THE ASSOCIATION BETWEEN SERUM VITAMIN D STATUS, BONE MINERAL DENSITY, AND FORCED EXPIRATORY VOLUME IN 1 SECOND IN PEDIATRIC CYSTIC FIBROSIS PATIENTS

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ABSTRACT

Cystic fibrosis (CF) currently affects around 30,000 individuals in the United States. Mucus obstructing the pancreas affects the production of digestive pancreatic enzymes causing pancreatic insufficiency, which leads to malabsorption, specifically of fat-soluble vitamins. New complications such as CF-related bone disease have also increased. Poor bone health is associated with malnutrition, inflammation, and vitamin D deficiency. CF patients generally have inadequate levels of 25-hydroxyvitamin D. Insufficient vitamin D status is linked with decreased bone mass, increased inflammation, decreased immunity, and is also believed to contribute to respiratory failure.

The purpose of this research is to examine the associations between serum vitamin D, bone mineral density (BMD) and lung function within the pediatric CF population. It is hypothesized that there will be a positive association between serum vitamin D and bone mineral density. It is further hypothesized there will be a positive association between serum vitamin D and lung function.

A retrospective chart review was conducted to evaluate the association of serum vitamin D by assessing pre and post serum vitamin D concentrations, BMD, and pulmonary function in 30 CF pediatric patients ages 8-18. Data was subjected to descriptive statistics, correlational analyses, and multiple linear regression to examine potential relationships between serum vitamin D levels with forced expiratory volume (FEV1) at baseline and with both BMD and FEV1 after 2 years of maximum dose

supplementation. Independent *t* tests were ran to compare differences in groups regarding supplementation doses.

No significant associations were found in serum vitamin D and BMD except in the baseline model. Furthermore, no significant associations were found between serum vitamin D and FEV1 values of lung function. Significant associations were seen in BMD and lean body mass at baseline and 2 years. Results also showed no significant differences between groups receiving either 2000 IU or 3000 IU vitamin D supplementation.

While there were no consistent associations with serum vitamin D and BMD, baseline associations show a link between low serum levels and BMD. Future research should focus on interventions for preventative care of maintaining adequate vitamin D serum levels and diets rich in calcium for optimal bone health.

DEDICATION

This thesis is dedicated with respect to the patients with cystic fibrosis at Children's of Alabama and their families. These patients have shown me true strength and persistence in the daily trials and tribulations they actively fight from this disease.

LIST OF ABBREVIATIONS AND SYMBOLS

ABPA	Bronchopulmonary aspergillosis
BMD	Bone mineral density
CF	Cystic fibrosis
CFBD	Cystic fibrosis related bone disease
CFTR	Cystic fibrosis transmembrane conductase regulator gene
DEXA	Dual energy x-ray absorptiometry scan
DRI	Dietary reference intakes
EER	Energy efficiency requirement
FEV1	Forced expiratory volume expelled in 1 second
FVC	Forced vital capacity
Hyp	Hypothesis
IU	International units
L2-L4	Lumbar spine region
LBM	Lean body mass
mg	Milligram, a unit of measure of mass
ng/ml	Nanogram per milliliter
nmol/L	Nanomole per liter
P	Probability associated with the occurrence under the null hypothesis of a value as extreme as or more extreme than the observed value.
PI	Pancreatic insufficiency
PTH	Parathyroid hormone

r	Spearman product-moment correlation
SD	Standard deviation
TNF- α	Tumor necrosis factor alpha
Z score	Standard scores; normal distribution
<	Less than
=	Equal to
±	Plus or minus
%	Percent

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Above all else I give all the credit and glory to God. Proverbs 16: 3 says commit your works to the Lord and your plans will be established.

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CHAPTER 1

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive genetic disorder that affects about 30,000 individuals in the United States.[1] CF is caused from a mutation in the cystic fibrosis trans-membrane conductance regulator (CFTR) gene. The CFTR protein functions as a chloride and bicarbonate channel, which is important for the salt and water balance on epithelial tissues in the lungs, pancreas, sinuses, skin, and gastrointestinal tract.[2] Mutations of the CFTR gene result in a dysfunctional CFTR protein, which causes chloride transport defects.[3] This changes the viscosity of hydration, thickening the secretions of mucus in the epithelial tissues causing functional problems in those organs. Mucus coating the lungs makes breathing difficult and can trigger serious infections and inflammation. Mucus obstructing the pancreas specifically affects the production of digestive pancreatic enzymes. This eventually leads to pancreatic insufficiency, which is defined as the loss of 90% of the ability to secrete digestive enzymes.[4]

About 88% of CF patients are pancreatic insufficient and are unable to digest fat and protein properly.[1] Pancreatic insufficiency (PI) leads to malabsorption of nutrients, specifically fat-soluble vitamins A, D, E, and K, greasy bowel movements, poor growth, decreased immune function, and difficulty gaining weight. Therefore, nutrition in the CF population is of utmost importance to support adequate health. Supplementing pancreatic enzymes in the pancreatic insufficient patients can help with digestion and reduce malabsorption symptoms. Supplementation of fat-soluble vitamins is also needed to compensate for the losses of

malabsorption secondary to PI. Due to the manifestations of this disorder, most patients require a high-calorie, high-protein, liberal fat, and high-sodium diet with up to 1.2-2.0 times energy efficiency requirement (EER) based on activity and severity of disease and 1.5-2.0 times the dietary reference intakes (DRI) for age of the DRI for protein.[5,6] Calories should not be restricted; however, no sweetened beverages are encouraged. Foods rich in calcium, iron, and zinc are also desired for optimal growth and development to reduce under-nutrition.

Life expectancy for cystic fibrosis patients has grown to 39.3 years, due to the increase in research and the successful CFTR modularity drugs.[1] With this increased life expectancy also comes the manifestation of new age-related complications such as CF-related bone disease (CFBD). As patients age, their bones weaken, leading to low bone mineral density and diseases like osteoporosis and osteopenia. The diagnosis of osteoporosis in pediatrics should not be made solely on the densitometry criteria. Osteoporosis is indicated by the presence of both a clinically significant fracture history and low bone mineral density. A significant fracture history is defined as two or more long bone fractures by age 10 or three or more long bone fractures by age 19.

Poor bone health is associated with malnutrition, inflammation, and vitamin D deficiency. Cystic fibrosis patients generally have inadequate levels of 25-hydroxyvitamin D.[7] Deficiency of vitamin D is reported to affect up to 95% of pediatric cystic fibrosis patients and 90% of adult cystic fibrosis patients.[7] Insufficient vitamin D status is linked with decreased bone mass, increased inflammation, decreased immunity, and is also believed to contribute to respiratory failure.[8] Maintaining adequate levels of vitamin D is thought to improve clinical outcomes and lessen complications associated with cystic fibrosis patients. Puberty is a critical period in adolescence for bone mineralization, and it is crucial to obtain optimal nutrition and

vitamin status to achieve peak bone mass to minimize potential problems of poor bone mineral density and CFBD.

The purpose of this study was to assess the relationship of serum vitamin D with bone mineral density and lung function within the pediatric cystic fibrosis population. The study will investigated the following hypotheses:

Hyp 1: There is a positive association between vitamin D serum status and bone mineral density. *($P<0.05$)*

Hyp 2: There is a positive association between vitamin D serum status and lung function. *($P<0.05$)*

CHAPTER 2

LITERATURE REVIEW

The following literature review focuses on current research that characterizes the pediatric and adult cystic fibrosis populations and examines the effects this disease produces on vitamin D status and bone mineral density. The insufficiency of serum vitamin D and low bone mineral density seen within the CF population shows a potential need for additional doses of vitamin D supplementation in cystic fibrosis patients. Further information regarding lung function and the effects supplementation has on pulmonary function tests within the cystic fibrosis population is also included.

VITAMIN D

Vitamin D is a fat-soluble vitamin that has long been known for its function in skeletal health.[9] Vitamin D is shown to be an important factor in calcium and phosphorous absorption for bone mineral regulation.[10] Aside from bone health, vitamin D is crucial for immune health and protection from multiple diseases. Recent studies investigating the functions of vitamin D have suggested that sufficient levels could decrease inflammation, increase immunity, and protect against respiratory infections, thereby overall decreasing mortality.[11] Deficiency of vitamin D creates risk for cardiovascular disease, diabetes, cognitive impairment, rickets, decreased respiratory function, and low bone mineral density.[10] Thus, keeping adequate vitamin levels is of vital importance for good health. If dietary intake of vitamin D is low or if serum

concentrations are inadequate, specifically in certain disease states such as cystic fibrosis patients, supplementation is considered the optimal treatment.

A retrospective study on 360 cystic fibrosis patients evaluated the effect of cholecalciferol supplementation on serum vitamin D concentrations.[12] Baseline vitamin D levels were analyzed and subjects were given clinical interventions of increased oral supplementation if baseline concentrations were below 50 nmol/L or given counseling on compliance with the supplement. Results of the study showed a significant increase in 92% of the subject's vitamin D concentrations from 25.5 to 62.5 nmol/L.[12] The subjects with baseline concentrations below 25 nmol/L showed the largest improvement in vitamin D concentrations (P=0.02).[12] The investigators concluded that vitamin D supplementation significantly increases serum vitamin concentrations, making it a therapeutic option to keep serum vitamin D at optimal levels.

BONE MINERAL DENSITY

Bone health is vital for overall health and growth. In order to build bones, calcium, phosphorus, magnesium, and fluoride are needed.[13] When bones lack these minerals they become porous, with low bone mineral density, and at risk for bone diseases such as osteopenia and osteoporosis.[13] This means the bones are more fragile and are prone to fractures. Bone mineral density is measured through a dual energy x-ray absorptiometry scan (DEXA) which is an enhanced form of an x-ray. DEXA scans particularly look at the lumbar spine L2-L4, total body, and, in adults, the hip.

Patients with cystic fibrosis are more disposed to low bone mineral density due to malabsorption of vitamin D, poor nutritional status, physical inactivity, glucocorticoid therapy, delayed puberty, or inflammation caused by lung infections.[14] A recent cross sectional study

aimed to find the prevalence of low bone mineral density in adolescent and adult cystic fibrosis patients. In a sample size of 58 subjects, they found a prevalence of 20.7%.[15] Another study done among 37 adolescents with cystic fibrosis found 54.1% of patients to have lower than normal lumbar spine bone mineral density and 32.5% to have lower than normal total body bone mineral density.[16]

The latest consensus for bone health in the cystic fibrosis population recommends targeting serum vitamin D concentrations at 30-60 ng/ml.[14] Aggressive supplementation of vitamin D may be needed to reach target levels. Based on the consensus, 1300-1500 mg calcium per day and 0.3-0.5 mg per day of vitamin K is also recommended.[14] Assessing bone mineral density in children prior to puberty and aggressively correcting insufficient vitamin levels in these children may reduce poor bone mineral density after puberty and within adulthood.[14]

VITAMIN D SUPPLEMENTATION AND ITS EFFECTS ON BONE MINERAL DENSITY

As indicated above, vitamin D is critical for calcium absorption and calcium is of vital importance for bone mineralization.[10] This draws to question the mechanism of how vitamin D affects bone mineral density. If there are insufficient vitamin levels, is calcium absorption decreased, thereby decreasing bone density? And, if this is true, would supplementation help improve bone density?

Vitamin D is activated in the body to its active form calcitriol. Calcitriol has multiple functions in the intestine, the kidney, and the bones. Specifically in the intestine, calcitriol functions to increase absorption of calcium and phosphorous. In the kidneys calcitriol is involved in the parathyroid hormone (PTH) stimulation of calcium phosphate reabsorption in the distal renal tubule. If calcium is low, the kidney will reabsorb and excrete less. In the bones calcitriol

triggers proliferation, differentiation and growth in the tissues. PTH and calcitriol direct the mobilization of calcium from bone to maintain a normal blood calcium concentration. Therefore, when vitamin D levels are low, calcitriol is low and the intestines have difficulty absorbing calcium. Low blood calcium concentrations are then corrected by taking calcium from the bones to the blood to correct the blood calcium concentration, decreasing bone density.

A meta-analysis assessed 6 different studies for the effects of bone mineral density with vitamin D supplementation in healthy children using DEXA scans. The main effects of vitamin D supplementation showed no significant effects on total body bone mineral content or in the bone mineral density of the hip or forearm. There was a slight change in the lumbar spine bone mineral density with supplementation, but it did not reach significance (P=0.07).[17] In the analysis of effects of supplementation from baseline serum vitamin D level there was a significant change in bone mineral density of the lumbar spine (P=0.05) and total body bone mineral content (P=0.04) in patients with a baseline vitamin D level less than 35 nmol/L.[17] From the results of this review vitamin D supplementation could improve bone mineral density in patients with low vitamin D concentrations.[17] Low vitamin D level is common in CF patients. Therefore, it is reasonable to suspect that vitamin D supplementation could help bone mineral density in cystic fibrosis patients.

A randomized, double blind, placebo controlled trial investigated the effect of calcium and vitamin D supplementation on bone mineral density and bone metabolism in adult cystic fibrosis patients.[18] All patients took 900 IU/day of vitamin D as regular supplementation. The study randomly assigned 15 patients to the placebo group and 16 patients to the treatment group to receive 1 gram of calcium/day and an additional 800 IU/day of vitamin D.[18] Patients were analyzed at baseline and after one year of treatment. Results showed a reduced rate of bone

turnover and bone loss in the treatment group, but changes did not reach statistical significance.[18] Investigators hypothesized that the lack of statistical significance may have due to low sample size and short intervention time.[18] There might also have a more marked effect if the intervention took place in younger patients, prior to pubertal growth spurt.

A retrospective study evaluated the prevalence of vitamin D insufficiency and its impact on bone and respiratory health in adults with CF. Of the 185 subjects, 70% reported taking CF-specific vitamins, 47.6% reported taking vitamin D supplementation through AquADEKs® vitamins or combined with calcium, 37% took both a CF-specific vitamin and vitamin D supplementation, and 88% received pancreatic enzymes.[19] Incidence of vitamin D insufficiency in this population was 76% and prevalence of deficiency was 23%. Of the BMD measurements, 52% had a t score <-1.0 and 10% had a t score < -2.5.[19] There was a positive significant association with vitamin D concentrations and lowest annual FEV1 ($P<0.05$).[19] Prevalence of vitamin D insufficiency and poor skeletal health is high in the US CF population.[19]

VITAMIN D SUPPLEMENTATION AND ITS EFFECTS ON PULMONARY FUNCTION

Spirometry is one of the most common lung function tests, measuring a variety of variables. Most studies analyze how much air in volume and speed can be inhaled and exhaled. Forced vital capacity (FVC) shows amount of air forcible blown out after full inspiration. Forced expiratory volume (FEV1) shows how much air you exhale in one second and depends on age and gender.

Wolfenden also examined lung function in the previous study mentioned. In this study, vitamin D status appears to be positively associated with lung function. There was a positive significant association with vitamin D concentrations and lowest annual FEV1 ($P<0.05$).[19] There

was a high prevalence of thoracic vertebral fractures was associated with lower FEV1 ($P<0.001$).[19] It is therefore hypothesized that vitamin D's anti-inflammatory property could possibly reduce respiratory stress and increase function when vitamin levels are optimal. Overall, further study is needed to assess the optimal level of vitamin D for skeletal health and the role of vitamins D in other functions such as respiratory health, immune health, and infections.

VITAMIN D SUPPLEMENTATION AND ITS IMPACT ON HOSPITALIZED PATIENTS

Researchers conducted a prospective observational study analyzing vitamin D in ICU patients hypothesized that deficiency in vitamin D would increase the patient's length of stay, mortality rate, and cost of hospitalization.[20] Of the 258 patients evaluated, 53.5% were severely deficient (serum vitamin D levels less than 13 ng/ml), 37.2% were moderately deficient (14-26 ng/ml), 7% were mildly deficient (27-39 ng/ml), and 1.2% presented normal serum vitamin D levels (>40 ng/ml).[20] The severely deficient group had a significantly greater mean treatment cost of $51,413.33 compared to the moderately deficient ($28,123.65) and mildly deficient group ($20,414.11)($P=0.027$). Length of hospitalization was significantly longer in the severely deficient group at 13.3 days compared to the moderately deficient (7.3 days) and mildly deficient (5.2 days)($P=0.002$).[20] Mortality rate for vitamin D deficiency was significantly greater in the severe and moderate groups compared to the mild and normal groups ($P=0.047$).[20] Thus, vitamin D status should be assessed and deficiency should be treated to provide the best outcomes for patients.

A randomized, placebo controlled, pilot study examines the influence of cholecalciferol supplements on hospitalized cystic fibrosis patients for pulmonary exacerbation. Researchers

randomly assigned 30 hospitalized cystic fibrosis patients to receive either 250,000 IU cholecalciferol or the control.[8] Outcome measures include plasma vitamin D, PTH, and calcium concentrations. Secondary outcome measures assessed patients one year after hospitalization on survival, hospitalization, antibiotic use, and lung function.

One week after the cholecalciferol intervention, plasma vitamin D increased significantly and the overall mean concentrations of vitamin D were significantly greater in the treatment group than seen in the control group ($P<0.001$).[8] Overall, subjects receiving the high cholecalciferol dose showed good tolerance with no reported adverse symptoms of vitamin D toxicity.[8] Six months post intervention, the vitamin D group had a greater number of hospital free days ($P=0.036$) and IV antibiotic therapy free days than the control.[8] An increase to 95% lung function was seen in 90% of the vitamin D group but only 50% of the control.[8] The vitamin D group overall had a lower mortality rate than the control group (one death vs. five deaths, respectively) which shows a significantly higher risk of mortality in the control group ($P=0.029$).[8] Researchers found that vitamin D treatment was associated with an overall better recovery and investigators concluded that a high dose of cholecalciferol could help improve plasma vitamin D levels and should be considered part of the treatment plan for patients with CF.[8]

Further interest in this topic leads to a more recent study of the effects of vitamin D on inflammatory markers in cystic fibrosis patients hospitalized for pulmonary exacerbation. A double blind, placebo controlled, randomized clinical trial was designed to further assess the intervention of a single, oral dosage of 250,000 IU cholecalciferol in hospitalized cystic fibrosis adults on the changes in antimicrobial peptide concentrations and inflammatory markers, interleukin-6 (IL-6) and interleukin-8 (IL-8).[7]

Vitamin D concentrations increased in the vitamin D group at week one and week 12 ($P<0.001$).[7] Tumor necrosis factor alpha (TNF-α), a cell signaling protein involved in systemic inflammation, was significantly decreased from week one to week 12 ($P=0.0002$) in the group who received vitamin D and was significantly lower than the control.[7] Pro-inflammatory cytokine IL-6 concentrations were significantly lower in the vitamin D group than the control. There was a significant decrease in IL-6 at week one from baseline ($P=0.004$) and week 12 ($P=0.4$) in the vitamin D group.[7] Significant changes were not found in the placebo group. The vitamin D and placebo groups both showed significant increase in IL-8, which is a mediator of inflammation. However, significant difference in the increase in IL-8 was not noted between the groups.[7]

Researchers found that supplementation of vitamin D can lower IL-6 and TNF-α concentrations and reduce inflammatory cytokines.[7] Thus, vitamin D may have anti-inflammatory properties to suppress pro-inflammatory cytokines and support immune functions. Results further support the concept that an increase of vitamin D is positively correlated with lung function and inversely correlated with inflammation.

While the previous studies examine an extremely high single dose given during hospitalization, the statistically significant associations shown question the possibility of high daily doses producing the same effect, diminishing the need for hospitalization.

After discussion of the studies above, it seems adequate vitamin D status is of even greater importance for cystic fibrosis patients, for this population is at greater risk for infection, decreased immunity, and poor lung function. Grossman et al stated that low vitamin D levels increase inflammation and respiratory infection leading to acute pulmonary obstructions.[8]

Therefore, if high doses of vitamin D supplementation were given and resulted in increased serum vitamin D, theoretically it could reduce poor clinical outcome, risks associated with infection, and also decrease in chances of pulmonary exacerbations. Therefore, it is possible increased vitamin D status will improve overall clinical outcome and health in individuals with cystic fibrosis.

CHAPTER 3

METHODS

INTRODUCTION

This study used a retrospective cohort design to evaluate the association of serum vitamin D levels with physiologic and functional outcomes by assessing differences in pre- and post-serum vitamin D concentrations, bone mineral density (BMD), and pulmonary function in pediatric cystic fibrosis patients. This chapter discusses the methodology used, including participants, selection criteria, vitamin D supplementation used, methods for evaluation of bone mineral density, serum vitamin D, lung function, and the plans for data analyses.

SUBJECTS

Thirty pediatric cystic fibrosis patients, ages 8 to 18 years old, were included in this study. All subjects have been patients at Children's of Alabama Hospital in Birmingham Alabama and were regularly seen in outpatient CF clinic between the years 2012 and 2015.

INCLUSION CRITERIA

To be included for this study, all patients must have had a diagnosis of CF, confirmed via a positive sweat chloride test and genotyping. Patients have exhibited pancreatic insufficiency and have been prescribed a high daily supplementation of 2000 IU or more vitamin D. Patients

must also have been consistently seen at least every 6 months in cystic fibrosis outpatient clinic or in hospital visits for follow-up labs and test.

Patients were excluded from the study if they had a diagnosis of liver disease (as evidenced by portal hypertension), or were diagnosed with Allergic Bronchopulmonary Aspergillosis (ABPA) and underwent steroid treatment. Patients were also excluded if they were chronically taking oral steroids for more than 6 weeks or if their disease state was too progressed, defined as FEV1 less than 30% predicted. Lastly, patients were excluded if their vitamin supplementation did not reach 2000 IU/day.

VITAMIN D SUPPLEMENTATION

In the CF population, optimal serum vitamin D concentrations are defined as 30-50 ng/ml.[22] Vitamin D deficiency is defined as serum levels less than 20 ng/mL and vitamin D insufficiency refers to serum levels in between 20-30 ng/mL. Patients with CF seen at Children's of Alabama are recommended to take a CF-specific vitamin, which contains vitamin D. The two most commonly prescribed multivitamins are AquADEKs® and MVW Complete Formulation™.

Table 1: Multivitamin recommendations for children with cystic fibrosis

Age	MVW Complete Formulation™	AquADEKs®
4-8 years old	1500 IU/ 1 chewable	For ages 4-10 800 IU/ 2 chewable
9-18 years old	3000 IU/ 2 soft gel	For ages 10 and up 600IU/ 2 soft gel

If serum levels remain under 30 ng/mL, patients are prescribed an increase in supplementation. The current recommended daily allowance for vitamin D intake for ages 1 to 70 years old or

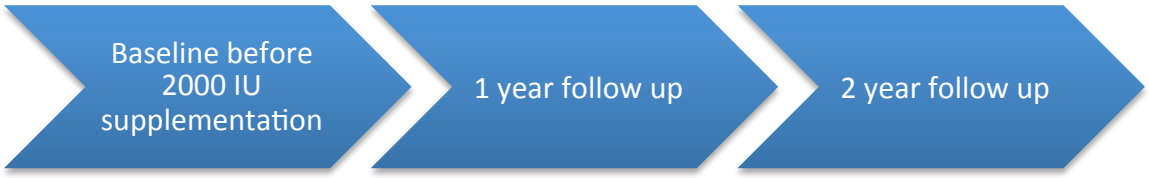
greater is 600 IU daily. It is noted that individuals with malabsorption may require two to three times more vitamin D than individuals without malabsorption. Thus, the Endocrine Society recommends 1,500-2,000 IU daily for individuals with CF.[22] This protocol used a higher dose of 2000-3000 IU/day vitamin D supplementation.

EVALUATION OF SERUM VITAMIN D

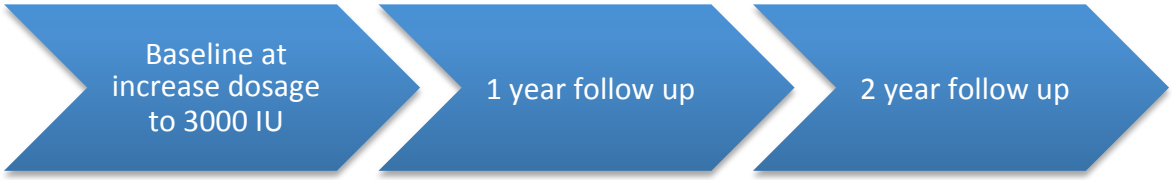
Serum vitamin D levels were obtained yearly for patients in this clinic as a standard of care. Patients participating in this study were categorized into two groups. The first group included patients who had vitamin D supplementation at a level of 2000 IU daily. In this group serum concentrations, BMD, and lung function were obtained prior to supplementation, post one year after starting supplementation of 2000 IU daily, and two years after starting supplementation. The second group included patients who required an increase in supplementation of vitamin D dose above the initial 2000 IU, secondary to not achieving serum level of 30 ng/mL. In this group, serum concentrations, BMD, and lung function were assessed at baseline of beginning a supplement, at time of the increase in supplementation, and then one and two years post increase in supplementation.

Figure 1: Flow chart with group descriptions

Group 1:



Group 2:



EVALUATION OF BMD

BMD, fat percentage, fat mass, and lean body mass (LBM) were measured by a dual energy X-ray absorptiometry (DEXA) scan. BMD regions evaluated were lumbar spine, L1-L4, and total body. BMD below expected range for age, race, and gender is defined by z-scores. Low bone mineral density is defined as less than or equal to -2.0 SD.[20]

EVALUATION OF LUNG FUNCTION

Lung function was evaluated through spirometry. Forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), and FEV1/FVC are all measured during clinic visits as standard of care. This study analyzed FEV1 values (at baseline) and at one and two years of supplementation to assess possible changes. In order to avoid confounding factors of differences in lung function severity, patients were assessed from individual changes in FEV1 values.

STATISTICAL ANALYSIS

This study included 15 female patients and 15 male patients. The majority of the patients were Caucasian, two of the patients were African American, two were Hispanic, and one reported having two or more races.

Descriptive statistics for the population were generated for the whole group and by sub-groups (2000 IU supplementation vs. 3000 IU supplementation groups) for all variables of interest. T-tests were used to describe differences in sample characteristics between the group supplemented with 2000 IU and the group supplemented with 3000 IU. Correlational analyses and multiple linear regression were used to examine potential relationships between serum vitamin D levels with FEV1 at baseline and with both BMD and FEV1 after 2 years of maximum dose supplementation, after adjusting for gender, age, ethnicity, and CF genotype (as appropriate.) Additionally, between-group comparisons between the two sub-groups after 2 years of maximum dose supplementation were made to compare BMD and FEV1. All statistical tests were two-tailed and performed using a significance level of <0.05. Analyses were performed using SAS, ver. 9.4 (SAS Institute, Inc., Cary, NC).

CHAPTER 4

RESULTS

The results of the study were generated from retrospective data collected from patient charts. Demographic information for the participants is shown in Table 2. The sample included 30 participants comprised of 15 girls and 15 boys with a mean age of 13.7 ± 2.39 years at baseline. Approximately 83.33% of the population was Caucasian, 6.67% African American, 6.67% Hispanic, and 3.33% reported 2 or more races. Genotype was verified and recorded, with 50% having two copies of ΔF508, 37% having one copy of ΔF508, and 13% having zero copies of ΔF508.

Mean serum vitamin D levels at baseline and post 2 years supplementation for each group are shown in Table 3. The patient group receiving 2000 IU of vitamin D daily had a mean vitamin D serum at baseline of 25.063 ± 5.196 ng/mL. After two years the mean serum level increased to 28.188 ± 6.01 ng/mL. The patient group receiving 3000 IU of vitamin D daily had a mean vitamin D serum at baseline 23.786 ± 7.465 ng/mL. After two years the mean serum level increased to 26 ± 11.891 ng/mL.

Correlation analyses were conducted. Supplement doses, gender, and genotype were not consistently significantly associated with bone mineral density or serum vitamin D and therefore are not reported. Relationships between bone mineral density with age and serum vitamin D are reported in Table 4.

At baseline, BMD of the L2-L4 was significantly associated with age (r=0.75, P<0.0001), weight (r=0.63, P=0.0002), height (r=0.50, P=0.005), BMI (r=0.445, P=0.014), fat mass (r=0.42, P=0.019), and lean body mass (r=0.61, P=0.0003). Serum vitamin D was not significantly correlated with BMD of the L2-L4. BMD L2-L4 z score was not significantly associated with any variable. BMD of the total body was significantly associated with age (r=0.83, P<0.0001), weight (r=0.64, P=0.0001), height (r=0.51, P=0.004), BMI (r=0.516, P=0.0035), fat mass (r=0.39, P=0.034), and lean body mass (r=0.72, P<0.0001). Serum vitamin D was not significantly correlated with BMD of the total body. BMD total body z score was significantly correlated with race (r=0.38, P=0.041) and serum vitamin D (r=0.40, P=0.028). FEV1 was significantly associated with race (r=0.40728, P=0.0255) and BMI z score (r=0.419, P=0.021). FEV1 was not significantly associated with vitamin D.

At year 2 BMD of the L2-L4 was significantly correlated with weight (r=0.48, P=0.007), weight z score (r=0.47, P=0.008), BMI (r=0.486, P=0.006), BMI z score (r=0.396, P=0.03) fat mass (r=0.36, P=0.048), and LBM (r=0.46, P=0.009). BMD L2-L4 z score was significantly associated with weight (r=0.45, P=0.011) and fat mass (r=0.42, P=0.02). BMD of the total body was significantly associated with age (r=0.49, P=0.006), weight (r=0.59, P=0.0006), weight z score (r=0.422, P=0.02), height (r=0.37, P=0.045), BMI (r=0.624, P=0.0002), BMI z score (r=0.399, P=0.028) and LBM (r=0.62, P=0.0002). BMD total body z score was significantly associated with weight (r=0.41, P=0.026) and fat grams (r=0.42P=0.021). FEV1 was significantly associated with weight z score (r=0.371, P=0.044), BMI (r=0.419, P=0.022, and BMI z score (r=0.543), P=0.002) after 2 years.

Using the variables that were significant in the correlations or were predicted in the hypotheses to be contributing variables, linear regression analyses were conducted to identify

predictors of BMD at the L2-L4, total body, and lung function measured through FEV1. Positive predictors baseline serum vitamin D (P=0.049) and age (P<0.0001) as well as negative predictor gender (P=0.018) explained 63.78% of the variance in baseline BMD of the L2-L4. Gender showed that females were associated with higher BMD than males. After 2 years of supplementation, positive predictor lean body mass (P=0.0001) and negative predictors race (P=0.006) and gender (P=0.001) explained 53.98% of the variance in BMD of the L2-L4. Race and gender showed that Caucasians and females had higher BMD than other races and males. Positive predictors baseline serum vitamin D (P=0.031), BMI z score (P=0.085), and age (P<0.0001) explained 78.66% of the variance in baseline BMD of the total body. After 2 years of supplementation, positive predictors weight (P=0.002) and age (P=0.016) explained 56.72% of the variance in BMD of the total body. Positive predictor weight (P=0.024) and negative predictor height (P=0.017) and fat mass (P=0.098) explained 20.85% of the variance in baseline FEV1 values. After 2 years supplementation, positive predictors BMI z score (P=0.003) and gender (P=0.082) and negative predictor serum vitamin D (P=0.166) explained 33.36% of the variance in FEV1 values.

Lastly, independent *t* tests were run on weight, height, genotype, BMD of the L2-L4, L2-L4 z-score, BMD of the total body, total body z-score, fat percentage, fat grams, lean body mass, serum vitamin D, and FEV1 values to compare the participants receiving 2000IU and 3000IU. There were no significant differences noted in any of the variables between the group given 2000 IU and the group given 3000 IU.

Table 2 Demographic characteristics of children with cystic fibrosis on 2000 IU and 3000 IU vitamin D supplementation

Variable	2000 IU	3000 IU	Total group
Age, years (mean± SD) at baseline	13.19 (±2.26)	14.29 (±2.49)	13.7 (±2.39)
Weight, kg (mean ± SD) at baseline	46.44 kg (±10.17)	48.69 kg (±11.83)	47.49 (±10.84)
Weight z score at baseline			
At risk of overweight (1.0-2.0)	1, 6.25%	1, 7.14%%	2, 6.67%
Normal (-1.0- 1)	11, 68.75%	9, 64.29%	20, 66.67%
Mild malnutrition (-1.0- -2.0)	4, 25%	2, 14.29%	6, 20%
Moderate malnutrition (-2.0- -3.0)	0, 0%	2, 14.29%	2, 6.67%
Height, cm (mean ±SD) at baseline	156.53 cm (±14.78)	157.23 cm (±16.09)	156.59 cm (±15.15)
BMI at baseline	18.85 kg/m^2 (±2.12)	19.46 kg/m^2 (±3.01)	19.14 (±2.547)
BMI z score at baseline			
At risk of overweight (1.0-2.0)	0, 0%	1, 7.14%	1, 3.33%
Normal (-1.0- 1)	14, 87.5%	10, 33.33%	24, 80%
Mild malnutrition (-1.0- -2.0)	1, 6.25%	2, 14.29%	3, 10%
Moderate malnutrition (-2.0- -3.0)	1, 6.25%	1, 7.14%	2, 6.67%
Gender (n, %)			
Male	8, 50%	7, 50%	15, 50%
Female	8, 50%	7, 50%	15, 50%
Ethnicity (n, %)			
Caucasian	13, 81.25%	12, 85.71%	25, 83.33%
African American	1, 6.25%	1, 7.14%	2, 6.67%
Hispanic	2, 12.5%	0	2, 6.67%
2 or more races	0	1, 7.14%	1, 3.33%
Genotype - percent of participants with specific number of copies of ΔF508			
2	10, 62.5%	5, 35.7%	15, 50%
1	5, 31.25%	6, 42.9%	11, 36.67%
0	1, 6.25%	3, 21.4%	4, 13.33%

Table 3 Serum vitamin D status of children with cystic fibrosis at baseline and following 2 years of supplementation; mean (±SD)

Vitamin D supplementation level	Baseline	2 years
2000 IU	25.063 (±5.196)	28.188 (±6.01)
3000 IU	23.786 (±7.465)	26 (±11.891)
Total group	24.467 (±6.274)	27.167 (±9.128)

Table 4 Spearman correlations of BMD with age and serum vitamin D at baseline and 2 years; r(p)

	Baseline		2 Year	
	Age	Serum Vitamin D	Age	Serum Vitamin D
BMD L2-L4	0.746 (<0.0001)	0.123 (0.517)	0.327 (0.078)	0.174 (0.366)
BMD L2-L4 z score	-0.092 (0.627)	0.189 (0.315)	-0.094 (0.623)	0.314 (0.091)
BMD total body	0.834 (<0.0001)	0.206 (0.276)	0.491 (0.006)	0.238 (0.205)
BMD total body z score	0.011 (0.954)	0.401 (0.028)	0.027 (0.889)	0.269 (0.151)

Table 5 Bone mineral density at baseline and following 2 years of vitamin D supplementation; mean (±SD)

	L2-L4 z score		Total Body z score	
Vitamin D supplementation level	Baseline	Post 2 years	Baseline	Post 2 years
2000 IU	-0.7 (±1.092)	-0.667 (±1.173)	-0.306 (±1.034)	-0.381 (±1.199)
3000 IU	-0.357 (±0.919)	-0.543 (±1.182)	-0.043 (±0.656)	-0.314 (±0.711)
Total	-0.54 (±1.012)	-0.609 (±1.158)	-0.183 (±0.874)	-0.35 (±0.986)

Table 6 Spearman correlations of BMD with LBM at baseline and 2 years; r(p)

	LBM (Baseline)	LBM (2 years)
BMD of the L2-L4	0.61097 (P=0.0003)	0.46474 (P=0.009)
BMD of the total body	0.71696 (P<0.0001)	0.62447 (P=0.0002)

CHAPTER 5

DISCUSSION

The purpose of this research was to examine the relationship of serum vitamin D, bone mineral density, and lung function in the pediatric cystic fibrosis population. This study was conducted to better describe and understand the vitamin D serum levels and BMD in the patient population attending the University of Alabama CF Center at Children's Hospital in Birmingham, Alabama. The results of the investigation provided evidence to reject both of the hypotheses, which were:

Hyp 1: There is a positive association between vitamin D serum status and bone mineral density. *($P<0.05$)*

Hyp 2: There is a positive association between vitamin D serum status and lung function. *($P<0.05$)*

In developing the hypotheses for the present study, there was expectation of finding statistically significant associations between serum vitamin D and BMD, and lung function. According to the CF Bone Consensus Statement, bone disease in CF is multifactorial with contributing factors of malabsorption of vitamin D, poor nutritional status, physical inactivity, glucocorticoid therapy, delayed pubertal maturation, and chronic pulmonary inflammation.[14] Specifically, the prevalence of vitamin D insufficiency and poor skeletal health is high in the US CF population. When vitamin D is low, calcium concentrations can become low. Calcium

concentrations are corrected by pulling calcium from the bones decreasing bone density. Therefore, an association between vitamin D serum levels and BMD seemed logical. Furthermore, because of vitamin D's anti-inflammatory property an association between vitamin D and lung function FEV1 values was also predicted.[19]

Evaluation of results of the current study showed no consistent significant associations between serum vitamin D and BMD. Associations with serum vitamin D were only seen at baseline in the total body z score of Spearman's correlation and in linear regressions at baseline of both total body and lumbar spine L2-L4. These associations suggest a greater vulnerability of vitamin D status at baseline than after 2 years. This is possibly due to not assessing calcium intake or because of the lower vitamin D serum levels seen at baseline before supplementation. Other studies compared in a meta-analysis showed similar results with associations at baseline in the patient's with low serum levels; however, there were no significant effects on BMD following supplementation.[17] This draws to question the strong emphasis on current guidelines on vitamin D. Is there another serum analyte that is more influential in the bone building process that would show greater associations with BMD? Another thought on the reasons behind the limited associations with BMD is the variety of different ages in the subjects and their different stages of growth. Subjects going through growth spurts experience longitudinal changes in bone before increased bone density takes place, which was not accounted for in the data collected in this population.

Furthermore, no significant associations were found in this study between serum vitamin D and FEV1 values of lung function. Although many studies support the idea of the anti-inflammatory property of vitamin D to increase lung function when serum levels are adequate, this study could not support this theory. This could likely be due to the overall mean serum

levels at baseline and post 2 years remaining low and not reaching the suggested adequate amount of 30 ng/mL. In Grossman's study, 90% of patients receiving vitamin D supplementation had improved lung functions measured by FEV1 values returning to 95% or greater from baseline.[8] These subjects receiving vitamin D supplementation had sufficient serum levels of 58.1 ng/mL after 1 week of treatment. Wolfenden's study also showed significant correlations with serum vitamin D and FEV1. Mean serum levels in this study were 58.8 ng/mL.[19] Both of these studies support the idea that sufficient vitamin D serum levels are needed to see any improvements in FEV1.

After supplementation, mean serum levels never reached the suggested adequate amount of 30 ng/mL in either group. Mean serum levels were actually higher in the 2000 IU group (28.188 ng/mL) than the 3000 IU group (26 ng/mL). Between group comparisons showed no significant differences in variables between groups either receiving vitamin D supplementation of 2000 IU or 3000 IU. This possibly suggests participants in the 3000 IU group may have a lower intestinal absorptive ability than the other group, and may require an even higher dose in order to be absorbed and increase serum levels.[14,23]

Pancreatic function decreases with age and with severity of obstruction.[23] Since age was not significantly different among groups it is also possible that patients were prescribed an increase in dose due to decreased compliance with medication, which in turn kept serum levels low, or an increased obstruction and severity of disease, which decreased absorption.

Additionally, this finding suggests that increasing the patient's vitamin D supplementation dose to 3000 IU was the correct treatment plan to increase serum levels and keep other variables of BMD in comparison to their peers on the lower 2000 IU supplementation.

Grossmann's study showed improved lung function in patient's receiving vitamin D supplementation during pulmonary exacerbations compared to placebos. However, unlike the present study, mean baseline serum levels were sufficient at 30.6ng/mL and increased to 58.1 ng/mL after 7 days of treatment.[8] Grossmann's study also used a single high dose of 250,000 IU, which is likely why serum levels increased quickly and showed positive changes in lung function. [8]

Apart from looking at serum vitamin D, fat mass and LBM showed associations with BMD in the data analysis. BMD at the L2-L4 and total body were associated with LBM and fat mass. The effects on LBM were greater than that of fat mass suggesting that LBM has a greater association with BMD. This shows that changes in body composition significantly impacts BMD. Specifically in CF patients, significant weight loss and malnutrition affecting body composition will be associated with lower levels of BMD. Of the patients studied at baseline, 6 were mildly malnourished and 2 were moderately malnourished based on weight z scores. When looking at BMI z scores 3 were mildly malnourished and 1 was moderately malnourished. After two years, 6 patients were mildly malnourished and 1 was severely malnourished based on weight z scores. When looking at BMI z scores 5 were mildly malnourished and 1 was severely malnourished.

Most of the literature focuses on recommendations to improve appetite and weight with an emphasis on a high calorie and high protein diet and increased weight bearing exercises for physical activity for CF patients to increase muscle mass.[13,14] This recommendation is supported with the results in the present study which shows the connection between BMD and LBM for the prevention of bone loss; however, results could additionally support the idea that LBM is of greater importance and in need of more attention in this area of care for these patients.

The present study contained limitations. First and foremost, the biggest limitation was not being able to record compliance of supplementation. This limitation was partially offset by keeping record of vitamin D serum levels and corresponding with Children's Rehabilitation Service (CRS) employees who track how often prescriptions are refilled. The lack of calcium intake data is a limitation in this study, as calcium plays a substantial role in vitamin D absorption. Another limitation was not being able to assess growth over the years of the study. The patient's ages varied throughout different phases of puberty and development, which is a time that greatly affects bone mass. Lastly, this study was limited by not addressing physical activity, which is a major contributor to bone mass.

While there were no consistent associations with serum vitamin D and BMD, baseline associations showed a link between low serum levels and BMD. Thus, supplementation for patients with deficient levels could result improvements in BMD, particularly in the lumbar spine and total body. Associations between BMD and LBM and fat mass support current recommendations of a weight bearing exercise to increase muscle mass. Other associations could possibly be identified, if additional data is collected and limitations are addressed to justify associations. Future research in this area should focus on interventions for preventative care of maintaining adequate vitamin D serum levels, high calorie high protein diets rich in calcium, and increased weight bearing exercise for optimal bone health.

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APPENDIX A

IRB APPROVAL FORMS

Office for Research

Institutional Review Board for the
Protection of Human Subjects

THE UNIVERSITY OF
ALABAMA
RESEARCH

January 12, 2016

Caroline Brantley
Dept. of Human Nutrition & Hospitality
The University of Alabama
Box 870311

Re: IRB # 16-OR-014-ME: "The Association between High Dose Vitamin D Supplementation and Bone Mineral Density in Pediatric Patients with Cystic Fibrosis"

Dear Ms. Brantley,

The University of Alabama Institutional Review Board has granted approval for your proposed research.

Your application has been given expedited approval according to 45 CFR part 46. You have also been granted a waiver of informed consent and waiver of HIPAA authorization. Approval has been given under expedited review category 5 and 7 as outlined below:

(5) Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected, solely for non-research purposes (such as medical treatment or diagnosis).

Your approval will expire on January 11, 2017. If the study continues beyond that date, you must complete the IRB Renewal Form within e-Protocol. If you modify the application, please complete the Revision Form. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants. When the study closes, please complete the Final Report Form.

Should you need to submit any further correspondence regarding this application, please include the assigned IRB approval number.

Good luck with your research.

Sincerely,

Carpantato T. Myles, MSM, CIM, CIP
Director & Research Compliance Officer
Office for Research Compliance

cc: Dr. Jeannine Lawrence

358 Rose Administration Building
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TOLL FREE (877) 820-3066

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 January 24, 2017. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56.

Principal Investigator:	GUTIERREZ, HECTOR H
Co-Investigator(s):	BRITTON, LACRECIA
Protocol Number:	**X080225007**
Protocol Title:	*Continuous Quality Improvement Changes Outcomes in Pediatric Cystic Fibrosis Patients*

The IRB reviewed and approved the above named project on 2-27-15. 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: 2-27-15

Date IRB Approval Issued: 2-27-15

IRB Approval No Longer Valid On: 2-27-16

HIPAA Waiver Approved?: Yes

Member - 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.

470 Administration Building
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irb@uab.edu

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Alabama at Birmingham
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AB 470
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THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

PI: GUTIERREZ, HECTOR H
Protocol # X080225007

UAB IRB Approval of Waiver of Informed Consent and/or Waiver of Patient Authorization

☑ **Approval of Waiver of Informed Consent to Participate in Research.** The IRB reviewed the proposed research and granted the request for waiver of informed consent to participate in research, based on the following findings:

1. The research involves no more than minimal risk to the subjects.
2. The research cannot practicably be carried out without the waiver.
3. The waiver will not adversely affect the rights and welfare of the subjects.
4. When appropriate, the subjects will be provided with additional pertinent information after participation.

Check one:
- ☑ **and** Waiver of Authorization (below)
- ☐ **or** Waiver of Authorization (below)
- ☐ Waiver of Authorization not applicable

☑ **Approval of Waiver of Patient Authorization to Use PHI in Research.** The IRB reviewed the proposed research and granted the request for waiver of patient authorization to use PHI in research, based on the following findings:

1. The use/disclosure of PHI involves no more than minimal risk to the privacy of individuals
   i. There is an adequate plan to protect the identifiers from improper use and disclosure.
   ii. There is an adequate plan to destroy the identifiers at the earliest opportunity consistent with conduct of the research, unless there is a health or research justification for retaining the identifiers or such retention that is otherwise required by law.
   iii. There is an assurance that the PHI will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study, or for other research for which the use or disclosure of PHI would be permitted.
2. The research cannot practicably be conducted without the waiver or alteration.
3. The research cannot practicably be conducted without access to and use of the PHI.

—OR—

☐ **Full Review**

The IRB reviewed the proposed research at a **convened meeting** at which a majority of the IRB was present, including one member who is not affiliated with any entity conducting or sponsoring the research, and not related to any person who is affiliated with any of such entities. The waiver of authorization was approved by the majority of the IRB members present at the meeting.

Date of Meeting

Signature of Chair, Vice-Chair or Designee

Date

☑ **Expedited Review**

The IRB used an **expedited review procedure** because the research involves no more than minimal risk to the privacy of the individuals who are the subject of the PHI for which use or disclosure is being sought. The review and approval of the waiver of authorization were carried out by the Chair of the IRB, or by one of the Vice-Chairs of the IRB as designated by the Chair of the IRB.

2-27-15
Date of Expedited Review

Signature of Chair, Vice-Chair or Designee

2-27-15
Date

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APPENDIX B

UAB IRB AMMENDMENT

UAB

Project Revision/Amendment Form

irb

Form version: June 26, 2012

18087

In MS Word, click in the white boxes and type your text; double-click checkboxes to check/uncheck.

- **Federal regulations require IRB approval before implementing proposed changes. See Section 14 of the IRB Guidebook for Investigators for additional information.**
- **Change means any change, in content or form, to the protocol, consent form, or any supportive materials (such as the Investigator's Brochure, questionnaires, surveys, advertisements, etc.). See Item 4 for more examples.**

1. Today's Date	November 3, 2015

2. Principal Investigator (PI)

Name (with degree)	Hector Gutierrez, MD	**Blazer ID**	Pulmon
Department	Pediatrics	**Division (if applicable)**	Pulmonary
Office Address	Lowder Bldg, Suite 620	**Office Phone**	638-9583
E-mail	hgutierrez@peds.uab.edu	**Fax Number**	975-5983

Contact person who should receive copies of IRB correspondence (Optional)

Name	LaCrecia Britton Thomas	**E-Mail**	LaCrecia.Thomas@childrensal.org
Phone	638-5489	**Fax Number**	975-5983
Office Address (if different from PI)			

3. UAB IRB Protocol Identification

3.a. Protocol Number	X080225007
3.b. Protocol Title	Continuous Quality Improvement Changes Outcomes in Pediatric Cystic Fibrosis Patients

3.c. Current Status of Protocol—Check ONE box at left; provide numbers and dates where applicable

- ☐ **Study has not yet begun** — **No participants, data, or specimens have been entered.**
- ☒ **In progress, open to accrual** — **Number of participants, data, or specimens entered:** 438
- ☐ **Enrollment temporarily suspended by sponsor**
- ☐ **Closed to accrual, but procedures continue as defined in the protocol (therapy, intervention, follow-up visits, etc.)**
  - **Date closed:**
  - **Number of participants receiving interventions:**
  - **Number of participants in long-term follow-up only:**
- ☐ **Closed to accrual, and only data analysis continues**
  - **Date closed:**
  - **Total number of participants entered:**

4. Types of Change

Check all types of change that apply, and describe the changes in Item 5.c. or 5.d. as applicable. To help avoid delay in IRB review, please ensure that you provide the required materials and/or information for each type of change checked.

- ☐ **Protocol revision (change in the IRB-approved protocol)**
  In Item 5.c., if applicable, provide sponsor's protocol version number, amendment number, update number, etc.
- ☐ **Protocol amendment (addition to the IRB-approved protocol)**
  In Item 5.c., if applicable, provide funding application document from sponsor, as well as sponsor's protocol version number, amendment number, update number, etc.
- ☒ **Add or remove personnel**
  In Item 5.c., include name, title/degree, department/division, institutional affiliation, and role(s) in research, and address whether new personnel have any conflict of interest. See "Change in Principal Investigator" in the IRB Guidebook if the principal investigator is being changed.
  - ☒ **Add graduate student(s) or postdoctoral fellow(s) working toward thesis, dissertation, or publication**
    In Item 5.c., (a) identify these individuals by name; (b) provide the working title of the thesis, dissertation, or publication; and (c) indicate whether or not the student's analysis differs in any way from the purpose of the research described in the IRB-approved HSP (e.g., a secondary analysis of data obtained under this HSP).
- ☐ **Change in source of funding; change or add funding**
  In Item 5.c., describe the change or addition in detail, include the applicable OSP proposal number(s), and provide a copy of the application as funded (or as submitted to the sponsor if pending). Note that some changes in funding may require a new IRB application.

☐ **Add or remove performance sites**
In Item 5.c., identify the site and location, and describe the research-related procedures performed there. If adding site(s), attach notification of permission or IRB approval to perform research there. Also include copy of subcontract, if applicable. If this protocol includes acting as the Coordinating Center for a study, attach IRB approval from any non-UAB site added.

☐ **Add or change a genetic component or storage of samples and/or data component—this could include data submissions for Genome-Wide Association Studies (GWAS)**
To assist you in revising or preparing your submission, please see the IRB Guidebook for Investigators or call the IRB office at 934-3789.

☐ **Suspend, re-open, or permanently close protocol to accrual of individuals, data, or samples (IRB approval to remain active)**
In Item 5.c., indicate the action, provide applicable dates and reasons for action; attach supporting documentation.

☐ **Report being forwarded to IRB (e.g., DSMB, sponsor or other monitor)**
In Item 5.c., include date and source of report, summarize findings, and indicate any recommendations.

☐ **Revise or amend consent, assent form(s)**
Complete Item 5.d.

☐ **Addendum (new) consent form**
Complete Item 5.d.

☐ **Add or revise recruitment materials**
Complete Item 5.d.

☐ **Other (e.g., investigator brochure)**
Indicate the type of change in the space below, and provide details in Item 5.c. or 5.d. as applicable.
Include a copy of all affected documents, with revisions highlighted as applicable.

5. Description and Rationale

In Item 5.a. and 5.b, check Yes or No and see instructions for Yes responses.
In Item 5.c. and 5.d, describe—and explain the reason for—the change(s) noted in Item 4.

☐ Yes ☐ No **5.a. Are any of the participants enrolled as normal, healthy controls?**
If yes, describe in detail in Item 5.c. how this change will affect those participants.

☐ Yes ☐ No **5.b. Does the change affect subject participation, such as procedures, risks, costs, location of services, etc.?**
If yes, FAP-designated units complete a FAP submission and send to fap@uab.edu. Identify the FAP-designated unit in Item 5.c.
For more details on the UAB FAP, see www.uab.edu/cto.

5.c. Protocol Changes: In the space below, briefly describe—and explain the reason for—all change(s) to the protocol.

Updated SIRP. dkw

- The following names should be removed from the protocol as they are no longer employees at Children's/UAB: Janet Brown, Sharan Robbins, and Sophie Burge.
- Add the following personnel: Isabel Virella-Lowell, MD (no conflict of interest), Jennifer Guimbellot, MD (no conflict of interest). Both are pediatric pulmonologist in the Department of Pediatrics at UAB.
- Add graduate student and nutrition trainee Caroline Brantley, RD to the protocol. She plans to use existing data that has been collected as part of this project to improve the nutritional status of patients with Cystic Fibrosis. The proposed title of her thesis is Vitamin D supplementation and the effects on bone mineral density in pediatric patients with Cystic Fibrosis (no conflict of interest).

5.d. Consent and Recruitment Changes: In the space below,
(a) describe all changes to IRB-approved forms or recruitment materials and the reasons for them;
(b) describe the reasons for the addition of any materials (e.g., addendum consent, recruitment); and
(c) indicate either how and when you will reconsent enrolled participants or why reconsenting is not necessary (not applicable for recruitment materials).

Also, indicate the number of forms changed or added. For new forms, provide 1 copy. For revised documents, provide 3 copies:
- **a copy of the currently approved document (showing the IRB approval stamp, if applicable)**
- **a revised copy highlighting all proposed changes with "tracked" changes**
- **a revised copy for the IRB approval stamp.**

Signature of Principal Investigator _____ Date NOV 03, 2015

FOR IRB USE ONLY

☐ Received & Noted ☑ Approved Expedited* ☐ To Convened IRB

Signature (Chair, Vice-Chair, Designee) 11-6-15 Date

DOLA 2/27/15

Change to Expedited Category Y / N / NA

*No change to IRB's previous determination of approval criteria at 45 CFR 46.111 or 21 CFR 56.111

UAB MEDICINE
PEDIATRICS

Children's of Alabama

November 3, 2015

TO: UAB IRB
FROM: Valerie Tarn, MS, RD
RE: Amendment Form - **X080225007**

I have included a project revision/amendment form for protocol: Continuous Quality Improvement Changes Outcomes in Pediatric Cystic Fibrosis Patients. If you have any questions, please contact me or Lacrecia Thomas at 205-638-9583 (main pulmonary office).

Thanks for your time,

Valerie Eubanks Tarn, MS, RD
PPC Nutrition Faculty and Training Director

UAB Department of Pediatrics, Division of Pulmonary and Sleep Medicine, Lowder Suite 620
1600 7th Avenue South, Birmingham, AL 35233 tel 205-638-9583 fax 205-975-5983 www.peds.uab.edu