Median	Range	Babies in hospital $\bar{X} \pm$ SD	Baker[1] median
Vitamin A, μg/100 ml	29 ± 13 (48)[2]	27	7–77	39 ± 14 (47)	44
Carotene, μg/100ml	28 ± 19 (48)	20	5–72	153 ± 46 (47)	145
Vitamin E, μg/100 ml	270 ± 155 (48)	242	4–586	759 ± 171 (41)	900
Cholesterol, mg/100 ml	102 ± 23 (31)	104	66–146		154

[1] Baker *et al.* [2]. Data based on 642 subjects.

[2] Number in parentheses is the number of subjects studied. In most cases of cystic fibrosis of the pancreas, the means calculated from two or more specimens analyzed at intervals of 6–12 months were used.

All parameters measured were considerably reduced in the plasma of CFP patients. A significant correlation was found between plasma levels of vitamins A and E ($r = 0.28$, $P < 0.05$) in the patients but not in the hospital controls. A significant correlation also was found between plasma levels of vitamin E and cholesterol ($r = 0.46$, $P < 0.01$).

Twelve of the forty-eight patients with CFP succumbed to their disease, permitting tissue analyses. Limited preliminary results on some of those patients have been reported [29]. Complete results of vitamin A and albumin levels in the plasma obtained from samplings before death and at autopsy, and the liver weight, lipid concentration, and vitamin A at the time of autopsy are presented in Table II. The values for vitamin A and lipid are compared with those of a control group of children of similar age, but without CFP, who died suddenly from accidental causes [31]. The serum values for the control group were obtained from heart blood taken at autopsy within 24 hr of death.

The mean vitamin A level in plasma of patients 10,000 international units (IU) vitamin A), pancreatic

Table II. Studies on plasma and liver in children with cystic fibrosis of the pancreas and in control subjects

Patient	Sex	Age, yr	Plasma			Liver		
			Time specimen obtained before death	Albumin, g/100 ml	Vitamin A, μg/100 ml	Total wt, g	Lipid, g/100 g	Vitamin A, μg/g
A	M	17	13 mos	4.4	77	900	3.8	482
B	F	14	4.5 mos	3.4	23			
			2.5 mos	2.7	14	1050	8.2	127
C	F	8				800	3.7	248
D	F	14	17.5 mos	3.9	20			
			3 mos	4.5	18			
			3 wks	3.1	18	850	6.4	68
E	F	9	0	2.3	10	1000	24.7	23
F	M	17	23 mos		38			
			9 mos	4.2	35			
			0	3.2	6	980	5.6	197
G	F	23	22 mos	4.1	29			
			14 mos		24			
			9 mos	4.2	26			
			1 wk	2.9	13		4.5	379
H	M	17	15 mos	3.2	22			
			7 mos	3.3	13			
			0	1.8	16		4.5	85
I	M	11	26 mos		31			
			12 mos	3.4	18			
			0	3.0	30	600	7.1	995
J	M	9	0	3.7	17	730	5.2	156
K	F	12	38 mos		23			
			4 mos		12			
			0	2.8	15	700	2.9	230
L	F	19	0	2.1	8		4.4	862
Mean ± SD				3.3 ± 0.7	22 ± 14		5.2 ± 1.5[1]	317 ± 300[2]
Median					18			197
Control subjects								
Mean ± SD				3.4 ± 0.5[3]	38 ± 11[3]		4.2 ± 0.5[4]	126 ± 164[4]
Median					39			57

[1] Value from *patient E* is excluded because this patient had extensive biliary cirrhosis.
[2] Concentration of vitamin A in liver was significantly higher in the children with CFP than in the control subjects ($P < 0.05$).
[3] Mean of 9 subjects.
[4] Mean of 18 subjects.

with CFP was significantly lower, and the mean vitamin A level in liver was significantly higher, than the values for the control group. In fact, the median vitamin A level in plasma of the patients was less than half that of the control group, whereas the median vitamin A level in liver was nearly 3.5 times higher. Lipid concentrations in liver were not significantly different if the value for one patient with extensive liver involvement is omitted (*patient E*). No correlation was found between the vitamin A concentrations in the plasma and the liver of either the subjects with CFP or the control subjects.

In contrast to vitamin A, concentrations of vitamin E in both the plasma and the liver of patients with CFP receiving no supplemental tocopherol were less than one-third that of a control group [29, 30] (Table III). Values from *patients E* and *G* were omitted because a tocopherol supplement had been given before death. The ratio of tissue to plasma vitamin E was variable and was not significantly different in the patients from that in the control group. Vitamin E in the plasma of the CFP patients correlated with liver α-tocopherol, both when the latter was expressed on the basis of tissue weight ($r = 0.64$, $P < 0.05$) and on the basis of tissue lipid ($r = 0.91$, $P < 0.01$). The correlation coefficients were higher in the patients than in the controls.

In a few patients who died, the α-tocopherol in skel-

etal muscle was measured (Table III). In general, muscle contained about the same amount of tocopherol as did the liver.

Discussion

By repeated measurements over a span of several years on the same patient with CFP, we have found the levels of vitamin A in plasma to vary from less than 10 μg/100 ml to 20–30 μg/100 ml (low normal). Vitamin A levels in plasma were seldom found to be above 35 μg/100 ml, and the fluctuations observed could not consistently be correlated with abnormal liver function tests, depressed serum albumin, or the clinical condition of the patient at the time of sampling. Samples taken near the time of death or at autopsy, however, usually had lower concentrations of vitamin A and albumin than samples obtained earlier in the clinical course of the disease. Vitamin E levels in plasma obtained at autopsy had not changed and showed less fluctuation than vitamin A in serially obtained specimens.

Median levels of vitamin A in plasma of school children in the United States range from 44 to 51 μg/100 ml [2, 14, 17, 18], except in depressed areas where a median of 26 μg/100 ml is reported [20]. In one study, only 6.5% of the normal children had vitamin A levels in plasma below 30 μg/100 ml [18], whereas over 50% of the children with CFP in our study had values below this figure (Table I). All of the CFP children received supplemental vitamin A, and one would expect their plasma levels to be above 30 μg/100 ml unless a defect in the absorption or transport of the vitamin existed.

Lipids are malabsorbed by many CFP patients. The low plasma levels of carotene and the low plasma and tissue concentrations of vitamin E found in the present study support this fact. The low plasma level of vitamin A, however, was not solely due to malabsorption inasmuch as 11 of the 12 patients studied at autopsy had liver stores of vitamin A which were considerably above the median for a normal population. Only one patient with extensive cirrhosis and fatty infiltration had a low hepatic concentration. Hence, a sufficient quantity of vitamin A had been absorbed and deposited in the liver to maintain stores even higher than those of age-matched controls, and considerably higher than those reported for an adult population [31].

Normally, stores of vitamin A in liver are exhausted before plasma levels decline. In 1939, Andersen [1] suggested a defect in the transport of vitamin A given to children with CFP which she attributed to low serum lipids. Recently, however, the transport of retinol was shown to be dependent upon two carrier proteins, retinol-binding protein (RBP) and prealbumin [11, 15], both of which are synthesized in the liver [25]. Most vitamin A is stored in the liver esterified with long chain fatty acid and presumably is freed prior to binding with transport proteins. Our data suggest that in children with CFP there is a defect in the ability to mobilize vitamin A from hepatic cells, perhaps owing to an inability to release retinol or to an inability to provide the transport proteins. This interpretation is consistent with reports that blood levels in CFP patients do rise after vitamin A absorption tests, but are not maintained at normal levels in spite of prolonged supplementation at high dosages [3].

Our data do not identify the true cause of the defect. Like albumin, the vitamin A carrier proteins are believed to be synthesized in the liver and liver metabolism is sometimes altered in CFP [6–8]. At autopsy, the liver from 3 of the 12 patients showed focal biliary cirrhosis as typically seen in some cases of CFP [7]. The liver of the remaining nine patients showed varying degrees of chronic passive congestion of the cardiac variety and some central necrosis and fibrosis, but there was no evidence of liver disease as gauged by liver function tests. Hence, this subclinical liver pa-

Table III. Vitamin E in plasma and α-tocopherol levels in liver and muscle in unsupplemented children with cystic fibrosis of the pancreas and in control subjects

Patient	No. of specimens	Plasma	Liver	Muscle
		Vitamin E, μg/100 ml	α-tocopherol, μg/g	α-tocopherol, μg/g
A	1	370	3.5	3.5
B	2	172 ± 9	4.5	5.6
C			6.6	
D	2	160 ± 34	4.0	
F	3	154 ± 54	3.2	
H	3	208 ± 76	3.4	2.0
I	3	226 ± 84	3.2	2.6
J	1	125	3.0	2.4
K	3	123 ± 69	1.0	1.4
L	1	12	2.6	0.9
Mean		171 ± 96	3.5 ± 1.4	2.9
Median		160	3.2	2.4
Control subjects				
Mean		708[1] ± 128	12.8[2] ± 3.4	

[1] Mean of 9 subjects.
[2] Mean of 18 subjects.

thology noted at autopsy might account for the reduced plasma levels of albumin noted in some patients, and possibly of transport proteins. However, Strober *et al.* [26] reported that rates of albumin synthesis in children with CFP are normal and that the hypoalbuminemia, sometimes seen in older children with CFP and in severe pulmonary disease, is primarily due to hemodilution. Hemodilution is not likely to be the cause of the low vitamin A levels in our studies, inasmuch as no correlation with albumin levels in serum was found. Also, we could not relate the blood level of vitamin A with the severity of respiratory disease as judged clinically.

In contrast to vitamin A, the content of vitamin E in both plasma and liver was low in the children who did not receive an α-tocopherol supplement. The ratio of the levels of α-tocopherol in plasma and tissue was constant, suggesting that both tissues were depleted in parallel. These low plasma values are similar to those reported by others for children with CFP [3, 12, 16] and probably reflect malabsorption. Several patients received 5 mg/kg/day of a water-dispersible vitamin E preparation for 4–11 months and exhibited an increase in plasma values in most cases, but the values usually remained below normal. *Patient G* had received this supplement for nearly 1 year before death and *patient E* was given 15 mg/kg/day of α-tocopherol for 3 months before death. As previously reported [29], plasma levels of both these subjects were normal; concentrations in the liver were normal in *patient G* and very high in *patient E* (180 μg/g tissue).

Summary

Concentrations of cholesterol, vitamins A and E, and carotene were determined in plasma from 48 randomly selected patients with CFP. Twelve patients died and, at autopsy, plasma and tissue were analyzed for vitamins A and E. The patients with CFP routinely received supplementary vitamin A (at least 10,000 IU daily), pancreatic enzymes, and a high protein, high caloric diet, but no supplementary vitamin E.

All lipids measured were lower in the plasma of children with CFP than in that of hospitalized control patients of comparable age and in that of normal children reported in the literature.

Although the median level of vitamin A in plasma was less than half that of the controls, the median level in liver for the 12 children who came to autopsy was 3.5 times that of the control group. The concentrations of vitamin E in both plasma and liver, however, were less than one-third those of the control group. Concentrations of vitamin E in muscle were also reduced in the seven patients with CFP in whom this tissue was studied.

The data suggest that children with CFP receiving oral vitamin A supplements are unable to maintain normal circulating levels of vitamin A because of a defect in mobilization or transport from storage tissue, rather than because of depleted tissue levels. In contrast, the low plasma levels of vitamin E reflect tissue depletion and not defective transport.

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