Diminished and erratic absorption of ergocalciferol in adult cystic fibrosis patients[1–3]

Robert K Lark, Gayle E Lester, David A Ontjes, Angelia D Blackwood, Bruce W Hollis, Margaret M Hensler, and Robert M Aris

ABSTRACT

Background: Osteoporosis diminishes the quality of life in adults with cystic fibrosis (CF). Vitamin D deficiency resulting from malabsorption may be a factor in the etiology of low bone mineral density (BMD) in patients with CF.

Objective: Absorption of oral ergocalciferol (vitamin D_2) and the consequent response of 25-hydroxyvitamin D in 10 adults with CF and exocrine pancreatic insufficiency was compared with that of 10 healthy control subjects.

Design: In this pharmacokinetic study, CF patients and control subjects were pair-matched on age, sex, and race. Each subject consumed 2500 µg oral vitamin D_2 with a meal. The CF group also took pancreatic enzymes that provided $\geq 80\,000$ U lipase. Blood samples were obtained at baseline and at 5, 10, 24, 30, and 36 h after vitamin D_2 consumption to measure serum vitamin D_2 and 25-hydroxyvitamin D concentrations.

Results: Vitamin D_2 concentrations in all subjects were near zero at baseline. CF patients absorbed less than one-half the amount of oral vitamin D_2 that was absorbed by control subjects ($P < 0.001$). Absorption by the CF patients varied greatly; 2 patients absorbed virtually no vitamin D_2. The rise in 25-hydroxyvitamin D in response to vitamin D_2 absorption was significantly lower over time in the CF group than in the control group ($P = 0.0012$).

Conclusions: Vitamin D_2 absorption was significantly lower in CF patients than in control subjects. These results may help explain the etiology of vitamin D deficiency in CF patients, which may contribute to their low BMD.   *Am J Clin Nutr* 2001;73:602–6.

INTRODUCTION

Cystic fibrosis (CF) is the most common lethal autosomal-recessive genetic disease in the white population, affecting $\approx 60\,000$ individuals worldwide. CF occurs in ≈ 1 in every 2500 live births in the white population and leads to death or lung transplantation in >1000 individuals annually (1, 2). Although respiratory disease is the greatest single cause of morbidity and mortality in CF, improvements in therapy for chronic pulmonary infection have markedly improved survival and have led to the recognition of other problems, including bone disease, that afflict CF patients as they age (2).

Low bone mineral density (BMD) in the CF population has gained the attention of many clinicians and researchers during the past 5 y (3–16). This condition starts early in life and worsens with age (3, 4). There are many factors that contribute to low BMD in patients with CF, including corticosteroid use, hypogonadism, chronic infections, malnutrition, calcium malabsorption, and poor absorption of fat-soluble nutrients including vitamin D. The limited data on bone histomorphometry have not clearly defined CF bone disease as osteoporosis or osteomalacia. Nonetheless, the term osteoporosis is most often used to describe low BMD in this population. Osteoporosis diminishes the quality of life in CF patients as they progress through adulthood by making them more prone to fractures and kyphosis (5).

Vitamin D insufficiency can be related to many factors, including insufficient absorption of the parent form of vitamin D from supplements. The animal form of vitamin D, cholecalciferol (vitamin D_3), is created by irradiation of 7-dehydrocholesterol in the skin by ultraviolet light. Vitamin D_3 is then converted to 25-hydroxyvitamin D_3 in the liver by the enzyme 25-hydroxylase. Ergocalciferol (vitamin D_2) is a plant-derived form of vitamin D created from irradiation of ergosterol (provitamin D_2) and is nearly identical to vitamin D_3 in structure. Ergocalciferol is rarely found in the human diet; therefore, baseline concentrations in most human subjects are near zero. This makes vitamin D_2 a suitable supplement for use in studies of vitamin D absorption. Additionally, vitamin D_2 is metabolized in much the same way as vitamin D_3, although some investigators found that vitamin D_3 was converted to 25-hydroxyvitamin D more easily (17).

TABLE 1
Subject characteristics[1]

	Control subjects	CF subjects
Age (y)	28.6 ± 7.9[2]	28.9 ± 8.1
Sex (M, F)	5, 5	5, 5
Race	10 White	10 White
Height (m)	1.74 ± 0.08	1.66 ± 0.11
Weight (kg)	65.0 ± 8.5	60.4 ± 11.9
BMI (kg/m^2)	21.5 ± 2.4	21.9 ± 3.2
FEV$_1$ (% of predicted)	—	56.3 ± 25.2

[1] CF, cystic fibrosis; FEV$_1$, forced expiratory volume in 1 s. None of the subject characteristics differed significantly between the CF and control groups.

[2] $\bar{x} \pm$ SD.

Many investigators have found low concentrations of 25-hydroxyvitamin D in the CF population (6). The pathophysiology of vitamin D insufficiency in CF is thought to be similar to that of other malabsorptive disorders, although decreased exposure to sunlight, increased vitamin D catabolism, or both may also contribute to the deficiency. Vitamin D is important in maintaining calcium homeostasis by increasing the intestinal absorption of calcium. Suboptimal 25-hydroxyvitamin D concentrations (<35 nmol/L) were shown to contribute to low BMD in other patient populations (18). We conducted the present study to characterize vitamin D absorption in CF patients and to investigate whether malabsorption contributes to deficiency of this vitamin in CF patients. To accomplish this, we compared the absorption of 2500 µg oral ergocalciferol (vitamin D$_2$) in 10 adults with CF and exocrine pancreatic insufficiency (PI) to absorption in 10 healthy control subjects.

SUBJECTS AND METHODS

Subjects

Ten CF patients aged 18–45 y were recruited from the Adult Cystic Fibrosis Center at the University of North Carolina at Chapel Hill. All the subjects were taking prescribed enzymes for clinically diagnosed PI and none had clinical evidence of liver disease (alanine transaminase <55 U/L). Of the 10 CF subjects studied, 8 had the genotype homozygous DF/508 and the other 2 had no genotype on record. Ten healthy control subjects were recruited to match the CF subjects according to race, age, sex, and body mass index (BMI; in kg/m^2) (**Table 1**). All subjects were studied as matched pairs (1 CF subject and 1 control subject) to minimize seasonal effects between January and August 1999. The study was approved by the Committee for the Protection of Human Subjects at the University of North Carolina. Written, informed consent was obtained from all subjects before enrollment in the protocol.

Protocol

Subjects were admitted to the General Clinical Research Center after fasting overnight. A baseline blood sample was obtained from each subject by venipuncture. The subject then consumed 2 pills, each of which contained 1250 µg ergocalciferol (vitamin D$_2$) (Drisdol; Sanofi Winthrop Pharmaceuticals, New York), with a meal. In addition, the CF subjects took their prescribed pan-

creatic enzymes in an amount that provided $\geq 80\,000$ U lipase. Blood samples were then obtained at 5, 10, 24, 30, and 36 h after the vitamin D$_2$ challenge for the measurement of vitamin D metabolites. The blood was allowed to clot and was then centrifuged at $2000 \times g$ for 12 min at $4\,°C$. The serum was decanted and stored at $-80\,°C$. One CF patient was unable to provide a 36-h sample and was consequently dropped, along with her match, from all area under the curve (AUC) analyses.

Measurement of vitamin D and its metabolites

Vitamin D$_2$ was measured by using direct ultraviolet quantitation after separation by HPLC as described by Hollis (19). Concentrations of 25-hydroxyvitamin D were measured with a commercially available assay kit (DiaSorin, Stillwater, MN). This equilibrium radioimmunoassay uses an antibody highly specific for 25-hydroxyvitamin D compared with other vitamin D metabolites and has a sensitivity of 0.60 nmol/L. The assay does not distinguish between 25-hydroxyvitamin D$_2$ and 25-hydroxyvitamin D$_3$ and interacts equally with either metabolite. Therefore, the 25-hydroxyvitamin D concentrations reported here represent a combination of both molecules; the normal range in our laboratory is 34–120 nmol/L.

Statistical analyses

All descriptive variables [age, height, weight, BMI, and forced expiratory volume in 1 s (FEV$_1$)] are presented as means $\pm$ SDs. Subject characteristics in the CF and control groups were compared with two-sample t tests. To test for group differences in vitamin D$_2$ and 25-hydroxyvitamin D concentrations over time, we used repeated-measures analysis of variance (ANOVA). We used two-sample t tests to compare the total vitamin D$_2$ absorption in the groups (AUC at 36 h) and to compare baseline and final (36-h) concentrations of 25-hydroxyvitamin D within each group. Pearson's product-moment correlation coefficients were determined to identify relations between subject characteristics and response to vitamin D supplementation. $P < 0.05$ was defined as statistically significant. Statistical analyses were performed with SAS (version 6.12) for the repeated measures ANOVA (20) and with SPSS SIGMASTAT (version 2.0) for the t tests, AUC calculations, and Pearson's product-moment correlation coefficients (21).

RESULTS

There were no significant differences between the 2 groups in any of the measured anthropomorphic variables (Table 1). CF subjects had a mean FEV$_1$ of $56.3 \pm 25.2\%$, indicating moderate lung disease. The mean ($\pm$SEM) serum 1,25-dihydroxyvitamin D concentrations at baseline were slightly higher in the CF group than in the control group (109.3 ± 11.5 and 81.5 ± 7.0 nmol/L, respectively; $P < 0.05$). Because vitamin D$_2$ is not readily available in the diet, all subjects had baseline vitamin D$_2$ concentrations near zero.

The differences in vitamin D$_2$ concentration between the control and CF groups at 5 h after vitamin D$_2$ consumption were not significant, but over time the vitamin D$_2$ concentrations were markedly lower ($P < 0.001$) in the CF group (**Figure 1**). There was wide variability in the individual absorption curves of the CF subjects. In 3 CF subjects, the peak concentrations of vitamin D$_2$ exceeded the average of that of the control subjects, whereas for other CF subjects, vitamin D$_2$ concentration never rose above 25 nmol/L (**Figure 2**). We analyzed AUCs for serum vitamin D$_2$
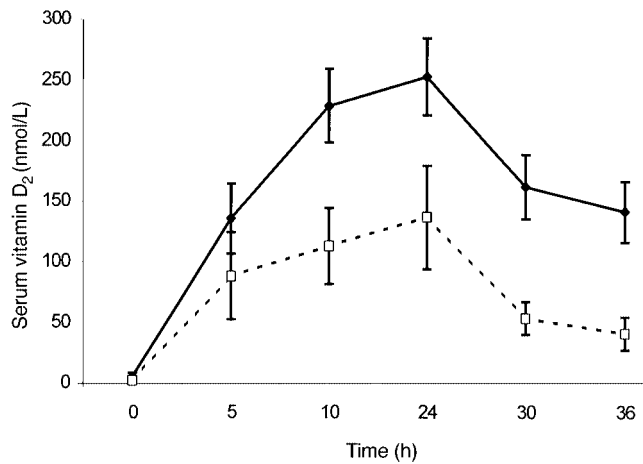
FIGURE 1. Mean (±SEM) serum vitamin D_2 concentrations over time in cystic fibrosis (□) and control (◆) subjects. The absorption response to vitamin D_2 administration was significantly lower in the cystic fibrosis group than in the control group, $P < 0.001$ (repeated-measures ANOVA; $n = 10$ in each group).

over time to quantify total vitamin D_2 absorption from baseline to 36 h. On average, the CF subjects absorbed only 45% of the amount absorbed by control subjects ($P < 0.001$; **Figure 3**). Because the peak vitamin D_2 concentration was reached in all subjects by 24 h, there was no indication that absorption was delayed beyond 36 h in the CF subjects.

For the CF group, the mean (±SEM) 25-hydroxyvitamin D concentration at baseline was 49.4 ± 7.8 nmol/L, which is in the low-normal range. Thirty percent of CF subjects were classified as deficient because they had 25-hydroxyvitamin D concentrations <35 nmol/L. The increase in the mean 25-hydroxyvitamin D concentration from baseline (49.4 ± 7.8 nmol/L) to 36 h (60.9 ± 6.5 nmol/L) was not significant in the CF group. However, the mean 25-hydroxyvitamin D concentration in the control group rose significantly from baseline to 36 h (64.2 ± 6.8 and 116.5 ± 15.0 nmol/L, respectively; $P < 0.002$). In the CF group, 25-hydroxyvitamin D concentrations did not increase significantly over baseline values at any time point. The conversion response over time, reflected by serum 25-hydroxyvitamin D concentrations, differed significantly between the CF and control groups ($P = 0.0012$; **Figure 4**).

None of the measured variables correlated positively with vitamin D_2 absorption in either group. However, a significant inverse correlation was found between baseline serum 25-hydroxyvitamin D concentration and the percentage change in serum 25-hydroxyvitamin D from baseline to 36 h in the CF group ($r^2 = -0.76$, $P < 0.02$). We did not perform multivariate correlation analyses because our sample size was not large enough.

DISCUSSION

Our data show that vitamin D_2 absorption is significantly lower and quite variable in adults with CF compared with control subjects pair-matched for age, sex, and race. This result is noteworthy given that each CF subject took a large dose of pancreatic enzymes with the vitamin D_2, and these enzymes should have lessened the severity of PI. Inefficient absorption of vitamin D may be an important contributor to the pathogenesis of bone dis-

ease in CF. As low BMD in the CF population becomes more common because of the increased average life span, methods for treating or preventing bone disease are becoming more important.

The imbalance between bone formation and bone resorption in CF patients continues to be puzzling. Much of the research on CF bone disease has focused on elevated bone resorption resulting from multiple factors, including increased circulating cytokines (especially tumor necrosis factor α, interleukin 6, and interleukin 1β), corticosteroid use, and possibly mild hyperparathyroidism (7, 8). Researchers have evaluated bisphosphonates for preventing bone resorption, but side effects in some patients have hindered this approach (7). Bone formation may also be diminished in patients with CF because of delayed growth, hypogonadism, calcium malabsorption, and deficient vitamin D concentrations. If some aspect of this pathogenesis is related to osteomalacia, vitamin D therapy would be a safe, inexpensive, and widely applicable treatment for CF bone disease.

An important finding of this study was that a large oral dose of vitamin D_2 did not raise serum 25-hydroxyvitamin D_2 concentrations significantly in the CF group. Because the subjects did not receive excess ultraviolet light during the short course of the study, we think that the 25-hydroxyvitamin D response was predominantly in the form of 25-hydroxyvitamin D_2, as it was in other vitamin D_2 studies (22). Although the CF group did not absorb as much of the vitamin D_2 as the control subjects did, the average serum vitamin D_2 concentration in the CF group did rise above 126 nmol/L at 24 h. This implies that the CF subjects had sufficient substrate (vitamin D_2) to increase their formation of 25-hydroxyvitamin D_2.

There are several possible reasons for the poor conversion response. When vitamin D_2 is absorbed from the intestine, it enters the enterohepatic circulation for hydroxylation by the hepatic enzyme, 25-hydroxylase. The vitamin D_2 molecules that do not get hydroxylated are then passed back through the intestine and should be reabsorbed by the enterohepatic circulation. Because CF patients do not absorb vitamin D_2 efficiently, there is less substrate for the 25-hydroxylase enzyme, resulting in less
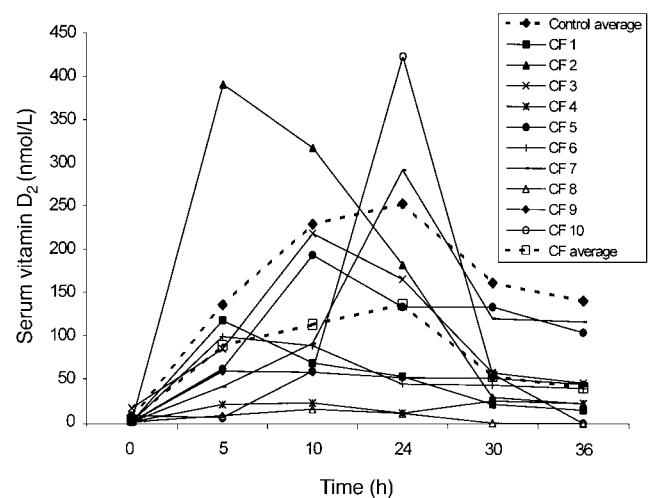

FIGURE 2. Serum vitamin D_2 concentrations of individual cystic fibrosis (CF) subjects over time showing the heterogeneity of responses to vitamin D_2 administration in the CF group. For reference, the group means for the control subjects and the CF subjects are shown ($n = 10$ in each group).
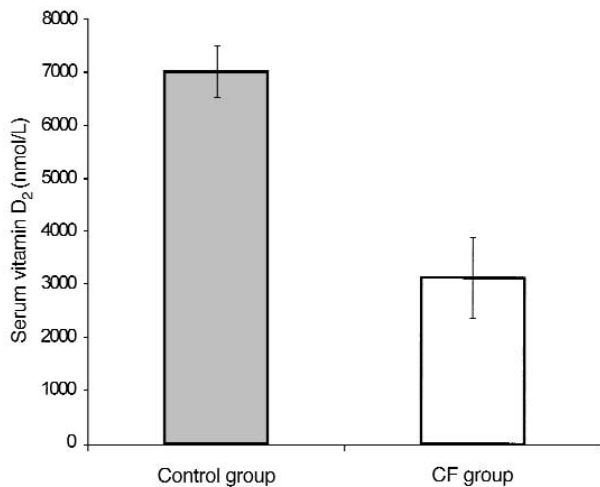
FIGURE 3. Mean (±SEM) area under the curve summation for serum vitamin D_2 concentrations from baseline to 36 h showing that the cystic fibrosis (CF) group had significantly lower absorption than did the control group, $P < 0.001$ ($n = 9$ in each group).

conversion to 25-hydroxyvitamin D_2 than occurred in control subjects over the same time period. The vitamin D_2 that is hydroxylated to 25-hydroxyvitamin D_2 is also circulated through the enterohepatic system and may not be reabsorbed efficiently.

Other possible reasons for the poor conversion response could be altered concentration or activity of the 25-hydroxylase enzyme, or higher rates of metabolic clearance of 25-hydroxyvitamin D. More rapid metabolic clearance of 25-hydroxyvitamin D would be a likely explanation for low 25-hydroxyvitamin D concentrations in this population. It is known that CF patients have high cytochrome P450 enzyme activity, which could lead to more rapid degradation of existing or newly formed hydroxylated vitamin D metabolites (23). The results of our study support those of other absorption studies done in the CF population with different fat-soluble vitamins. Studies that examined the effects of vitamins A, E, and K have found results similar to ours. For example, Winklhofer-Roob et al (24) used a 24-h AUC analysis and found that CF patients absorbed 37% less vitamin E than the control group after each subject consumed a large dose of vitamin E. Other investigators found that vitamin A and E concentrations were lower in CF patients with PI than in control subjects, even though the CF patients were taking pancreatic enzymes and vitamin supplements (25). These studies also could not identify a specific physical characteristic that correlated with vitamin absorption.

We investigated possible reasons why the CF patients had such variable vitamin D_2 absorption curves and we tried to identify factors that might explain why the concentrations of 25-hydroxyvitamin D did not increase significantly in the CF group. Our data support the conclusions of other investigators, who found a negative correlation between baseline 25-hydroxyvitamin D concentration and percentage change in 25-hydroxyvitamin D in patients who did not have CF; patients with very low baseline 25-hydroxyvitamin D concentrations showed increased concentrations after vitamin D_2 therapy (26). However, we found no other variables that correlated significantly with vitamin D_2 absorption. We expected that BMI might correlate well with vitamin D_2 absorption, because BMI can be a good reflection of the

degree of PI or illness, but our data did not support this theory ($r^2 = 0.07$, $P = 0.85$). FEV_1, which is often used as an indicator of disease severity, did not correlate with vitamin D absorption in our study population ($r^2 = -0.113$, $P = 0.77$). Our small sample size prevented us from examining possible multivariate relations that might have explained the variance in vitamin D_2 absorption. With such a small group and the lack of data on intraindividual differences in vitamin D_2 absorption, it is difficult to reach general conclusions about vitamin D_2 handling and conversion to 25-hydroxyvitamin D. A pharmacokinetic study in which CF patients received intravenous vitamin D_2 would be necessary to distinguish actual differences in the rate of 25 hydroxylation from variations in absorption. This type of study might better elucidate the factors involved in the abnormal handling of the vitamin in CF patients.

Studies in populations that did not have CF showed that the 25-hydroxyvitamin D response to vitamin D therapy was small when the dosage of vitamin D was ≤250 µg/d, however, these studies found a dramatic response at a vitamin D dose of 2500 µg (as reviewed in reference 26). Trials in elderly adults showed that dosages >1000 µg/d are required to induce vitamin D toxicity resulting in hypercalcemia (26). Most vitamin D supplementation trials that use dosages in the normal range (5–20 µg/d) are done in elderly populations and take place over a period of several months. One of these studies, which was done in elderly women, found a 54% increase in 25-hydroxyvitamin D concentrations in response to a daily dosage of 10 µg vitamin D_3 for a period of 1 mo (27). A study by Dawson-Hughes et al (28) showed an 83% increase in 25-hydroxyvitamin D concentration after subjects received 17.5 µg cholecalciferol/d for a period of 3 y. Few studies have used ergocalciferol as an oral vitamin D supplement in amounts smaller than pharmacologic dosages. However, one recent study by Harris et al (22) found that vitamin D–deficient young men had a 94% increase in their 25-hydroxyvitamin D concentrations in response to therapy with 45 µg vitamin D_2/d for 3 wk. Not surprisingly, Hanly et al (29) reported that supplements of vitamins A, D, E, and K (including 20 µg
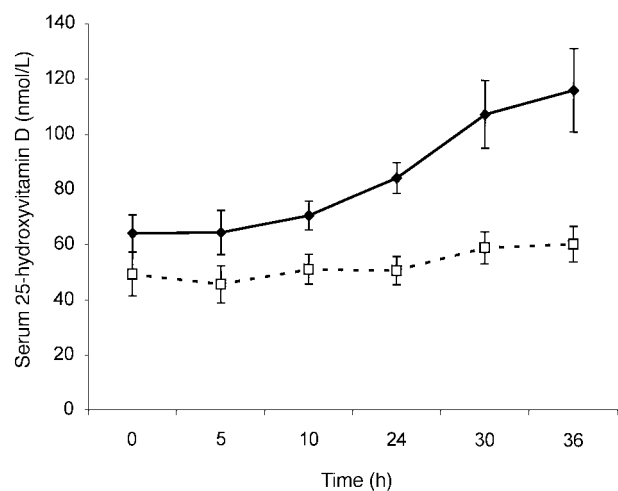

FIGURE 4. Mean (±SEM) serum 25-hydroxyvitamin D concentrations over time in cystic fibrosis (□) and control (◆) subjects showing a significantly lower conversion response to vitamin D_2 administration in the cystic fibrosis group than in the control group, $P = 0.0012$ (repeated-measures ANOVA; $n = 10$ in each group).

vitamin D_3) did not increase 25-hydroxyvitamin D concentrations in 25–50% of CF patients. Haworth et al (11) published data showing that CF patients in the United Kingdom had low 25-hydroxyvitamin D concentrations even though they received 22.5 μg ergocalciferol/d. No studies have been carried out in CF patients receiving >250 μg vitamin D/d.

Our data clearly indicate that there are differences in the way that CF patients absorb vitamin D as compared with control subjects. Our findings support the hypothesis that CF patients have vitamin D malabsorption. Additionally, CF patients do not convert the absorbed vitamin D_2 into the more active form, 25-hydroxyvitamin D, in a manner similar to that occurring in control subjects. Taken together, these data suggest that adults with CF may need >25 μg oral vitamin D/d for adequate repletion; this dosage includes a margin of safety. Our data, in addition to those from other trials, indicate that the currently recommended amount of 20 μg vitamin D/d for adults with CF may be grossly inadequate. This amount may be especially inadequate in extreme northern and southern latitudes where ultraviolet radiation from the sun is not adequate to stimulate conversion of 7-dehydrocholesterol (provitamin D_3) to vitamin D_3 in the skin.

The heterogeneity of vitamin D_2 absorption in the CF subjects in the present study suggests that dosage individualization is necessary to adequately replete vitamin D concentrations. All CF patients should have a baseline 25-hydroxyvitamin D measurement before they start a tailored treatment regimen. Dosages >125 μg/d may require close monitoring of 25-hydroxyvitamin D concentrations on a monthly basis to prevent hypervitaminosis D. Clinicians should remember that high dosages of vitamin D should not be achieved by increasing the dosage of supplements containing vitamins A, D, E, and K, because toxicity of vitamins A, E, and K could result. More active vitamin D analogs, such as calcidiol or calcitriol, which are also more polar, may be more easily absorbed from the gut and may offer advantages in repleting vitamin D concentrations in this population. In patients treated with more potent vitamin D analogs, serum calcium concentrations and urinary ratios of calcium to creatinine should also be monitored to detect the complications of hypercalcemia or hypercalcuria. Further research is needed to better understand the sequelae of vitamin D deficiency and to optimize vitamin D therapy in CF patients.

REFERENCES

1. FitzSimmons SC. The changing epidemiology of cystic fibrosis. J Pediatr 1993;122:1–9.
2. Cystic Fibrosis Foundation Patient Registry 1995 Annual Data Report. Bethesda, MD: Cystic Fibrosis Foundation, 1996:1–2.
3. Bachrach LK, Loutit CW, Moss RB, Marcus R. Osteopenia in adults with cystic fibrosis. Am J Med 1994;96:27–32.
4. Henderson RC, Madsen CD. Bone density in children and adolescents with CF. J Pediatr 1996;128:28–34.
5. Aris RM, Renner JB, Winders AD, et al. Increased rate of fractures and severe kyphosis: sequelae of living into adulthood with cystic fibrosis. Ann Intern Med 1998;128:186–93.
6. Ott SM, Aitken ML. Osteoporosis in patients with cystic fibrosis. Clin Chest Med 1998;19:555–67.
7. Haworth CS, Selby PL, Webb AK, Mawer EB, Adams JE, Freemont TJ. Severe bone pain after intravenous pamidronate in adult patients with cystic fibrosis. Lancet 1998;352:1753–4.
8. Bonfield TL, Panuska JR, Konstan MW, et al. Inflammatory cytokines in cystic fibrosis lungs. Am J Respir Crit Care Med 1995; 152:2111–28.
9. Aris RM, Neuringer IP, Weiner MA, Egan TM, Ontjes DA. Severe osteoporosis before and after lung transplantation. Chest 1996;109: 1176–83.
10. Bhudhikanok GS, Lim J, Marcus R, Harkins A, Moss R, Bachrach LK. Correlates of osteopenia in patients with cystic fibrosis. Pediatrics 1996;97:103–11.
11. Haworth CS, Selby PL, Webb AK, Adams JE. Osteoporosis in adults with cystic fibrosis. J R Soc Med 1998;91(suppl):14–8.
12. Merkel PA, Herlyn K, Lapey A, et al. Osteoporosis in adults with cystic fibrosis. Pediatr Pulmonol Suppl 1999;19:294 (abstr).
13. Elkin SL, Fairney A, Burnett S, et al. Osteoporosis in adults with CF: a cross-sectional study. Pediatr Pulmonol Suppl 1999;19:299 (abstr).
14. Donovan DS, Papadopoulos A, Staron RB, et al. Bone mass and vitamin D deficiency in adults with advanced cystic fibrosis lung disease. Am J Respir Crit Care Med 1998;157:1892–9.
15. Elkin S, Compston J, Kemp M, Fairney A, Hodson ME. Hormones, bone mineral density, and cystic fibrosis. Pediatr Pulmonol Suppl 1998;26:366 (abstr).
16. Aris RM, Lester GE, Dingman S, Ontjes DA. Altered calcium homeostasis in adults with cystic fibrosis. Osteoporos Int 1999;10:102–8.
17. Trang HM, Cole DE, Rubin LA, Pierratos A, Siu S, Vieth R. Evidence that vitamin D_3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D_2. Am J Clin Nutr 1998;68:854–8.
18. Hollick MF. Vitamin D: photobiology, metabolism, mechanism of action and clinical applications. In: Favus MJ, ed. Primer on the metabolic bone diseases and disorders of mineral metabolism. 3rd ed. Philadelphia: Lippincott-Raven, 1996:74–81.
19. Hollis BW. Detection of vitamin D and its major metabolites. In: Feldman DF, ed. Vitamin D. Boston: Academic Press, 1997:587–606.
20. SAS Institute Inc. SAS/STAT user's guide, version 6. 4th ed. Cary, NC: SAS Institute Inc, 1989:891–996.
21. Jandel Scientific Software. SIGMASTAT statistical software, version 2.0. San Rafael, CA: Jandel Scientific Software, 1995.
22. Harris SS, Dawson-Hughes B, Perrone GA. Plasma 25-hydroxyvitamin D responses of younger and older men to three weeks of supplementation with 1800 IU/day of vitamin D. J Am Coll Nutr 1999;18:470–4.
23. Rey E, Treluyer JM, Pons G. Drug disposition in cystic fibrosis. Clin Pharmacokinet 1998;35:313–29.
24. Winklhofer-Roob BM, Tuchschmid PE, Molinari L, Shmerling DH. Response to a single oral dose of *all-rac*[hyphen]α-tocopheryl acetate in patients with cystic fibrosis and in healthy individuals. Am J Clin Nutr 1996;63:717–21.
25. Lancellotti L, D'Orazio C, Mastella G, Mazzi G, Lippi U. Deficiency of vitamins E and A in cystic fibrosis is independent of pancreatic function and current enzyme and vitamin supplementation. Eur J Pediatr 1996;155:281–5.
26. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr 1999;69:842–56.
27. Van Der Klis FRM, Jonxis JHP, Van Doormaal JJ, Sikkens P, Saleh AEC, Muskiet FA. Changes in vitamin-D metabolites and parathyroid hormone in plasma following cholecalciferol administration to pre- and postmenopausal women in the Netherlands in early spring and to postmenopausal women in Curacao. Br J Nutr 1996;75: 637–46.
28. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med 1997;337: 670–6.
29. Hanly JG, McKenna MJ, Quigley C, Freaney R, Muldowney FP, Fitzgerald MX. Hypovitaminosis D and response to supplementation in older patients with cystic fibrosis. Q J Med 1985;56: 377–85.