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Comparison of vitamin D supplementation regimens in pediatric patients with cystic fibrosis

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COMPARISON OF VITAMIN D SUPPLEMENTATION REGIMENS IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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CLINICAL NUTRITION

ABSTRACT

The objective of this randomized prospective study was to determine the most efficacious form of vitamin D supplementation for pediatric patients with Cystic Fibrosis (CF). We hypothesized that 50,000 IU monthly cholecalciferol would be as effective in raising serum 25-hyrdroxyvitamin D [25(OH)D] concentrations as routine daily supplementation of 1000-2000 IU cholecalciferol in pediatric patients with CF, birth to 20 years of age, over a period of 3 months. Data collected at baseline included serum 25(OH)D concentration, gender, genotype, race, age, FEV1, height, weight, BMI, previous vitamin D supplementation, sunlight exposure, and vitamin D intake from food and beverage, oral nutritional supplements, and CF fat soluble vitamins. A survey that included a food frequency questionnaire and questions on time spent in the sunlight during the weekdays and weekends was used to obtain data on vitamin D intake and sunlight exposure. Three month follow-up data collected included serum 25(OH)D concentration, FEV1, height, weight, BMI, and calcium creatinine ratio. Thirty-four participants were enrolled, and age ranged from four to 20 years of age. Twenty-nine participants were Caucasian (85%), two were African American (6%), two were Hispanic (6%), and one was classified other (3%). The mean serum 25(OH)D was 23.5±4.6 ng/mL at baseline. Six participants (38%) in group A and six participants in group B (33.3%) achieved vitamin D sufficiency, considered serum 25(OH)D levels of ≥30 ng/mL. However, t-tests revealed no significant difference between the treatment groups

regarding serum 25(OH)D at three months. A significant increase in serum 25(OH)D was observed for the sample as a whole, including both groups (p-value = 0.0004). No significant trends were observed for BMI status or FEV1 over the course of the treatment period. Calcium creatinine ratio was not significantly different between groups and the mean ratio for both groups was within normal limits. These results suggest that treatments were equivalent. However, both methods were minimally effective in correcting vitamin D insufficiency, with only 35% of participants achieving sufficient 25(OH)D concentrations. Future research should be conducted using higher dosages of cholecalciferol to determine the best vitamin D supplementation method for pediatric patients with CF and vitamin D insufficiency.

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INTRODUCTION

Cystic Fibrosis (CF) is a complex and lethal inherited genetic disorder that occurs with a frequency of 1 in every 3,500 live births (1). The genetic defect is an abnormality of the chloride channel, which causes thick secretions from epithelial cells in the lungs, pancreatic duct, intestines, sweat ducts, and the biliary tree (2). The main cause of death in CF is respiratory failure (3). While CF manifests primarily as a respiratory disease, the GI tract is also compromised, resulting in severe malabsorption of fat and fat soluble vitamins (4).

When CF was first identified in 1938, patients rarely lived to six months of age (5). With advances in treatment, patients with CF now have a median life expectancy of 36 years of age (1). With increased longevity, CF related bone disease has become a common and concerning comorbidity. Osteopenia occurs in 85% and osteoporosis is present in 10% to 34% of adults with CF (6). Low bone mineral density is also a concern in the pediatric population because childhood and adolescence mark crucial periods for the accrual of peak bone mass(6).

While bone disease in CF is multifactorial, vitamin D malabsorption is hypothesized to play a large role in the development of low bone mineral density (7). The main function of vitamin D is to promote calcium absorption in the intestine and to facilitate optimal bone mineralization (8). Vitamin D deficiency is present in 40% of

patients with CF, and up to 80% of patients in some studies present with vitamin D insufficiency (9,10).

Due to the high prevalence of suboptimal vitamin D concentrations and low bone mineral density in patients with CF, the Cystic Fibrosis Foundation (CFF) set forth guidelines for vitamin D supplementation (3). Vitamin D supplements are available in two forms: ergocalciferol (D2) and cholecalciferol (D3) (8). The CFF favors the use of ergocalciferol due to cost and safety reasons (3). However, several studies have found that cholecalciferol is preferable in patients with CF due to a higher rate of absorption (7). Subsequent studies have also shown that the treatment regimens following the CFF guidelines are not effective in achieving optimal vitamin D status (7). Recent studies of vitamin D supplementation have shown conflicting results, with very few achieving significant increases in serum vitamin D concentrations (7). The best method of vitamin D supplementation in patients with CF still remains to be determined (7). It is important to ascertain the most effective vitamin D supplementation regimen in pediatric patients with CF in order to identify preventative measures and adequate therapy for CF related bone disease (7).

Objective of Study

The objective of this study is to determine the most efficacious cholecalciferol (D3) supplementation regimen in pediatric patients with Cystic Fibrosis.

Research Hypothesis

Supplementation with 50,000 IU monthly cholecalciferol will be as effective in raising serum 25 hydroxyvitamin D concentrations as routine supplementation with 1,000-2,000 IU daily cholecalciferol in pediatric patients with CF, birth to 20 years of age, over a period of 3 months.

REVIEW OF LITERATURE

CYSTIC FIBROSIS OVERVIEW

With an incidence of 1 in every 3,500 live births, Cystic Fibrosis (CF) is the most common life threatening genetic disease affecting the Caucasian race (1). CF is an autosomal recessive disorder caused by a gene deletion in the long arm of the 7q chromosome. This gene codes for a protein known as CF transmembrane conductance regulator (CFTR). CFTR is part of a cAMP regulated chloride channel that controls sodium and chloride transport across the apical membrane of epithelial cells (2). This defect in CFTR causes decreased sodium and chloride resorption, resulting in extremely thick mucus secretions (2). The most common mutation in CFTR is F508del, but there are over 1,600 other possible mutations (7). CFTR is expressed in the airways, sweat ducts, pancreatic duct, biliary tree, intestines, and vas deferens (5).

CF primarily manifests as a respiratory disease because the thick mucus cannot be effectively cleared from the lungs and causes severe, chronic respiratory infection. The gastrointestinal (GI) tract is also affected by thick pancreatic secretions that clog organs and ducts. Mucus plugs can obstruct the pancreas, rendering it unable to secrete digestive enzymes and bicarbonate (4). Roughly 90% of patients with CF are pancreatic insufficient and suffer from severe malabsorption of protein, fat, and fat soluble vitamins (1). Additionally, the defect in CFTR can cause intestinal obstruction, biliary cirrhosis,

and infertility (5). Common symptoms of CF include salty tasting skin, bulky, malodorous stools, poor growth, and poor pulmonary function.

In recent decades, treatment for CF has advanced to the point that the median life span has increased from 6 months in 1938 to 36 years of age in 2009 (1). Once thought of as a pediatric disease, adults now make up 47% of patients with CF (1). While this steady increase in longevity is promising, adults with CF are faced with additional comorbid conditions caused by disease progression. Two of the most common adult complications in CF are CF Related Diabetes (CFRD) in 40-50% of adults and low bone mineral density in 50-70% of adults (6, 11).

Diagnosis of Cystic Fibrosis

Early and accurate diagnosis of CF is important in order to initiate appropriate therapies that optimize quality of life and longevity. According to the CFF consensus panel on the diagnosis of CF, criteria for diagnosis include: having one or more of the phenotypic features of CF, a sibling with CF, or a positive new born screen with evidence of abnormally elevated sweats chloride concentrations (12). A diagnosis of CF has been traditionally been confirmed by a sweat chloride test. For a definitive CF diagnosis, a pilocarpine ionotophoresis sweat test should be performed. Results of the test must show a chloride concentration greater than 60 mmol/L to be indicative of CF. The diagnosis can only be made if the patient has two positive sweat tests of greater than 60 mmol/L, and has either one or more phenotypic markers of CF or a history of a sibling diagnosed with CF (5). The CF gene was discovered in 1989, and this led to newborn screening initiatives that measure immunoreactive trypsinogen concentrations to identify patients

that are at risk for CF (13). It is to be noted that newborn screening is not a diagnostic test and should be confirmed by sweat chloride test (13). Some patients with CF genotypes will have normal sweat tests, making the appropriate diagnosis difficult. In these cases, assessment of the functionality of systems affected by CF is warranted in making a firm diagnosis of CF (5).

Complications and Treatment of Cystic Fibrosis

Respiratory

Although CF affects multiple organ systems, it manifests primarily as a respiratory disease with respiratory failure being the most typical cause of death in patients with CF (14). In fact, CF has been recognized as the leading cause of death due to respiratory failure in the Uniteds States (3). The defect in CFTR manifests in the lungs as a depletion of airway surface liquid. This causes abnormally thick mucus secretions and also causes the cilia in the airways to collapse, thus inhibiting mucus transport and clearance from the airways. Mucus is retained in the lungs, causing chronic infection which leads to inflammation. This becomes a cycle when inflammatory products are released from neutrophils that cause increased mucus secretion and breakdown (15). The most common organism that causes pulmonary infections in Patients with CF is *pseudomonas aeruginosa* (14). Other common pathogens that cause respiratory infection in CF include *staphylococcus aureus, burkholderia cepacia,* and *haemophilus influenzae* (16).

The goals of treatment are to preserve lung function by prevention of pulmonary infections and bacterial colonization of the lungs and to eradicate existing bacterial

colonization (16). Clinical monitoring of lung function on a regular basis is essential in determining pulmonary status. Monitoring includes tests such as chest x-rays, sputum cultures, and pulmonary function tests such as spirometry (16). Antibiotics are an essential part of CF treatment and are used both prophylactically as "maintenance" medications to prevent colonization with bacteria, and to treat existing infections.

In addition to antibiotic therapy, general infection control is also a crucial part of the management of CF lung disease. This can be achieved through isolation of patients when they have an active infection, recommending decreased socialization with other Patients with CF, and hand hygiene (16). The CFF also recommends the use of inhaled hypertonic saline, anti-inflammatory drugs, and mucolytics for management of pulmonary disease in children over the age of six (14).

Non-medical treatment for the pulmonary aspect of CF, known as airway clearance therapy (ACT), is also recommended by the CFF to help manage disease progression. There are many different techniques of ACT which loosen mucus so that it may be cleared from the lungs, and include: percussion or tapping on the chest with hands, a vibrating vest known as high frequency chest compression, postural drainage, positive expiratory pressure (PEP), active-cycle-of-breathing technique (ACBT), autogenic drainage , oscillatory positive expiratory pressure, and exercise (17). Since none of the techniques listed have been proven to be superior, ACT should be selected and modified based on each individual patient's needs (17).

Gastrointestinal and Pancreatic Involvement

Both exocrine and endocrine pancreatic functions are altered by CF. Roughly 90% of patients with CF have exocrine pancreatic insufficiency (PI) (1). PI occurs when thick secretions obstruct the pancreatic ducts causing acinar cell atrophy, fatty replacement, and eventually pancreatic fibrosis (18). This means that digestive enzymes and bicarbonate are unable to be released into the small intestine in sufficient amounts to facilitate digestion of nutrients. Decreased production of trypsin and lipase causes a reduction in protein and triglyceride hydrolysis into absorbable peptides, amino acids, and long chain fatty acids (19). Therefore, patients with CF and PI experience maldigestion of protein, fat, and fat soluble vitamins. Decreased bile acid resorption and the presence of excessive mucus lining in the small intestine also contribute to fat malabsorption. Symptoms of PI include oily, malodorous stools, diarrhea, flatulence, and abdominal distention and cramping (20).

PI is managed by the use of pancreatic enzyme replacement therapy (PERT) to compensate for malabsorption. Pancreatic enzymes are available as capsules which contain enteric-coated microencapsulated enzymes. Enteric coating prevents inactivation of enzymes in the acidic environment of the stomach (21). Enzymes should be taken before each meal and snack to facilitate optimal absorption. Since there is a high degree of variation in the response to PERT, dosage should be individualized to meet each patient's needs. In some cases, the decreased secretion of bicarbonate causes the intestine to remain acidic and leads to inactivation of pancreatic enzymes. In this case, histamine 2-receptor blockers or proton pump inhibitors can be prescribed (5). High-strength pancreatic enzyme supplements have been associated with colonic

strictures and fibrosing colonopathy. For this reason, the CFF recommends that enzyme dosage should be limited to 2,500 lipase units per kilogram of body weight per meal or less than 10,000 lipase units per kilogram of body weight per day (22).

As CF progresses, the endocrine function of the pancreas may also be affected. CF related diabetes affects 20% of adolescents and 40-50% of adults with CF (11). The insulin producing beta cells of the pancreas can be damaged by tissue scarring, resulting in decreased insulin production and secretion. This is postulated to be the primary cause of CFRD, although insulin resistance related to chronic illness may also play a role in CFRD development (23). CFRD is linked to poor weight status, decreased pulmonary function, protein catabolism, and increased mortality. For this reason, the CFF recommends that patients over the age of ten be screened annually using a two-hour oral glucose tolerance test. Management of CFRD includes insulin therapy, self-monitoring of blood glucose concentrations, ongoing diabetes education, routine quarterly visits with a multidisciplinary CF team, and regular aerobic exercise for at least 150 minutes per week (11).

In addition to pancreatic involvement, up to 11% of patients experience CF related liver abnormalities (1). Increased viscosity and decreased flow of bile cause obstruction in the biliary ducts and collagen deposition in the portal tracts. This can result in portal fibrosis, which may progress to cirrhosis (24). The goals of CF related liver disease treatment are to minimize liver damage and disease progression, prevent complications of cholestasis, and to manage portal hypertension and cirrhosis. Ursodeoxychoic acid is a common treatment in CF related liver disease because it improves biochemical alterations in liver injury, improves bile flow, and may stimulate

bicarbonate excretion into bile (24). Nutrition therapy is also an important component of the treatment of CF related liver disease, and seeks to preserve normal nutrition status and prevent deficiencies. Patients with cholestasis may require medium chain triglycerides (MCT) in order to facilitate optimal fat absorption in the intestine. Further, these patients may require increased energy intake 20 – 40% higher than the typical recommendations for patients with CF. It is crucial to monitor fat soluble vitamin concentrations every six to 12 months in patients with CF and liver dysfunction, and extra supplementation should be provided when patients are found to have deficiencies (24).

Bone Health

The increase in life span of patients with CF has also brought about new consequences for those patients who survive into adulthood. One of the most common complications is low bone mineral density which occurs in 50-70% of adults with CF (3). Recent evidence from pediatric studies in patients with CF suggests that low bone mineral density may begin in childhood. In the pediatric population, osteopenia is defined as bone mineral density that falls one standard deviation below normal. Osteoporosis is defined as bone mineral density that falls two or more standard deviations below normal. Osteopenia is found in 28% and osteoporosis is found in 10-34% of children with CF (6). Bone disease in CF is multifactorial and may be due to a range of issues including altered sex hormone production, inflammation, physical inactivity, glucocorticoid therapy, malnutrition, low calcium intake, and malabsorption of vitamin D (25). Recommendations for management of CF related bone disease include increased

weight bearing exercise, and supplementation with calcium, vitamin D, and vitamin K. More research is needed to determine the best method of treatment for CF related bone disease (3).

Nutrition in Cystic Fibrosis

The primary goal of medical nutrition therapy in pediatric patients with CF is to promote normal growth and development through the provision of adequate macro and micronutrients. Secondary goals of nutrition therapy in CF include facilitating optimal gastrointestinal (GI) and pulmonary function, and promoting the achievement of full genetic potential (9). There is good evidence that normal weight-for-age, height-for-age, weight-for-height, and BMI-for-age are associated with better pulmonary function and survival in patients with CF (22). Therefore, maintaining optimal BMI or weight-for-length (infants through children two years of age) status is crucial for extending life expectancy and reducing disease severity. Weight, length (measured lying down) or height (measured standing), and head circumference (for infants) should be measured quarterly and plotted on 2000 National Center for Health Statistics/ Centers for Disease Control growth charts. Additionally, weight for length should be plotted for children under age two, and BMI should be plotted for children age two to twenty. In order to maintain optimal health, the CFF recommends that children maintain a BMI status that is at or greater than the 50^{th} percentile (22). For adults, the CFF recommends that females have an actual BMI greater than or equal to 22 kilograms per meter squared (kg/m^2) and males have an actual BMI greater than or equal to 23 kg/m^2 (22). Mid arm circumference and triceps skin fold measurements may be obtained and provide

information regarding lean body mass and adipose tissue reserves (9). The goal is for children to reach and maintain optimal BMI status and to follow a normal growth curve. The CFF recommends that children with CF have growth and nutrition status checked every three months (9). Annually, Patients with CF should have their diets analyzed to make sure that they are consuming enough energy and micronutrients (9).

Energy Intake

Patients with CF have increased energy needs due to malabsorption, chronic infection/inflammation, and increased work of breathing. For this reason, the recommended energy intake for patients with CF is 110-200% of the energy needs of the healthy population (22). This goal can be achieved by increasing both the amount of food and the energy density of foods consumed. Due to fat malabsorption, patients with CF have increased requirements for fat intake, and it is recommended that 30-45% of total calories come from fat (9). Since fat is the most concentrated source of calories, increased energy needs may be met by adding foods that are high in fat, such as: butter, gravy, cheese, and extra dressing to salad, eggs to hamburger meat and casseroles, and half and half to whole milk. Snacks that are high in energy include: cheese or peanut butter and crackers, cookies and milk, cream cheese and bagels or muffins, chips or vegetables and dip, French fries, yogurt made from whole milk, ice cream, pizza, and puddings (9). For children with poor growth, the CFF recommends intensive behavioral intervention in combination with nutrition counseling in order to facilitate weight gain (22). If the addition of high energy foods does not promote weight gain, the use of oral supplements is warranted. However, patients using oral supplements should be

monitored to make sure that they are not substituting supplements for actual food intake. When oral supplements fail to achieve weight gain, enteral feedings should be initiated as a supportive and ongoing therapy (22). Nocturnal feedings are recommended in order to promote normal intake of food and beverages during the day. Feedings should provide between 30-50% of the patient's estimated energy needs. Pancreatic enzymes should be taken at the beginning of enteral feedings, and may be given midway through or after feedings, to promote optimal absorption of nutrients (9).

Micronutrient Supplementation

Fat malabsorption also impairs the absorption of fat soluble vitamins (A, D, E, and K) in patients with CF, even in the presence of PERT (9). For this reason, it is recommended that Patients with CF take supplemental multivitamins containing water miscible and water soluble forms of fat soluble vitamins. Fat soluble vitamin concentrations should also be obtained annually to screen for deficiencies (26).

Vitamin A plays a role in vision, epithelial cell function and proliferation, and immunity. Deficiency of vitamin A is common in CF and has been documented in 15-40% of patients (9). Vitamin D functions to increase calcium absorption in the intestine and is essential for optimal bone health. Deficiency of vitamin D is postulated to play a large role in the development of low bone mineral density and can also lead to delayed tooth eruption in patients with CF. Vitamin D deficiency occurs in up to 40% of patients with CF (9). Vitamin E functions as an antioxidant in multiple systems and is an important component of cell membranes (26). Deficiency can cause retinal and cognitive

impairments. Low serum vitamin E concentrations occur in five to ten percent of patients with CF (9).

Vitamin K can be synthesized in the intestinal flora and functions as a coenzyme in the synthesis of clotting factors and plays a role in bone metabolism. Deficiency of vitamin K may result in coagulation disorders and decreased bone mineral density (26). Chronic antibiotic therapy in CF may impair vitamin K synthesis in the intestinal bacteria. However, the prevalence of vitamin K deficiency in CF remains unknown (9). Essential fatty acid deficiency (EFAD) may also occur in CF, even in patients that are pancreatic sufficient. While EFAD primarily manifests biochemically, clinical symptoms may been seen in infants with failure to thrive. It is unclear whether specific fatty acid supplementation is warranted in CF. However, vegetable oils including soy, canola, flax, and cold-water marine fish oils may be recommended as they are also a good source of calories and protein (9).

Fat malabsorption is also suspected to affect calcium absorption (27). Close attention should be paid to dietary intake of calcium due to the increasing prevalence of CF related bone disease (9). Additionally, the Institute of Medicine increased the calcium requirements for all populations in 2010. Patients with CF should receive at least the current recommended dietary allowance for calcium which is 700-1,300 milligrams depending on age (28).

Patients with CF are at increased risk for hyponatremia due to decreased sodium and chloride resorption which causes excessive salt losses through the skin (9). For this reason, it is recommended that patients with CF consume a high sodium diet. Additional supplemental sodium should be added during the summer months, in hot climates, and

during activities that cause perfuse sweating (9). Before the addition of solid foods, breast-fed and formula fed infants should receive additional sodium supplementation. This may be achieved by adding 1/8 teaspoon of salt per day, or through the use of prescription sodium chloride solutions (9).

VITAMIN D OVERVIEW

Vitamin D is a fat soluble, hormone precursor that is vital for maintaining bone health. Vitamin D is synthesized in the skin when the UVB rays in sunlight combine with 7-dehydrocholesterol, which is then converted to precholecalciferol. Precholecalciferol is then isomerized into cholecalciferol (D3) in the skin and moves to the dermal capillary to be bound with Vitamin D Binding Protein (29). Vitamin D3 is converted to 25 hydroxyvitamin D [25(OH)D] in the liver and then is converted to its biologically active form of 1,25 dihydroxyvitamin D, or calcitriol, in the kidney (29).

Vitamin D's main function is in calcium and phosphorus homeostasis. In the intestine, vitamin D facilitates optimal absorption of calcium. Studies suggest that Vitamin D may also help prevent certain types of cancer, autoimmune diseases, type 1 diabetes, high blood pressure, and cardiovascular disease (29). Vitamin D is also thought to play a part in overall immunity (7). From a pulmonary standpoint, vitamin D deficiency has been linked to increased risk for upper respiratory tract infection and to decreased forced expiratory volume (FEV1) (29). Additionally, there is data from the NHANES III that indicates a correlation between vitamin D insufficiency and decreased FEV1 (30).

Vitamin D deficiency causes serum calcium to decrease, resulting in increased parathyroid hormone (PTH) secretion. PTH causes increased resorption of calcium and increased secretion of phosphorus in the kidney. Prolonged PTH secretion can lead to

decreased bone mineral density (29). In children, deficiency of vitamin D leads to rickets, a painful bone disease that causes growth retardation, decreased bone mineralization, and deformed long bones in the legs. Deficiency in adults causes decreased bone mineralization resulting in osteomalacia (29). The best clinical marker of vitamin D status is serum 25(OH)D. Vitamin D deficiency is defined as a serum 25(OH)D level of < 20 ng/mL. Concentrations between 20 and 29 ng/mL indicate vitamin D insufficiency and optimal concentrations are between 30 and 60 ng/mL (31). Concentrations higher than 150 ng/mL may induce symptoms of vitamin D toxicity which include: hypercalcemia, nausea, vomiting, fatigue, and weakness (32). Hypervitaminosis D can result in calcification of soft tissues, the production of urinary calcium phosphate crystals, and renal damage (28).

Sunlight exposure is the primary source of vitamin D in healthy individuals (28). Exposure of the limbs for five to 30 minutes between 10am and 3pm twice weekly can be adequate to prevent vitamin D deficiency (32). However, an individual's ability to synthesize vitamin D can be affected by age, season, latitude, skin pigmentation, and the use of sun screens. The level of 7-dehydrocholesterol in the skin, and thus the ability to make vitamin D endogenously, decreases with age. UVB rays with frequencies between 290 and 315 nm are required for optimal vitamin D production in the skin. Therefore, vitamin D is poorly synthesized during the winter months and in northern regions as UVB rays are not in the desired range in these situations (29). Increased melanin in skin also impairs vitamin D biosynthesis through UVB absorption. Individuals with darker skin pigmentation are at increased risk for vitamin D deficiency for this reason (32).

Sunscreens also effectively absorb UVB radiation, and thus inhibit the synthesis of vitamin D in the skin (8).

Vitamin D can be obtained exogenously through the diet in two forms: ergocalciferol (D2) from plant sources such as shitake mushrooms, and cholecalciferol (D3) from animals sources including salmon, sardines, mackerel, tuna, cod liver oil, and egg yolk (29, 32). Individuals unable to meet vitamin D requirements through sunlight exposure alone should consume at least an additional 1000 IU of vitamin D from the diet or from supplements (8). Recently, the Institute of Medicine released increased Dietary Reference Intakes for vitamin D. The recommended dietary allowance is now 600 IU per day for children, adolescents, and young adults. Upper intake concentrations range from 1,000 IU per day in infants up to 4,000 IU per day in children nine years of age and older (28).

Vitamin D Status in Cystic Fibrosis

Vitamin D status has become a topic of interest in the CF population due to the mounting literature indicating that 50-70% of adults with CF have low bone mineral density (3). Development of bone disease in CF is multifactorial, however, the malabsorption of Vitamin D is postulated to play a large role in its etiology. Vitamin D deficiency has been well documented in CF and is present in 30-40% of patients with CF. Further, Vitamin D insufficiency has been noted to be present in as high as 80% of Patients with CF in some studies (33). These findings, in combination with the prevalence of low bone mineral density prompted the CF Foundation (CFF) to make recommendations for Vitamin D supplementation in the 2005 Guide to Bone Health and

Disease. The CFF guidelines define optimal Vitamin D status as serum 25(OH)D level of 30-60 ng/mL. If concentrations fall below 30 ng/mL, the CFF recommends Vitamin D supplementation of 800 IU ergocalciferol (D2) per day in patients older than 1 year of age. If this fails to correct serum 25(OH)D, high dose supplementation of 50,000 IU of D2 per week for 8 weeks is suggested. If concentrations remain less than 30 ng/mL, this warrants 50,000 IU of D2 twice weekly for 8 weeks. Phototherapy and calcitriol supplementation should be considered when serum vitamin D concentrations remain below 30 ng/mL (3, 33). These recommendations were developed based on healthy individuals as there were no studies addressing the topic in patients with CF at the time. Therefore, they are to be considered a "starting point," and call for research of Vitamin D supplementation in CF (7).

Vitamin D Supplementation in Cystic Fibrosis

Since the publication of CFF recommendations for vitamin D supplementation to correct D insufficiency, several studies have been conducted to assess the effectiveness of D2 supplementation in replenishing vitamin D status. Boyle et al found that 94% of adult Patients with CF treated with the recommended 50,000 IU D2 weekly for eight weeks failed to reach optimal serum 25(OH) D concentrations. Further, none of the patients receiving the second round of twice weekly D2 supplementation for eight weeks were able to reach sufficient concentrations (33). Green et al found similar results when testing the CFF guidelines with only 54% of the patients achieving serum 25(OH)D in the optimal range. Results also showed that only 43% were able to maintain optimal concentrations six to 18 months after the study, displaying that the limited effectiveness

of the recommended D2 supplementation was poorly sustained (34). However, in a prospective cohort study that assessed the efficacy of very high dose ergocalciferol supplementation, Boas et al found that 50,000 IU of ergocalciferol over a period of 2 weeks significantly raised serum 25(OH)D concentrations in 17 out of 18 children with CF and 100% of participants achieved optimal serum vitamin D concentrations (10). A possible explanation for the poor results seen with the recommended D2 dosage is poor absorption of this form of vitamin D. Lark et al observed in a pharmokinetic study that one time supplementation of 2,500 micrograms of D2 did not raise serum 25(OH)D concentrations in 10 adults with CF. Additionally, the CF participants only absorbed 45% of the D2 that the healthy controls absorbed (35).

Although the CFF guidelines favor ergocalciferol (D2) due to cost and safety reasons, cholecalciferol (D3) is emerging as the preferred form of supplementation in the CF population. In a small study comparing the effectiveness of D2 and D3 in healthy adults, Armas et al discovered that participants receiving D3 supplements showed an increase three times higher in serum 25(OH)D concentrations than did those receiving D2 supplementation (36). However, in a randomized controlled trial exploring the effect of calcium and vitamin D supplements on bone health in 30 adults with CF, Haworth et al observed no significant increase in serum 25(OH)D concentrations over the 12 month study period despite the intervention group receiving 800 IU D3 (37). Similar results were found in a double blind randomized controlled trial in which 15 children (age 7-13 years) each received, in three 6-month phases, 1600 IU supplemental D3, 1 gram calcium plus 1600 IU D3, 1 gram calcium, and placebo. No changes were observed in serum 25(OH)D concentrations with either the D3 or the Calcium plus D3 group (27). A

retrospective cohort study of 360 adult patients with CF tested an intervention involving compliance counseling and increasing D3 dosage on an individual basis by either 400 IU, 800 IU, 1000 IU, and >1000 IU per day over a period of 3 months. Results showed a significant increase in 25(OH)D concentrations in 92% of the participants with the mean dose of 1800 IU D3. However, only 17% reached the suggested level of 30 ng/mL (38). While these lower doses of D3 supplementation did not achieve high 25(OH)D repletion rates, Khazai et al observed that 100% of adult patients with CF achieved optimal 25(OH)D concentrations after taking 50,000 IU D3 weekly for 12 weeks. This comparative study of D2, D3, and UV light therapy showed that while both D3 and D2 significantly raised serum 25(OH)D concentrations, D3 supplementation was more effective than D2 supplementation. Of the patients receiving D3 therapy, 100% reached optimal concentrations compared to 60% in the D2 group (39).

UV light and the more polar analogs of Vitamin D, calcidiol and calcifediol, are recommended by the CFF when other high dose therapy fails. In Khazai's study, UV light therapy did not achieve a significant increase in serum 25(OH)D. However, it is important to note that less than 50% of the participants were compliant with the treatment by self-report (39). In a 24-week study assessing the effectiveness of UV light therapy, 30 adults and children (median age 17 years) were randomized to either UVB or control groups. The study found that after 24 weeks, the UVB radiation had significantly raised serum vitamin D concentrations to a mean of 50.4 ng/mL compared to 25 ng/mL in the control group. It is to be noted that true randomization did not occur as patients were selected to be in the UVB group if they had a history of high compliance (40). The results of both of these studies suggest that while UVB therapy may be effective in

repleting vitamin D, it is likely only a viable option for highly compliant patients. A study assessing the effect of calcitriol on calcium homeostasis in adults with CF found that 0.5 micrograms of Calcitriol twice daily for 14 days did not cause significant raises in 25(OH)D concentrations. However, it increased fractional absorption of calcium in both the CF and healthy control groups. The two-week treatment time may not have been a long enough treatment window in which to observe significant increases in serum 25(OH)D concentrations (41).

Overall, the literature sends mixed messages on vitamin D supplementation. In a recent Cochrane review of Vitamin D in Cystic Fibrosis, authors conducting the review of literature on the topic conclude that more trials are necessary in order to ascertain the best mode of supplementation (42).

METHODOLOGY

A prospective, randomized quality improvement trial was conducted to determine the best of two cholecalciferol supplementation regimens in pediatric patients with CF. Participants were recruited from Children's Hospital of Alabama/University of Alabama at Birmingham Cystic Fibrosis Center's outpatient clinics and from the inpatient pulmonary unit. Informed consent was obtained from a parent or legal guardian and assent was obtained from children over the age of seven years during their routine quarterly clinic visits or during hospital admissions. Participants were enrolled from November, 2010 through February, 2011. Inclusion criteria included: diagnosis of CF, a serum 25 hydroxyvitamin D level of less than 30 ng/mL, and age 20 years or younger. Participants were excluded if they did not have a serum 25-hydroxyvitamin D measurement from the year of 2010. Approval to conduct this study was obtained from the Institutional Review Board of the University of Alabama at Birmingham, protocol # X100910009 (Appendix).

Data Collection

The participant's last measured serum 25-hydroxyvitamin D level during the year of 2010 served as the baseline. This data was obtained from the electronic medical record software, Logician. Age, race, gender, CF genotype, previous vitamin D supplementation, brand and dosage of CF specific fat soluble vitamins, forced expiratory

volume (FEV1), height, weight, and BMI were also obtained at baseline. A survey was administered at baseline to collect the amount of dietary vitamin D consumed and level of sun exposure.

At 3-month follow-up visits, blood was drawn to obtain endpoint serum 25-hydroxyvitamin D level. Formation of kidney stones can be a side effect of high dose vitamin D supplementation. Therefore, urine calcium creatinine ratio was measured at follow up since it is a marker of renal stones, a possible side effect of high dose vitamin D supplementation. Height, weight, BMI, and FEV1 were also obtained at follow up.

Treatment Dosage

Upon enrollment, participants were randomized to one of two treatment groups. Group A received 50,000 IU monthly oral cholecalciferol, regardless of age. In treatment group B, participants less than ten years of age received 1,000 IU daily oral cholecalciferol and participants ten to 20 years of age received 2,000 IU daily oral cholecalciferol. Treatment duration was 3 months for both groups.

Clinical Status

Nutritional status was determined by height (centimeters), weight (kilograms), and BMI (kilograms per meter squared). Pulmonary function was defined by measurement of FEV1, and obtained by an experienced respiratory therapist. FEV1 was expressed as percent predicted for age, weight, height, gender, and race.

Intake and Sun Exposure

Dietary vitamin D intake from food and beverages was obtained by using a food frequency questionnaire, administered by a Registered Dietitian. For each food item, participants were informed of portion sizes of each food, and asked how many servings they consume each day and how many servings each week. Patients were asked if they drink oral nutritional supplements, the brand name of the supplement, and how many servings they consume each day and each week. Compliance with CF fat soluble vitamin supplements and frequency of missed dosage was assessed during questionnaire administration by patient self-report. Total amount of vitamin D in international units was calculated from the sum of intake from food and beverages, oral supplements, and CF specific fat soluble vitamins. Totals were calculated per day and per week. Participants were asked questions about sunlight exposure in order to quantify the amount of time that they spent in the sunlight on weekdays and weekends during winter months. Sunlight exposure was measured in minutes and coded on a scale of one to four.

Statistical Analyses

Statistical analyses were performed using the statistical package SAS version 9.2. Alpha level was set equal to 0.05. A paired t-test was performed to determine whether a significant increase in serum 25-hydroxyvitamin D occurred within groups. A t-test was performed to determine whether there was a significant difference in endpoint serum 25-hydroxyvitamin D concentrations between treatment groups. T-tests were also used to examine differences between groups with regard to secondary endpoints of calcium

creatinine ratio, FEV1, and BMI status. Chi square tests were used to compare categorical variables.

RESULTS

Description of Subjects

Forty-seven participants met the inclusion criteria for this study and were enrolled. Two participants were dropped from the study because they never received their treatment. Eleven participants were not included in the final analysis because they missed their follow up visit and did not have a final 25(OH)D measurement at the time of the analysis. Of the 34 subjects studied, 21 patients were male and 13 were female. They ranged in age from four to 20 years old, with a mean of 11.2 years of age. Twenty-nine participants were Caucasian (85%), two were African American (6%), two were Hispanic (6%), and one was classified as other (3%). Fifteen different genotypes were identified with twenty participants (59%) being ΔF508 homozygous, seven (20.5%) being ΔF508 heterozygous, and seven (20.5%) did not have any copies of ΔF508. Categorical variables by group are presented in Table 1.

All participants were determined to be pancreatic insufficient and were on pancreatic enzyme replacement therapy. The average initial serum 25(OH)D was 23.5±4.6 ng/mL. All participants were taking CF fat soluble vitamins. Seventeen participants (50%) had been on prior oral vitamin D supplements outside of their CF fat soluble vitamins. The mean total daily amount of vitamin D consumed at baseline through food and beverage, oral nutritional supplements, and CF fat soluble vitamins was 1760±726 IU. At baseline measurement the mean BMI was 18.3±2.5 kg/m² and the

mean FEV1 for all 34 participants at baseline was 81.5±27.5. Descriptive statistics for all continuous variables are presented by group in Table 2. There were no significant differences between groups at baseline.

Table 1. Demographic Data by Group

	Group A	**Group B**
Females [n (%)]	5 (31)	8 (44)
Males [n(%)]	11 (69)	10 (56)
Race [n (%)]		
White	14 (88)	15 (82)
Black	1 (6)	1 (6)
Hispanic	1 (6)	1 (6)
Other	-	1 (6)
Genotype [n (%)]		
2 copies ΔF508	11 (69)	9 (50)
1 copy ΔF508	2 (12)	5 (28)
no copies ΔF508	3 (19)	4 (22)
Sun Exposure Week Day [n(%)]		
0-15 minutes	4 (27)	5 (29)
16-30 minutes	5 (33)	6 (35)
31-60 minutes	5 (33)	2 (12)
> 60 minutes	1 (7)	4 (24)
Sun Exposure Weekend [n (%)]		
0-15 minutes	0 (0)	5 (29)
16-30 minutes	4 (27)	4 (24)
31-60 minutes	4 (27)	2 (12)
> 60 minutes	7 (46)	6 (35)

Table 2. Summary Statistics of Clinical Features by Group

	Group A (n=16)			Group B (n=18)		
	Mean ± SD	Median	Range	Mean ± SD	Median	Range
Age (years)	11.3 ±4.72	12.50	4-19	11.1±4.7	11.00	4-20
Prior Vit D dosage (IUs)	575±789	0.00	0-2000	577.7±657.6	600.00	0-2000
Initial 25(OH)D (ng/mL)	22.7±4.9	23.50	13-29	24.2±4.5	25.50	40876.00
Initial Height (cm)	144.4±24.0	152.20	102-175.3	140.6±23.0	148.00	106.2-169.7
Initial Weight (cm)	40.7±16.5	44.90	17-73.2	36.6±13.5	35.7	17.4-55.3
Initial BMI (kg/m²)	18.6±3.2	18.50	14.8-25.5	18.0±1.8	17.4	14.9-21.1
Initial FEV1	79.1±31.2	80.00	27-128	83.5±24.7	88.50	39-118
Daily Vit D intake (IUs)	1744.9±722.0	1820.00	600-2895	1759.9±769.3	1808.00	380-2904
3 month 25(OH)D (ng/mL)	27.3±8.8	26.00	10-44	27.8±6.4	26.5	17-42
3 month Height (cm)	145.9±24.3	152.80	102.4-176	141.9±23.2	149.2	104.5-172.1
3 month Weight (kg)	41.9±17.6	45.30	17.8-78.2	37.7±14.3	39	17.9-58
3 month BMI (kg/m²)	18.8±3.6	18.10	14.2-27.3	17.9±1.7	18.0	15.3-20.8
3 month FEV1	76.7±31.9	84.00	26-127	83.9±23.7	92	35-130
3 month Ca:Cr ratio	0.16±0.10	0.12	0.03-0.42	0.11±0.05	0.10	0.04-0.22

Serum 25-hydroxyvitamin D

The primary outcome variable of interest in this study was serum 25(OH)D. An unpaired t-test was performed to assess differences in initial 25(OH)D at baseline. A statistically significant difference was not found between groups at baseline with group A having a mean value of 22.7±1.2 ng/mL and group B having a slightly higher mean of 24.2±1.1 ng/mL (t ratio = 0.9601, p = 0.3442).

An unpaired t-test was also performed to determine if there were differences between groups with respect to follow up 25(OH)D. One-way ANOVA of 3 month 25(OH)D concentrations revealed that there was no significant difference in the mean follow-up 25(OH)D between groups (t ratio = 0.1783, p-value = 0.8596) with group A having mean of 27.3±1.9 ng/mL and group B having a mean of 27.6±1.8 ng/mL.

A difference score was calculated by subtracting initial 25(OH)D from 3 month follow up 25(OH)D. A paired t-test was then utilized to determine if there were differences between mean difference scores for groups. Analysis revealed that there was not a significant difference between group A and group B regarding the increase in 25(OH)D concentrations from the initial measurement to the three month follow-up. Group A had a mean increase of 4.6±1.8 ng/mL and group B had a slightly lower mean increase of 3.6±1.7 ng/mL. However, this did not achieve statistical significance (t ratio = 0.4206, p-value = 0.6768). It is also to be noted that while group B experienced a smaller increase in 25(OH)D concentrations, both baseline and follow up mean 25(OH)D were higher than group A. See figure 1 for details.

The overall difference score, including both groups, was examined to determine whether treatment improved vitamin D status for the group as a whole. This test showed that over the treatment time of 3 months, there was a significant global improvement in serum 25(OH)D for all participants, regardless of group assignment (t ratio = 3.239, p-value = 0.0027). There was one outlier observed in the distribution of the difference score which was a decrease of 19 ng/mL in serum 25(OH)D. This was confirmed to be a valid data point and was therefore included in analysis. Nonparametric testing was robust to the outlier (Wilcoxin signed rank = 175.5, p-value = 0.0004). See figure 1 for details.

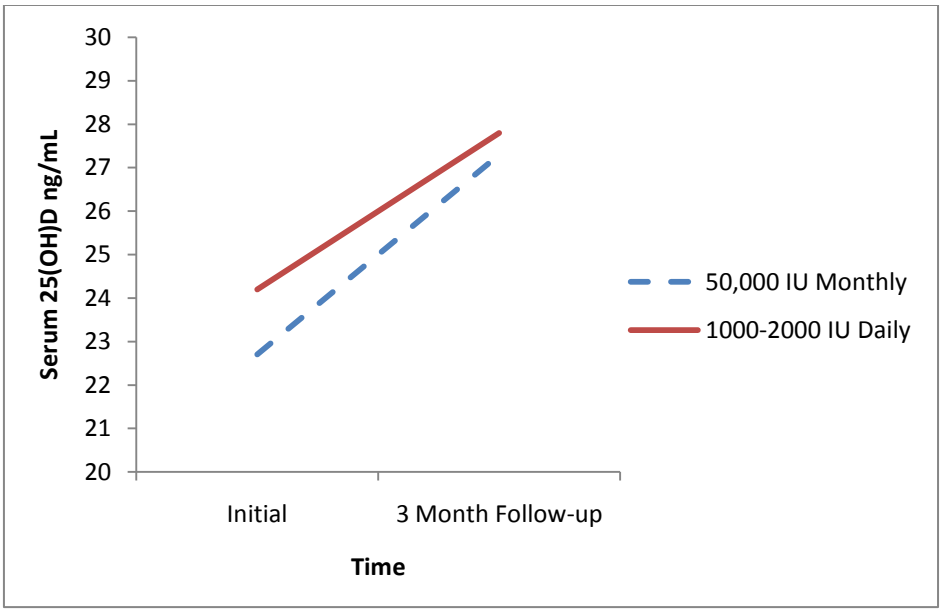

Figure 1. Trends in serum 25(OH)D over study time period.

Overall, six participants (38%) in group A and six participants in group B (33.3%) achieved vitamin D sufficiency, considered serum 25(OH)D concentrations of ≥30 ng/mL, in the three-month study period.

Calcium Creatinine Ratio

In order to determine whether a significant difference in calicum creatinine ratio existed between treatment groups, an unpaired t-test was performed. While group A had a slightly higher calcium creatinine ratio at 0.16±0.02 than group B at 0.11±0.03, there was no statistical difference found between groups (t ratio = -1.4308, p-value = 0.1654). Clinically, the means for both groups were within normal limits of < 0.20.

Pulmonary Function

T tests revealed that there was no statistically significant difference between groups at baseline or follow up with respect to FEV1 (t ratio baseline = 0.4484, p-value

baseline = 0.6570 and t ratio (follow up = 0.7378, p-value follow-up = 0.4662). Refer to Table 1 for means±SD for baseline and follow up FEV1. A difference score was then calculated by subtracting initial FEV1 from three month follow-up FEV1. Globally, there was no statistically significant difference in FEV1 over the course of the study (t ratio = -0.3717, p-value = 0.7126). An outlier of 60 was observed in the overall distribution of difference score for FEV1. Therefore, nonparametric tests were employed to control for the outlier and results showed that there was still no significant difference between groups (Wilcoxin signed rank = -68.0, p-value = 0.1865). An unpaired t test was used to determine whether the difference in FEV1 was different between groups. Group A had a mean difference of -2.4±3.5 and group B had a mean difference of 0.39±3.2. Results showed that there was not a significant difference in the trend in FEV1 over the course of the study (t ratio = 0.5812, p-value = 0.5653). See Figure 2 for graphic description of trends in FEV1.

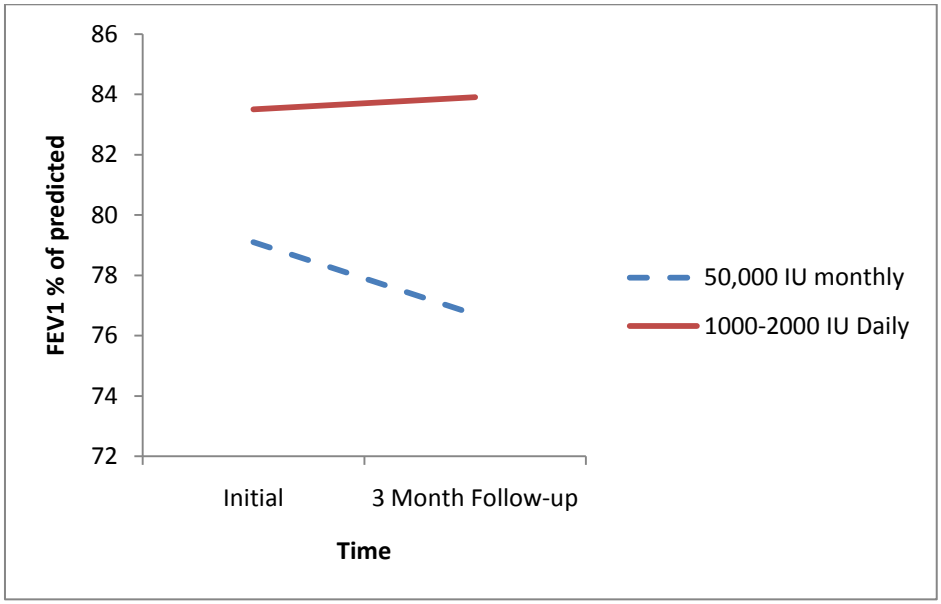

Figure 2. Trends in FEV1 over study time period.

BMI Status

There were no significant differences in BMI between groups at baseline or follow-up (t ratio baseline = -0.6749, p-value = 0.5046; t ratio follow-up = -0.9777, p-value follow-up = 0.3355). Mean±SD for BMI by group at baseline and follow-up are presented in Table 2. A difference score was calculated by subtracting initial BMI kg/m² from three month follow-up BMI. Group A had a mean difference of 0.2±0.2 BMI points and group B had a mean difference of -0.1±0.2. Results of t-test revealed that there was no significant difference in change in BMI between groups over the course of the study (t ratio = -0.9841, p-value = 0.3325). The overall change in BMI, without considering group assignment, also proved to show no significance (t ratio = 0.1587, p-value = 0.8749). Trends in BMI are depicted in figure 3.

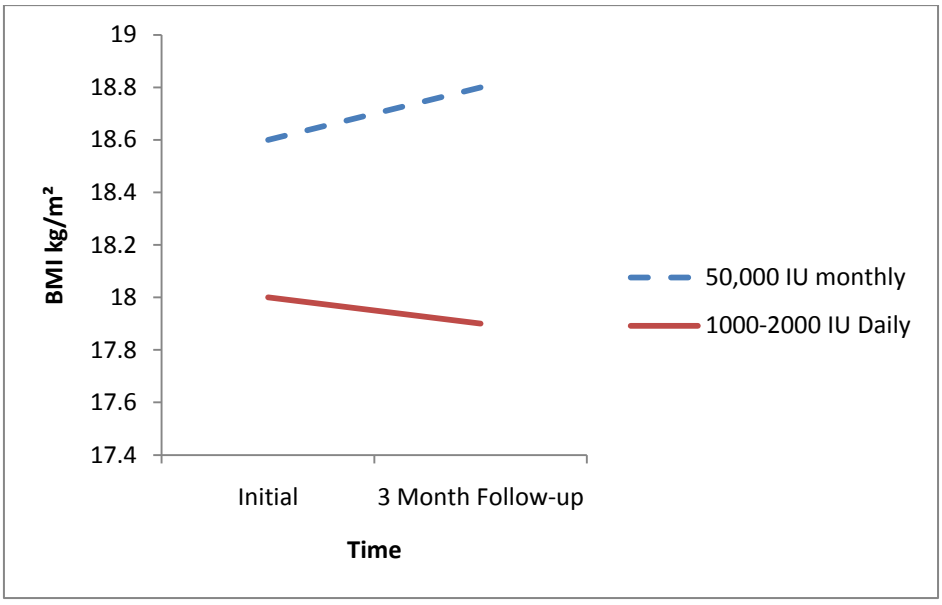

Figure 3. Trends in BMI status over study time period.

DISCUSSION

The objective of this randomized, prospective trial was to determine the most effective regimen of cholecalciferol supplementation in pediatric patients with CF. This study was conducted as part of a quality improvement initiative to update the protocol for treating vitamin D insufficiency at Children's Hospital of Alabama/University of Alabama at Birmingham Cystic Fibrosis Center.

No studies have examined the efficacy of a monthly dose of 50,000 IU oral cholecalciferol in this patient population. We hypothesized that a monthly dose of 50,000 IU D3 would be as effective in correcting 25(OH)D insufficiency as daily doses of 1000 IU for children less than ten years of age and 2000 IU for children ten to 20 years of age. This study failed to show a statistically significant difference between treatment groups, suggesting that monthly high dose supplementation is as effective but not superior to daily high dose supplementation with cholecalciferol. The proportion of participants in each group achieving vitamin D sufficiency was 38% and 33.3% for monthly and daily dose groups, respectively.

These findings are consistent with the literature regarding daily high dose cholecalciferol supplementation. Stephenson et al found when supplementing adult Patients with CF with greater than 1000 IU of cholecalciferol daily that only 17% achieved optimal concentrations (38). Hillman et al supplemented with 1600 IU daily cholecalciferol and found that this did not significantly change serum 25(OH)D

concentrations (27). No previous studies have examined the efficacy of using a monthly dose of 50,000 IU of cholecalciferol. However, Khazai et al was able to show 100% sufficiency when using 50,000 IU cholecalciferol on a weekly basis (39). This suggests that a more frequent dosage of 50,000 IU may be necessary to correct insufficiency in this patient population.

When the data was examined without considering group assignment, there was a significant increase in serum 25(OH)D concentration from initial measurement to follow-up measurement. However, while this observation was statistically significant, it has limited clinical relevance as the increase in mean 25(OH)D was not large enough to produce a mean 25(OH)D greater than or equal to 30 ng/mL. In fact, only 35% of all thirty-four participants were able to achieve vitamin D sufficiency. This low rate of vitamin D sufficiency in response to supplementation is likely due to malabsorption experienced by patients with CF which is postulated to play a large role in the etiology of low vitamin D status in this patient population. Additionally, the overall increase observed may be reflective of seasonal variations in serum 25(OH)D concentrations since some follow-up measurements were obtained during the summer months.

Calcium creatinine ratio was examined as a secondary endpoint in this study because it is a marker of renal stone formation, a possible side effect of high dose vitamin D therapy. Results from this study suggest that both daily high doses of 1000 IU and 2000 IU, and monthly dose of 50,000 IU do not cause elevated calcium creatinine ratios, with both groups having a mean value within normal limits. Further, for the three-month study duration, there were no significant differences in pulmonary function or BMI status by group or globally over the course of the study.

It is also important to note that the mean daily dietary intake of vitamin D of 1760±726 IU observed in this study exceeds the current dietary reference intake (DRI) of 600 IU daily (28). This finding displays that vitamin D insufficiency persists in this patient population despite reported intake that is roughly three times higher than the DRIs for vitamin D. This further highlights the need to find the most efficacious D3 supplementation for pediatric patients with CF.

Limitations

A small sample size was a major limitation and may have resulted in decreased statistical power. This was due to participants either not receiving their treatment or not returning for follow-up measurements. Other limitations include participant's self-report of dietary intake and sunlight exposure, lack of control for seasonal variation in 25(OH)D concentrations, and relatively short duration of treatment.

Suggestions for Future Research

Future studies should continue to focus on ascertaining the best method of cholecalciferol supplementation in children with CF. Since low rates of sufficiency were seen with both methods of supplementation in this study, subsequent studies should examine higher daily doses or more frequent doses of 50,000 IU cholecalciferol. A multicenter prospective study would be useful because it could likely achieve sample sizes large enough to have statistical power and could also help determine vitamin D insufficiency treatment guidelines for pediatric patients with CF. Additional research should also be conducted to identify predictors of vitamin D insufficiency and to further

examine the effect of vitamin D insufficiency on bone mineral density in this patient population.

CONCLUSIONS

Increased life span in patients with CF has brought about the common and serious complication of CF related bone disease, including osteopenia and osteoporosis. While adults with CF are affected by this comorbidity at a higher rate, childhood marks a crucial point in optimizing bone health and preventing the development of osteoporosis and osteopenia. Vitamin D malabsorption leading to insufficiency is hypothesized to be a key factor in the development of CF related bone disease. Findings from the literature have shown that age is a significant predictor of vitamin D status, with serum 25(OH)D concentrations decreasing as age increases (10). This further highlights the need to develop effective interventions and clinical protocols for the treatment and prevention of vitamin D insufficiency in pediatric patients with CF.

Results from this study warrant the conclusion that a monthly dosage of 50,000 IU cholecalciferol is as effective as daily high dose D3 therapy of 1000 to 2000 IU (dose depending on age). However, findings also lead to the conclusion that both methods are minimally effective in correcting vitamin D insufficiency, with only 35% of participants achieving sufficient 25(OH)D concentrations. These findings are consistent with the literature, and emphasize the need for further research in the area of cholecalciferol supplementation to correct vitamin D insufficiency in children with CF. While an overall increase in serum 25(OH)D was observed over the course of the study for the entire sample population, this may have been due to seasonal variation in 25(OH)D

concentrations. Renal stone formation is not likely with either treatment method as the mean calcium creatinine ratio for both groups was within normal limits. Since no significant trends were observed globally or between groups with regard to pulmonary function and BMI status, no conclusions may be drawn concerning the effect of vitamin D supplementation on these factors.

Clinicians involved in the care of children with CF should be aware that routine high dose cholecalciferol supplementation is most often not effective in correcting vitamin D insufficiency. Additionally, it is to be noted that despite a relatively high reported intake and varying degrees of sunlight exposure, initial serum 25(OH)D concentrations were still below optimal at baseline. The need to establish the most effective method of vitamin D supplementation in pediatric patients with CF still exists. Future research should focus on testing higher and more frequent doses of cholecalciferol supplementation for effectiveness and safety. Further clarification of the role of vitamin D in CF related bone disease is also needed to refine screening, prevention, and treatment protocols for vitamin D insufficiency. Development of effective vitamin D supplementation regimens could enable clinicians to help prevent and attenuate CF related bone disease.

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APPENDIX A

INSTITUTIONAL REVIEW BOARD FORMS

UAB THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The Assurance number is FWA00005960 and it expires on September 29, 2013. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56.

Principal Investigator: HOOVER, WYNTON C.

Co-Investigator(s):

Protocol Number: **X100910009**

Protocol Title: *Comparison of Vitamin D Supplementation Regimens*

The IRB reviewed and approved the above named project on 11/29/10. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.

IRB Approval Date: 11-29-10

Date IRB Approval Issued: 11/29/10

Marilyn Doss

Marilyn Doss, M.A.
Vice Chair of the Institutional Review
Board for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

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AB 470
1530 3RD AVE S
BIRMINGHAM AL 35294-0104

c. List all staff who will be involved with the design, conduct, and reporting of the research, their degree(s) and job title, and any additional qualifications. Include individuals who will be involved in the consent process. *Repeat the table below for each individual.*

Note. For studies involving investigational drugs, include all investigators who will be listed on FDA Form 1572 and attach a copy, if applicable. Send the IRB a copy

Role:	☒Co- *-OR-* ☐Other *–AND/OR-* ☐Consent Process
Full Name:	**LaCrecia Britton**
Primary UAB Dept.: (Employer if not UAB)	**CHS Pediatric Pulmonary**
Degree(s) / Job Title:	**RN, MSN, CRNP- AC/PC / CF Center Coordinator, Children's Hospital**
Additional Qualifications pertinent to the study:	**Research coordinator in EPIC protocol as well as numerous experiences with studies in the CF population.**
Role:	☒Co- *-OR-* ☐Other *–AND/OR-* ☐Consent Process
Full Name:	**Heather Searcy, Pharm D**
Primary UAB Dept.: (Employer if not UAB)	**CHS Pediatric Pulmonary Pharmacist**
Degree(s) / Job Title:	**Pharm. D**
Additional Qualifications pertinent to the study:	**Direct patient care of CF patients with medication treatment recommendations.**
Role:	☒Co- *-OR-* ☐Other *–AND/OR-* ☐Consent Process
Full Name:	**Erica Krogsgard, Pharm D**
Primary UAB Dept.: (Employer if not UAB)	**CHS Pediatric Pulmonary Pharmacist**
Degree(s) / Job Title:	**Pharm. D**
Additional Qualifications pertinent to the	**Direct patient care of CF patients**

study:	
Role:	☒Co- -*OR*- ☐Other –*AND/OR*- ☐Consent Process
Full Name:	**Nancy Wooldridge, MS, RD**
Primary UAB Dept.: (Employer if not UAB)	**Pediatric Pulmonary Nutritionist**
Degree(s) / Job Title:	**MS,RD**
Additional Qualifications pertinent to the study:	**Direct patient care of CF patients and previous involvement in clinical studies.**
Role:	☒Co- -*OR*- ☐Other –*AND/OR*- ☐Consent Process
Full Name:	**Julianna Bailey**
Primary UAB Dept.: (Employer if not UAB)	**Pulmonary Nutritionist PPC**
Degree(s) / Job Title:	———
Additional Qualifications pertinent to the study:	**Student trainee**
Role:	☒Co- -*OR*- ☐Other –*AND/OR*- ☐Consent Process
Full Name:	**Allison Pavey, MS, RD, LD**
Primary UAB Dept.: (Employer if not UAB)	**Pediatric Pulmonary Nutrionist**

APPENDIX B

VITAMIN D STATUS SURVEY

Vitamin D Status Survey

Food Frequency

Vit D units	Food	Serving Size	Servings/day	Servings/week
~100	Milk	8 oz		
25	Eggs	1 egg		
1000	Salmon (wild)	3oz		
275	Salmon (farmed)	3oz		
231	Sardines (in oil)	3oz		
425	Catfish	3 oz		
120	Shrimp	3 oz		
135	Tuna (in water)	3 oz		
545	Oysters	3oz		
453	Cod-liver oil	1 teaspoon		
375	Trout (farmed)	3oz		
35	Bass (farmed)	3oz		
45	Pudding/custard made with milk	½ cup		
20	Biscuits, cornbread	2" cube		

Are you currently drinking a **nutritional supplement**? Yes □ No □

Vit D units	Brand	Serving Size	Servings/d	Servings /week

CF Vitamin Supplement:

Vit D units	Brand	Dosage

How many times per week do you remember to take the Vitamin?

Exposure to Sunlight:

On average, how many minutes do you spend outside in the sunshine on school days during the winter months?

On average, how many minutes do you spend outside in the sunshine on a Saturday or Sunday during the winter months?

Reference: Taylor C, Lamparello B, Kruczek K, Anderson EJ, Hubbard J, Misra M. Validation of a Food Frequency Questionnaire for Determining Calcium and Vitamin D Intake by Adolescent Girls with Anorexia Nervosa. *J Am Diet Assoc*. 2009; 109: 479-485.