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Relationship Between Estrogen Treatment and Skeletal Health in Women with Cystic Fibrosis

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Abstract

Background—Patients with cystic fibrosis (CF) are at risk for CF-related bone disease. Women with CF may use estrogen supplementation for reasons other than skeletal health. It is unknown whether estrogen therapy has a beneficial impact on skeletal health in women with CF.

Methods—In this retrospective cohort study of women with CF followed at a single CF center, the lumbar spine bone mineral density (BMD) of women with CF exposed to supplemental and not exposed to supplemental estrogen were compared. Spline function models included the main effect of estrogen exposure and the interaction between the age and estrogen supplementation.

Results—Of the 145 subjects analyzed: 44 subjects were exposed to supplemental estrogen. The baseline characteristics of estrogen exposed and unexposed subjects were similar except for use of CFTR modulators and anti-osteoporosis medications. Women exposed to estrogen reached peak BMD around 21 years of age, but women not exposed to estrogen reached peak BMD around 25

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Authors Contributions Statement:

M.W. and V.T. conceived the project; M.W. and N.A. collected data; M.W., V.T., E.L.B., and C.M. designed the analysis; M.W., E.L.B., and C.M. performed analysis; M.W. and V.T. wrote the first draft; all authors reviewed and edited the manuscript; V.T. supervised.

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years of age. A significant interaction of age and estrogen supplementation indicated that the lumbar spine BMD trajectories differed by exposure group.

Conclusions—Our study demonstrates that few women with CF of reproductive age are prescribed estrogen therapy. Furthermore, estrogen exposure up to age 21 is associated with improved BMD, but estrogen exposure after age 21 does not appear to be associated with improved BMD. Further studies are needed to understand the reasons for the low rates of estrogen use in young women with CF and the optimal timing, dose and formulation of estrogen prescription.

Keywords

Adolescent; Estradiol; Hypogonadism/drug therapy; Vitamin D; Women's Health/statistics and numerical data

Introduction

Advancements in therapies for cystic fibrosis (CF) have increased the median expected survival of patients with CF to 44 years.¹ With this improved survival, the prevalence of extrapulmonary manifestations such as CF-related bone disease (CFBD) have also increased. CFBD increases the risk for low-trauma fractures² and vertebral fractures.³ Vertebral compression fractures diagnosed by chest x-ray may affect up to 27% of patients with CF.⁴ Thoracic vertebral fractures limit the ability of patients to perform daily therapies necessary to maintain optimal lung health.³

CFBD is a multifactorial disease affected by CF transmembrane conductance regulator (CFTR) dysfunction, vitamin D and K deficiency relating to exocrine pancreatic insufficiency, calcium deficiency, malnutrition, delayed puberty and hypogonadism, decreased physical activity, chronic infections, systemic inflammation, use of chronic glucocorticoids and CF-related diabetes.^{5,6} Primary prevention of osteoporosis includes optimizing these factors contributing to CFBD. Estrogen supplementation is used by females for treatment of delayed puberty and hypogonadism, but also for other indications including contraception, menses regulation and symptoms of hyperandrogenism. There are no published investigations of the association of estrogen supplementation for bone health in women with CF or estrogen treatment for hypogonadal women with CFBD.

We sought to examine the BMD of women followed in our center classified by estrogen status using a retrospective cohort study design. We identified women with CF who have been prescribed estrogen therapy and those who have not been prescribed estrogen therapy over the past 19 years in our center. We hypothesized that women prescribed estrogen would have higher lumbar spine BMD than women not prescribed estrogen in this 19-year retrospective cohort analysis of women followed in our CF center.

Methods

Study Design

We conducted a retrospective cohort study of women with CF seen at a single adult CF clinic investigating the association of estrogen exposure on bone mineral density measured by dual x-ray absorptiometry (DXA). This study was approved by the Emory University Institutional Review Board (IRB) and Children's Healthcare of Atlanta IRB.

Subject selection

Female subjects were included if they had a diagnosis of CF and had a DXA performed as part of their care. Subjects were excluded if they were followed for a non-CF diagnosis or had no DXA testing. Subjects' data was censored after their most recent DXA.

Data Sources

Subjects were identified from the Emory University Clinical Data Warehouse (CDW) as having an encounter with a cystic fibrosis related diagnosis code and/or an encounter with one of the healthcare providers in the Emory CF clinic between 01/01/2000 and 06/30/2019. The CDW is a repository that integrates data from multiple business and clinical applications within the Emory Healthcare system including electronic health record (EHR) used inpatient, outpatient and for radiologic studies as well as billing platforms. The clinic notes of the subjects were reviewed to confirm diagnosis of CF. Subjects were considered to have CF if the patient was being treated in Emory CF Center and had typical clinical characteristics including CF comorbidities and typical treatments such as CFTR modulator, combined with diagnostic testing with a positive sweat test and/or genetic testing consistent with CF. Subjects with non-CF diagnoses such as CFTR-RD, CRMS and bronchiectasis were excluded.

All DXA reports, laboratory results pertaining to bone health and prescribed medication lists were extracted from the CDW. Two instances of vitamin D results were below the lower limit of the assay and were analyzed as half the lower limit. Subjects' name and date of birth were used to match to records in the pediatric clinic of the same CF center. If there were pediatric records in the EHR, DXA reports were extracted from the EHR as well. The first clinic visit of each year was reviewed to extract information about comorbid diagnoses (CF-related diabetes, exocrine pancreatic insufficiency, transplantation status), demographic information (including CF genotype, height, weight, age, race, ethnicity) and patient-reported medication list. If there was a change in a covariate of interest, for example initiation of estrogen supplementation, the interval clinic notes were reviewed to identify when the health status changed. Additionally, the most recent clinic visit in the EHR was reviewed. If the clinic notes were unclear, a second investigator also reviewed the chart to clarify covariate status. Subjects with CF are recommended to have a follow-up four times per year in their CF center; however, acute pulmonary exacerbations and hospitalizations may affect the number of visits each year.

Estrogen exposure

The index date of estrogen exposure was the first note documenting estrogen exposure in the EHR. Some subjects had used multiple formulations of estrogen during the follow-up period. Each documented product was given equal weight in calculating the average dose of estrogen used by each subject.

Bone mineral density

The Z-score and areal BMD measured in g/cm^2 of the lumbar spine (L1-L4), femoral neck and total hip were extracted from the DXA reports. As our CI- center encompasses a wide catchment area, there were multiple facilities with DXA scanners available to patients. DXAs were measured by both Hologic programs (Hologic, Inc., Malborough, MA, USA) and Lunar programs (GE Healthcare, Madison, WI, USA). As more DXAs were performed on GE scanners, aBMD of Hologic scans was converted to GE equivalent by industry accepted conversion formulas.⁷ Changes in protocol over time at different facilities resulted in the BMD of different body sites reported. All DXAs analyzed had measured BMD at the lumbar spine. Double entry by two people was performed for 78% of DXA reports to verify validity of manually extracted data. In the remaining 22% of DXA reports, double entry was performed by a single person at two different times.

Statistical Analysis

Descriptive statistics were calculated for all variables of interest. Categorical variables were reported as counts and percentages, and continuous variables were reported as median and interquartile range (25th – 75th). Demographic and clinical characteristics were compared between groups (e.g., estrogen exposed and unexposed using Chi-square tests or Wilcoxon rank sum tests).

Prior to analysis, lumbar spine Z-scores and lumbar spine BMD were plotted across a range of patient ages. The best fit line of the data points was determined with a local nonparametric regression function. Given the non-linear relationship between age and lumbar spine measures, data were analyzed using polynomial spline functions to accurately model the non-linear trajectory. Linear, quadratic and cubic functions were considered, but ultimately linear functions were chosen as it provided the best fit to the data with the most parsimonious model. Furthermore, because some subjects were evaluated multiple times over the study period and entered the study at different ages, a subject-specific random intercept was included to account for subject-level variation in lumbar spine measurement.

To determine the impact of estrogen exposure on these outcomes, models included the main effect of estrogen exposure and the interaction between the spline function and estrogen. A significant interaction indicated that the lumbar spine trajectories differed by exposure group. Post-hoc comparisons were made to determine ages where the two groups differed. Models were adjusted for BMI, DelF508 mutation, pancreatic insufficiency and cystic fibrosis-related diabetes. Predicted values were estimated from the spline function with respective confidence intervals and were overlaid on raw data. Models also adjusting for vitamin D status were performed in a subset of subjects with known vitamin D status. As vitamin D status fluctuates with treatment and supplementation, vitamin D results measured

for routine clinical care do not capture the subjects' vitamin D status over time. Therefore, subjects were included in the models as "ever" vitamin D deficient (<20 ng/mL), insufficient (20 – 29 ng/mL) and sufficient (≥ 30 ng/mL).

Some women in the study had exposure to anti-osteoporosis medications which may confound the results. Therefore, a sensitivity analysis excluding subjects who were ever prescribed anti-osteoporosis medications (bisphosphonates or PTH analogs) was also performed in our primary models. Analysis was conducted using SAS v. 9.4 (Cary, NC) and statistical significance was assessed at the 0.05 level.

Results

Subjects

A total of 145 subjects with CF were analyzed (Figure 1). Twenty subjects were identified to have estrogen exposure at the start of the observation period and an additional 24 subjects were initiated on estrogen during the follow-up period. Forty-four subjects were prescribed estrogen in the cohort.

Subject characteristics at baseline and time of last DXA

Women with CF who were exposed to estrogen and not exposed to estrogen at the beginning of the observation period were similar in age, BMI, race, ethnicity, CFTR mutation, FEV1, pancreatic status, presence of CFRD, transplantation status and vitamin D status (Table 1). These demographics were similar at the end of the observation period (Table 2). Use of CFTR modulators and anti-osteoporosis therapy (bisphosphonates and PTH analogs) was slightly different between the two groups at baseline (Table 1), but not at the end of the observation period. BMI was 1 kg/m² statistically significantly lower in the estrogen-exposed group at the end of the observation period (Table 2). The estrogen-exposed group had lower lumbar spine BMD Z-score at baseline and at the last DXA (Tables 1 and 2). Baseline characteristics of subjects who were younger than or older than 21 years of age were similar (Table 3).

Estrogen use

The majority of subjects who were exposed to estrogen used oral combination ethinyl estradiol with progesterone. The median ethinyl estradiol dose was 30 mcg/day, range 10 – 50 mcg/day. Three women used transvaginal combination ethinyl estradiol and progesterone. One woman used transdermal combination ethinyl estradiol and progesterone. One woman used transdermal estradiol without progesterone; this same woman had also used oral conjugated estrogens. The median age at initiation of estrogen supplementation in subjects younger than 45 years old was 23.9 years (interquartile range 19.8 – 27.3 years).

Bone mineral density

Subjects' lumbar spine bone mineral density vs age by estrogen supplementation exposure is shown in Figure 2. The mean number of DXAs per subject was 2.9 (minimum 1, maximum 8). The median number of DXAs per subject was 2 (interquartile range 1 – 4).

Anti-osteoporosis medications

Thirteen of the 145 subjects in the cohort were prescribed an antiresorptive (bisphosphonates: alendronate, ibandronate, risedronate or zoledronate) or osteoanabolic (PTH analogs: teriparatide or abaloparatide) medication. Of these subjects, 7 subjects (53.8%) were prescribed bisphosphonates only, 3 subjects (23.1%) were prescribed PTH analogs only, and 3 subjects (23.18%) were prescribed both antiresorptive and osteoanabolic medications during the observation period. The median age at first prescription of bisphosphonate was 28.8 years (interquartile range 21.1 – 31.8). The median age at first prescription of PTH analog was 55.4 years (interquartile range 44.2 – 63.5).

Vitamin D status

Of the 145 subjects in the cohort, 89 subjects (61.3%) had a documented 25-hydroxyvitamin D result. Of these subjects with a 25(OH)D result, the median number of serum 25(OH)D results per subjects was 2 (interquartile range 1 – 4). The mean documented serum 25(OH)D result was 33.0 ng/mL (SD 14.9, range <5 – 99 ng/mL). Of all documented serum 25(OH)D results, 58.1% of results were at least 30 ng/mL. Of subjects with a documented serum 25(OH)D result, 78.7% subjects had at least one measured serum 25(OH)D result that was at least 30 ng/mL (Table 4).

Spline model

Polynomial spline functions were used to model estrogen supplementation and the interaction between age and estrogen exposure. There was statistically significant interaction between estrogen and age (Table 5). The model was adjusted for BMI, homozygous and heterozygous DelF508, exocrine pancreatic insufficiency and CF-related diabetes. Predicted values from the spline models were overlaid on raw data (Figures 3 and 4). A sensitivity analysis was performed using our primary models and excluding patients who had been prescribed anti-osteoporosis medications (bisphosphonates or PTH analogs). Similar results were found in that the interaction between estrogen and age were significant in younger patients (17 – 24 years old, p-value <0.001, and 17 – 27 years old, p-value <0.001).

When vitamin D status was included in the model, interaction of age and estrogen remained statistically significant in models with younger patients (17 – 24 years old, p-value < 0.001, and 17 – 27 years old, p-value < 0.001). Estrogen also had a significant contribution to models of patients 17 – 27 years old when adjusting for subjects ever being vitamin D deficient (p-value 0.031) and vitamin D insufficient (p-value 0.029).

Discussion

This study focused on the effect of supplemental estrogen exposure on lumbar spine bone mineral density in women with CF. This retrospective cohort analysis of women with CF followed at a single center suggests that the timing of estrogen exposure affects lumbar spine BMD as measured by DXA. The model suggests that the most benefit of estrogen supplementation comes from early supplementation before the age of 21. Like healthy adolescents, the majority of bone accrual in adolescents with CF occurs during puberty.⁸

However, women with CF have puberty later than healthy peers, although still within normal limits.^{9,10} Our study suggests that women with CF continue to accrue bone until age 25.

Previous studies of estrogen therapy in women with CF have focused on outcomes including lung inflammation measured in sputum (IL1beta, IL8, leukocytes, percentage of neutrophils)¹¹, lung function (FEV1, CFQ-R respiratory domain scores)^{11–13}, number and frequency of pulmonary exacerbations.^{11,13,14} These studies have investigated oral estrogens or observed subjects' use of their own chosen estrogen-containing medication. Overall, these studies have suggested beneficial effects of supplemental estrogen in regards to inflammation, exacerbations and lung function.^{11–14} Estrogen supplementation association with bone mineral density in women with CF has not been published.

Our study suggests that women exposed to supplemental estrogen reached their peak bone mass four years earlier than women not exposed to supplemental estrogen. Supplemental estrogen may enable these women to reach this peak at an earlier time. However, these estrogen-exposed women did not maintain their peak BMD and had a decline in BMD. This may reflect the estrogen therapy used by the subject was inadequate for maintaining BMD. Estrogen therapy may have been inadequate due to the route (oral, transdermal, or transvaginal), dose, or formulation (ethinyl estradiol, estradiol or even conjugated estrogens). Recent studies have raised concerns in other adolescent and young adult females with hypogonadism due to Turner Syndrome¹⁵ and endurance exercise¹⁶ that transdermal estradiol may be superior to oral estrogen formulations for accruing bone mass.

The 2005 CFF guidelines for CFBD³ recommend screening for CFBD with DXA starting at age 8 for patients with risk factors of CFBD and universally at age 18. Patients with a low BMD Z-score < -1 and > -2 should be treated for hypogonadism or delayed puberty. Similar screening is recommended by the 2011 European CFBD guidelines.² In the CFF guidelines, Aris et al do not make recommendations regarding dose, route or formulation of estrogen to be used to treat hypogonadism or delayed puberty to optimize bone health. In the European guidelines, SermetGaudalas et al recommend evaluating for female hypogonadism with estradiol and sex hormone binding globulin levels without diagnostic criteria. Hughan et al have made recommendations for evaluation of menstrual irregularity with luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estradiol into evaluation for other secondary causes, and presumably standards of care for non-CF women would be applied to diagnose hypogonadism.¹⁷ Putman et al further reinforces the CFBD guidelines to treat hypogonadism and highlights the lack of data to support a particular formulation of estrogen over another in women with CF for optimization of bone health.⁵

A strength of this study was a relatively large cohort of 145 subjects for a rare disease, although it was limited by the small number of subjects exposed to estrogen (30.3% of subjects). A cross-sectional study conducted at our center found an estrogen prescription rate of 24.5%.¹⁸ A study of 42 women with CF in the UK found that 60% had at any time used a combined oral contraceptive; however, only 16.7% of participants were using combined oral contraceptive at the time of the survey.¹⁹ These low prescription rates highlight the need to screen and treat CF-related hypogonadism.

Another strength of this study was that 78.7% of subjects with a measured 25-hydroxyvitamin D result had at least one test within goal (at least 30 ng/mL), and the mean 25(OH)D result was also greater than 30 ng/mL. The fluctuations in vitamin D status within each subject in our cohort reflects that our cohort was being treated for vitamin D deficiency during the observation period. Vitamin D sufficiency plays a key role in optimizing bone health,^{20,21} and vitamin D status may serve important roles in optimizing extra-skeletal health.²⁰ The 2012 CFF guidelines for vitamin D Deficiency recommend an annual assessment of vitamin D status by measuring a serum 25-hydroxyvitamin D level and a goal of at least 30 ng/mL.²¹ When vitamin D status was included in models of 17 – 27 year olds, estrogen did have a significant effect on lumbar spine trajectory. This further underscores the importance of treating vitamin D deficiency and insufficiency according to the CFF guidelines.

Limitations of this study include potential misclassification bias regarding estrogen supplementation status due to reliance on documentation in the electronic health record. The gonadal status and reason for estrogen supplementation use was unknown. Systemic glucocorticoid use, a known modifier of bone health, could not be reliably extracted from the EHR and was not included in the models. As our CF center encompasses a wide catchment area, there were multiple locations with DXA scanners available to patients using Hologic and GE programs. Changes in protocol over time at different locations resulted in the BMD of different body sites reported. This study was further limited as it relied on documentation obtained as part of routine healthcare. In 2005, CFF CFBD guidelines recommended screening all patients with CF older than 18 years with a DXA at least every 5 years or more frequently if risk factors for CFBD or low bone mineral density. The median 5-year DXA rate of subjects in the CFF Patient Registry remains 55% which is consistent with our center's data. Furthermore, our study examined patients in a 19 year follow up period during which significant advancements in therapies available for CF were made. However, in two similar cohorts of patients with CF from 1995–1999 and 2011–2013 at a single CF center, areal BMD of the spine was similar in both cohorts.²²

Conclusion

Our study demonstrates that few women with CF of reproductive age are prescribed estrogen therapy. Furthermore, estrogen exposure up to age 21 is associated with improved bone density. Estrogen exposure after age 21 does not appear to be associated with improved bone density. Further studies are needed to understand the reasons for the low rates of estrogen use in young women with CF and the optimal timing, dose and formulation of estrogen prescription.

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Figure 1.
Flow diagram of subjects analyzed.

Figure 2. Lumbar spine bone mineral density Z-score in women exposed to estrogen and not exposed to estrogen by age.

Subjects who had been previously exposed to supplemental estrogen (red circles) with superimposed best fit line (red solid line) overlap with subjects who had not been exposed to supplemental estrogen (blue triangles) with superimposed best fit line (blue dashed line). The best fit lines are significantly different ($p < 0.001$).

Figure 3. Lumbar spine BMD trajectories in women 17 – 24 years old exposed to estrogen and not exposed to estrogen.

Predicted lumbar spine BMD of subjects 17–24 years old exposed to estrogen (red long dashed line with red dotted line indicating 95% confidence intervals) and not exposed to estrogen (blue solid line with blue dotted line indicating 95% confidence intervals) were statistically significantly different ($p < 0.001$). Predicted models are superimposed over data of estrogen exposed subjects (red circle) and unexposed subjects (blue triangle).

Figure 4. Lumbar spine BMD trajectories in women 25 – 35 years old exposed to estrogen and not exposed to estrogen.

Predicted lumbar spine BMD of women 25 – 35 years old exposed to estrogen (red long dashed line with red dotted line indicating 95% confidence intervals) and not exposed to estrogen (blue solid line with blue dotted line indicating 95% confidence intervals) were statistically significantly different ($p = 0.043$). Predicted models are superimposed over data of estrogen exposed subjects (red circle) and unexposed subjects (blue triangle).

Table 1:

Baseline characteristics of subjects

Variable	Level	N	Overall N = 145	Estrogen Unexposed N = 130	Estrogen Exposed N = 15	P-Value
Patient Age		145	25.20 (19.30, 32.90)	24.9 (18.80, 33.40)	26.8 (20.90, 32.90)	0.189
BMI (kg/m ²)		144	21.32 (19.70, 23.90)	21.40 (19.70, 24.40)	20.40 (19.80, 21.94)	0.237
FEV1 (% predicted)		129	75.0 (52.0, 85.0)	75.0 (50.0, 86.0)	75.0 (62.0, 84.0)	0.483
Race	Caucasian	144	131 (90.97%)	118 (91.47%)	13 (86.67%)	0.539
	Not Caucasian		13 (9.03%)	11 (8.53%)	2 (13.33%)	
	Homozygous		58 (40.00%)	53 (40.77%)	5 (33.33%)	
F508 Mutation	Heterozygous	145	60 (41.38%)	55 (42.31%)	5 (33.33%)	0.302
	None		27 (18.62%)	22 (16.92%)	5 (33.33%)	
CFTR Modulator	No	145	141 (97.24%)	128 (98.46%)	13 (86.67%)	0.008
	Yes		4 (2.76%)	2 (1.54%)	2 (13.33%)	
CF-Related Diabetes	No	145	117 (80.69%)	104 (80.00%)	13 (86.67%)	0.536
	Yes		28 (19.31%)	26 (20.00%)	2 (13.33%)	
Exocrine Pancreatic Insufficiency	No	144	28 (19.44%)	25 (19.38%)	3 (20.00%)	0.954
	Yes		116 (80.56%)	104 (80.62%)	12 (80.00%)	
Previous Lung or Liver Transplant	No	145	143 (98.62%)	129 (99.23%)	14 (93.33%)	0.064
	Yes		2 (1.38%)	1 (0.77%)	1 (6.67%)	
Anti-osteoporosis Therapy*	No	145	140 (96.55%)	128 (98.46%)	12 (80.00%)	0.008
	Yes		5 (3.45%)	2 (1.54%)	3 (20.00%)	
	Ever Deficient	89	38 (42.70%)	36 (45.00%)	2 (22.22%)	0.291
Vitamin D Status**	Ever Insufficient	89	52 (58.43%)	47 (58.75%)	5 (55.56%)	1.000
	Ever Sufficient	89	70 (78.65%)	63 (78.76%)	7 (77.78%)	1.000
Lumbar Spine Z-Score		145	-0.20 (-1.10, 0.50)	-0.10 (-1.00, 0.50)	-0.90 (-1.80, -0.60)	0.035

Continuous variables are described as median (interquartile range), and categorical variables are described as subjects (proportion).

* Anti-osteoporosis therapy includes bisphosphonates and PTH analogs.

** Vitamin D status groups are not mutually exclusive. Therefore, overall percentages will not add up to 100%. Ever deficient includes any subjects who had a 25(OH)D result < 20 ng/mL. Ever insufficient includes any subjects who had a 25(OH)D result between 20 – 29 ng/mL. Ever sufficient includes any subjects who had a 25(OH)D result ≥ 30 ng/mL.

Table 2:

Characteristics of subjects at time of their last DXA

Variable	Level	N	Overall N = 145	Estrogen Unexposed N = 101	Estrogen Exposed N = 44	P-Value
Patient Age		145	28.6 (23.7, 37.6)	29.3 (24.3, 38.3)	27.9 (22.95, 33.85)	0.227
BMI (kg/m ²)		144	21.55 (19.7, 24.05)	21.9 (19.7, 25.15)	20.85 (19.65, 22.7)	0.004
FEV1 (% predicted)		140	72.5 (46.0, 85.0)	71.0 (43.0, 85.0)	75.0 (53.0, 84.0)	0.549
Race	Caucasian	145	132 (91.03%)	90 (89.11%)	42 (95.45%)	0.219
	Not Caucasian		13 (8.97%)	11 (10.89%)	2 (4.55%)	
	Homozygous		59 (40.69%)	42 (41.58%)	17 (38.64%)	
F508 Mutation	Heterozygous	145	58 (40.00%)	38 (37.62%)	20 (45.45%)	0.633
	None		28 (19.31%)	21 (20.79%)	7 (15.91%)	
CFTR Modulator	No	145	133 (91.72%)	94 (93.07%)	39 (88.64%)	0.373
	Yes		12 (8.28%)	7 (6.93%)	5 (11.36%)	
CF-Related Diabetes	No	145	105 (72.41%)	73 (72.28%)	32 (72.73%)	0.956
	Yes		40 (27.59%)	28 (27.72%)	12 (27.27%)	
Exocrine Pancreatic Insufficiency	No	145	18 (12.41%)	13 (12.87%)	5 (11.36%)	0.8
	Yes		127 (87.59%)	88 (87.13%)	39 (88.64%)	
Previous Lung or Liver Transplant	No	145	140 (96.55%)	98 (97.03%)	42 (95.45%)	0.633
	Yes		5 (3.45%)	3 (2.97%)	2 (4.55%)	
Anti-osteoporosis Therapy*	No	145	132 (91.03%)	93 (92.08%)	39 (88.64%)	0.505
	Yes		13 (8.97%)	8 (7.92%)	5 (11.36%)	
	Ever Deficient	89	38 (42.70%)	27 (44.26%)	11 (39.29%)	0.658
Vitamin D Status**	Ever Insufficient	89	52 (58.43%)	34 (55.74%)	18 (64.29%)	0.447
	Ever Sufficient	89	70 (78.65%)	47 (77.05%)	23 (82.14%)	0.586
Lumbar Spine Z-Score		145	-0.20 (-1.10, 0.50)	-0.10 (-1.00, 0.60)	-0.70 (-1.50, 0.20)	0.039

Continuous variables are described as median (interquartile range), and categorical variables are described as subjects (proportion).

* Anti-osteoporosis therapy includes bisphosphonates and PTH analogs.

** Vitamin D status groups are not mutually exclusive. Therefore, overall percentages will not add up to 100%. Ever deficient includes any subjects who had a 25(OH)D result < 20 ng/mL. Ever insufficient includes any subjects who had a 25(OH)D result between 20 – 29 ng/mL. Ever sufficient includes any subjects who had a 25(OH)D result ≥ 30 ng/mL.

Table 3:

Baseline characteristics of subjects less than and older than 21 years of age

Variable	Level	N	Overall N = 145	< 21 years old N = 50	21 years old N = 95	P-Value
BMI (kg/m ²)		144	22.69 (19.70, 23.60)	21.05 (19.7, 24.40)	21.46 (19.7, 23.60)	0.5065
FEV1 (% predicted)		129	75.0 (52.0, 85.0)	79.0 (56.0, 97.0)	73.5 (50.0, 84.0)	0.108
Estrogen Exposure	Unexposed	145	130 (89.66%)	46 (92.00%)	84 (88.42%)	0.501
	Exposed		15 (10.34%)	4 (8.00%)	11 (11.58%)	
Race	Caucasian	144	131 (90.97%)	48 (96.00%)	83 (88.30%)	0.1247
	Not Caucasian		13 (9.03%)	2 (4.00%)	11 (11.70%)	
	Homozygous		60 (41.38%)	25 (50.00%)	35 (36.84%)	
F508 Mutation	Heterozygous	145	58 (40.00%)	19 (38.00%)	39 (41.05%)	0.198
	None		27 (18.62%)	6 (12.00%)	21 (22.11%)	
CFTR Modulator	No	145	141 (97.24%)	48 (96.00%)	93 (97.89%)	0.508
	Yes		4 (2.76%)	2 (4.00%)	2 (2.11%)	
CF-Related Diabetes	No	145	117 (80.69%)	42 (84.00%)	75 (78.95%)	0.4638
	Yes		28 (19.31%)	8 (16.00%)	20 (21.05%)	
Exocrine Pancreatic Insufficiency	No	144	28 (19.44%)	6 (12.24%)	22 (23.16%)	0.117
	Yes		116 (80.56%)	43 (87.76%)	73 (76.84%)	
Previous Lung or Liver Transplant	No	145	143 (98.62%)	50 (100.00%)	93 (97.89%)	0.302
	Yes		2 (1.38%)	0 (0.00%)	2 (2.11%)	
Anti-osteoporosis Therapy *	No	145	140 (96.55%)	50 (100.00%)	90 (94.74%)	0.165
	Yes		5 (3.45%)	0 (0.00%)	5 (5.26%)	
	Ever Deficient	89	38 (42.70%)	10 (38.46%)	28 (44.44%)	0.604
Vitamin D Status **	Ever Insufficient	89	52 (58.43%)	13 (50.00%)	39 (61.90%)	0.200
	Ever Sufficient	89	70 (78.65%)	18 (69.23%)	52 (82.54%)	0.164
Lumbar Spine Z-Score		145	-0.20 (-1.10, 0.50)	-0.50 (-1.20, 0.30)	-0.10 (-1.00, 0.70)	0.0527

Continuous variables are described as median (interquartile range), and categorical variables are described as subjects (proportion).

* Anti-osteoporosis therapy includes bisphosphonates and PTH analogs.

** Vitamin D status groups are not mutually exclusive. Therefore, overall percentages will not add up to 100%. Ever deficient includes any subjects who had a 25(OH)D result < 20 ng/mL. Ever insufficient includes any subjects who had a 25(OH)D result between 20 – 29 ng/mL. Ever sufficient includes any subjects who had a 25(OH)D result ≥ 30 ng/mL.

Table 4:

Summary of 25-hydroxyvitamin D measurements

25-hydroxyvitamin D range	25 hydroxyvitamin D test results in this range	Subjects with a 25-hydroxyvitamin D result in this range
< 10 ng/mL	8 (3.4%)	12 (13.5%)
< 20 ng/mL (Deficient)	34 (14.4%)	38 (42.7%)
20 – 29 ng/mL (Insufficient)	65 (27.5%)	52 (58.4%)
> 30 ng/mL (Sufficient)	137 (58.1%)	70 (78.7%)
Total	236 (100%)	89 (100%)

Of the 145 subjects in the cohort, 89 subjects (61.3%) had a total of 236 documented 25-hydroxyvitamin D levels. The majority of subjects (78.7%) had at least one documented 25(OH)D level that was in goal as per the Cystic Fibrosis Foundation's Vitamin D guidelines' goal of at least 30 ng/mL. Note that the lowest category of vitamin D results (< 10 ng/mL) is included in the next lowest category of < 20 ng/mL. Subjects had vitamin D results in multiple ranges over time reflecting ongoing vitamin D treatment and supplementation.

Table 5:

Interaction of age and estrogen supplementation on lumbar spine bone mineral density trajectories

	Modelled Lumbar Spine BMD D	Covariate	p-value
Model 1	BMD of subjects age 17 – 24 years	Estrogen	0.229
		Interaction of Age and Estrogen	< 0.001
	BMD of subjects age 25 – 35 years	Estrogen	0.408
		Interaction of Age and Estrogen	0.043
Model 2	BMD of subjects age 17 – 27 years	Estrogen	0.873
		Interaction of Age and Estrogen	<0.001
	BMD of subjects age 28 – 35 years	Estrogen	0.903
		Interaction of Age and Estrogen	0.871

The interaction of the effects of age and estrogen on the lumbar spine bone mineral density (BMD) trajectory together is different from the sum of the separate effects of age and estrogen on lumbar spine BMD trajectory in the spline models except for the oldest subjects (ages 28–35 years). Estrogen by itself did not have a statistically significant contribution to the model. The model was adjusted for BMI, homozygous Delf508, heterozygous Delf508, exocrine pancreatic insufficiency and CF-Related Diabetes. Two age cut-points were used in two separate models. Age cut-point of 25 years was selected as healthy individuals attain peak bone mass at 25 – 30 years of age. Age cut-point of 28 years was selected as this was the age peak bone mass was attained in our total cohort including subjects older than 35 years of age.