Some biochemical indices of nutrition in treated cystic fibrosis patients[1, 2]

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ABSTRACT Postprandial levels of copper, ceruloplasmin, iron, total iron binding capacity, cholesterol, vitamin A, carotene, folic acid, vitamin C, albumin, and total globulins in plasma, of 25-OH-vitamin D in serum, and of glutathione reductase activity, an index of riboflavin status, in erythrocytes were determined in a group of 18 juvenile cystic fibrosis patients receiving specialized outpatient care with attention to diet, vitamin supplementation, and pancreatic enzyme replacement. Bone mineralization was assesed by radiographic and photon beam technique. In the plasma of cystic fibrosis patients, levels were elevated for copper, ceruloplasmin, total globins, and total proteins and were depressed for iron, vitamin D, vitamin A, carotene, and albumin. Cortical thickness was diminished in the patients, but bone density was not. For patients with cystic fibrosis, a relation was established between forced vital capacity and certain biochemical indices in plasma. As forced vital capacity decreased, plasma levels increased for copper, total globulins and total proteins and decreased for albumin. *Am. J. Clin. Nutr.* 34: 462–474, 1981.

KEY WORDS Cystic fibrosis, pancreatic enzymes, vitamin supplements, copper, ceruloplasmin, 25-OH-vitamin D, bone mineralization

Introduction

Cystic fibrosis, a hereditary, idiopathic disease, alters exocrine gland secretion, and is characterized by recurrent pulmonary infections and pancreatic insufficiency with maldigestion and malabsorption. It is also associated with malnutrition and growth retardation. Advances in therapy, particularly of the pulmonary disease, with the introduction of chest physiotherapy and antibiotics (1–3) had increased life expectancy from less than 1 yr in 1948 (4) to 21 yr in 1964 (5). Despite progress in arresting the pulmonary deterioration and improving survival, impaired growth and undernutrition continue to affect a substantial number of patients. The specific relationship between impaired nutrition and growth retardation in cystic fibrosis is incompletely defined. Several studies have suggested growth failure is more closely related to the severity of the pulmonary disease than to the pancreatic insufficiency (6, 7). However, as a broad interrelationship between nutrition and infection has been demonstrated by Scrimshaw et al. (8), it seems likely that the nutritional status of a patient with cystic fibrosis influences his or her resistance to respiratory infection.

Malabsorption of many specific nutrients has been reported (7, 9–20) and thus, despite the documentation of protein and energy intakes well in excess of the Recommended

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Dietary Allowance in one report (21), nutritional deficiencies of protein (22, 23), fat (7, 10, 11, 24–26), fat-soluble vitamins (11, 12, 14, 24, 27–33), vitamin B_{12} (34, 35), and various minerals (36–38) have been reported in patients with cystic fibrosis Recent attention has been given to the use of dietary supplementation with easily digested artificial diets in the management of cystic fibrosis Results have been variable with both encouraging (39–41) and discouraging (42–44) findings Most modern treatment regimens include a high-protein, high-energy, moderate- to low-fat dietary intake, supplemental vitamins, and individualized use of pancreatic enzymes The techniques used to measure adequacy of replacement and supplementation therapy are rarely comprehensive We now present the results of a broad biochemical nutritional assessment in a group of juvenile cystic fibrosis patients attending a specialized outpatient clinic, and review the literature on nutritional status in this disease

Patients and methods

Eighteen patients, ages 6 to 18 yr (mean age 11 yr) with cystic fibrosis were randomly selected from among registrants attending an outpatient appointment in the Cystic Fibrosis and Immunology Clinic of the Wyler Children's Hospital Evidence of an acute infectious process was a criterion for exclusion from the study, and no patient who was taking any antibiotic which might interfere with the microbiological assay for folate was included The diagnosis of cystic fibrosis had been established in all patients included in the study on the basis of a quantitative pilocarpine iontophoresis sweat test and analysis of sweat electolytes (abnormal test, >60 mEq/l for Na^+ and Cl) and a typical clinical evolution The protocol was approved by the Human Investigation Committee of the University of Chicago Hospital and Clinics Informed consent was obtained from the patients and from the parents or guardian after the nature and purpose of the studies were fully explained

Patients were interviewed and studied during a regularly scheduled clinic visit As part of routine care, individualized attempts at optimizing nutritional status for all of the patients had been made Patients all received individual instructions for a high-protein, high-energy, moderate- to low-fat diet from the clinic dietitian In addition, for the majority of patients, a multivitamin preparation, Poly-vi-sol (Mead Johnson Laboratories, Evansville, IN) had been prescribed Pancreatic function, e g , stool trypsin, secretin test fecal balance of fat and nitrogen, etc , was not evaluated in our clinic Pancreatic enzymes are prescribed, however with the enzyme dosage titrated to achieve the maximum clinical improvement in the odor consistency, and frequency to stools The pancreatic enzyme preparation was Viokase (Viobin Corp , Monticello, IL) in five patients and Cotazyme (Organin Inc , West Orange, NJ) in 11 others Two patients were not taking pancreatic enzymes, one because of noncompliance with her physicians' recommendations (patient 13) and the other because he showed no clinical evidence of pancreatic insufficiency (patient 18) Regular pulmonary function tests, including forced vital capacity (FVC) and 1-second forced expiratory volume (FEV_1), performed on a Pulmonar spirometer (Jones Medical Instrument Co , Oakbrook, IL), are used along with ausculation and x-ray to evaluate the respiratory function and health of the patients Patients in this clinic may receive brief courses of antibiotic therapy when indicated by changes in sputum production, in ausculatory or radiographic findings, or in clinic condition, but no patient was receiving antibiotics at the time of study The current height and weight measurements were obtained from patients' clinic records and height-for-age and weight-for-age percentiles were calculated from the anthropometric scales of the Iowa Child Welfare Station or the Stuart curve of the Harvard School of Public Health

Forty simultaneous adolescent controls, 16 girls and 24 boys, aged from 10 to 17 yr (mean age = 13 yr) from a private day-school were studied after their parents gave written informed consent Information on vitamin pill consumption and diet is not available for the adolescent control group The control values for 25-hydroxycholecalciferol (25-OH-vitamin D) were obtained from 17 normal adult volunteers, none of whom were taking supplemental vitamin D results of skeletal mineralization determinations for a group of normal children and adolescents were obtained from the archives of the Nuclear Medicine Department

Postprandial blood samples for plasma and erythrocytes were taken between 10 AM and noon from all subjects, patients and controls Samples were drawn into plastic syringes and handled in plastic, trace metal-free containers After withdrawal, the whole blood was anticoagulated promptly with oxalate and centrifuged Plasma and packed red cells were stored separately at −20°C until analysis Hemolyzed samples were excluded from determinations for copper, iron, and iron binding capacity (TIBC) Samples for serum 25-OH-vitamin D were drawn from the cystic fibrosis patients, blood was placed in clotting tubes, centrifuged after coagulation, and stored in the dark at −20°C until analyzed Sera from controls were handled in an identical manner

For the analysis of copper concentration, plasma was diluted 5-fold in trace metal-free water and the copper content measured by atomic absorption spectrophotometry on a Varian Techtron Model AA5 spectrophotometer (45) Plasma ceruloplasmin was measured after the method of Ravin (46) Plasma iron and iron-binding capacity were determined in the 5-fold plasma dilutions by a modification of the Zaino (47) method and atomic absorption spectrophotometry Serum 25-OH-vitamin D was determined by the competitive binding method of Haddad and Chyu (48), with the following modifications kidney cytosol binder was prepared from nonrachitic rats, the specific activity of the labeled 25-OH-vitamin D was 2 9 curie/m*M*, and the samples were dissolved in sufficient ethanol to provide aliquots of 500 and 100 μl after the silicic acid chromatography Plasma carotene and vitamin A were by a modified Carr-Price procedure, trifluoroacetic acid was substituted as the chromogenic

reagent (49) Folic acid was measured by a *Lactobacillus casei* microbiological assay (50) A modified dinitrophenyl hydrazine method (51) was used to measure plasma ascorbic acid The level of vitamin B_2 (riboflavin) was determined in hemolysates of red blood cells Riboflavin status was determined in hemolysates of red blood cells Riboflavin status was expressed as activity units of erythrocyte glutathione reductase (52) performed on a Honeywell Diclan 240 Digital Clinical Analyzer (Honeywell Corp , Minneapolis, MN) Total cholesterol (free cholesterol, cholesterol esters, and protein-bound cholesterol) was determined after the method of Abell et al (53) Cellulose acetate paper electrophoresis was used to determine albumin and total globulin Total plasma proteins were measured by a modified Gilford microprocedure (54)

Two techniques, measurement of metacarpal cortical thickness and proton beam absorption, were used to assess skeletal mineralization in each patient and control The Garn index (55), an expression of combined cortical thickness of the second metacarpal of the left hand, was determined from fine detail radiographs of the hand on fine grain industrial film, Kodak type M (Eastman Kodak Co Rochester, NY) that was reviewed with optical magnification of 4 to 10× by the method of Genant et al (56) Bone mineral content for both cortical and medullary phalangeal bone was determined by measuring the transmission, through the diaphysis of the middle phalanx of the third right digit of the left hand, of a narrow, collimated beam of photon radiation emitted from an I^{125} source after the method of Lanzl and Strandjord (57) The transmitted beam is detected by a sodium iodide scintillation crystal connected to a photomultiplier, and bone density is expressed as the linear absorption coefficient, μ bone, cm^{-1}, after adjustment for bone width and correction for soft tissue absorption

The Student's *t* test was used for statistical comparison of values between patient and control groups The product moment correlation coefficients were also computed for each clinical laboratory variable against all other variables This process resulted in an "experiment-wise" significance level that was substantially larger than the "test-wise ' significance level reported herein for individual intergroup comparisons Simple linear regression was used to compare values with a group

Results

Individual values for age, sex, height-for-age, weight-for-age, pulmonary function, pancreatic enzyme replacement, and vitamin supplement intake are given for the 18 patients with cystic fibrosis in **Table 1**. Also listed are individual and grouped data for biochemical assays Values for the cystic fibrosis patients falling 2 SD or more outside of the control mean are indicated For the purposes of our analysis, "deficient" levels of the biochemical indices are those which are 2 SD or more from the control mean in a direction which indicates nutritional impairment

Patient profile

Mean ages did not differ significantly between the patients and the adolescent controls ($0\ 1 > p > 0\ 05$) The patients with cystic fibrosis tended to show a greater degree of retardation of weight than of linear growth, 12 of 18 patients' weights were at or less than 10th percentile, with 10 of those at or below the 3rd percentile, while nine patients had a height-for-age equal to or less than the 10th percentile with only three of these at or below the 3rd percentile Eight patients showed clear evidence of restrictive lung disease, with forced vital capacities below 75% of predicted levels

Mineral nutrients

Plasma copper and ceruloplasmin were significantly higher in the patients, means 131 0 μg/dl and 22 6 mg/dl, respectively, than in the adolescent controls, means 102 7 μg and 18.8 mg/dl, respectively Values for TIBC did not significantly differ between the groups, but the mean plasma iron for patients, 67 3 μg/dl, was significantly lower than the corresponding mean for controls, 101 5 μg/dl Two individuals with cystic fibrosis had iron levels that were 2 SD below the control mean

Lipids and fat soluble vitamins

The mean plasma cholesterol for the cystic fibrosis group of 118 6 mg/dl was significantly lower than the 152 9 mg/dl observed in controls (Table 1) Mean serum 25-OH-vitamin D concentrations were significantly lower in patients, 17.4 ng/ml, than the winter values of controls, 23 8 ng/ml When the abnormally outlying value in patient 4 was eliminated, the difference between patients and controls was significant for plasma vitamin A with mean concentrations of 30 7 and 36 9 μg/dl, respectively Three individuals within the patient group had plasma vitamin A levels that were more than 2 SD below the control mean Mean plasma carotene concentration of the controls, 128 8 μg/dl, was significantly higher than the 33 9 μg/dl mean of the patients and was deficient in 10 to 18 patients

Water soluble vitamins

Levels of the three water soluble vitamins, folic acid, ascorbic acid, and riboflavin, did

TABLE 1
Individual clinical and biochemical data on the cystic fibrosis patients

			Profile data						Mineral nutrients and their binding proteins			
No.	Sex	Age	Height percentile	Weight percentile	FVC % predicted	$\frac{FEV}{FVC}$ %	No. enzyme capsules/day	No. vitamin capsules/day	Copper	Ceruloplasmin	Iron	TIBC
									μg/dl	*mg/dl*	*μg/dl*	*μg/dl*
1	M	6	90th	75th	81	78	7 (v)*	2	136	20.8	56	417
2	M	7	80th	90th	82	71	5 (c)†	2	106		100	349
3	F	8	10th	50th			10 (v)	1	122	18.9	80	432
4	M	9	<3rd	<3rd	71	75	10 (c)	2	90		91	414
5	M	9	35th	30th	79	93	12 (c)	2	109		98	370
6	M	9	25th	3rd	100	55	3 (c)	1	123		77	287
7	M	11	15th	<3rd	48	67	26 (c)	0	176‡	30.4‡	49	322
8	F	11	10th	<3rd	92	76	10 (v)	2	126	22.5	92	380
9	F	11	5th	<3rd	70	66	20 (c)	1	136		74	286
10	M	11	50th	25th	88	75	12 (c)	2		22.6		
11	M	12	10th	10th	63	71	28 (v)	2	149‡	23.8	46	317
12	F	12	50th	<3rd	41	54	16 (c)	2	192‡	26.4‡	36§	349
13	F	12	10th	20th	98	77	0	1	132		59	357
14	M	12	3rd	<3rd	78	43	9 (c)	2	109		35§	290
15	M	13	15th	10th	80	78	15 (c)	3	150‡	20.7	58	284
16	F	15	<3rd	<3rd	61	53	3 (v)	1	132		64	312
17	F	16	15th	<3rd	54	45	10 (c)	0	111	17.0	62	446
18	M	17	10th	3rd	95	88	0	0				
				Cystic fibrosis group mean ± SD					131.0 ±26.2	22.6 ±4.0	67.3 ±21.0	350.8 ±54.9
				Control group mean ± SD					102.7 ±21.2 (n = 38)	18.8 ±2.7 (n = 17)	101.5 ±33.2 (n = 38)	333.5 ±62.5 (n = 37)
				p value					$p < 0.05$	$p < 0.05$	$p < 0.05$	NS
				No. of patients with "deficient"‖ level					0/16	0/9	2/16	0/18

TABLE 1 (continued)

Lipids and fat soluble vitamins				Water-soluble vitamins			Plasma proteins				No of "deficient"§ indices
Cholesterol	Vitamin A	Carotene	25 OH Vit. D ng/ml	Folate	Vitamin C	Glutathione reductase (riboflavin)	Total protein	Albumin	Total globulins	A.G ratio	
mg/dl	*μg/dl*	*μg/dl*	*ng/ml*	*ng/ml*	*mg/dl*	*AU*	*g/dl*	*g/dl*	*g/dl*		
150	31.1	22.4§		30.0‡	1.27	0.85	7.23	4.18	3.16‡	1.37	1/13
100	40.0	9.6§	12.7§			0.93	7.34	3.97§	3.05‡	1.18	3/11
111	48.5	23.2§	12.4§	19.6‡	1.61	0.87	7.66‡	4.60	3.37‡	1.50	2/14
115	(61.4)‡¶	13.6§		6.9	0.48§	0.96	6.74	3.72§	3.02‡	1.23	3/12
122	28.5	25.6§	14.1			1.01	6.92	3.92§	3.00‡	1.31	2/11
124	21.4§	65.8	21.0		1.47	1.05	7.47‡	3.63§	3.85‡	0.94	2/12
106	32.4	40.1	17.8	30.0‡	1.15	0.90	7.99‡	3.84§	4.16‡	0.92	1/14
117	43.6	41.7	32.2	26.2‡		0.87	7.41	4.05§	3.36‡	1.21	1/13
118	32.0	62.6	28.7		1.46	0.87	8.08‡	3.77§	4.31‡	0.87	1/12
96	26.8	48.1	16.1	8.8		0.92	6.63	3.30§	3.33‡	0.99	1/10
108	18.9§	24.8§	12.7§	12.2	0.97	0.99	7.36	3.45§	3.91‡	0.88	4/14
127	23.6§	14.4§	15.2	9.2	0.53§	0.95	8.21‡	3.47§	4.74‡	0.73	5/14
139	32.6	48.1	15.3	7.4	1.16	1.08	7.54‡	4.22	3.32‡	1.27	0/13
116	28.3	17.6§		9.6	1.08	0.89	7.36	3.94§	3.42‡	1.15	3/12
123	28.7	13.6§		8.2	0.38§	1.09	7.14	3.70§	3.44‡	1.07	3/13
141	30.2	56.1	25.7			1.00	7.30	3.74§	3.56‡	1.05	1/11
85§	16.7§	19.2§	1.9§	11.9	1.00	0.94	8.10‡	3.52§	4.59‡	0.77	5/14
136	37.5	64.2		2.2§		0.97	7.58‡	4.42	3.16‡	1.40	1/8
118.6 ±16.8	30.7 ±8.5	33.9 ±19.4	17.4 ±8.0	13.2 ±9.9	1.04 ±0.40	0.95 ±0.07	7.44 ±0.45	3.85 ±0.34	3.59 ±0.55	1.10 ±0.22	
152.9 ±28.8 (n = 40) p < 0.05 1/18	36.9 ±6.0 (n = 33) p < 0.05 3/18	128.8 ±50.9 (n = 34) < 0.05 10/18	23.8 ±5.6 (n = 17) < 0.05 4/16	7.7 ±2.7 (n = 20) NS 1/13	1.26 ±0.27 (n=23) NS 3/12	0.99 ±0.14 (n = 32) NS 0/18	6.95 ±0.30 (n = 40) p < 0.05 0/18	4.61 ±0.25 (n = 40) p < 0.05 4/18	2.33 ±0.30 (n = 40) p < 0.05 0/18	2.00 ±0.30 (n = 40) p < 0.05	

* Viokase.
† Cotazyme.
‡ ≥ 2 SD of control mean.
§ ≤ 2 SD of control mean.
| "Deficient" level is equal to or greater than 2 SD from the control in the direction of nutrient deficiency.
¶ Not included in the mean.

not significantly differ between patients and controls. One individual among the 13 patients for whom folate was determined had values that were more than two standard deviations below the control mean. Three deficient ascorbic acid values were encountered among the 12 patients in whom this vitamin was measured.

Plasma proteins

The group mean for total plasma proteins, 7.44 g/dl, was significantly higher among cystic fibrosis patients than the 6.95 g/dl of the controls. The difference reflected the greatly elevated globulin concentrations of the patients, 3.59 g/dl versus 2.33 g/dl for controls. The concentration of albumin were lower in patients, 3.85 g/dl, than in controls, 4.61 g/dl; 14 of 18 patients had albumin levels that were more than 2 SD below the control mean. The albumin globulin ratio was significantly higher in the controls than in patients.

Skeletal mineralization

The mean Garn index of metacarpal cortical thickness was 3.03 mm for cystic fibrosis patients and 4.09 mm in controls ($p < 0.005$). The photon beam absorption technique, on the other hand, revealed no significant differences between the groups as mean linear absorption coefficients were 1.89 and 1.99 for patients and controls, respectively.

Clinical correlations

It has not been the practice in our clinic to assess the status of patients with global clinical scores such as the ones proposed by Shwachman and Kulczycki (58), by Huang et al. (59), or by Taussig et al. (60). Rather, emphasis has been placed on the degree of restrictive and obstructive lung disease as an objective index of pulmonary status, on the empirical requirement for pancreatic enzymes to achieve maximum improvement in stool characteristics, as an index of pancreatic exocrine function, and on growth attainment. With regard to anthropometry, height-for-age and weight-for-age reflect approximately the recent and long-term protein and energy nutrition of the patients. Linear regression of the biochemical nutrient assays and the A:G ratio against forced vital capacity, FEV_1/FVC, daily dosage of enzyme capsules, weight percentile and height percentile were performed. The correlations which achieved statistical significance have been listed in **Table 2.**

TABLE 2
Correlations of clinical and biochemical indices which achieved statistical significance

Clinical/biochemical correlation	Correlation coefficient	Significance level (p)
Forced vital capacity versus plasma copper	−0.517	<0.05
Forced vital capacity versus total plasma proteins	−0.504	<0.05
Forced vital capacity versus plasma albumin	+0.489	<0.05
Forced vital capacity versus plasma globulins	−0.663	<0.01
Forced vital capacity versus A:G ratio	+0.656	<0.01
Enzyme capsules/day versus plasma copper	+0.538	<0.05
Enzyme capsules/day versus ceruloplasmin	+0.701	<0.05
Enzyme capsules/day versus plasma globulins	+0.494	<0.05
Enzyme capsules/day versus A:G ratio	−0.497	<0.05
Weight percentile versus plasma globulins	−0.473	<0.05
Weight percentile versus A:G ratio	+0.483	<0.05

Discussion

Two factors focus the importance of nutritional considerations in cystic fibrosis: *1*) the nutritional determinants of growth failure in this disease are incompletely understood; *2*) the increasing longevity of patients raises the possibility, as yet unproven, that improved early nutrition might improve the quality of life in late adolescence and early adulthood. Over the years, various biochemical indices for specific nutrients have been evaluated in cystic fibrosis patients, but our study has been more comprehensive than the foregoing reports in terms of simultaneous variables. We have attempted a broad evaluation of multiple, biochemical indices of nutritional status in a group of cystic fibrosis patients who were receiving modern, concerted, specialized management to determine the nature and prevalence of any nutritional deficiencies that might persist despite such therapy and management.

As previously described in other populations of cystic fibrosis patients (6), the deficit in anthropometric indices was greater for weight than for height. No systematic inquiry into the dietary or supplemental vitamin intake of the control adolescent group was undertaken. Controls had unrestricted diets and presumably a higher fat intake than the patients. An unspecified number may have been using nutrient supplements, as well. Moreover, as postprandial blood samples were used in this study, they might be expected to reflect a rise in cholesterol concentration. Other nutrient levels may be affected by the morning meal also, but the design was consistent for both groups of subjects. Controls for vitamin D were neither simultaneous nor age-equivalent. They were purposely studied during the winter, however, when seasonal fluctuations in vitamin D levels would be expected to be at their lowest (61, 62). Moreover, no age-specific differences among children (63) or between adolescents and adults (64) in vitamin D have been identified beyond early infancy.

Minerals

Iron. Smith (18) demonstrated diminished absorption of organic iron from hemoglobin in cystic fibrosis patients treated with pancreatic supplementation as compared to untreated patients. Tönz and coworkers (65, 66) found no difference in the absorption of hemoglobin iron between patients and normals, but reported that the absorption of inorganic iron was elevated in the patients. They also suggested that pancreatic extract decreased iron absorption. Recent studies from Germany, using inorganic ^{59}Fe and whole-body counting techniques, demonstrated that patients with cystic fibrosis but normal iron stores absorbed iron in a manner identical to that of iron-repleted controls; iron-deficient patients with cystic fibrosis increased their iron absorption in a similar compensatory fashion to that of iron-deficient patients without cystic fibrosis (67). The range of absorption of biosynthetically-labeled ^{59}Fe hemoglobin, and pork meat and hog liver intrinsically-labeled with ^{59}Fe by cystic fibrosis children was also identical to values reported in adults with comparable iron stores (20). The presence or absence of pancreatic enzymes in the intestinal lumen did not influence the absorption of either inorganic or organic iron in patients with cystic fibrosis (20, 67).

In studies of tissue levels of iron, hepatic iron stores at autopsy of cystic fibrosis patients were normal (68). Iron levels in bone marrow, however, were variable, from depressed to elevated, in the series reported by Gilly et al. (69). In a similar pattern to that previously reported (18), plasma iron levels in our patients were strikingly depressed without a corresponding increase in TIBC. Such a pattern could be attributed to an impairment of transferrin synthesis paralleling that of albumin, or to redistribution of plasma iron. Moreover, as a majority of our patients were receiving pancreatic supplements, if the observations of Tönz et al. be correct, the absorption of food iron might have been reduced.

Copper. Copper content in fingernail clippings was elevated in cystic fibrosis patients as compared to controls in one study (70), while no difference was observed in another (L. Kopito, personal communication). In the present study, plasma copper was elevated in cystic fibrosis. Moreover, correlations were significant between plasma copper, and restrictive lung disease or pancreatic enzyme requirements; apparently copper levels rise as the pulmonary and pancreatic deterioration advances. The association between increased tissue and plasma copper levels and cystic fibrosis remains to be explained.

Among the mineral nutrients, a plasma pattern of diminished iron, borderline to normal zinc (N. W. Solomons, unpublished observations), and elevated copper was observed in our subjects with cystic fibrosis. Leukocytic endogenous mediator (LEM) is a polypeptide hormone released from phagocytizing cells during infections or inflammatory stress. It mediates a number of the serum changes that are characteristic of infections, such as diminished circulating zinc and iron (71, 72), increased circulating copper and ceruloplasmin (73), as well as increased amino acid turnover and augmented synthesis of acute phase macroglobulins (74). None of the patients in this study was acutely ill with respiratory infections at the time of sampling. It is tempting to speculate, as a unifying explanation for the trace mineral pattern, that

chronic stimulation of lung macrophages might chronically elevate LEM activity and mediate the changes in circulating mineral concentrations which have been observed.

Zinc. Evidence of impaired zinc nutriture in patients with cystic fibrosis has been discovered in the present subjects (N. W. Solomons, unpublished observations), in another series (37) and in one well-studied case (38). Palin et al. (75), however, were unable to show any beneficial nutritional effects of zinc supplementation in a cohort of patients with cystic fibrosis; these individuals, however, had no evidence of zinc deficiency in their pretreatment biochemical studies. Mineral balance studies in six patients taking pancreatic supplements showed impairment of apparent zinc absorption (19).

Selenium. The other trace mineral with a possibly precarious nutritional situation in cystic fibrosis is selenium. In the German study cited above, impaired absorption of ^{74}Se as selenomethionine incorporated into bioorganically-labeled pork meat (20). Convincing data on selenium nutriture have not yet appeared.

Lipids and fat-soluble vitamins

A major pathophysiological disturbance in cystic fibrosis is exocrine pancreatic insufficiency and the malabsorption of lipids and fat-soluble vitamins (7, 10, 13). Steatorrhea improved, but was not corrected with pancreatic enzyme replacement (7). Bile salt malabsorption in cystic fibrosis patients has also been described (76). Abnormalities of fatty acid patterns (9, 10) and essential fatty acid deficiencies have been reported (25, 26). In this study, we were able to measure concentrations of the fat-soluble vitamins, A and D, but have no indices of vitamin E or K status. Deficiency of the latter two fat-soluble vitamins, however, has been reported sporadically in cystic fibrosis patients (12, 13, 24, 31-33). A relationship has been proposed between various pathological sequelae such as neuroaxonal dystrophy (30) and focal necrosis of striated muscle (14) and deficiency of vitamin E in this disease. Hypoprothrombinemia and hemorrhagic phenomena, responsive to vitamin K administration, have also been reported (12, 33, 34). Because pancreatic enzyme replacement does not completely restore normal fat absorption, water miscible preparations of fat-soluble vitamins, such as Poly-vi-sol have been proposed to improve vitamin absorption in the presence of fat malabsorption and steatorrhea.

Cholesterol. In cystic fibrosis patients, cholesterol availability is influenced by fat malabsorption and by the prescription of low fat diets. Lapey et al. (7) found that cholesterol concentration increased only slightly, from 119 to 126 mg/dl with the addition of Cotazym replacement enzymes, despite a substantial reduction in fecal fat excretion in the same period. Our patients' mean cholesterol concentration of 118 mg/dl is comparable to the range, from 102 to 145 mg/dl, previously reported for patients with cystic fibrosis (7, 9, 10, 77). Since the level of circulating cholesterol is a function not only of intake and absorption, but also of endogenous heptic synthesis (78), the narrow range of cholesterol concentrations seen in cystic fibrosis might primarily be due to basal hepatic sterol production.

Vitamin A and carotene. A consistent finding in cystic fibrosis has been decreased plasma levels of vitamin A (24, 77, 79, 80) and carotene (77). Clinical signs of vitamin A deficiency, nightblindness and xerophthalmia, that responded to parenteral doses of vitamin A (28) or simply to the addition of pancreatic enzyme replacement (11), have been reported in patients with cystic fibrosis. Underwood and Denning (77, 79) observed that despite diminished plasma levels of vitamin A in vitamin-supplemented cystic fibrosis patients, liver concentrations of the vitamin were up to 4-fold greater in patients than in unsupplemented controls. They postulated that defective mobilization and transport of vitamin A from liver stores was the explanation. This hypothesis was confirmed by Smith et al. (80) who subsequently demonstrated depression of retinol binding protein (RBP), the transport protein for vitamin A, in patients with cystic fibrosis. RBP synthesis is influenced by zinc deficiency. Given the evidence for zinc deficiency in cystic fibrosis (37, 38), we have examined the interrelationship among vitamin A, RBP, and zinc in this disease. Our findings (81) suggest that zinc deficiency may play a determinant role in the transport of vitamin A in cystic fibrosis.

Despite supplementation with 2500–5000 IU of vitamin A per day in most patients as Poly-vi-sol,[7] circulating vitamin A levels were below control levels in our patient group. Two of three individuals with frankly deficient vitamin A levels were taking Poly-vi-sol. It should be noted, however, that the doses prescribed in our clinic are below the 10,000 to 25,000 IU of vitamin A recommended by the Cystic Fibrosis Foundation handbook (82). It is possible that if the higher levels had been administered to our patients, their vitamin A levels might have been comparable to those of control subjects.

25-OH-vitamin D. 25-OH-vitamin D is the hepatic metabolite and the principal circulating form of vitamin D. Only recently have techniques become available to measure 25-OH-vitamin D as an index of vitamin D adequacy. Hahn et al. (36) studied 21 patients with cystic fibrosis. The subjects were taking 400 IU of vitamin D per day on a chronic basis. Nonetheless, a 36% reduction in 25-OH-vitamin D levels as compared to a control group not receiving supplementary vitamin was found among the patients. On the other hand, Hubbard et al. (83) found no difference in 25-OH-vitamin D concentrations between 17 cystic fibrosis patients receiving 800 IU of daily vitamin D supplements and a group of age-matched, unsupplemented healthy controls. The daily vitamin D intake by our patients receiving Poly-vi-sol supplementation was in the range of 400 to 800 IU.[7] The present findings are in agreement with the findings of Hahn et al. (36) in so far as significantly diminished plasma 25-OH-vitamin D concentrations were observed in our patient group.

In the Hahn et al. study (36), bone density was measured by photon beam densitometry and found to be reduced by 14% as compared to controls. The average age of these patients was 20.9 yr. Mischler et al. (84) used the same technique to estimate bone mineralization in cystic fibrosis patients. They found demineralization in 37% of male patients and 63% of females, but patients under 10 yr of age had normal bone mineral content. Our densitometry evaluation in a population with a mean age of 11 yr showed no decreased bone density although cortical thickness appeared to be reduced. In addition to malabsorption of ingested vitamin D, interruption of the enterohepatic circulation might chronically deplete vitamin D stores. The serum 25-OH-vitamin D assay should be useful for monitoring the adequacy of the dosage of replacement vitamin D.

Water-soluble vitamins

Of the water-soluble vitamins, only vitamin B_{12} has received any investigative attention in cystic fibrosis research. Several cases of macrocytic anemia have responded to the administration of vitamin B_{12} (34, 35). Deren et al. (17) found that absorption of crystalline vitamin B_{12} was impaired in cystic fibrosis due to exocrine pancreatic insufficiency. Normal absorption could be restored partially by the administration of sodium bicarbonate and fully with pancreatic extract (17). In the present study, biochemical status with regard to folic acid, riboflavin, and ascorbic acid was determined; mean levels were equivalent in patients and controls. Isolated instances of deficient plasma levels were noted in some patients. Vitamin supplements were being taken by the three patients in whom deficient vitamin C levels were noted; the single deficient folic acid value was present in an unsupplemented patient. Since pancreatic secretions presumably are not involved in the absorption of water-soluble vitamins, except for vitamin B_{12}, and since most patients receive supplemental as well as dietary vitamins, deficiency of water-soluble vitamins would not be likely to occur commonly in cystic fibrosis. Increased utilization or increased requirements due to stress of intercurrent infection (85), however, could cause deficiency in some individuals.

Plasma proteins

West et al. (86) observed normal postprandial amino acid patterns in cystic fibrosis patients, and concluded that protein digestion and amino acid absorption were normal. Subsequent studies showed increased fecal loss of nitrogen in patients (7). This azotorrhea could

[7] Each tablet of Poly-vi-sol contains: vitamin A, 2500 IU; vitamin D, 400 IU, vitamin E, 15 IU; vitamin C, 60 mg; folic acid, 0.3 mg; thiamin, 1.05; riboflavin, 1.2 mg; niacin, 13.5 mg; vitamin B_6, 1.05 mg; and vitamin B_{12}, 150 μg.

be diminished by ingestion of pancreatic supplements. Excessive quantities of free amino acids have also been detected in the stools of patients with cystic fibrosis (15, 16). Hypoproteinemia has been reported in a number of studies, and hypoalbuminemia is a frequent finding. The classical pattern of serum proteins in cystic fibrosis, described by Green et al (87), displays depressed albumin levels, elevated globulins, and a slightly increased overall protein concentration. The pattern becomes more pronounced with increasing age and with increased severity of disease. The mean values for albumin, total globulins, and total proteins, reported by Green et al., (87), 3.42, 3.64, and 7.06 g/dl, respectively, are remarkably similar to our values in Table 1. The increase in globulins has been explained on the basis of recurrent pulmonary infection; the decrease in albumin is less completely understood. Pittman et al. (88) found normal total body albumin reserves, normal turnover rates, and expanded plasma volumes in three patients studied with radiolabeled albumin and concluded that hemodilution was the basis of hypoalbuminemia. All three patients had evidence of corpulmonale. Strober et al. (89) also found normal catabolic and synthetic rates for albumin in cystic fibrosis, and detected increased plasma volumes in hypoalbuminemic patients compared to the patients with normal albumin concentrations. Albumin concentration was positively correlated with forced vital capacity in our study. Thus, in cystic fibrosis, the use of albumin concentration as an index of protein nutriture may be misleading.

Conclusion

Comparison of many biochemical indices of nutrition between a group of cystic fibrosis patients and a group of normal controls revealed an overall reduction in the mean concentration of many nutrients in the patients and a number of apparently *deficient levels* among the patients. Water-soluble vitamins were minimally affected whereas levels of the fat-soluble vitamins, vitamin A and 25-OH-vitamin D, were significantly depressed. These effects occurred despite modern, outpatient management that included pulmonary physio-therapy, intermittent antibiotics, a low-fat, high-protein diet, pancreatic enzyme replacement, and multivitamin supplementation with a product (Poly-vi-sol) containing water-miscible, fat-soluble vitamins. The net intake of some fat-soluble vitamins was below that recommended by some (82), but the reduced plasma levels of vitamin A could also reflect defective synthesis of binding proteins in the mobilization from liver stores. The biochemical technology to measure vitamin A, retinol-binding protein, and 25-OH-vitamin-D now available should enable researchers to determine the optimal replacement doses necessary to maintain normal fat-soluble vitamin concentrations in most out-patients with cystic fibrosis.

Plasma copper and ceruloplasmin levels were significantly elevated. Moreover, the increase in copper seemed to parallel the clinical deterioration as reflected by the correlation with advancing restrictive lung disease and with increasing requirements for pancreatic enzyme replacement to optimize fecal characteristics. It is a matter of speculation whether or not the copper concentration may be increasing in response to chronic stimulation of lung macrophages to secrete leukocytic endogenous mediator. If so, that hormone may also mediate other changes in biochemical indices such as iron and zinc.

With an increasingly optimistic prognosis for longevity in patients with cystic fibrosis, but with a persistent incidence of growth impairment, renewed attention to the details of optimal nutrition may be merited. There is no conclusive evidence by which to assess the potential impact of nutrition on growth retardation in cystic fibrosis, nor is there firm evidence to indicate that improved early nutrition will improve the quality of life in late adolescence or early adulthood. These, however, are two important questions that the present observations and the review of the literature would suggest are worthy of specific and direct attention through clinical investigation. The present paper has shown that recognition of the pathophysiological mechanisms that are specific to cystic fibrosis would aid in the meaningful interpretation of biochemical indices of nutritional status.

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