Cholecalciferol significantly increases 25-hydroxyvitamin D concentrations in adults with cystic fibrosis1,2

Anne Stephenson, Michelle Brotherwood, Ronalee Robert, Eshetu Atenafu, Mary Corey, and Elizabeth Tullis

ABSTRACT
Background: Vitamin D deficiency is increasingly being recognized and treated in patients with cystic fibrosis, although the treatment guidelines are not proven and the effectiveness of vitamin D preparations is untested.

Objectives: The aims of this study were to determine the prevalence of 25-hydroxyvitamin D [25(OH)D] deficiency in a large cohort of adults with cystic fibrosis and to evaluate the effectiveness of supplementation with cholecalciferol.

Design: In this retrospective cohort design, baseline 25(OH)D concentrations were measured, and the effects of clinical interventions that involved either counseling on compliance or increasing supplemental cholecalciferol on serum 25(OH)D concentrations in those subjects with baseline concentrations ≤ 50 nmol/L were evaluated.

Results: Of 360 adults with cystic fibrosis, 249 (69%) had baseline 25(OH)D concentrations ≤ 50 nmol/L, despite similar levels of supplementation. The lowest 25(OH)D concentrations were seen in younger subjects who had lower body mass indexes and less pulmonary function. Serum 25(OH)D concentrations increased significantly (P < 0.001)—from 35.5 ± 10.1 to 62.5 ± 19.1 nmol/L—in 92% of the subjects after the intervention. The subjects with baseline 25(OH)D concentrations < 25 nmol/L had the largest increase in serum 25(OH)D (P = 0.02).


KEY WORDS 25-Hydroxyvitamin D, cystic fibrosis, supplementation

INTRODUCTION

Low bone mineral density has been well documented in persons with cystic fibrosis (CF), and, although bone disease in CF likely is multifactorial, vitamin D deficiency has been implicated as a causative factor (1). Vitamin D is necessary to maintain bone health, because it maximizes calcium absorption from the lumen of the gut. Several studies have documented 25-hydroxyvitamin D [25(OH)D] deficiency in CF patients despite routine supplementation (2–5). Typically, a serum 25(OH)D concentration < 50 nmol/L (20 ng/mL) is considered deficient; however, more recently, data in the non-CF literature suggested that vitamin D concentrations > 75 nmol/L are needed to maximize gastrointestinal calcium absorption and normalize parathyroid hormone (6, 7). Possible causes of low vitamin D in CF include decreased gastrointestinal absorption, impaired hydroxylation of vitamin D, reduced sun exposure, increased use of sunscreens, seasonal influence, and geographical location. Vitamin D is a fat-soluble vitamin that has an effect beyond bones (8). It has been linked to conditions such as diabetes (7), multiple sclerosis (9), and cancer (10) and more recently low vitamin D has been associated with lower lung function (11).

Several forms of vitamin D supplementation have been used to correct vitamin D deficiency; however, controversy exists as to the most effective way of administering vitamin D to persons with CF. Hanly et al (12) assessed the response to 800 IU vitamin D3 (cholecalciferol)/d in 20 adolescents and young adults with CF and found that serum concentrations of 25(OH)D increased from 10.3 to 21.3 nmol/L; however, concentrations in many subjects remained well below the target range. Haworth et al (13) reported that calcium and vitamin D supplementation reduced the rate of bone turnover and bone loss in 15 adults with CF; however, this reduction was not statistically significant. Lark et al (14) studied the effect of vitamin D2 (ergocalciferol) and found absorption to be significantly (P < 0.001) lower and highly variable in adults with CF than in control subjects; they found that poor conversion of vitamin D2 to 25(OH)D increased the possibility of impaired hydroxylation. Boyle et al (15) found that 50 000 IU ergocalciferol/wk failed to increase serum 25(OH)D concentrations significantly. Although short-term calcitriol increases calcium absorption, Brown et al (16) found that serum 25(OH)D concentrations were not significantly increased in CF subjects so treated. The purposes of this study were to determine the prevalence of 25(OH)D deficiency in a large cohort of subjects with CF and to evaluate the effectiveness of vitamin D supplementation with cholecalciferol in the adult CF population followed at a large CF center.

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SUBJECTS AND METHODS

Sample population

A retrospective cohort design was used, and 360 adults aged 17–62 who were attending the Adult Cystic Fibrosis Clinic at St Michael’s Hospital in Toronto (the 360 adults represent the patient population regularly followed at the clinic) were included in the study between 1998 and 2005. All subjects had been diagnosed with CF on the basis of sweat chloride testing, genotyping, or both. Pancreatic status was determined by either 3-d fecal fat measurements, serum trypsinogen, clinical evidence of steatorrhea, or all 3 measures. Patients at the CF clinic at St Michael’s Hospital who have pancreatic insufficiency are routinely supplemented with 800 IU cholecalciferol/d, whereas patients who have pancreatic sufficiency receive 400 IU cholecalciferol/d. After patients underwent lung transplantation, subsequent serum vitamin D concentrations were not included in the analysis.

All CF patients followed at the clinic provided written informed consent for their data to be entered into the CF patient data registry and used for such research purposes as outlined in the present study. The study was approved by the research ethics board at St Michael’s Hospital.

Outcome measurements

Serum 25-OH vitamin D

Serum 25(OH)D concentrations were measured by using a radioimmunoassay (DiaSorin; Stillwater, MN). Concentrations were measured in all subjects at baseline and yearly thereafter as part of routine clinical care.

Seasonal variation

Mean serum 25(OH)D concentrations were categorized by season. Seasons were defined as follows: winter: December 22nd–March 21st; spring: March 22nd–June 21st; summer: June 22nd–September 21st; and fall: September 22nd–December 21st.

Intervention

All baseline serum 25(OH)D results were monitored by the CF dietitian. Oral intake of cholecalciferol was increased in 1 of 2 ways: either the patient was counseled on compliance, or additional oral cholecalciferol was added to the patient’s regimen. If the concentration was ≤ 50 nmol/L (20 ng/mL), the dietitian contacted the patient, reviewed the dosage of vitamin D supplementation the patient was taking, and inquired about compliance. For those subjects who admitted to nonadherence to the prescribed vitamin regimen, the dietitian encouraged compliance and reviewed the importance of adequate vitamin supplementation, paying particular attention to bone health. However, if the patient was compliant with the regimen, additional oral cholecalciferol was prescribed with increases as follows: 17% added 400 IU/d, 5% added 800 IU/d, 61% added 1000 IU/d, and 17% added >1000 IU/d. The amount added was not standardized but was at the discretion of the dietitian and treating physician. Repeat 25(OH)D concentrations were measured ≥3 mo after this intervention and recorded.

Statistical analysis

Descriptive statistics for continuous variables are given as means and SDs unless otherwise noted. Categorical variables were compared by using a chi-square test. Student’s t test was used to compare mean values between 2 groups for continuous variables, and a paired t test was used to compare measures before and after intervention. When comparing > 2 groups, generalized linear modeling (analysis of variance) was used. Subsequent to this, in a subgroup analysis, we used Tukey’s correction factor for multiple comparisons. The binomial distribution was used to assess the change in the proportion of patients with 25(OH)D concentrations > 50 and > 75 nmol/L before and after intervention. P < 0.05 was considered significant. Statistical analysis was performed with SAS software (version 9.1; SAS Institute Inc, Cary, NC).

RESULTS

Baseline serum 25(OH)D concentrations were measured in 360 subjects. Overall descriptive characteristics of the subjects are given in Table 1. At baseline, 249 subjects (69%) had serum 25(OH)D concentrations ≤ 50 nmol/L (20 ng/mL), and only 44 (12%) had concentrations > 75 nmol/L (30 ng/mL). Eighty-six percent of subjects were taking vitamin D supplementation at baseline. Of the 52 subjects not taking supplementation, 17 had pancreatic insufficiency and 35 had pancreatic sufficiency. The overall mean concentration of 25(OH)D at baseline in the cohort was 47 ± 22 nmol/L (median: 43 nmol/L).

Sex, pancreatic status, forced expiratory volume in 1 s (FEV1), body mass index (BMI; in kg/m²), and age were used in the univariate regression analysis, and baseline 25(OH)D concentrations were used as the outcome variable. Age was the only factor found to be significant (P = 0.01). In a multiple regression model, age continued to be a significant (P = 0.011) predictor of baseline 25(OH)D after adjustment for FEV1, BMI, sex, and pancreatic status.

Baseline 25(OH)D concentrations had a seasonal pattern: mean values were highest in the summer (60.4 nmol/L), and fall (42.6 nmol/L), winter (44.9 nmol/L), and spring (46.0 nmol/L) were lower. We collapsed the values for winter, spring, and fall and compared that value with the value for summer; the difference was significant (P < 0.0001). There was no difference in vitamin D supplementation between seasons.
Vitamin D concentrations were categorized as <25 nmol/L, 25–50 nmol/L, and >50 nmol/L in groups 1, 2, and 3, respectively. Group mean 25(OH)D concentrations were 18 ± 4.7, 39 ± 7.0, and 73 ± 19.1 nmol/L, respectively (Table 2). With the use of analysis of variance (ANOVA) to compare multiple groups (Table 2), no significant between-group differences were noted for baseline vitamin D supplementation or sex. There was a significant difference between groups with respect to age (P = 0.003) and BMI (P = 0.018). The subjects with the lowest serum 25(OH)D concentrations were younger and had lower BMI than did other subjects. FEV1 was not significant, but there was a trend toward increasing lung function with higher serum vitamin D (P = 0.06). FEV1 was significantly (P = 0.048) different between group 1 and group 3. Subjects with pancreatic insufficiency were significantly (P = 0.003) more likely to have low serum 25(OH)D concentrations.

Of the 249 patients with baseline 25(OH)D concentrations ≤ 50 nmol/L, 5 underwent no intervention because they died or received a transplant before the intervention began. The remaining 244 subjects were contacted by the CF dietitian. Postintervention serum 25(OH)D concentrations were measured in 215 of the 244 potential subjects. Twenty-nine subjects did not have repeat vitamin D testing because of death, transplantation, failure to return to clinic, or relocation. The overall mean serum vitamin D concentration increased significantly (P < 0.0001) after intervention, from 35.5 ± 10.1 nmol/L to 62.5 ± 19.1 nmol/L.

### Table 2

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>18 ± 4.72</td>
<td>39 ± 7.0</td>
<td>73 ± 19.1</td>
<td></td>
</tr>
<tr>
<td>Cholecalciferol supplementation (IU)</td>
<td>568 ± 363</td>
<td>652 ± 304</td>
<td>668 ± 389</td>
<td>0.266</td>
</tr>
<tr>
<td>FEV1 (L)</td>
<td>1.98 ± 0.9</td>
<td>2.31 ± 1.0</td>
<td>2.44 ± 1.1</td>
<td>0.061</td>
</tr>
<tr>
<td>FVC (% of predicted)</td>
<td>56.6 ± 23.3</td>
<td>61.6 ± 24.0</td>
<td>66.6 ± 24.3</td>
<td>0.061</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.3 ± 3.3</td>
<td>21.9 ± 3.4</td>
<td>22.9 ± 3.7</td>
<td>0.018</td>
</tr>
<tr>
<td>Age (y)</td>
<td>25 ± 6.5</td>
<td>27 ± 8.6</td>
<td>30 ± 10.1</td>
<td>0.003</td>
</tr>
<tr>
<td>PI (%)</td>
<td>80</td>
<td>83</td>
<td>67</td>
<td>0.003</td>
</tr>
<tr>
<td>Male (%)</td>
<td>48</td>
<td>62</td>
<td>57</td>
<td>0.187</td>
</tr>
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</table>

1 25(OH)D, 25-hydroxyvitamin D; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; PI, pancreatic insufficiency. Categorical variables were compared by using a chi-square test; ANOVA was used to evaluate significant differences in continuous variables between the groups.

2 x ± SD (all such values).

### Table 3

25-Hydroxyvitamin D [25(OH)D] concentrations before and after the intervention.

<table>
<thead>
<tr>
<th></th>
<th>Before intervention</th>
<th>After intervention</th>
<th>Difference</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Overall 25(OH)D concentration</td>
<td>35.5 ± 10.12</td>
<td>62.5 ± 19.1</td>
<td>26.9 ± 20.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Group 1 (n = 35)</td>
<td>17.9 ± 4.8</td>
<td>53.1 ± 16.0</td>
<td>35.2 ± 16.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Group 2 (n = 180)</td>
<td>38.7 ± 7.1</td>
<td>63.8 ± 19.2</td>
<td>25.2 ± 20.3</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

All subjects in intervention arm

<table>
<thead>
<tr>
<th></th>
<th>Before intervention</th>
<th>After intervention</th>
<th>Difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D &gt;50 nmol/L (n = 215)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (%)</td>
<td>0</td>
<td>82</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No (%)</td>
<td>100</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25(OH)D &gt;75 nmol/L (n = 215)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (%)</td>
<td>0</td>
<td>17</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No (%)</td>
<td>100</td>
<td>83</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 25(OH)D concentrations were <25 and 25–50 nmol/L in group 1 and group 2, respectively. Paired Student’s t test was used to evaluate differences in mean 25(OH)D concentrations before and after the intervention; binomial distribution was used to evaluate the change in proportions before and after the intervention.

2 x ± SD (all such values).
D concentration, and older subjects had higher concentrations. However, within the group whose baseline vitamin D concentrations were 25–50 nmol/L, pancreatic status became the significant predictor ($P = 0.01$) and age was no longer significant ($P = 0.468$). In other words, subjects with pancreatic sufficiency had higher vitamin concentrations after the intervention than before.

**DISCUSSION**

To the best of our knowledge, the CF cohort in the present study is the largest to date in which the prevalence of vitamin D deficiency and the effectiveness of cholecalciferol supplementation have been examined. Our findings confirm that a significant proportion (69%) of adults with CF have 25(OH)D concentrations ≤ 50 nmol/L, despite baseline supplementation with oral cholecalciferol. This is strong evidence that the current dosage of 400 to 800 IU cholecalciferol/d is inadequate to normalize serum concentrations. In addition, the present study shows that additional cholecalciferol can significantly increase 25(OH)D concentrations to >50 nmol/L in most subjects (82%) and in some subjects (18%), a concentration above 75 nmol/L was achieved.

At the time of this study, a serum 25(OH)D concentration above 50 nmol/L was felt to be adequate, although we recognize that the most recent recommendation by the Cystic Fibrosis Foundation (CF) Consensus Guidelines on bone health is a serum 25(OH)D concentration > 75 nmol/L (17). What is considered a vitamin D–deficient state varies widely, depending on the threshold used. Consequently, the prevalence of this condition increases as the cutoff to define deficiency increases. A recent study by Boyle et al (15) also reported vitamin D concentrations < 75 nmol/L in 81% of CF subjects. Despite high-dose ergocalciferol supplementation (up to 50,000 IU 2 times/wk), serum 25(OH)D concentrations remained abnormally low in CF patients. In contrast to the findings of Boyle et al, however, the present study found that additional cholecalciferol significantly increased serum 25(OH)D in CF patients. Several studies have shown that cholecalciferol (vitamin D3) is more efficacious than is ergocalciferol (vitamin D2) in correcting vitamin D deficiency in the non-CF population. Armas et al (18) found a significant difference in potency between vitamin D2 and D3 in healthy males; vitamin D3 was more potent. Trang et al (19) found that vitamin D2 increased serum 25(OH)D concentrations by 13.7 nmol/L, whereas vitamin D3 increased concentrations by 23.3 nmol/L (95% CI: 1.4, 17.8 nmol/L). Possible mechanisms for this differential response include differences in the affinity of vitamin D–binding protein for the 2 calciferols or increased affinity for the hepatic 25-hydroxylase for D3. Given these findings, consideration should be given to recommending cholecalciferol as the supplement for CF patients.

Compared with subjects in the present study with baseline 25(OH)D concentrations > 25 nmol/L, those with 25(OH)D concentrations <25 nmol/L were younger, had lower BMI, and had a trend toward a lower FEV1, which may suggest that those at the highest risk of vitamin D deficiency are persons with more severe disease. Those persons also could have less sun exposure because they may be less mobile and less likely to participate in outdoor activities. Age was significantly associated with both baseline and postintervention vitamin D concentrations. The older subjects in group 1 (baseline 25(OH)D concentrations < 25 nmol/L) had higher postintervention vitamin D concentrations than did the younger subjects. Age was not a significant predictor of postintervention vitamin D status in group 2 (baseline: 25–49 nmol/L), and pancreatic sufficiency was a significant predictor of higher concentrations. Moreover, the group with the largest increase in 25(OH)D had the lowest baseline concentrations, despite similar increases in cholecalciferol supplementation. Although regression to the mean may explain some of this relation, Trang et al (19) found a similar phenomenon in healthy volunteers, whereby the change in 25(OH)D concentration was dependent on the baseline measurement. When baseline 25(OH)D concentrations were > 50 nmol/L, the increases in serum concentrations seemed to plateau. The mechanism for this phenomenon may be the fact that increased vitamin D supplementation inhibits 25-hydroxylase in the liver, although that possibility has not been studied in CF patients. If that fact is true in CF patients, achieving concentrations > 75 nmol/L through oral vitamin D supplementation may require extremely high doses or may be impossible to attain. An alternative to oral supplementation is sun exposure, because most vitamin D is made in the skin. Brief casual exposure of the arms and face is equivalent to ingesting ≈200 IU/d, although that is dependent on the season and the angle of the sun (20). Gronowitz et al (21) compared serum calcidiol concentrations in CF subjects who received up to 10 min of ultraviolet B exposure 3 times/wk for 6 mo with concentrations in age- and sex-matched CF control subjects. Mean serum vitamin D concentrations in the treatment group increased significantly, from a baseline of 54 nmol/L to 109 and 124 nmol/L at 8 and 24 wk, respectively. Although this was a small study (only 15 subjects/group), it suggests that UVB exposure may be an effective and efficient way to maintain high serum 25(OH)D concentrations.

It is important to recognize the limitations of our study. First, it is a retrospective study design and not a randomized controlled trial, which limits the conclusions that can be drawn from the results. It is not possible to say with certainty how much supplementation is needed to correct vitamin D concentrations to >50 nmol/L, but our results suggest that, on average, an additional 1000 IU/d appears to increase concentrations in most subjects. Second, the exact date of the intervention was not accurately recorded; therefore, it was not possible to determine with accuracy how long subjects needed to take additional vitamin D before their serum concentrations rose. A randomized clinical trial is needed to determine the dosage and duration of treatment required to achieve 25(OH)D concentrations above the target goal of 50 (or 75) nmol/L in the CF population. Furthermore, we did not record information on sun exposure or sunscreen use in our subjects, which could potentially confound our results. We attempted to account for sun exposure in the analysis by looking at the season in which the concentration was measured. Season did not seem to play a significant role in the increased concentrations; however, this possibility should be studied in a more systematic fashion to confirm the results. So that specific therapeutic recommendations can be made, planned prospective controlled trials are needed to clarify the most effective formulation of vitamin D and to determine the dose that is required to normalize serum concentrations.

In conclusion, vitamin D concentrations <50 nmol/L are very common in CF patients. Bringing low concentrations within the normal range may mitigate the deleterious effects of chronic
fibrosis. are needed to evaluate the efficacy of cholecalciferol in cystic minimize vitamin D synthesis in the skin. Future randomized studies needed to evaluate the use of ultraviolet light exposure to max-

concentrations in CF patients; however, further studies are future studies. Sun exposure may be required to achieve higher nmol/L although that possibility should be formally evaluated in future studies. The results of the present study show that cholecalciferol is capable of increasing serum concentrations to > 50 nmol/L for most subjects, which makes it a more effective therapeutic option for persons with CF. Furthermore, our data imply that young CF patients with low lung function and a low BMI may be at the highest risk of vitamin D deficiency and should be monitored closely. Subjects with the lowest concentrations had the greatest response to supplementation, and, as baseline concentrations approached 50 nmol/L, the efficacy of supplementation diminished. It may be that oral supplementation with cholecalciferol alone may be insufficient to maintain serum concentrations > 75 nmol/L although that possibility should be formally evaluated in future studies. Sun exposure may be required to achieve higher concentrations in CF patients; however, further studies are needed to evaluate the use of ultraviolet light exposure to max-
imize vitamin D synthesis in the skin. Future randomized studies are needed to evaluate the efficacy of cholecalciferol in cystic fibrosis.

The authors’ contributions are as follows—AS, ET, and MC: participation in all stages of the research project; RR and MB: the design of the study, the collection of data, and the writing of the manuscript; and EA: data analysis and writing of the manuscript. None of the authors had a personal or financial conflict of interest

REFERENCES