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Estrogen Supplementation and Bone Health of Women with Cystic Fibrosis

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Estrogen Supplementation and Bone Health of Women with Cystic Fibrosis

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MD, Jefferson Medical College, 2014

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Abstract

Estrogen Supplementation and Bone Health of Women with Cystic Fibrosis

By Malinda Wu

Cystic fibrosis-related bone disease (CFBD) affects 25% of adults with cystic fibrosis (CF). The cause of CFBD is multifactorial, but sex steroid deficiency likely plays a key role. National guidelines recommend treating sex steroid deficiency in women with CF who have low bone mineral density (BMD), but the role of estrogen in CFBD has not been well studied.

In this retrospective cohort study, the association of estrogen supplementation and lumbar spine BMD was examined in women with CF and BMD assessed by dual-energy x-ray absorptiometry (DXA). Of the 145 women studied, 43 women were prescribed estrogen supplementation; the most common formulation was oral ethinyl estradiol. Exposure to estrogen supplementation was associated with lower lumbar spine BMD compared to no exposure to estrogen supplementation. Data from 104 subjects with multiple assessments of lumbar spine BMD were included in logistic regression models of estrogen supplementation exposure and other factors affecting CFBD for predicting increasing lumbar spine BMD. Subjects exposed to estrogen had an odds ratio of 0.607 (95% CI 0.253 – 1.456) of having increasing lumbar spine BMD compared to the subjects not exposed to estrogen. Findings were similar when adjusted for age, pancreatic insufficiency, vitamin D deficiency and body mass index. Findings were similar in sensitivity analysis excluding subjects who had used anti-osteoporosis therapy and younger subgroup examining DXAs obtained when subjects were less than 30 years old. However, in the older subgroup (between 30 and 50 years old), subjects exposed to estrogen supplementation had an odds ratio of 1.072 (95% CI 0.284 – 4.046) of having increasing lumbar spine BMD compared to subjects not exposed to estrogen supplementation.

Recent published literature raises concern that ethinyl estradiol, a synthetic estrogen commonly found in hormonal contraception, impairs bone accrual in premenopausal women at risk for low BMD. This study's results further support these concerns. Estradiol, a physiologic estrogen, was only used by patients older than 50 years in this retrospective cohort study. Prospective studies are needed to examine different doses, routes and formulations of estrogen supplementation to understand the role of estrogen in treating and preventing CFBD.

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Table of Contents:

INTRODUCTION

Cystic fibrosis (CF) is a progressive life-limiting genetic disease caused by mutations of the CF transmembrane conductance regulator (CFTR). It affects more than 31,000 people in the US. Traditionally considered a pediatric disease, recent advancements in therapies for people with CF including highly effective modulator therapies have increased the median predicted survival into the 5th decade of life (1). With these increases in lifespan, people with CF are surviving to have extrapulmonary manifestations such as CF-related bone disease (CFBD).

CFBD affects 25% of adults with CF (1). CFBD is characterized by low bone mineral density (BMD) and is associated with increased risk for thoracic and vertebral fractures which can limit patients' abilities to perform crucial daily airway clearance therapies (2). CFBD can be a barrier to lung transplant eligibility which can be a life-saving treatment option for patients with CF who have end-stage lung disease. There are many factors affecting bone health of people with CF including sex steroid deficiency, pancreatic insufficiency, malnutrition, vitamin D deficiency, CF-related diabetes, decreased physical activity, chronic inflammation, glucocorticoid use, and CFTR dysfunction (2, 3). These variables have been summarized in the causal diagram (Figure 1). Lung function in patients with CF is often assessed with the surrogate marker of forced expiratory volume in 1 second (FEV_1, percent predicted). Similarly, nutritional status is often assessed with the surrogate marker of body mass index (BMI). There are many mutations known to cause CF, but the Delta F508 mutation is the most common seen in Caucasians and is the most common affecting patients in the US.

Estrogen is a key hormone affecting the accrual of bone mass (4). Young adults with CF continue to accrue bone mass until 30 years of age. In females without CF, the bulk of bone accrual occurs under the influence of estrogen during and after puberty. Within one year of menopause, a physiologic hypogonadal state, loss of trabecular bone and increased bone resorption without compensatory increase in bone formation is apparent (5). As the dual-energy x-ray absorptiometry (DXA) site with the most trabecular bone, the lumbar spine is the most sensitive to sex hormone status. This loss of BMD in post-menopausal women has been reversed with estrogen therapy (6).

Historically, patients with CF have had delayed puberty which is the manifestation of sex steroid deficiency in girls. A 1976 observational study describing adolescent and young adults with CF in London, UK, found that females with CF had delayed puberty, with onset of breast development occurring after 14 years of age (7). Pubertal delay in adolescents with CF has improved in parallel with advancements in CF care, nutrition, overall clinical status and survival. While pubertal delay is improving, pubertal timing is not yet equal to healthy controls. In two recent surveys, one in the United States published in 2019 and the other in Poland in 2010, subjects with CF had a statistically significant later onset of menarche than healthy subjects, suggesting inadequate estrogen status (8, 9). Symptoms of hypoestrogenism in post-pubertal women can include irregular menstrual cycles and menopausal symptoms. Recent cross-sectional studies of adolescent and young adult women with CF highlighted that they frequently experience urinary incontinence (8, 10), sexual dysfunction and dyspareunia (8) which can be symptoms of hypoestrogenism.

The CF Foundation guidelines recommend sex steroid replacement for patients with CFBD (2). However, no prospective study has evaluated estrogen for augmenting bone mass in women with CF.

This retrospective cohort study sought to understand the role of estrogen therapy in bone health for women with CF by examining covariates affecting bone health and estrogen supplementation in addition to bone mineral density as the surrogate marker of bone health.

BACKGROUND

The role of estrogen for CF-related bone disease has not been well studied. In our recent cross-sectional study of 49 adult women with CF followed at our CF center, 12 women taking estrogen supplementation in the form of ethinyl estradiol had lower mean lumbar spine BMD than 37 women with CF not taking estrogen (Figure 2) (11). Estrogen supplemented women had lower BMD at the femoral neck and total hip which trended towards significance.

Most of research of estrogen for women with CF has focused on the association of estrogen with inflammation and infection. There have been concerns regarding the safety of estrogen therapy for women with CF, in part stemming from the "gender gap" observation, where females with CF had earlier mortality compared to males with CF (12-14). Notably, in recent years this gender gap has shrunk, with females and males having a similar age at mortality (15-18). However, in comparison to the general population of the US in which females have 5 years longer life expectancy than males, that women with CF have a similar life expectancy as men with CF still suggests a relative loss in life expectancy for women with CF. Girls with CF have an earlier conversion to chronic *Pseudomonas aeruginosa* infection (12). *Pseudomonas aeruginosa* is a respiratory pathogen associated with increased morbidity and mortality in people with CF. In *vitro* models have shown that increased estrogen is associated with decreased airway surface liquid which would result in more viscous sputum that is harder to effectively clear thereby increasing the risk of pathologic microbial growth in the lungs (13, 14).

However, retrospective studies of estrogen supplementation via oral contraceptives detected no changes in morbidity (lung function, body mass index, CF exacerbation frequency) (19-21). In contrast, a retrospective study by Chotirmall et al. in Irish women with CF found decreased incidence of CF exacerbations with oral contraceptive use, which was affirmed in their 2-year prospective observational study which found that women taking oral contraceptives had less exacerbations per year than regularly or irregularly menstruating women with CF (20).

In the menstrual cycle, estradiol levels surge upwards in the days leading up to ovulation and peak a few days before ovulation. Jain's and Chotirmall's studies which relate markers of health to timing of the menstrual cycle found that in the days leading up to ovulation compared to the days during menses, women with CF had decreased patient-reported lung function, increased markers of inflammation in sputum (leukocytes, percentage of neutrophils, IL-8 and IL-1β) and increased CF exacerbation frequency. These findings were reversed when the same patients were treated with oral contraceptives containing estrogen (20, 22).

These studies suggest that surges in estradiol, rather than absolute estradiol level, are harmful. Treatment with supplemental estrogen suppresses the hypothalamic-pituitary-ovarian axis and thereby prevents the cyclic swings in estradiol levels.

Many of the factors affecting CFBD are affected or caused by the underlying CFTR dysfunction. Highly effective CFTR modulator therapies for CF showed promise for improving BMD in a small retrospective study of 7 patients (23), but a recent prospective study following 52 people with CF identified changes in bone microarchitecture with highly effective modulator therapy but no improvements in BMD

(24). The other factors influencing CFBD such as sex steroid deficiency must be investigated and optimized even as therapies for CF continue to advance, improving morbidity and mortality for people with CF.

METHODS

Hypothesis:

The primary aim of this retrospective cohort study of women with cystic fibrosis was to compare the lumbar spine BMD trajectory of women with CF prescribed estrogen supplementation to the lumbar spine BMD trajectory of women with CF not prescribed estrogen supplementation. We hypothesized that women with CF prescribed estrogen supplementation would have consistently higher lumbar spine BMD than women with CF not prescribed estrogen supplementation.

The secondary aim of this retrospective cohort study was to describe the patterns in the use of estrogen supplementation by women with CF at a single center. We hypothesized that women with CF would be using estrogen supplementation for optimization of bone health and/or contraception.

Study Design:

This was a retrospective cohort study of women with CF followed at a single CF center. This study was approved by the Emory University Institutional Review Board (IRB) and the IRB of the Children's Healthcare of Atlanta.

Subject Selection:

Subjects were eligible if they were female, had been seen in the Adult CF clinic and had a DXA report documented in their electronic medical record. Subjects who did not have a diagnosis of CF were excluded; exclusion diagnoses included CFTR-related disorder, CFTR-related metabolic syndrome and bronchiectasis.

Potential subjects were identified from the Emory Clinical Data Warehouse (CDW), a data repository that integrates data from multiple business and clinical

applications within the Emory Healthcare system, as having an encounter with a CF-related diagnosis code or an encounter with one of the Adult CF clinic providers between January 1, 2000, and June 30, 2019. The clinic notes of subjects were reviewed to confirm the diagnosis of CF.

Variables:

Data was collected from the Emory CDW and the electronic health record of both adult and pediatric clinics in the CF center. Subjects' date of birth and name were used to match records from the adult CF clinic to the local pediatric CF clinic (Children's Healthcare of Atlanta) and additional clinic notes and DXA reports were extracted.

The first CF clinic visit of each calendar year was reviewed to extract additional information about covariates (CFTR mutations, CF-related diabetes, exocrine pancreatic insufficiency, lung transplantation status, height and weight to calculate body mass index (BMI), and medication lists). CFTR mutation status was analyzed as Delta F508 homozygous, Delta F508 heterozygous or no copies of Delta F508 mutation; Delta F508 is the most common mutation found in patients with CF in the US. BMI was dichotomized as at goal for women with CF according to CF Foundation Guidelines (at least 22 kg/m^2), or not at goal. Medication lists from the CDW and clinic notes were also reviewed for prescription of anti-osteoporosis therapy (bisphosphonates and parathyroid hormone analogues), oral glucocorticoids, CFTR modulators and estrogen supplementation. Patients' electronic health records were reviewed to collect race and ethnicity, and DXA results.

Laboratory results (25-hydroxyvitamin D) were extracted from the CDW. 25-hydroxyvitamin D (25(OH)D) levels that were below the limits of detection were

analyzed as half the lower limit for the assay. Subjects were classified as ever vitamin D deficient according to Endocrine Society guidelines if a 25(OH)D level was ever measured < 20 ng/mL (25).

The primary exposure of interest was estrogen supplementation. Information about the formulation, dose, route and reason for prescription of estrogen supplementation was extracted from the CDW and clinic notes. If a subject started estrogen supplementation, the interval clinic notes were reviewed to clarify when estrogen supplementation was first documented. Patients with CF are recommended to have follow-up four times per year in their CF center; however, additional visits for acute care or hospitalizations may affect the number of visits each year in their CF center. Once exposed to estrogen supplementation, a subject was considered exposed for the remainder of their observations.

The primary outcome was lumbar spine BMD as measured by DXA. Additional data from the DXA report including lumbar spine BMD Z score and BMD measured at other body sites (if performed) was also collected. As these DXAs were performed for routine clinical care, there were multiple facilities with DXA scanners available to patients. DXAs were measured by both Hologic programs (Hologic Inc., Malborough, MA, USA) and GE programs (GE Healthcare, Madison, WI, USA). As more DXAs were performed on GE scanners, the BMD of Hologic scans were converted to GE equivalent by industry accepted conversion formulas (26).

The primary outcome in the logistic regression model was increasing lumbar spine BMD trajectory (g/cm^2/year), which was defined as the annualized change in lumbar spine BMD and which was calculated for subjects who had at least two

consecutive DXAs performed while the subject was exposed to supplemental estrogen or not exposed to supplemental estrogen. This annualized trajectory was averaged if they had multiple trajectories between DXAs. This outcome was dichotomized in the logistic regression model as greater than zero (> 0) or not (≤ 0) to reflect an increasing lumbar spine BMD trajectory (g/cm^2/year).

Missing Data:

Data was missing in BMI, 25(OH)D and ever vitamin D deficient status. The last observation was carried forward. If the earliest BMI or 25(OH)D level was obtained within 12 months of the DXA, this value was input. The pattern of missing data was found to be arbitrary. Missing data in BMI status or ever vitamin D deficient status was included in the model as a separate level.

To examine the selection bias of excluding patients with CF who did not have a DXA report and therefore their BMD was unknown due to this missing data, clinical data and demographics of women with CF presenting to the same CF center during a 12-week period (01/24/2019 – 04/16/2019) was collected and analyzed. If subjects had multiple clinic visits during that time, data was used from the earliest clinic visit in that period. This data was collected under a different Emory IRB approval.

Statistical Analysis:

Analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics were calculated for all variables of interest. Categorical variables were reported as counts and percentages. Continuous variables were reported as median and interquartile ranges. Demographic and clinical characteristics were compared

between the subjects prescribed estrogen and not prescribed estrogen using Kruskal Wallis, Chi-square or Fisher's Exact tests.

Lumbar spine BMD was plotted against subjects' age. The best fit line of the data points was determined with a non-parametric regression function. Repeated measures ANOVA was used to compare the lumbar spine BMD measurements by exposure to supplemental estrogen.

To examine the association of estrogen supplementation on lumbar spine BMD trajectory, a logistic regression model was performed with the dependent variable being increasing lumbar spine BMD trajectory. The independent variables were exposure to supplemental estrogen and additional variables were chosen by plausibility, including median age (years), pancreatic insufficiency, BMI at goal and ever vitamin D deficient. The calculated odds ratio and 95% confidence intervals (CI) were reported.

Additional covariates considered included F508 Delta mutation (homozygous, heterozygous, no copies), CF-related diabetes, previous lung transplant as surrogate for chronic systemic glucocorticoid use and use of anti-osteoporosis therapy (defined as bisphosphonates and/or parathyroid hormone analogues). Missing data was treated as a separate level, but models were also analyzed in which missing data was input as each level. Models were also analyzed with different thresholds for dichotomization of the outcome examining the upper tertile and upper quartile in addition to the threshold of zero which was felt to be the most clinically significant to examine. Ultimately these models were not selected as the final model reported as they had very similar results.

A sensitivity analysis excluding subjects who were ever prescribed anti-osteoporosis therapy (bisphosphonates and/or parathyroid hormone analogues) was also performed.

Separate models examining the lumbar spine BMD trajectory during periods that women with CF are expected to be accruing bone (before age 30 years) and maintaining BMD (between 30 to 50 years) were also analyzed. Models examining BMD trajectory in later life (older than 50 years) were not performed as the few observations all had declining BMD trajectory.

RESULTS

A total of 417 subjects were assessed for eligibility (Figure 3). A total of 145 subjects with CF were analyzed. Twenty subjects at the time of their first BMD assessment by DXA were exposed to estrogen supplementation; during the observation period 23 additional subjects had a DXA measurement while exposed to estrogen supplementation. The subjects exposed to estrogen supplementation and unexposed to estrogen supplementation had similar baseline demographics (Table 1) except for exposure to anti-osteoporosis therapy (bisphosphonates and/or parathyroid hormone analogues). However, only 5 of 145 women were using these medications at the time of their first DXA during the observation period.

The 145 subjects had a total of 419 DXA measurements (Table 2). At baseline, exposed and unexposed subjects had similar numbers of DXA scans performed. Subjects exposed to estrogen had lower baseline lumbar spine BMD which was significant when comparing lumbar spine BMD Z score (*P* 0.03) but not lumbar spine BMD (g/cm^2). There were also significant differences at baseline left total hip BMD with estrogen exposed subjects again having lower BMD measurements than unexposed subjects.

When comparing lumbar spine BMD measurements, the mean lumbar spine BMD measurements obtained after the subject had been exposed to estrogen (1.104 g/cm^2, SD 0.164 g/cm^2) was lower than lumbar spine BMD of subjects who had not been exposed to estrogen supplementation (1.114 g/cm^2, SD 0.164 g/cm^2), (Table 3, Figure 4). The difference in the mean lumbar spine BMD of women exposed to estrogen compared to women not exposed to estrogen was not statistically significant (*P* 0.6). Similarly, the mean lumbar spine BMD Z score obtained after the subject had been exposed to estrogen

(-0.4, SD 1.6) was lower than the mean lumbar spine BMD Z score of subjects who had not been exposed to estrogen supplementation (-0.3, SD 1.2), (Figure 5). The difference in the mean lumbar spine BMD Z scores of women exposed to estrogen compared to women not exposed to estrogen was not statistically significant (*P* 0.4).

Lumbar spine BMD was plotted against age with best fit line of the data points superimposed (Figure 6).

Lumbar spine BMD trajectories were calculated for 104 subjects. The subjects analyzed in the logistic regression models had similar baseline demographics (Table 4). The odds ratio of subjects exposed to estrogen supplementation having an increasing lumbar spine BMD trajectory compared to subjects not exposed to estrogen supplementation was 0.607 (95% CI 0.253 – 1.456). There were similar findings when adjusting for age; for age and vitamin D deficiency; as well as for age, pancreatic insufficiency and BMI status (Table 5). When excluding subjects who had been exposed to anti-osteoporosis therapy, the crude odds ratio of having an increasing lumbar spine BMD trajectory was 0.564 (95% CI 0.223 – 1.435) in subjects exposed to supplemental estrogen compared to subjects not exposed to supplemental estrogen. The adjusted odds ratios in this sensitivity analysis excluding subjects who were ever prescribed anti-osteoporosis therapy were similar (Table 6).

Models examining DXAs obtained when subjects were < 30 years old had similar results. The odds of subjects exposed to estrogen supplementation having an increasing lumbar spine BMD trajectory were 0.431 (95% CI 0.140 – 1.326) times the odds among subjects not exposed to estrogen supplementation (Table 7). However, in models examining DXAs obtained when subjects were ≥ 30 years and ≤ 50 years old, the odds of

subjects exposed to estrogen supplementation having an increasing lumbar spine BMD trajectory were 1.072 (95% CI 0.284 – 4.046) times the odds among subjects not exposed to estrogen supplementation (Table 7).

In a 3-month period, 98 women with CF were seen in the adult CF clinic (Table 8). Women who had a DXA were significantly older with a median age of 30.6 years compared to women who did not have a DXA with a median age of 26.5 years (*P* 0.02). Women who had a DXA were more likely to have been prescribed estrogen supplementation (21.1%) compared to 4.9% of women who did not have a DXA (*P* 0.02). Other demographics including BMI, vitamin D level and lung function as estimated by forced expiratory volume in 1 second (FEV_1, percent predicted) were similar.

Data regarding estrogen supplementation was extracted for 50 subjects, including 7 subjects who were exposed to estrogen after their most recent DXA was performed in the observation period. Combination oral ethinyl estradiol and progesterone was the most common formulation and route of estrogen supplementation (Table 9). Some women were also using transdermal and transvaginal routes. The four women who were using estradiol or conjugated estrogens were all older than 50 years old. The median ethinyl estradiol dose was 30 mcg/day, range 10 – 50 mcg/day. The reason for prescription of estrogen supplementation was very rarely stated in the electronic health record. Estrogen supplementation was not prescribed by the healthcare providers in the CF clinic.

DISCUSSION

This retrospective cohort study does not support the hypothesis that estrogen supplementation promotes increased lumbar spine bone mineral density in women with CF. The compared means of lumbar spine BMD of subjects exposed to estrogen supplementation and not exposed to estrogen supplementation were similar. Subjects exposed to estrogen supplementation were less likely than subjects not exposed to estrogen to have increasing lumbar spine BMD trajectories. While a small benefit is suggested in the older population when analyzing BMD measurements of subjects between 30 and 50 years among subjects exposed to estrogen supplementation compared to subjects not exposed to estrogen supplementation, it likely does not represent a clinically significant odds ratio. Furthermore, these differences were not statistically significant with wide confidence intervals in the odds ratios overlapping one. Data was not available to address the hypothesis that women with CF were taking estrogen supplementation for the purposes of improving bone health or contraception.

A strength of this study is the large number of subjects analyzed for a rare disease and longitudinal observations with a median of 2 (interquartile range 1-4) DXAs per subject. While this longitudinal follow-up is a strength, during this time period significant advancements in therapies available for treating CF were discovered and have significantly increased the health and lifespan of patients with CF. 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 suggesting that our data is not weakened by this long follow-up (27).

There is very little data published about the role of estrogen for the bone health of women with CF. Similar to this study, our recent cross-sectional study of 49 adult women with CF, women taking estrogen supplementation in the form of ethinyl estradiol also had lower mean lumbar spine BMD than women with CF not taking estrogen (11). These women who were supplemented with estrogen were taking ethinyl estradiol.

As a retrospective cohort study relying on data obtained for routine clinical care, this data is subject to misclassification bias and selection bias from missing data. Current CF Foundation guidelines recommend assessment of BMD in adults > 18 years of age at least every 5 years, and more frequent if the BMD is low or declining quickly (2). As subjects were identified who attended the adult CF clinic, where the typical minimum age is 18 years, the rate of DXA screening would be expected to be 100% if adherent to the national recommendation. However, the rate of DXA screening of 47.3% in this population is consistent with performance of other CF centers. A median of 59.3% of adults with CF followed at CF centers in the US had had a DXA in the previous 5 years (1). There could be selection bias in healthcare providers only ordering DXA screening on patients in whom there was a clinical concern for bone health.

When examining the 3 month cross-section of women with CF regardless of DXA performed status, it is not surprising that older individuals were more likely to have had a DXA performed and more likely to have been exposed to estrogen supplementation as they are older and have had more time to have these events occur. Additionally, data about lung function as estimated by the FEV_1 and inflammation as estimated by hospitalizations for CF in the previous 12 months which was not collected in the primary data set, was similar in women who did and did not have a DXA.

As a retrospective cohort study, this study cannot address causation. It is unknown if women with CF were prescribed estrogen supplementation to improve their BMD, in which case it would not be surprising that their BMD is lower than women with CF not prescribed estrogen supplementation. However, it may be that the use of estrogen supplementation is detrimental to bone health for women with CF or that unmeasured factors which influenced these women to have been prescribed estrogen supplementation were detrimental to their bone health. Future prospective trials would be needed to understand the role of estrogen in the bone health of women with CF and their overall health.

Furthermore, recent studies of premenopausal women without CF have raised concerns that low doses of estrogen used by subjects is detrimental to bone accrual. The Women's Health Initiative which demonstrated benefit in post-menopausal women for bone health and fracture prevention used relatively high doses of estrogen in the form of conjugated estrogens (6). Only two women in this study were prescribed conjugated estrogens. Older studies that showed benefit of oral ethinyl estradiol in women examined higher doses of ethinyl estradiol. A small study of adolescent and young adults with functional hypothalamic amenorrhea were randomized to receive 35 mcg of ethinyl estradiol with progesterone, depo medroxyprogesterone or placebo found that the 5 subjects receiving 35 mcg ethinyl estradiol had small improvement in BMD compared to the 5 subjects who received placebo (28).

There has been a shift towards ever smaller doses of ethinyl estradiol to minimize symptoms of estrogen supplementation such that "high" dose ethinyl estradiol is now considered 20 or 30 mcg ethinyl estradiol. The benefits of ethinyl estradiol for bone

health may be lost at these doses typically used currently. In a retrospective cohort study of adolescents who were using combination oral ethinyl estradiol and progesterone, who all used ≤ 35 mcg of ethinyl estradiol, they had smaller increases in BMD than matched adolescents who had not used estrogen preparations (29). There are no studies in women with CF studying different doses of ethinyl estradiol.

This study further adds to the literature as descriptions of estrogen use in women with CF have focused on contraception (30-33). Thus, previous studies have excluded formulations of estrogen supplementation that are not used for contraception such as physiologic estradiol or conjugated estrogens which may promote bone health.

In addition, recent studies of premenopausal women without CF have raised concerns that oral ethinyl estradiol is detrimental to bone accrual. Studies instead favor physiologic transdermal estradiol instead of oral ethinyl estradiol in these populations. In an open-label randomized crossover trial, 18 women ages 18-39 years with primary ovarian insufficiency due to Turner Syndrome, oophorectomy, or idiopathic cause took one year of combined oral contraceptive containing 30 mcg ethinyl estradiol or one year of transdermal estradiol with transvaginal progesterone before crossing over to the other treatment arm for another year. During treatment with transdermal estradiol, subjects accrued more lumbar spine BMD and had increased markers of bone formation than during treatment with combined oral contraceptive, suggesting that transdermal estrogen may be more beneficial on bone accrual than oral estrogen (34). Similarly, in a study of oligo-amenorrheic athletes ages 14-25 years, the subjects randomized to 100 mcg of transdermal estradiol had higher BMD at the lumbar spine and femoral neck after one year compared to those randomized to a combined oral contraceptive with 30 mcg ethinyl

estradiol or no treatment (35). However, there are no studies in girls or women with CF comparing the effects of transdermal estradiol to oral estradiol on bone health.

In this retrospective cohort study, subjects who were prescribed estrogen supplementation compared to subjects who were not prescribed estrogen supplementation had lower odds of increasing lumbar spine bone mineral density. The median dose used by subjects was 30 mcg/day of oral ethinyl estradiol which has not been sufficient to protect bone accrual in women without CF, unlike transdermal estradiol. To understand the role of estrogen supplementation for promoting bone health, prospective, randomized placebo-controlled studies of transdermal estradiol conducted in young adult premenopausal women with CF are needed.

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Table 1: Baseline demographics of subjects

Variable	Level	N	Overall N = 145	N	Estrogen Unexposed N = 125	N	Estrogen Exposed N = 20	*P* value
Age (years)		145	25.2 (19.3, 32.9)	125	24.4 (18.6, 21.7)	20	28.1 (21.4, 34.3)	0.09
BMI (kg/m^2)		101	21.30 (19.5, 24.0)	84	21.5 (19.9, 25.3)	17	20.3 (19.4, 21.3)	0.09
BMI at goal	Yes	101	40 (39.6%)	84	36 (42.9%)	17	4 (23.5%)	0.18
25-hydroxy vitamin D (ng/mL)		66	34.5 (25.0, 42.0)	54	33.5 (25.0, 42.0)	12	36.5 (31.0, 44.0)	0.45
Delta F508 Mutation	Homozygous	145	60 (41.3%)	125	54 (43.2%)	20	6 (30%)	0.50
	Heterozygous		58 (40%)	125	49 (39.2%)	20	9 (45%)	
	None		27 (18.6%)	125	22 (17.6%)	20	5 (25%)	
CF-related diabetes	Yes	145	28 (19.3%)	125	25 (20%)	20	3 (15%)	0.77
Exocrine pancreatic insufficiency	Yes	145	117 (80.7%)	125	103 (82.4%)	20	14 (70%)	0.22
Previous lung or liver transplant		145	2 (1.4%)	125	1 (0.8%)	20	1 (5%)	0.26
Anti-osteoporosis therapy	Yes	145	5 (3.4%)	125	2 (1.6%)	20	3 (15%)	0.02
Vitamin D status	Ever insufficient (< 30 ng/mL)	89	65 (73%)	75	54 (72%)	14	11 (78.6%)	0.75
	Ever deficient (<20 ng/mL)	89	40 (44.9%)	75	36 (48%)	14	4 (28.6%)	0.24
	Ever severely deficient (< 10 ng/mL)	89	12 (13.4%)	75	10 (13.3%)	14	2 (14.3%)	1

Table 1 abbreviations: BMI (body mass index), CF (cystic fibrosis)

Anti-osteoporosis therapy included the use of bisphosphonates or parathyroid hormone analogues. Subjects with the different vitamin D statuses do not sum to 100% as these

statuses (ever insufficient (<30 ng/mL), ever deficient (<20 ng/mL), ever severely deficient (<10 ng/mL)) are not exclusive. Continuous variables are reported as median (interquartile range) and were compared with Kruskal Wallis test. Categorical variables are reported as count (percent) and were compared with Chi-square test or Fisher's Exact if rare.

Table 2: Baseline characteristics of first bone mineral density measurement

Variable	Overall N	Overall N = 145	Estrogen Unexposed N	Estrogen Unexposed N = 125	Estrogen Exposed N	Estrogen Exposed N = 20	*P* value
Total DXA per subject	145	2 (1, 4)	125	3 (1, 4)	20	2 (1, 3.5)	0.61
Lumbar spine BMD (g/cm^2)	145	1.130 (1.013, 1.215)	125	1.132 (1.029, 1.208)	20	1.069 (0.953, 1.302)	0.59
Lumbar spine BMD (Z score)	145	-0.2 (-1.1, 0.5)	125	-0.1 (-1.0, 0.5)	20	-0.9 (-1.8, -0.2)	0.03
Left femoral neck BMD (g/cm^2)	100	0.842 (0.730, 0.964)	86	0.847 (0.777, 0.970)	14	0.764 (0.657, 0.900)	0.13
Left femoral neck BMD (Z score)	93	-0.1 (-1.0, 0.4)	80	-0.1 (-0.7, 0.5)	13	-0.7 (-1.3, 0.0)	0.09
Right femoral neck BMD (g/cm^2)	34	0.978 (0.810, 1.061)	27	0.955 (0.817, 1.061)	7	1.006 (0.767, 1.088)	0.92
Right femoral neck BMD (Z score)	31	0.1 (-1.1, 0.9)	24	0.1 (-0.7, 0.9)	7	0.0 (-1.2, 0.9)	0.65
Left total hip BMD (g/cm^2)	95	0.950 (0.830, 1.030)	81	0.954 (0.844, 1.039)	14	0.837 (0.732, 1.001)	0.05
Left total hip BMD (Z score)	88	0.1 (-0.8, 0.8)	75	0.1 (-0.6, 0.8)	13	-0.8 (-1.3, -0.3)	0.01
Right total hip BMD (g/cm^2)	30	0.890 (0.997, 1.088)	24	0.997 (0.901, 1.08)	6	0.982 (0.887, 1.088)	0.82
Right total hip BMD (Z score)	27	0.4 (-0.5, 0.8)	21	0.4 (-0.2, 0.8)	6	-0.2 (-0.5, 0.6)	0.40
Total body less head BMD (g/cm^2)	19	0.945 (0.845, 1.015)	18	0.934 (0.845, 1.015)	1	1.010 (1.010, 1.010	0.47
Total body less head BMD (Z score)	19	-0.3 (-1.2, 0.3)	18	-0.4 (-1.2, 0.3)	1	-0.1 (0.1, 0.1)	0.71

Table 2 abbreviations: DXA (dual-energy x-ray absorptiometry), BMD (bone mineral density)

Continuous variables are reported as median (interquartile range) and were compared with Kruskal Wallis test. Categorical variables are reported as count (percent) and were compared with Chi-square test or Fisher's Exact if rare.

Table 3: Comparison of lumbar spine bone mineral density by estrogen supplementation status

	Estrogen unexposed	**Estrogen exposed**	***P* value**
Lumbar spine BMD (g/cm^2)	1.114 (SD 0.164)	1.104 (SD 0.181)	0.60
Lumbar spine BMD (Z score)	-0.3 (SD 1.2)	-0.4 (SD 1.6)	0.40

Table 3 abbreviations: BMD (bone mineral density), SD (standard deviation)

Annualized lumbar spine trajectory was calculated as the annualized change in lumbar spine BMD in successive BMD assessments. There were a total of 313 DXAs performed in subjects not exposed to estrogen and 106 DXAs performed in subjects exposed to estrogen. Results are reported as mean (standard deviation) and were compared by repeated ANOVA.

Table 4: Baseline demographics of observations analyzed in logistic regression models

Variable	Level	N	Overall N = 104	N	Estrogen Unexposed N = 76	N	Estrogen Exposed N = 28	*P* value
Age (years)		104	26.1 (20.5, 33.8)	76	25.0 (19.8, 32.4)	28	27.7 (22.1, 34.8)	0.17
BMI (kg/m^2)		96	21.2 (19.7, 23.5)	68	21.2 (19.8, 23.8)	28	21.2 (19.5, 23.0)	0.48
BMI	Mean BMI ≥ 22	104	28 (26.9%)	76	28 (36.8%)	28	10 (35.7%)	0.18
	Mean BMI < 22		58 (55.8%)		40 (52.6%)		18 (64.3%)	
	Missing		8 (7.7%)		8 (10.5%)		0 (0.0%)	
25-hydroxy vitamin D (ng/mL)		71	31.0 (23.0, 39.0)	50	29.6 (22.0, 38.0)	21	32.0 (29.0, 39.0)	0.35
Vitamin D status	Ever vitamin D deficient	104	33 (31.7%)	76	25 (32.9%)	28	8 (28.6%)	0.67
	Not ever vitamin D deficient		41 (39.4%)		28 (36.8%)		13 (46.4%)	
	Missing		30 (28.8%)		23 (30.3%)		7 (25.0 %)	
Annualized LS BMD trajectory (g/cm^2/year)		104	0.001 (-0.016, 0.012)	76	0.002 (-0.010, 0.012)	28	-0.003 (-0.029, 0.007)	0.05
LS BMD trajectory increasing	Yes	104	54 (51.9%)	76	16 (32.0%)	28	12 (22.2%)	0.26
Delta F508 mutation	Homozygous	104	46 (44.2%)	76	37 (48.7%)	28	9 (32.1%)	0.32
	Heterozygous		42 (40.4%)		28 (36.8%)		14 (50.0%)	
	None		16 (15.4%)		11 (14.5%)		5 (17.9%)	
CF-related diabetes	Yes	104	31 (29.8%)	76	22 (29.0%)	28	9 (32.1%)	0.75
Exocrine pancreatic insufficiency	Yes	104	84 (80.8%)	76	61 (80.3%)	28	23 (82.1%)	0.83
Previous lung or liver transplant	Yes	104	4 (3.8%)	76	3 (4.0%)	28	1 (3.6%)	1.00
Anti-osteoporosis therapy	Yes	104	12 (11.5%)	76	9 (11.8%)	28	3 (10.7%)	1.00

Table 4 abbreviations: BMI (body mass index), LS BMD (lumbar spine bone mineral density), CF (cystic fibrosis)

Baseline characteristics of observations included in the logistic regression models comparing the odds of increasing lumbar spine BMD in subjects exposed to estrogen supplementation and not exposed to estrogen supplementation.

Table 5: Logistic regression adjusted odds ratios for factors affecting bone health for increasing lumbar spine bone mineral density

Model	OR	95% CI
Crude	0.607	(0.253, 1.456)
Adjusted for age	0.696	(0.265, 1.824)
Adjusted for age and vitamin D deficiency	0.760	(0.280, 2.061)
Adjusted for age, pancreatic insufficiency and BMI	0.705	(0.265, 1.872)

Table 5 abbreviations: BMI (body mass index), OR (odds ratio), CI (confidence interval)
The odds of increasing lumbar spine bone mineral density trajectory among patients exposed to estrogen supplementation compared to the odds of increasing lumbar spine bone mineral density trajectory among patients not exposed to estrogen supplementation is adjusted for the covariates listed in the model. Observations from 104 subjects are included.

Table 6: Logistic regression adjusted odds ratios for factors affecting bone health for increasing lumbar spine bone mineral density, excluding subjects exposed to anti-osteoporosis therapy

Models excluding subjects exposed to anti-osteoporosis therapy	**OR**	**95% CI**
Crude	0.564	(0.223, 1.425)
Adjusted for age	0.617	(0.225, 1.691)
Adjusted for age and vitamin D deficiency	0.652	(0.232, 1.822)
Adjusted for age, pancreatic insufficiency and BMI	0.674	(0.245, 1.855)

Table 6 abbreviations: BMI (body mass index), OR (odds ratio), CI (confidence interval)
The odds of increasing lumbar spine bone mineral density trajectory among patients exposed to estrogen supplementation compared to the odds of increasing lumbar spine bone mineral density trajectory among patients not exposed to estrogen supplementation is adjusted for the covariates listed in the model. Subjects who were exposed to anti-osteoporosis therapy (bisphosphonates and/or parathyroid hormone analogues are excluded). Observations from 92 subjects are included.

Table 7: Logistic regression adjusted odds ratios for factors affecting bone health for increasing lumbar spine bone mineral density, by age groups

	Age < 30 years (n = 66)		Age ≥ 30 and ≤ 50 years (n = 40)	
Models by age groups	**OR**	**95% CI**	**OR**	**95% CI**
Crude	0.431	(0.140, 1.326)	1.072	(0.284, 4.046)
Adjusted for age	0.652	(0.1920, 2.208)	1.099	(0.287, 4.205)
Adjusted for age and vitamin D deficiency	0.699	(0.2030, 2.41)	1.317	(0.286, 6.054)
Adjusted for age, pancreatic insufficiency and BMI	0.592	(0.1650, 2.124)	1.196	(0.304, 4.704)

Table 7 abbreviations: BMI (body mass index), OR (odds ratio), CI (confidence interval)
The odds of increasing lumbar spine bone mineral density trajectory among patients exposed to estrogen supplementation compared to the odds of increasing lumbar spine bone mineral density trajectory among patients not exposed to estrogen supplementation is adjusted for the covariates listed in the model.

Table 8: Characteristics of women with CF who did and did not have DXA

Variable	N	Overall N = 98	N	Has a DXA N = 57	N	No DXA N = 41	*P* value
Age (years)	98	28.7 (23.6, 38.0)	57	30.6 (26.9, 40.4)	41	26.5 (22.2, 33.6)	0.02
BMI (kg/m^2)	98	22.1 (19.7, 25.7)	57	22.1 (19.7, 25.7)	41	22.0 (19.7, 25.4)	0.83
FEV_1 (% predicted)	96	68.0 (41.0, 86.0)	57	62.0 (37.0, 83.0)	39	74.0 (46.0, 87.0)	0.12
CF hospitalizations in the previous 12 months	89	0 (0, 1)	51	1 (0, 2)	38	0 (0, 1)	0.23
Lumbar spine BMD (g/cm^2)	57	1.035 (0.934, 1.087)	57	1.035 (0.934, 1.087)		x	x
Lumbar spine (Z score)	57	-0.1 (-0.9, 0.5)	57	-0.1 (-0.9, 0.5)		x	x
25-hydroxyvitamin D (ng/mL)	91	29.0 (20.0, 38.0)	55	29.0 (21.0, 37.9)	36	26.5 (19.0, 44.0)	0.80
F508del mutation status:							
Homozygous		40 (40.8%)		23 (40.3%)		17 (41.5%)	0.76
Heterozygous	98	37 (98%)	57	23 (40.3%)	41	14 (34.1%)	
None		21 (21.4%)		11 (19.3%)		10 (24.4%)	
CF-related diabetes	98	27 (27.6%)	57	17 (29.8%)	41	10 (24.4%)	0.55
Has exocrine pancreatic insufficiency	98	88 (89.8%)	57	54 (94.7%)	41	34 (82.9%)	0.09
Use of anti-osteoporosis therapy	98	1 (1%)	57	1 (1.8%)	41	0 (0%)	1.00
Taking estrogen supplementation	14	14 (14.2%)	12	12 (21.1%)	2	2 (4.9%)	0.02

Table 8 abbreviations: CF (cystic fibrosis), DXA (dual-energy x-ray absorptiometry), BMI (body mass index), FEV_1 (forced expiratory volume in 1 second), BMD (bone mineral density)

Baseline characteristics of women with CF in another cross-sectional study seen at the same adult CF clinic to compare women with CF who did have and did not have a DXA. Anti-osteoporosis therapy included the use of bisphosphonates or parathyroid hormone analogues. Continuous variables are reported as median (interquartile range) and were

compared with Kruskal Wallis test. Categorical variables are reported as count (percent) and were compared with Chi-square test or Fisher's Exact if rare.

Table 9: Description of estrogen formulations used by subjects

Estrogen type	**N (%)**
Ethinyl estradiol (oral, transvaginal and transdermal)	49 (98%)
Estradiol (oral and transdermal)	3 (6%)
Conjugated estrogens (oral)	2 (4%)
Oral route	47 (94%)
Transvaginal route	3 (6%)
Transdermal route	3 (6%)

Most subjects who were exposed to estrogen used combination oral ethinyl estradiol and progesterone. The median ethinyl estradiol dose was 30 mcg/day, range 10 – 50 mg/day. The four women who were prescribed estradiol and conjugated estrogen were all older than 50 years old (one woman was prescribed both estradiol and conjugated estrogens). Data from 50 subjects is reported because some women were first exposed to estrogen supplementation after their most recent DXA was performed during the observation period.

Figure 1: Causal diagram for bone mineral density in women with cystic fibrosis

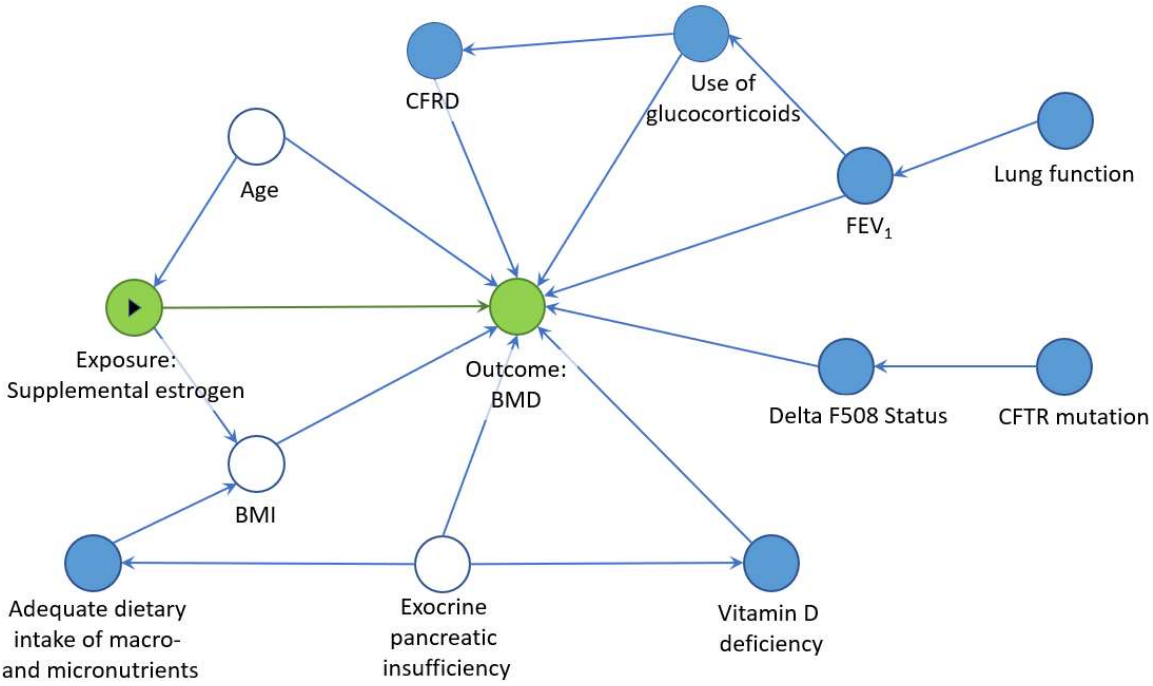

Figure 1 abbreviations: BMD (bone mineral density), CF (cystic fibrosis), CFRD (CF-related diabetes), FEV1 (forced expiratory volume in 1 second), CFTR (CF transmembrane conductance regulator), BMI (body mass index)

Causal diagram for BMD in women with CF with primary exposure as supplemental estrogen shown in green. Variables adjusted for in the final logistic regression model are shown in white (age, BMI and exocrine pancreatic insufficiency) and other covariates considered are shown in blue. Diagram is adapted from causal diagram for low BMD in people with CF by Aris et al (2).

Figure 2: Bone mineral density of women with CF by use of estrogen supplementation

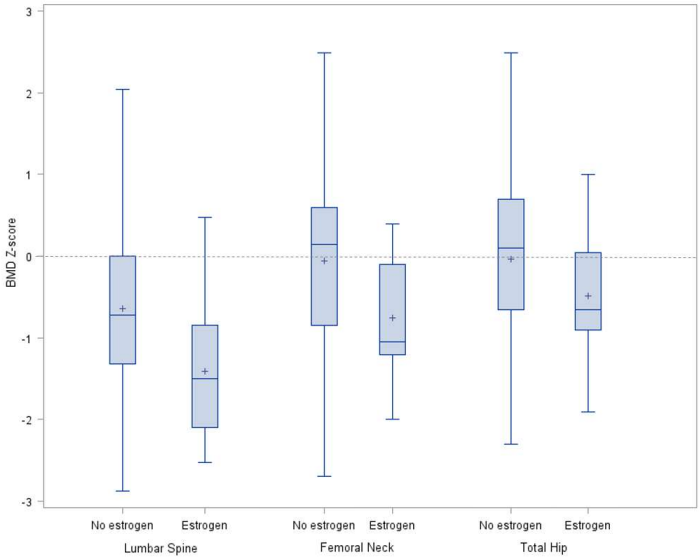

Figure 2 abbreviations: BMD (bone mineral density)

In a cross-sectional study of 49 adult women with CF, 12 women taking estrogen supplementation in the form of ethinyl estradiol had lower mean lumbar spine BMD than 37 women with CF not taking estrogen ($P < 0.05$) (11). Women taking estrogen supplementation compared to women not taking estrogen also had lower mean BMD at the femoral neck ($P < 0.05$) and total hip ($P > 0.05$).

Figure 3: Consort diagram

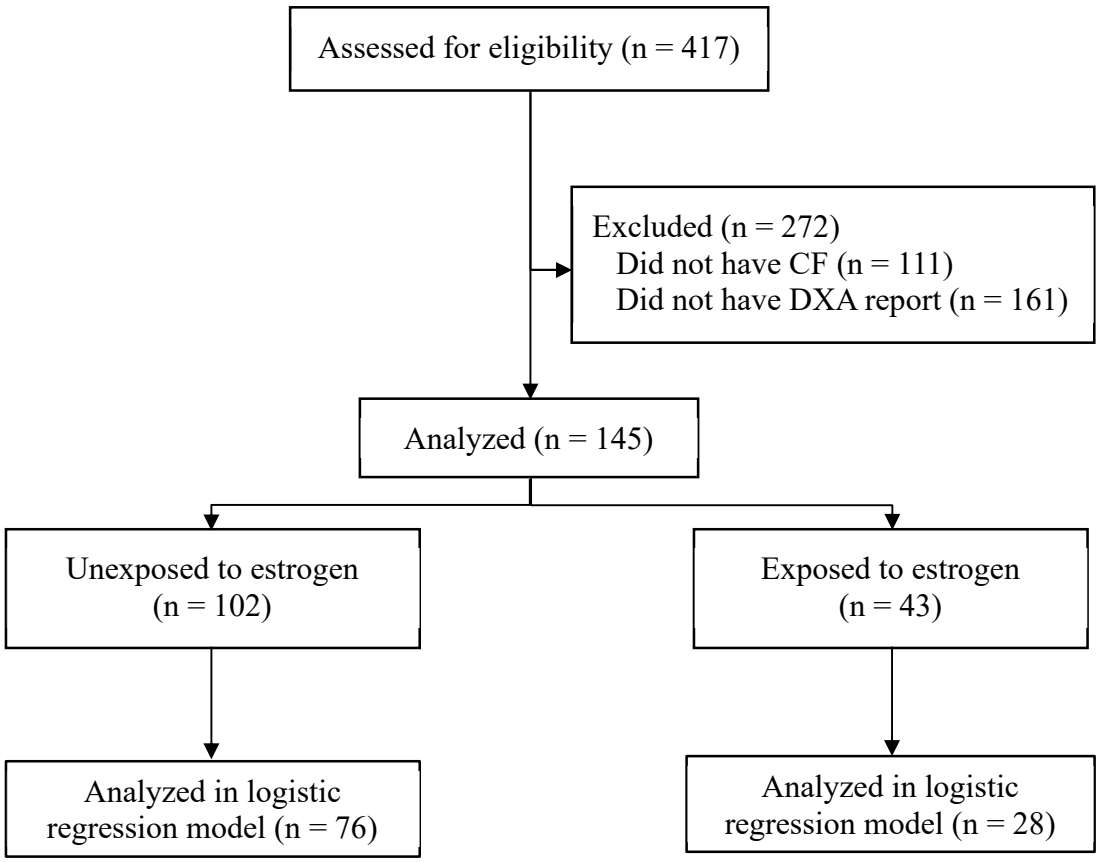

Figure 3 abbreviations: CF (cystic fibrosis), DXA (dual-energy x-ray absorptiometry)

Figure 4: Lumbar spine bone mineral density (g/cm^2) by exposure to supplemental estrogen status

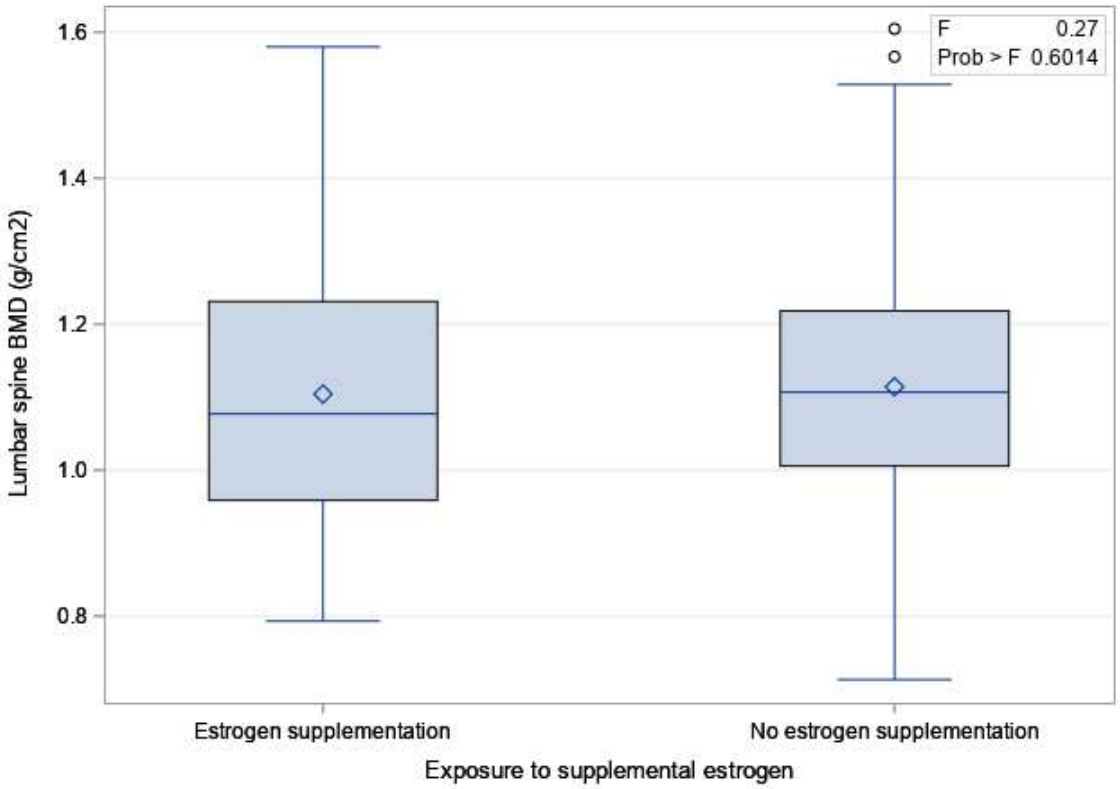

Figure 4 abbreviations: BMD (bone mineral density)

Boxplot comparing lumbar spine BMD (g/cm^2) by exposure to supplemental estrogen status.

Figure 5: Lumbar spine bone mineral density (Z score) by exposure to supplemental estrogen status

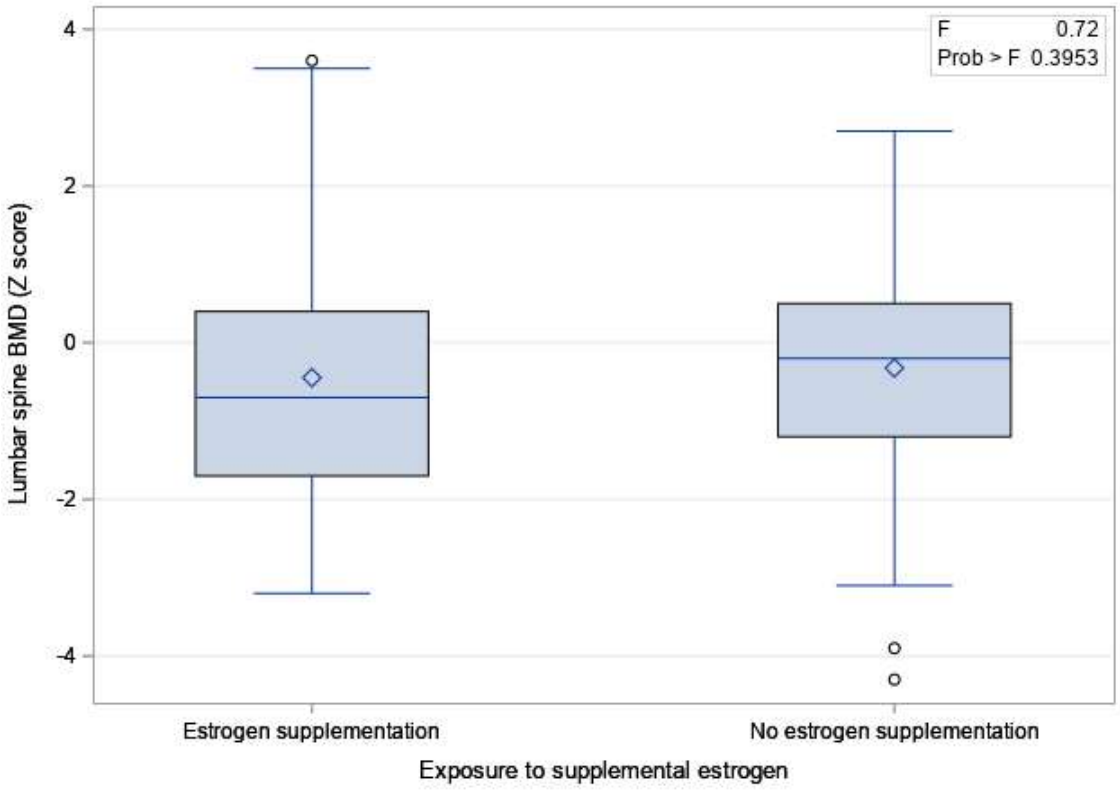

Figure 5 abbreviations: BMD (bone mineral density)

Boxplot comparing lumbar spine BMD (Z score) by exposure to supplemental estrogen status.

Figure 6: Lumbar spine bone mineral density of women with cystic fibrosis by estrogen supplementation status

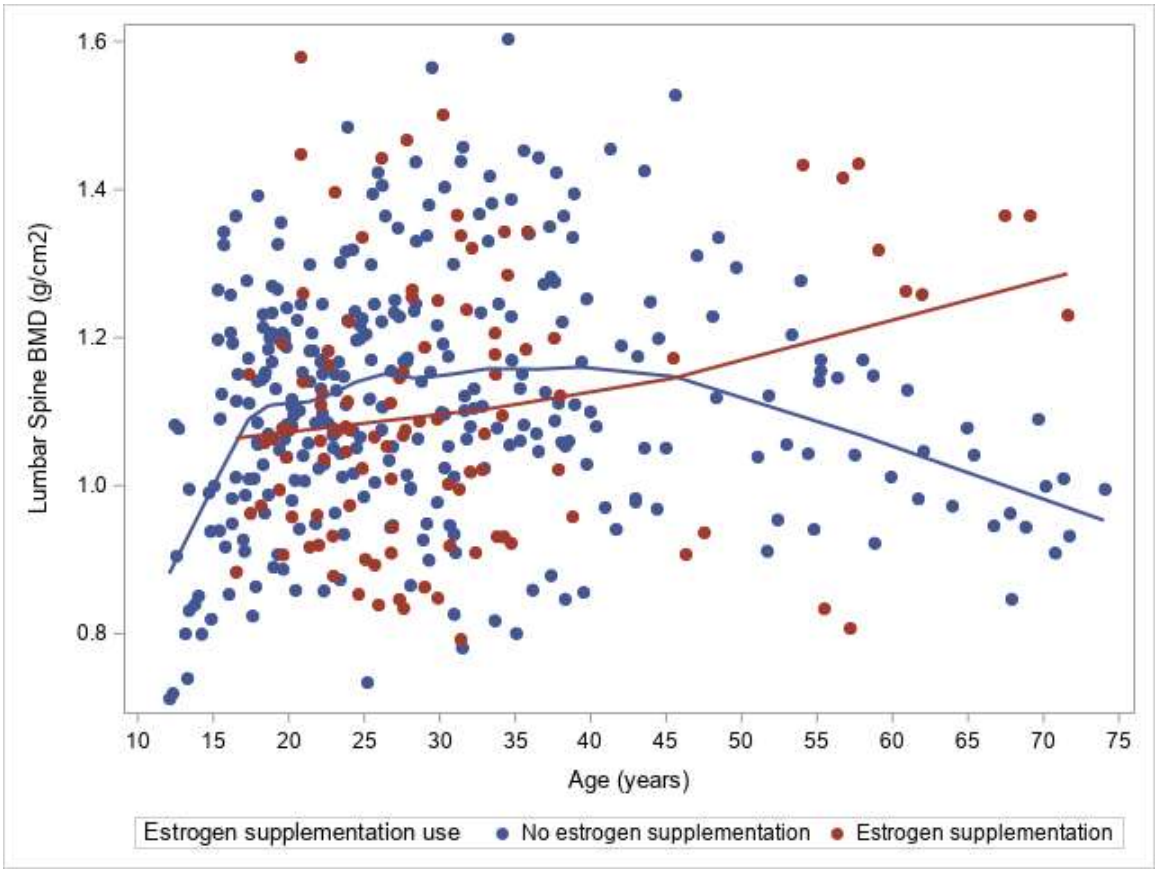

Figure 6 abbreviations: BMD (bone mineral density), DXA (dual-energy x-ray absorptiometry)

The lumbar spine BMD of women with cystic fibrosis is plotted against age at time of BMD measurement by DXA. If the subject had been exposed to estrogen supplementation at the time of DXA, the data point is shown in red. If the subject had not been exposed to estrogen supplementation at the time of DXA, the data point is shown in blue. The best fit line of BMD measurements is superimposed over the scatterplot.