Vitamin status in treated patients with cystic fibrosis

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SUMMARY The water-soluble (B1, B2, B6, C, folic acid) and fat-soluble vitamin (A, carotene, E, and D) status of 36 patients with cystic fibrosis was assessed and compared with a control group of 21 age-matched normal children. Twenty-seven of the patients were receiving vitamin supplements (except folic acid and vitamin E) at the time of investigation. Vitamin B1, B2, and B6 status was adequate in all patients, and there was little evidence of folic acid deficiency. Vitamin C stores might not have been adequate in some of these patients, despite daily supplements with 50 mg of the vitamin. Steatorrhoea, often severe, was present in most of them. Serum carotene and vitamin E concentrations were low in over 90% of patients and were related to the severity of steatorrhoea. Vitamin A was low in over 40% of the patients despite daily vitamin supplements of 4000 IU and correlated with the serum retinol-binding protein level. Serum 25-OH cholecalciferol was low in some patients whether or not they were receiving a daily supplement of 400 IU vitamin D. In a short-term supplementation trial with water-miscible preparations of vitamins A and E in 14 patients, the serum levels of both vitamins responded well to 2 weeks of treatment with 50 mg vitamin E and 4000 IU vitamin A. Except for serum vitamin A, which was lowest in patients with the poorest clinical grading, the other vitamins were not influenced by the clinical grade of the patients.

Cystic fibrosis occurs in about 1/2000 of live births in white Europeans, the majority of new cases now being diagnosed soon after birth. Life expectancy has been greatly improved by early vigorous treatment,¹⁻⁴ and has resulted in an increasing population of patients who will require life-long medical care. Pancreatic insufficiency is present in over 80% of patients with cystic fibrosis.¹ The resulting fat malabsorption is often severe and may not respond well to pancreatic replacement.^{5,6} Although gastrointestinal absorption of fat-soluble vitamins is impaired in patients with steatorrhoea and correlates with its severity,⁷ clinical deficiency, manifested by bone disease (rickets) or severe bleeding, is rare in cystic fibrosis, although it does occur.^{8,9}

Symptoms are mainly a consequence of pulmonary infection and pancreatic insufficiency and there is great variation in the presentation and severity of symptoms.¹⁰ Malnutrition can be a major complication of cystic fibrosis,^{11,12} resulting in the main from pancreatic insufficiency,¹³ and in younger children is manifested in poor weight gain and growth. It has been stressed that improvement of nutritional status is important for growth into adolescence¹⁴ and the

subsequent quality of life. Many different dietary manipulations have been recommended and it is generally accepted that vitamin deficiencies should be avoided. This is especially important with regard to the fat-soluble vitamins.¹⁵ Almost all patients with cystic fibrosis, from the time of diagnosis, are given a daily multivitamin preparation containing water-soluble vitamins as well as the fat-soluble vitamins A and D. Vitamins E and K are not consistently supplemented. The quantity of vitamins given is generally twice the recommended intake for age,^{16,17} but this appears to be quite arbitrary, and there is little evidence that these supplements are adequate except for vitamin A, which has been widely studied. Despite prolonged supplementation, deficiencies of vitamin A are common.^{18,19} A need therefore exists to assess the vitamin status of patients with cystic fibrosis during treatment, and to determine if present practice of vitamin supplementation is adequate.

The purpose of the present study was to measure a wide spectrum of blood vitamin levels in patients with cystic fibrosis. The adequacy of conventional vitamin supplements was assessed, and the level of fat-soluble vitamins was related to the severity of fat

malabsorption. The effect of short-term supplementation with water-miscible forms of vitamins A and E was also assessed.

Patients studied

Thirty-six patients with cystic fibrosis (19 girls and 17 boys) were studied. Age range was 10 months to 16 years (girls 10 months–15 years 3 months; boys 2 years 1 month–16 years). All patients were diagnosed by positive sweat tests, and recurrent pulmonary symptoms. Steatorrhoea was present in 23 of 26 patients tested. None of the patients was in hospital at the time of investigation and all were attending a regular outpatient clinic. At the time of investigation, each patient was graded clinically by a modification of the grading system of Shwachman and Kulczycki²⁰ as described by Doershuk *et al.*²¹ based on (1) case history, (2) pulmonary findings, (3) growth and nutrition, and (4) radiology of chest. Each category was graded 0–25 in fives, the best grading attainable in each category being 25. Thus the highest grading possible was 100.

All patients were receiving treatment at the time of investigation; 32 patients were receiving pancreatic supplements in varying quantities dependent on age and symptoms; 28 were receiving continuous antibiotics, usually cloxacillin or flucloxacillin, or both, and 27 were receiving vitamin supplements to provide twice the recommended intake for age (24 Abidec and 3 Ketovite). Twenty-six patients were on a low dietary fat intake and 15 of them were receiving medium chain triglycerides as a vegetable oil for cooking in the older children or as a medium chain triglyceride milk (Portagen) in younger children.

Results in patients with cystic fibrosis were compared with a group of 21 control children in good nutritional state, as judged by height and weight for age and without evidence of gastrointestinal or pancreatic disease. These children were the offspring of the authors, or were children attending the outpatient clinic who had had a venepuncture for some other purpose. There were 10 boys and 11 girls in this group, age range 1 to 16 years.

Materials and methods

As these children were outpatients, some of whom had travelled long distances to attend the clinic, it was felt unreasonable to expect them to fast. They were advised to take a light breakfast excluding any vitamin-rich foods or drink on the day of investigation. Vitamin supplements were not taken until after they had attended the clinic; thus no patient received a vitamin supplement within 18 hours of the

time of venepuncture. Blood was collected using heparin and sequestrene as anticoagulants. Specimens from control children were collected at similar times and using the same dietary restrictions. Owing to the range and complexity of the analytical assays, only 2 subjects could be copied with each week, thus the specimens from patients and controls were collected over a period of almost 12 months from October 1977 to August 1978.

Vitamin assays.

Ascorbic acid (vitamin C)

Vitamin C was measured in leucocytes plus platelets by the method of Denson and Bowers.²²

Folic acid

Plasma and erythrocyte folic acid was assayed by radioimmunoassay using a commercially available kit (Becton Dickinson UK Ltd).

Vitamin B1, B2, and B6

These vitamins were assessed by assaying the activity of erythrocyte enzymes requiring them as co-factors before and after addition of the appropriate co-factor *in vitro*. The value is expressed as a ratio of the activity after and before addition of the co-factor. Thus a high ratio is an indication of possible vitamin deficiency or depletion. Transketolase (EC 2.2.1.1) was assayed in the presence and absence of thiamine pyrophosphate, to determine vitamin B1 status, by the method of Schouten *et al.*²³ Glutathione reductase (EC 1.6.4.2) was assayed in the presence and absence of added flavin adenine dinucleotide, to determine vitamin B2 status, by the method of Nichoalds.²⁴ Glutamic-oxaloacetic transaminase (EC 2.6.1.1) was assayed in the presence and absence of pyridoxal phosphate to determine vitamin B6 status, by the method of Stanolovic *et al.*²⁵

Vitamin A

Vitamin A was assayed spectrofluorimetrically after hexane extraction.²⁶

Carotene

Plasma carotene was assayed spectrophotometrically at 520 nm after petroleum ether extraction.²⁷

25-OH-cholecalciferol

Plasma 25-OH-cholecalciferol was assayed by competitive protein binding assay.²⁸

Vitamin E

Plasma tocopherol was measured spectrophotometrically after reaction with bathophenanthroline

by a modification of the procedure of Fabianek *et al.*²⁹

Retinol binding protein and low-density (β) lipoprotein These were assayed by radial immunodiffusion on commercially available agar plates (Hoechst Pharmaceuticals Ltd).

Faecal fat

Polyethylene glycol 4000 was used as a non-absorbable marker and determined by the method of Hyden.³⁰ Polyethylene glycol 4000 was administered in a dose of 250 mg 3-times daily one dose with each main meal for 7 days and faeces collected on day 6–7. Faecal fat was determined on 2-day faecal collections³¹ by the method of van de Kamer *et al.*³² and corrected for polyethylene glycol recovery.

Assays for vitamins B2, B6, and C were performed on the same day as the specimen was received; all other assays were performed within one week of collection.

Water-miscible vitamin A/E supplementation

This was given to 14 patients, aged 1 year 8 months to 17 years, who continued with their usual treatment including vitamin supplements. A blood sample was obtained at the beginning of the treatment period. The patients then received 50 mg of water-miscible α -tocopherol as an emulsion in cremophor (Roche Products Ltd, Welwyn Garden City, UK) and 4000 IU of water-miscible vitamin A acetate as gelatin-coated beadlets (Roche Products Ltd) a day for 2 weeks. On the 15th day (24 hours after the last vitamin dose) a second blood sample was obtained after an overnight fast.

Results

Clinical grade. This was available in 35 out of the 36 patients with cystic fibrosis. The gradings were as follows: <75 in nine, 75–80 in nine, 85–90 in ten, and at least 95 in seven. Pulmonary findings and growth and nutrition were the most common abnormal grading categories. For growth and nutrition, 17 of 35 patients had a grading of 15 or less, while for pulmonary symptoms 11 of 35 had a grading of 15 or less.

Vitamins B1, B2, and B6. The Table shows the enzyme activation coefficients found for the control and patient groups; there is close similarity between them. For vitamins B2 and B6, all values were within 2 SD of the control mean. Minor abnormalities of vitamin B1 were found in 3 patients who had enzyme activation ratios of 1.15, 1.15, and 1.16 which were just outside the upper limit of 1.14 found

Table Enzyme activation tests (mean $\pm$ 1 SD)

	Vitamin B1	Vitamin B2	Vitamin B6
Controls (n=20)	1.02 $\pm$ 0.06	1.15 $\pm$ 0.13	1.63 $\pm$ 0.19
Cystic fibrosis (n=29)	1.04 $\pm$ 0.07	1.04 $\pm$ 0.15	1.49 $\pm$ 0.13
Significance	NS	NS	NS

in the control group. Thus B1, B2, and B6 status in this group of patients was apparently adequate. Vitamin B1, B2, and B6 status was also normal in 7 patients who were not receiving vitamin supplements.

Folic acid. Except for the 3 receiving Ketovite, patients were not receiving folic acid supplements and little evidence of deficiency was found. Only one patient had a low serum folate level (2.5 ng/ml, lower limit of normal 3.0) and this subject had a normal erythrocyte folate (178 ng/ml, lower limit of normal 122). Two further patients had low erythrocyte folate levels, 70 and 90 ng/ml in the presence of normal serum folate, 3.2 and 3.4 ng/ml respectively. The haemoglobin concentration and blood films were normal in these 3 subjects.

Vitamin C. Leucocyte plus platelet vitamin C levels in the control and cystic fibrosis groups are shown in Fig. 1. Only 3 patients out of 30 were below the control range. Two of them had low levels despite receiving vitamin C supplements of 50 mg/day. Several other patients were at the lower limit of the normal range. Five patients out of the 30 in whom vitamin C values were available were not receiving

Fig. 1 Leucocyte vitamin C levels in control children and patients with cystic fibrosis. Vitamin C supplemented patients are shown as (●) and those not receiving supplements as (○). Horizontal bar shows the mean for each group.

Fig. 2 Leucocyte vitamin C levels in two groups of patients with cystic fibrosis. The groups were selected on the basis of clinical grading (see text). Vitamin C supplemented patients are shown as (●) and those not receiving supplements as (○). Horizontal bars show the mean for each group.

additional vitamin C and their levels were similar to those of the supplemented group.

The clinical state as assessed by the Shwachman score had little relation to leucocyte vitamin C levels (Fig. 2). Patients were arbitrarily divided into two groups, above and below a score of 75, and the range of values in each group was similar (Fig. 2).

The clinical state of the patients had little, if any, relationship to the blood levels of any of the other water-soluble vitamins.

Faecal fat. Only 4 of 26 patients tested had a normal faecal fat (<17.6 mmol/24 h; 5 g/24 h) despite regular pancreatic enzyme replacement treatment in all patients except one. Steatorrhoea was often severe; 10 patients had values greater than 100 mmol/24 h (28.4 g/24 h), and 17 had values above 50 mmol/24 h (14.2 g/24 h).

Carotene. Serum carotene was normal in only one patient of 28 tested. Normal range in 20 control children was 2.10 ± 0.21 1 SE $\mu\text{mol/l}$. Ten patients had values below $0.4 \mu\text{mol/l}$, while 23 patients (82% of total) had values below $0.8 \mu\text{mol/l}$. The correlation between serum carotene and faecal fat, although significant ($r = 0.41$ $P < 0.05$), was not close.

Vitamin A. Despite supplementation with up to 4000 IU a day, a high proportion of patients had low serum vitamin A levels (Fig. 3). The normal range

Fig. 3 Serum vitamin A levels in control children and children with cystic fibrosis. Horizontal bars show the mean for each group.

Fig. 4 Serum vitamin E levels in control children and children with cystic fibrosis. Horizontal bars show the mean for each group.

found was $2.83 \pm 0.20 \mu\text{mol/l}$, the levels in patients with cystic fibrosis (1.98 ± 0.16 1 SE $\mu\text{mol/l}$) being significantly lower ($t = 3.7$ $P < 0.01$). Thirteen (43%) of 30 patients had values more than 2 SD below the mean for the controls. Retinol-binding protein (RBP) was present in significantly lower concentrations ($t = 3.99$ $P < 0.01$) in the patients ($34 \pm 1.8 \mu\text{g/ml}$) than in the controls ($60.7 \pm 4.3 \mu\text{g/ml}$), 18 (67%) of 27 being more than 2 SD below the control mean. Vitamin A correlated significantly with RBP ($r = 0.62$ $P < 0.01$). There was no correlation between vitamin A and the severity of steatorrhoea ($r = 0.18$ $P > 0.1$). The clinical state of the patients, as assessed by the Shwachman score, showed a relationship to plasma vitamin A and RBP. In those with a Shwachman score <75 , serum vitamin A levels (mean 1.19 ± 0.16 1 SE $\mu\text{mol/l}$) were significantly lower ($t = 4.08$ $P < 0.01$) than in those with a score above 75 (mean 2.13 ± 0.10 1 SE $\mu\text{mol/l}$). The RBP level was also significantly lower ($t = 2.97$ $P < 0.01$) in the <75 group (mean 26.0 ± 2.9 1 SE $\mu\text{g/ml}$) compared with the >75 group (mean 36.0 ± 1.80 1 SE $\mu\text{g/ml}$).

Vitamin E. Serum vitamin E concentration in the patients (mean 7.7 ± 0.81 1 SE $\mu\text{mol/l}$) was significantly lower ($t = 8.6$ $P < 0.01$) than in the control group (mean 17.6 ± 0.87 1 SE $\mu\text{mol/l}$) (Fig. 4). Twenty-eight (93%) of the 30 patients had values more than 2 SD below the control mean; 8 (27%) had values lower than $5 \mu\text{mol/l}$, and 21 (70%) had values lower than $10 \mu\text{mol/l}$. Concentrations of low density lipoproteins (β -lipoproteins), which in

most conditions are the major carrier proteins for vitamin E in serum, were significantly lower ($t = 3.9$ $P < 0.01$) in patients (mean 358 ± 11.8 1 SE mg/100 ml) than in controls (mean 440 ± 21.3 1 SE mg/100 ml). Surprisingly, there was no significant correlation between serum vitamin E and β -lipoprotein ($r = 0.16$). Serum vitamin E correlated significantly with faecal fat ($r = 0.55$ $P < 0.01$). The clinical grading showed no significant correlation with serum vitamin E, the mean value in the <75 group ($8.02 \mu\text{mol/l}$) being similar to that in the >75 group ($6.88 \mu\text{mol/l}$).

Vitamin D. 25-OH-cholecalciferol was measured in 25 patients, 20 of whom were receiving 400 IU vitamin D supplements a day. Twenty-two of the 25 samples were collected between the months of October and April. Four patients had values lower than 10 ng/ml, 2 of whom were not receiving vitamin D supplements. Eight further patients had 25-OH-cholecalciferol levels between 10 and 15 ng/ml. These values may be considered at the lower end of the normal range;³³ 3 of these 8 were not receiving vitamin D supplements. Thus all 5 patients not receiving vitamin D supplements had serum values lower than 15 ng/ml and in 2 the values were lower than 10 ng/ml. Of the 20 patients receiving vitamin D supplements, 7 (35%) had values lower than 15 ng/ml and 2 (10%) had values lower than 10 ng/ml. There was no significant correlation between serum 25-OH-cholecalciferol and severity of steatorrhoea ($r = 0.17$ $P < 0.1$).

Response to supplements. The response of the serum vitamin A and E to oral supplementation for 2 weeks with fairly small doses of water-miscible preparations is shown in Fig. 5. The level of both vitamins rose in all 14 patients. The mean serum vitamin A increased from 1.68 to 3.09 $\mu\text{mol/l}$, while the mean serum vitamin E level increased from 7.33 to 13.0 $\mu\text{mol/l}$, both increases being highly significant ($P < 0.01$). The increase in vitamin A in individual patients was not related either to the severity of steatorrhoea or to the initial level of RBP. The increase in serum vitamin E was not related to the severity of steatorrhoea or to the level of β -lipoprotein.

Discussion

Patients with cystic fibrosis are routinely given water-soluble and fat-soluble vitamin supplements. Administration of the fat-soluble vitamins is readily rationalised by the fact that such patients often have severe fat malabsorption⁵ and consequently fat soluble-vitamin malabsorption.¹ Dietary intake in children with cystic fibrosis is often well maintained owing to an increased appetite³⁴ and absorption of water-soluble vitamins would be expected to be normal so that severe deficiency of water-soluble vitamins seems unlikely.

The roles of vitamin C and the B vitamins in human nutrition are complex, and they play many essential roles in maintaining intermediary metabolism³⁵ and the immunological defences may depend on adequate nutrition.¹⁵ The importance of marginal or subclinical water-soluble vitamin deficiency is not understood. Among the many roles ascribed to vitamin C is its capacity to promote the immune response³⁶ and possibly aid in the prevention of infection.³⁷ The presence of low or marginally low leucocyte vitamin C levels in the patients reported here, despite oral supplementation, suggest depleted or inadequate tissue stores.³⁸ It has also been stated that patients receiving long-term broad spectrum antibiotics should receive additional vitamins.¹⁶

To our knowledge there is little, if any, information to suggest that deficiency of water-soluble vitamins occurs in cystic fibrosis, and we do not know of any previous studies on blood levels of vitamin B1, B2, B6, or vitamin C in cystic fibrosis. Reviews of the subject have without exception stated the need for multivitamin supplementation at twice the recommended intake.^{13 17 34 39} Chase *et al.*¹² have stressed the continuing lack of information on the water-soluble vitamin needs of patients with cystic fibrosis. Based on blood analysis, the patients with cystic fibrosis in the present study showed little, if any, evidence of water-soluble vitamin deficiency and

Fig. 5 Response of serum vitamin A and E in 14 patients with cystic fibrosis to short-term (14 days) supplementation with water miscible preparations of vitamin A (4000 IU/day) and vitamin E (50 mg/day). The mean for each group is shown by the horizontal bars and the lines connect the results from individual patients.

this applied equally to those not receiving vitamin supplements.

Severe fat malabsorption is present in a high proportion of patients with cystic fibrosis,^{5 6 40} and this was confirmed in the present study. Fat malabsorption is widely accepted as the cause of the fat soluble vitamin deficiency,^{1 9 40} but the relationship has not always been close.

Vitamin A in serum is present as the free alcohol retinol, almost entirely attached to a specific transport protein, the RBP,^{41 42} which is synthesised in the liver.⁴³ It has previously been shown that levels of RBP in serum are often low in cystic fibrosis and that a good correlation exists between serum vitamin A and RBP levels¹⁹ which may help to explain low serum vitamin A levels. In the present study, although most patients had daily supplements of 4000 IU of vitamin A, a high proportion had low serum levels and there was a good correlation between vitamin A and RBP levels in serum. It is not possible however, to decide the relative contribution of low levels of RBP and inadequate absorption. Adding a small vitamin A supplement as a water-miscible formulation resulted in a prompt and significant increase in serum levels in all patients tested, suggesting perhaps that absorption of the vitamin may have been limiting. Further studies will be required to determine the minimum daily water-miscible vitamin A supplement required to maintain normal serum levels. Because of the dangers of vitamin A toxicity, even at fairly low daily intakes,^{41 44} it will be important to determine this minimal daily requirement of water-miscible preparations.

Serum vitamin E levels were low in most of our patients in agreement with previous studies,^{40 45 46} and this was correlated with the severity of fat malabsorption. Fat malabsorption is probably the major cause of the vitamin E deficiency although the level of β lipoproteins, the major transport protein for vitamin E,⁴⁷ was sometimes low. The clinical significance of vitamin E in human nutrition is debatable, although in the USA it is considered an essential nutrient.⁴⁸ Several reports in cystic fibrosis suggest that supplements should be administered to achieve normal serum levels.^{40 48} There is little doubt that water-miscible preparations are more efficiently absorbed than fat-soluble preparations.⁴⁶ Vitamin E is an antioxidant and may also protect against oxidative destruction of vitamin A and other compounds within the gastrointestinal tract.^{49 50}

Severe deficiencies of vitamin D and vitamin K are rare in cystic fibrosis although rickets⁸ and life-threatening bleeding^{9 51} have been described. Assay of serum vitamin K to assess subclinical deficiency is not available, but by competitive protein binding

assay of the hydroxylated metabolites of vitamin D, it is now possible to assess the frequency of sub-clinical vitamin D deficiency. Reports of serum 25-OH-cholecalciferol levels in cystic fibrosis are somewhat conflicting. Thus Hubbard *et al.*⁵² reported that levels were generally normal during the summer/autumn season in the USA in patients receiving 800 IU orally a day. In contrast, Hahn *et al.*⁵³ also in the USA, found that values for 25-OH-cholecalciferol during March and April were only 60% of control values despite oral supplements, and 30% had unequivocally low levels. In the absence of faecal fat measurements in either of these studies, the possible contribution of steatorrhoea cannot be assessed.

The results of the present study by comparison with the values in normal English schoolchildren reported by Poskitt *et al.*³³ tend to support those of Hahn *et al.*⁵³ There is, therefore, some indication from the present study that subclinical deficiency of vitamin D may exist in cystic fibrosis even though routine oral supplementation was being given.

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