Eur J Pediatr (2006) 165: 540–545
DOI 10.1007/s00431-006-0132-1

ORIGINAL PAPER

Polyxeni Nicolaidou · Ilias Stavrinadis · Ioanna Loukou · Anna Papadopoulou · Helen Georgouli · Konstantinos Douros · Kostas N. Priftis · Dimitrios Gourgiotis · Yiannis G. Matsinos · Stavros Doudounakis

The effect of vitamin K supplementation on biochemical markers of bone formation in children and adolescents with cystic fibrosis

Received: 31 January 2006 / Accepted: 8 March 2006 / Published online: 19 April 2006

Abstract *Introduction:* Impaired vitamin K status in cystic fibrosis (CF) has been considered as a newly emerged pathogenetic factor for reduced bone mineral density (BMD). *Objectives:* Our aim was to evaluate the effectiveness of vitamin K supplementation in managing bone formation abnormalities in children and adolescents with CF. *Materials and methods:* The statuses of vitamins K and D in relation to biochemical markers of bone metabolism and BMD were examined in 20 CF children receiving vitamin D supplements but not vitamin K supplements. Laboratory tests were carried out at the beginning of the study period and after 1 year of vitamin K supplementation (10 mg single oral dose/week) and the results were compared; the results were also compared with those of 25 healthy controls. *Results and discussion:* Ten of the CF patients had BMD z-score ≤2.5 (n=5) or between −1 and −2.5 (n=5). Biochemical tests on patients before vitamin K supplementation revealed that the levels of osteoblastic activity markers, namely, bone alkaline phosphatase (BAP), serum osteocalcin (Gla-OC), serum carboxy-terminal propeptide of type I procollagen (PICP) and serum amino-terminal propeptide of type I procollagen (PINP), were significantly reduced compared with those of the controls. These patients had also lower 25-hydroxy-vitamin D (25(OH)D) and vitamin K serum levels, higher undercaboxylated osteocalcin (Glu-OC) and parathormone (PTH) levels and a higher calcium to creatinine ratio (Ca/Cr) than the controls. Vitamin K intake was associated with an increase in Gla-OC, PINP, PICP levels and a decrease in Glu-OC levels. PTH levels were lower after vitamin K supplementation without any difference in BMD z-scores. *Conclusion:* Our data indicate that vitamin K supplementation may have a beneficial role in bone health in CF children.

Keywords Cystic fibrosis · Osteopenia · Osteoporosis · Vitamin D · Vitamin K

P. Nicolaidou · A. Papadopoulou · K. Douros
Third Department of Pediatrics,
Athens University Medical School,
University General Hospital "Attikon",
Athens, Greece

I. Stavrinadis · H. Georgouli · D. Gourgiotis
Second Department of Pediatrics,
Athens University Medical School,
"P. and A. Kyriakou" Children's Hospital,
Athens, Greece

I. Loukou · S. Doudounakis
Cystic Fibrosis Unit, "Aghia Sophia" Children's Hospital,
Athens, Greece

K. N. Priftis
Allergy-Pneumonology Department,
Penteli Children's Hospital,
Athens, Greece

Y. G. Matsinos
Department of Environmental Sciences,
University of the Aegean,
Mytilene, Greece

P. Nicolaidou (✉)
Third Department of Pediatrics,
University of Athens Medical School, "Attikon" Hospital,
1 Rimini St.,
Chaidari 12464, Greece
e-mail: pedatt@med.uoa.gr
Tel.: +30-210-5832233
Fax: +30-210-5832229

Introduction

Cystic fibrosis (CF) is the most common autosomal recessive lethal disease in Caucasians and is characterized by abnormal secretions in the respiratory, gastrointestinal and genitourinary tracts. Improved survival of patients with CF due to new strategies of treatment has resulted in an increased prevalence of late complications, such as osteopenia/osteoporosis. Reduced bone mineral density (BMD) has been documented in adults and reported in children and adolescents with CF [6–8, 10, 15]. Pathogenetic factors of osteoporosis in CF patients include chronic inflammation, delayed puberty, hypogonadism, glucocorticoid therapy, limited physical activity, poor tolerance to sun exposure,

malnutrition and vitamin D malabsorption. Recent evidence suggests that vitamin K deficiency also plays an important role in the pathogenesis of decreased bone mineralization in CF patients [2, 28, 29].

The purpose of the present study was to evaluate the effectiveness of vitamin K supplementation in managing bone formation abnormalities in children and adolescents with CF.

Materials and methods

Twenty children and adolescents with CF, aged 6–17 years, were recruited from the Cystic Fibrosis Unit of "Aghia Sophia" Children's Hospital. The diagnosis of CF had been established by a positive sweat test and genetic analysis. Seven children were found to be homozygotes for the ΔF508 Cystic Fibrosis Transmembrane Regulator (CFTR) gene mutation, four were characterized as compound heterozygotes for the ΔF508 and a non-ΔF508 CFTR gene mutation, six were characterized as compound heterozygotes for non-ΔF508 CFTR gene mutations and three children were heterozygotes for a non-ΔF508 mutation although the other mutation of the CFTR gene could not be detected.

The control group comprised 25 healthy children and adolescents aged 8–17 years. All patients were clinically stable for at least 6 months before recruitment. No participant in the study was suffering from acute infection, was currently taking antibiotics or had taken them for at least 3 weeks prior to the blood samples being taken or was currently taking or had ever taken oral or intravenous corticosteroids. All patients were suffering from pancreatic insufficiency and were taking pancreatic enzyme supplementation on a regular basis in doses adjusted to their individual needs. They were also receiving vitamin D in a dose of 20 μg (800 IU) daily since, according to the consensus recommendations, this is the minimum vitamin D supplementation that should be taken by CF patients over the age of 1 year [3]. A questionnaire of their dietary habits was used to estimate their daily intake of calories and calcium. Both calories and calcium were found to be within the recommended limits (150% of recommended calories for age and sex; 800 mg calcium for children and 1200 mg calcium for adolescents). Nutritional status was assessed by the basal metabolic index (BMI; weight in kilograms/height in square meters) and was expressed as the BMI z-score. The patients exhibited normal daily activity which was similar to that of healthy controls. Tanner staging was performed in all participants. Pulmonary function assessment included measurements of forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and flow-volume loops. All measurements were expressed as a percentage of the predicted value (Spirometer: Morgan type of Flexiflo). In all cases, patients were able to produce technically acceptable flow-volume curves. The severity of CF was evaluated by the Shwachman-Kulczycki (SK) score [24].

This was a two-step study. In the first step, blood and urine samples were obtained from CF patients during their usual weekly vitamin D supplementation (group A1: baseline measurements) and from the healthy control subjects (group B). At the same time, BMD was measured in the CF patients. In the second step, blood and urine samples were collected from the same patients and their BMD was assessed after 1 year of vitamin K supplementation (group A2). During this year vitamin K was administered orally in a single weekly dose of 10 mg. The supplementation with vitamin K commenced soon after we had taken the baseline samples. Blood and spot urine samples of the second urination in the morning from all participants were obtained after overnight fasting.

BMD was measured in the lumbar spine (L1–L4) by dual-energy X-ray absorbiometry (DEXA) with a Hologic QDR-1000 upgraded unit and was expressed as z-scores. A z-score is defined as the number of standard deviations (SDs) below or above the mean BMD. It is calculated as (patient's BMD–mean)/SD, where the mean and SDs are determined using age- and sex-specific reference BMD data from healthy populations (as reference data we used those provided for Caucasians by the manufacturer of the Hologic densitometer). The expected z-score for a healthy population is 0. A patient was considered to be osteopenic if the z-score was between −1 and −2.5 and osteoporotic if the z-score was <−2.5.

Calcium (Ca) and phosphate (P) in the plasma and Ca and creatinine (Cr) in the urine were measured using standard methods. Vitamin K serum concentrations were determined by means of high performance liquid chromatography. Serum 25-hydroxy-vitamin D [25(OH)D] and parathormone (PTH) levels were measured by radioimmunoassay (RIA; Nichols Institute Diagnostics, San Clemente, Calif.), as was serum bone alkaline phosphatase (BAP; Beckman Coulter, Fullerton, Calif.) and osteocalcin (Gla-OC; Myria OC; Techno Genetics, Italy). Undercarboxylated osteocalcin (Glu-Oc) was determined by ELISA (Takara, Shyzoco, Shiga, Japan). Serum carboxy-terminal propeptide of type I procollagen (PICP), serum amino-terminal propeptide of type I procollagen (PINP) and serum cross-linked carboxy-terminal telopeptide of type I collagen (ICTP) were measured using specific RIAs (Orion Diagnostica, Espoo, Finland). The first two peptides are generated from newly synthesized type I collagen and are considered to be reliable markers of early osteoblastic function, whereas the latter has been shown to reflect degradation of collagen type I and is deemed to be a marker of bone resorption. Deoxypyridinoline (DPD), which is a bone breakdown product, was measured in urine by a RIA (Gamma-BTC, DPD, RIA, IDS). Measurements of 25(OH) D, PTH, BAP, Gla-OC, Glu-OC, PICP, PINP and ICTP in the serum and DPD and Ca in the urine were performed on patients of groups A1 and A2 and in the controls of group B, whereas vitamin K was measured only in patient group A1 and in control group B. DPD and Ca in the urine are expressed as a ratio to creatinine.

The project was approved by the Ethics Committee of Aghia Sophia Children's Hospital and informed consent was obtained from all participants.

Statistical analysis

Because some of the data were distributed in a non-normal fashion, we expressed our results as median, 25th percentile (p25), and 75th percentile (p75). In order to compare distributions of variables between groups, we used Wilcoxon sign-ranks and Mann-Whitney U tests for continuous distributions and the Fisher exact test for discrete distributions. We used Pearson's coefficient for correlations, provided that both variables were normally distributed. In all other cases we used Spearman's coefficient. We investigated the role of vitamin K intake in the levels of bone turnover markers with ANCOVA models, allowing for potential confounders. The backward elimination procedure was used for the selection of the best models. All reported p values are based on two-sided tests and are compared with a significance level of 5%. SPSS 10 software (SPSS, Chicago, Ill.) was used for the statistical calculations.

Results

The clinical characteristics and biochemical values of the patients and the controls are shown in Tables 1 and 2. No difference in age distribution was found between groups A1 and B (p=0.405 and p=0.595 for girls and boys, respectively) and between groups A2 and B (p=0.330 and p=0.489 for girls and boys, respectively). The pubertal development of both the patients and the controls was compatible with their chronological age. No statistically significant differences were found in the distribution of Tanner stages between groups A1 and B (p=0.662 and p=0.884 for the girls and boys, respectively) and between groups A2 and B (p=0.330 and p=0.489 for the girls and boys, respectively).

In the assessment of the clinical status of the CF patients at the beginning of the study (group A1), five children were assessed to be suffering from mild disease (SK score: 56–70), three from moderate disease (SK score: 41–55) and three from severe disease (SK score <40), while nine children were judged to be in good or excellent clinical condition (SK score: >70). In the second assessment, 1 year later (group A2), three children were assessed to have mild forms of the disease, three to have moderate forms and five to have severe forms, while the same nine children as 1 year earlier were in good or excellent clinical condition. No statistical significant difference was found in the distributions of SK scores between groups A1 and A2 (p=0.70). We also found no difference in the distributions of FEV1 and FVC between groups A1 and A2 (p=0.22 and p=0.27, respectively). Although z-scores for BMI were within normal limits for all participants, they were higher in control group B than in patient groups A1 and A2 (p=0.012 and p=0.047, respectively).

Results from the initial measurements of BMD in the CF patients (group A1) showed that half of the children suffered from osteopenia and/or osteoporosis. Specifically, there were five children with z-scores between −1 and

Table 1 Clinical characteristics[a] of participants in patient groups A1 and A2 and in control group B

	Sex	Age (years)	BMI z-score	SK-score	FEV_1 (% predicted)	FVC (% predicted)	Tanner stage	BMD z-score
Group A1 (patients before vitamin K supplementation)	Boys (n=10)	15 (14, 16)	−0.42 (−1.03, −0.03)	66 (45, 70)	71.5 (45, 85)	74 (56, 86)	3 (2, 4)	−1.82 (−3.03, −1.12)
	Girls (n=10)	14.5 (11, 15)	−0.19 (−0.76, 0.32)	77.5 (60, 90)	75.5 (62, 104)	80 (60, 109)	4 (3, 5)	−0.57 (−2.11, 0.04)
Group A2 (patients 1 year after vitamin K supplementation)	Boys (n=10)	16 (15, 17)	−0.04 (−1.03, −0.31)	62.5 (40, 70)	81.5 (36, 84)	82.5 (39, 92)	3 (3, 4)	−1.60 (−2.75, −1.22)
	Girls (n=10)	15.5 (12, 16)	−0.19 (−0.76, 0.32)	77.5 (55, 90)	85 (52, 89)	96.5 (57, 103)	4 (4, 5)	−0.54 (−1.22, −0.31)
Group B (healthy subjects)	Boys (n=13)	14 (12, 16)	0.01 (−0.25, 0.27)				3 (2, 5)	
	Girls (n=12)	11 (9, 14)	0.03 (−0.08, 0.11)				2.5 (1, 4)	

[a]Data are expressed as median (p25, p75). BMI, Body mass index; SK-score, Shwachman-Kulczycki score; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; BMD, bone mineral density

Table 2 Biochemical values[a] in patient groups A1 and A2 and in control group B

Biochemical characteristics[b]	Group B	Group A1	Group A2	*p* (B vs A1)	*p* (B vs A2)	*p* (A1 vs A2)
Ca (mg/dl)	9 (9, 9.4)	9.25 (8.85, 9.5)	9.1 (8.7, 9.3)	0.822	0.699	0.875
P (mg/dl)	4.5 (3.9, 5.1)	4.8 (3.9, 5.3)	4.8 (4.1, 5.1)	0.863	0.872	0.901
Vitamin K (ng/ml)	0.277 (0.140, 0.631)	0.156 (0.059, 0.590)		0.021		
25(OH)D (ng/ml)	38.0 (33.0, 46.0)	18.0 (14.0, 25.0)	15 (9.7, 20.5)	<0.001	<0.001	0.005
PTH (pg/ml)	22.0 (17.0, 29.0)	32.7 (27.0, 49.2)	29.5 (19.0, 42.5)	<0.001	0.059	0.020
BAP (μg/l)	70 (58.0, 91.5)	46.2 (30.0, 63.2)	49.5 (41.5, 65.5)	<0.001	0.001	0.052
Gla-OC (ng/ml)	22 (16.10, 27.60)	13.75 (9.75, 16.50)	22.5 (15.00, 35.50)	0.002	0.522	0.005
Glu-OC (ng/ml)	4.00 (3.38, 6.52)	8.08 (4.25, 10.77)	1.25 (0.53, 2.14)	0.017	<0.001	<0.001
ICTP (μg/l)	11.00 (8.50, 12.50)	9.35 (7.25, 14.00)	10.95 (9.25, 13.00)	0.521	0.790	0.204
PICP (μg/l)	385.0 (300.0, 460.5)	274.8 (190.5, 394.5)	339.0 (191.0, 466.0)	0.012	0.360	0.009
PINP (μg/l)	850.0 (517.0, 1215.0)	219.0 (150.0, 525.5)	530.0 (373.0, 820.5)	<0.001	0.020	<0.001
DPD/Cr (n*M*/m*M*)	11.6 (7.50, 22.90)	12.10 (9.20, 19.85)	11.9 (10.25, 19.25)	0.819	0.623	0.910
Ca/Cr (mg/mg)	0.090 (0.060, 0.120)	0.125 (0.100, 0.190)	0.145 (0.095, 0.220)	0.005	0.011	0.260

[a]Data are expressed as a median (p25 and p75)
[b]Ca, Calcium; P, phosphorus; 25(OH)D, 25-hydroxy-vitamin D; PTH, parathormone; BAP, bone alkaline phosphatase; Gla-OC, osteocalcin; Glu-Oc, undercarboxylated osteocalcin; PICP, carboxy-terminal propeptide of type I procollagen; PINP, amino-terminal propeptide of type I procollagen; ICTP, cross-linked carboxy-terminal telopeptide of type I collagen; DPD, deoxypyridinoline; Cr, creatinine

−2.5, and 5 whose z-score was lower than −2.5. No difference was found between groups A1 and A2 with respect to BMD z-scores (p=0.935). The BMD z-score was positively correlated with the BMI z-score in patient groups A1 and A2 (r=0.45, p=0.045 and r=0.56 p=0.009, respectively), and it was also associated with the pulmonary function tests and SK scores. More specifically, the BMD z-score was positively correlated with FEV_1 in groups A1 (r=0.7, p<0.001) and A2 (r=0.66, p=0.001), with FVC in groups A1 (r=0.69, p<0.001) and A2 (r=0.7, p<0.001) and with SK score in groups A1 (r=0.67, p=0.001) and A2 (r=0.67, p<0.001).

The levels of vitamin K, 25(OH)D, BAP, Gla-OC, PICP and PINP were significantly lower in patient group A1 in comparison with control group B (p=0.021, p<0.001, p<0.001, p=0.002, p=0.012, p<0.001, respectively), whereas, Glu-OC, PTH and Ca/Cr values were higher (p<0.001, p=0.005, p=0.017, respectively). A comparison of patient group A2 and control group B revealed that although the levels of 25(OH)D, BAP and PINP remained lower (p<0.001, p=0.001, p=0.020, respectively) and Ca/Cr remained higher (p=0.011) in the patient group, there were no differences between the two groups with respect to the levels of Gla-OC, PICP and PTH at this time. Furthermore, the direction of the difference in Glu-OC levels had been reversed, with the levels in the patients being lower than those in the controls (p=0.001). Finally, a comparison between the patients before and after 1 year of vitamin K supplementation showed that Gla-OC, PINP and PICP levels were higher in group A2 than group A1 (p=0.005, p<0.001, p=0.009, respectively); conversely, 25(OH)D, PTH and Glu-OC levels were higher in group A1 (p=0.005, p=0.020, p<0.001, respectively) (Table 2).

In patient group A1, 25(OH)D was found to be associated with some of the biochemical markers of bone turnover. More specifically, Gla-OC, PINP, and PICP were all positively correlated with 25(OH)D (r=0.47, p=0.033; r=0.64, p=0.002; r=0.51, p=0.032, respectively). 25(OH)D was not associated with the BMD z-score in either group A1 (p=0.54) or in group A2 (p=0.45).

For multivariate analysis we used successive ANCOVA models which, each time, had as response variable one of the bone turnover markers, i.e., BAP, Gla-OC, Glu-OC, ICTP, PICP, PINP, DPD/Cr, Ca/Cr and P/Cr. In each model we controlled for potential confounders which may have influenced the relation between markers and vitamin K, namely, age, gender, Tanner stage, BMI, FEV, FVC, SK score, PTH and levels of 25(OH)D.

Vitamin K intake was found to be an independent predictor of Glu-OC (p<0.001; the decrease, on average, in Glu-OC levels with vitamin K intake was 6.17 ng/ml), of Gla-OC (p=0.013; the increase, on average, in Gla-OC levels with vitamin K intake was 10.77 ng/ml), of PICP (p<0.001; the increase, on average, in PICP levels with vitamin K intake was 108.2 μg/l) and of PINP (p<0.001; the increase, on average, in PINP levels with vitamin K intake was 297.2 μg/l).

Discussion

Although the majority of our patients had normal pubertal development, normal daily activity and acceptable BMI z-scores, the initial measurements of BMD at the lumbar spine (group A1) revealed that half of them had osteopenia or osteoporosis. This is not surprising since the pathogenesis of low BMD in individuals with CF is multifactorial and remains largely uncertain. Furthermore, serum and urine analysis of the same group of patients showed that their osteoblastic activity, as expressed by BAP, Gla-OC, PICP and PINP levels, was reduced relative to that of the control group. The markers of osteoclastic activity, ICTP

and DPD/Cr, did not differ between these groups, whereas the Ca/Cr ratio was higher in the patient groups than in the controls. Of these three markers only ICTP can be considered to be a true marker of osteoclastic activity: Ca/Cr is a non-specific marker of bone resorption. Consequently, it was not possible to come to a conclusion with respect to osteoclastic activity in our patients although our data suggest that our CF patients had impaired bone formation. Numerous articles have reported that children and adolescents with CF may have lower BMD as well as reduced levels of biochemical markers of osteoblastic activity [6–8, 10, 15]. In addition, some authors have reported increased bone osteoclastic activity in CF patients, suggesting an imbalance between bone formation and degradation in these patients [1, 4, 9], although others have measured normal levels of bone resorption markers [19]. Moreover, as already been demonstrated [4, 8], our patients' BMD z-scores were positively associated with their BMI z-scores and disease severity indexes (SK score, FEV_1 and FVC), suggesting that nutritional status and disease severity may affect bone mineralization in children and adolescents with CF.

The complex pathogenesis of CF bone disease complicates experimental procedures [6–8, 10, 15]. In our study, the confounding effect of some of the known pathogenetic factors – i.e., inflammation, glucocorticoid therapy, limited activity – was minimized by the selection criteria which excluded those who were exposed to any of the aforementioned factors. Unfortunately, since only a limited number of patients fulfilled the above criteria we were able to recruit 20 subjects only.

A notable finding of our study was that CF patients of group A1 were found to have lower serum levels of 25(OH) D than the controls despite vitamin D supplementation (20 μg daily). Similar findings have also been reported by other authors [8, 10, 13, 18, 19] .These results suggest that the minimum recommended dose of vitamin D supplementation for CF patients [3] may not be adequate even in Greece where solar exposure is high. Although no correlation between serum 25(OH)D levels and BMD z-scores was found, we observed a weak, nevertheless positive, correlation between serum 25(OH)D levels and biochemical markers of osteoblastic activity (Gla-OC, PINP, PICP) in our patients. These findings suggest that vitamin D insufficiency may contribute to reduced bone mass [10, 12, 14]. Furthermore, despite no correlation having been found between serum levels of 25(OH)D and PTH in our study, the hypovitaminosis D reported in our CF patients of group A1 may have resulted in elevated levels of PTH. The increase in PTH secretion in these patients may contribute to their diminished BMD z-scores through an enhancement of the osteoclastic process, as illustrated in our study by the higher Ca/Cr ratio.

In addition to having a vitamin D deficiency, patients of group A1 were also found to have lower levels of vitamin K and higher levels of Glu-OC than the control subjects. This finding was expected given the malabsorption and lack of vitamin K supplementation of the patients of group A1 at that time. Vitamin K is an essential cofactor in the post-translational carboxylation of γ-glutamyl acid residues [30]. The family of vitamin K-dependent carboxylated proteins include clotting factors and Gla-OC, the latter being produced by osteoblasts during bone matrix formation. The carboxylation state of osteocalcin has been shown to be responsive to changes in vitamin K status, and a deficiency of this vitamin leads to increased serum levels of Glu-OC, which is both indicative and a particularly reliable marker of poor bone status [5, 23]. Some studies have reported an association between hypovitaminosis K and reduced BMD [11, 25]. Therefore, although we found no correlation between serum vitamin K levels and the BMD z-score or biochemical markers of bone turnover, we propose that vitamin K insufficiency may contribute to the diminished bone turnover of our patients, thereby resulting in a lower BMD.

Although the old guidelines support oral supplementation with 5 mg/week of vitamin K when the patients are older than 1 year and on antibiotics or have liver disease and the new guidelines advise 0.3–0.5 mg/day of vitamin K, these dosages are not based on prospective, randomized studies [3, 22, 26]. Our decision to administer a relatively high dose of vitamin K, namely 10 mg/week, was based on the results of a prospective study which suggested that patients with CF have compromised vitamin K status while receiving 5 mg/week of vitamin K supplementation [5]. Following 1 year of vitamin K supplementation in a dose of 10 mg orally per week, we repeated all laboratory tests in the same patients. The problem of hypovitaminosis D was more accentuated in CF patients of group A2 than in those of group A1 since the vitamin D dose, which was apparently insufficient, had not changed in the period between two measurements. Unfortunately, we were unable to measure vitamin K levels in group A2. Although this is a limitation of our study, we evaluated vitamin K status in patients of this group indirectly via Glu-OC concentrations [30]. In this way, we also avoided interferences from exogenous intake of vitamin K during the year of supplementation. Another possible limitation of our study is the low levels of 25(OH)D in patients, which may have obscured the effect of vitamin K supplementation. Patients of group A2 had lower values of Glu-OC compared with patients of groups A1, suggesting an improved vitamin K status in CF patients following vitamin K supplementation. Moreover, biochemical markers of osteoblastic activity (OC, PICP, PINP) in the CF patients were significantly increased after the completion of 1 year of vitamin K supplementation [20, 25, 27]. Since CF patients of group A2 had more severe hypovitaminosis D after 1 year of vitamin K supplementation, we had to attribute the amelioration in the biochemical markers of bone formation to the improved vitamin K status. Multivariate analysis confirmed these results.

The most striking finding in our study was the improvement of serum PTH levels in CF patients of group A2 compared with those of group A1, despite the more pronounced vitamin D deficiency in the former group. PTH can promote both the formation and resorption of bone. Although intermittent stimulation by PTH

promotes bone formation through the local production of growth factors, continuous stimulation by PTH promotes bone resorption [21]. Recent experimental findings have shown that vitamin K supplementation of vitamin K-deficient animals can induce a reduction in PTH secretion via an increase in calcium re-absorption in the urinary tubules [16, 17]. Although we did not find any reduction of the urine Ca/creatinine ratio in patients of group A2 compared with group A1, we suggest that vitamin K supplementation of our vitamin K-deficient CF patients may correlate with serum PTH reduction.

Despite the improvement in bone formation markers and the reduction in PTH levels in group A2 patients compared with those of group A1, BMD z-scores remained virtually the same. Given that a dose of 10 mg of vitamin K per week is considered to be too high for CF children [28] and that a 1-year treatment is too short to improve BMD, we suggest that long-term vitamin K supplementation may ultimately result in an improved BMD in CF patients.

References

1. Aris RM, Ontjes DA, Buell HE, Blackwood AD, Lark RK, Caminiti M, Brown SA, Renner JB, Chalermskulrat W, Lester GE (2002) Abnormal bone turnover in cystic fibrosis adults. Osteoporos Int 13:151–157
2. Aris RM, Ontjes DA, Brown SA, Chalermskulrat W, Neuringer I, Lester GE (2003) Carboxylated osteocalcin levels in cystic fibrosis. Am J Respir Crit Care Med 168:1129
3. Aris RM, Merkel PA, Bachrach LK, Borowitz DS, Boyle MP, Elkin SL, Guise TA, Hardin DS, Haworth CS, Holick MF, Joseph PM, O'Brien K, Tullis E, Watts NB, White TB (2005) Guide to bone health and disease in cystic fibrosis. J Clin Endocrinol Metab 90:1888–1896
4. Baroncelli GI, De Luca F, Magazzu G, Arrigo T, Sferlazzas C, Catena C, Bertelloni S, Saggese G (1997) Bone demineralization in cystic fibrosis: evidence of imbalance between bone formation and degradation. Pediatr Res 41:397–403
5. Beker LT, Ahrens RA, Fink RJ, O'Brien ME, Davidson KW, Sokoll LJ, Sadowski JA (1997) Effect of vitamin K1 supplementation on vitamin K status in cystic fibrosis patients. J Pediatr Gastroenterol Nutr 24:512–517
6. Bhudhikanok GS, Lim J, Marcus R, Harkins A, Moss RB, Bachrach LK (1996) Correlates of osteopenia in patients with cystic fibrosis. Pediatrics 97:103–111
7. Bhudhikanok GS, Wang MC, Marcus R, Harkins A, Moss RB, Bachrach LK (1998) Bone acquisition and loss in children and adults with cystic fibrosis: a longitudinal study. J Pediatr 133:18–27
8. Conway SP, Morton AM, Oldroyd B, Truscott JG, White H, Smith AH, Haigh I (2000) Osteoporosis and osteopenia in adults and adolescents with cystic fibrosis: prevalence and associated factors. Thorax 55:798–804
9. Conway SP, Wolfe SP, Brownlee KG, White H, Oldroyd B, Truscott JG, Harvey JM, Shearer MJ (2005) Vitamin K status among children with cystic fibrosis and its relationship to bone mineral density and bone turnover. Pediatrics 115:1325–1331
10. Donovan DS Jr, Papadopoulos A, Staron RB, Addesso V, Schulman L, McGregor C, Cosman F, Lindsay RL, Shane E (1998) Bone mass and vitamin D deficiency in adults with advanced cystic fibrosis lung disease. Am J Respir Crit Care Med 157:1892–1899
11. Douglas AS, Robins SP, Hutchison JD, Porter RW, Stewart A, Reid DM (1995) Carboxylation of osteocalcin in post-menopausal osteoporotic women following vitamin K and D supplementation. Bone 17:15–20
12. Hahn TJ, Spuires AE, Halstead LR, Strominger DB (1979) Reduced serum 25-hydroxyvitamin D concentrations and disordered mineral metabolism in patients with cystic fibrosis. J Pediatr 94:38–42
13. Hanly JG, McKenna MJ, Quigley C, Freaney R, Muldowney FP, FitzGerald MX (1985) Hypovitaminosis D and response to supplementation in older patients with cystic fibrosis. Q J Med 56:377–385
14. Henderson RC, Madsen CD (1996) Bone density in children and adolescents with cystic fibrosis. J Pediatr 128:28–34
15. Ionescu AA, Nixon LS, Evans WD, Stone MD, Lewis-Jenkins V, Chatham K, Shale DJ (2000) Bone density, body composition, and inflammatory status in cystic fibrosis. Am J Respir Crit Care Med 162:789–794
16. Iwamoto J, Yeh JK, Takeda T, Ichimura S, Sato Y (2003) Comparative effects of vitamin K and vitamin D supplementation on prevention of osteopenia in calcium-deficient young rats. Bone 33:557–566
17. Iwamoto J, Yeh JK, Takeda T, Sato Y (2005) Effects of vitamin K2 administration on calcium balance and bone mass in young rats fed normal or low calcium diet. Horm Res 63:211–219
18. Leifke E, Friemert M, Heilmann M, Puvogel N, Smaczny C, von zur Muhlen A, Brabant G (2003) Sex steroids and body composition in men with cystic fibrosis. Eur J Endocrinol 148:551–557
19. Mortensen LA, Chan GM, Alder SC, Marshall BC (2000) Bone mineral status in prepubertal children with cystic fibrosis. J Pediatr 136:648–652
20. Nakashima A, Yorioka N, Doi S, Masaki T, Ito T, Harada S (2004) Effects of vitamin K2 in hemodialysis patients with low serum parathyroid hormone levels. Bone 34:579–583
21. Potts TJ (2005) Parathyroid hormone: past and present. J Endocrinol 187:311–325
22. Ramsey BW, Farrell PM, Pencharz P (1992) Nutritional assessment and management in cystic fibrosis: a consensus report. The Consensus Committee. Am J Clin Nutr 55:108–116
23. Schoon EJ, Muller MC, Vermeer C, Schurgers LJ, Brummer RJ, Stockbrugger RW (2001) Low serum and bone vitamin K status in patients with longstanding Crohn's disease: another pathogenetic factor of osteoporosis in Crohn's disease? Gut 48:473–477
24. Shwachman H, Kulczycki LL (1958) Long-term study of 105 patients with cystic fibrosis. Am J Dis Child 96:6–15
25. Takahashi M, Naitou K, Ohishi T, Kushida K, Miura M (2001) Effect of vitamin K and / or D on undercarboxylated and intact osteocalcin in osteoporotic patients with vertebral or hip fractures. Clin Endocr 54:219–224
26. UK Cystic Fibrosis Trust, Nutrition Working Group (2002) Nutritional management of Cystic Fibrosis. A consensus report. UK Cystic Fibrosis Trust, Bromley, Kent, UK
27. Ushiroyama T, Ikeda A, Ueki M (2002) Effect of continuous combined therapy with vitamin K(2) and vitamin D(3) on bone mineral density and coagulofibrinolysis function in postmenopausal women. Maturitas 41:211–221
28. van Hoorn JH, Hendriks JJ, Vermeer C, Forget PP (2003) Vitamin K supplementation in cystic fibrosis. Arch Dis Child 88:974–975
29. Wilson DC, Rashid M, Durie PR, Tsang A, Kalnins D, Andrew M, Corey M, Shin J, Tullis E, Pencharz PB (2001) Treatment of vitamin K deficiency in cystic fibrosis: effectiveness of a daily fat-soluble vitamin combination. J Pediatr 138:851–855
30. Zittermann A (2001) Effects of vitamin K on calcium and bone metabolism. Curr Opin Clin Nutr Metab Care 4:483–487