# Cystic Fibrosis Related Bone Disease

The Harvard community has made this article openly available. **Please share** how this access benefits you. Your story matters.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Citable link</td>
<td><a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:17613728">http://nrs.harvard.edu/urn-3:HUL.InstRepos:17613728</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a></td>
</tr>
</tbody>
</table>
Cystic Fibrosis Related Bone Disease

Introduction

Over the past several decades, life expectancy for patients with cystic fibrosis (CF) has increased significantly, from an average of 10 years of age in 1962 to more than 39 years of age in 2011 (1). As patients live longer, other nonpulmonary co-morbidities related to CF have become increasingly prevalent, including CF-related bone disease (2). Because CF related bone disease has only recently emerged as a clinical problem, and the underlying bone alterations and pathogenesis of this condition have not been established.

Multiple clinical studies have reported that bone mineral density (BMD) measured by dual energy X-ray absorptiometry (DXA) is low in patients with CF (3-8). Fracture rates in CF patients are also up to two times higher than in healthy populations (8-11). Rib and vertebral fractures may lead to pain, chest wall deformities, reduced lung volumes, ineffective cough and airway clearance, and ultimately compromised lung function. In addition, severe bone disease may obviate lung transplantation. Due to this high morbidity, CF-related bone disease can significantly affect the well-being of these patients.

Patients with CF have multiple risk factors for low BMD, including malnutrition, vitamin D deficiency, delayed puberty, pancreatic insufficiency, glucocorticoid use, inflammation, and physical inactivity (2). In addition, CF-related bone disease is characterized by an unusual pattern of increased bone resorption and decreased bone formation (6; 12; 13), and the underlying cause of this altered bone turnover is unclear. Interestingly, recent evidence has also suggested that CFTR itself plays a causative role in low bone density observed in CF patients (14-17).

Over the past few decades, advances in treatments for the pulmonary and GI manifestations of CF along with increased attention to screening for osteoporosis and treating identifiable risk factors have contributed to improved clinical care of CF patients (18). However, it is unclear if these improvements have had a similar impact on the bone health of adults with CF. The first manuscript submitted with this thesis, which is currently under review for publication at the Journal of Cystic Fibrosis, directly addresses this question. We compared clinical characteristics and BMD measured by DXA in adults with CF evaluated in 1995-1999 to age-, race-, and gender matched patients with CF evaluated in 2011-2013 at the same center on calibrated DXA machines. Interestingly, despite improvements in pulmonary function and lower rates of vitamin D deficiency and secondary hyperparathyroidism in present-day CF patients, BMD in young adults with CF remains as low today as it was in patients in the late 1990s. These sobering results suggest that our current efforts to prevent this complication may be inadequate and illustrate the need for further progress in this area.

Current tools for assessing bone health, namely DXA and standard peripheral quantitative computed tomography (pQCT), have significant limitations. DXA is the primary method for measuring BMD and assessing fracture risk in both children and adults. DXA-based BMD values are two-dimensional rather than volumetric and are therefore influenced by bone size, overestimating BMD for large bones and underestimating BMD for small bones (19), as are
seen in many patients with CF. Moreover, DXA cannot distinguish between trabecular and cortical bone (20). Standard pQCT can provide volumetric BMD and some aspects of bone structure; however the significant radiation exposure limits its utility, and the resolution is 0.4 mm, which is not ideal for assessing cortical and trabecular microarchitecture or detecting small changes in bone architecture. In contrast, high resolution peripheral quantitative computed tomography (HR-pQCT) is a new modality may be a superior measure of assessing changes in BMD. HR-pQCT has a resolution (voxel size) of 82 um, which allows for the assessment of the microstructure of bone in three dimensions. It measures true volumetric BMD, rather than areal BMD, of the total bone segment as well as the individual cortical and trabecular compartments (22-24). Furthermore, HR-pQCT is not limited by projection artifacts caused by bone size and adipose tissue as can occur with DXA measurements (25), which is of particular concern in CF patients who tend to be shorter and weight less than their healthy peers. In addition, HR-pQCT also offers the advantage over standard pQCT of smaller voxel size (82um vs. 0.4mm), lower radiation dose, and faster scanning time. HR-pQCT is currently being studied as an alternative method of measuring bone density, predicting fracture, and assessing response to medications used to treat osteoporosis (34). HR-pQCT also has the potential to provide a more accurate estimation of bone strength using microfinite element analysis (μFEA) (26-31).

The second manuscript presented for this thesis was published last month in the Journal of Clinical Endocrinology and Metabolism and represents the first study to utilize this new technology in patients with CF. This manuscript presents novel data in our understanding of the alterations in bone micro-architecture and strength in CF patients and supporting the utility of HR-pQCT in this patient population (21). We performed HR-pQCT scans of the radius and tibia in 30 young adults with CF ages 18-40 years, compared with 60 healthy volunteers matched by age (± 2 years), gender, and race. At the radius and tibia, young adults with CF had smaller bone cross-sectional area and lower vBMD. Cortical and trabecular micro-architecture were compromised at both sites. These differences translated into 23% and 22% lower estimated bone strength at the radius and tibia, respectively. After accounting for BMI differences, young adults with CF had lower bone area and estimated bone strength at the radius and had compromised trabecular micro-architecture and lower total and trabecular vBMD and estimated bone strength at the tibia. Furthermore, deficits in bone strength persisted at the tibia even after adjustment for BMI and DXA areal BMD, suggesting that HR-pQCT provides unique information beyond DXA.

This thesis presents novel data regarding the underlying bone structural abnormalities observed in CF related bone disease and also illustrates that compromised bone health continues to remains an important clinical problem even despite recent advances in the care and treatment of patients with CF. Given the recent emergence of this clinical problem, there is still a great deal to learn about the pathogenesis and treatment of this condition. These manuscripts set the stage for our ongoing clinical study, “Cystic Fibrosis Related Bone Disease: the Role of CFTR,” which is a longitudinal multiple cohort study evaluating the effects of CFTR potentiation using a new FDA-approved medication, ivacaftor, on bone microarchitecture as measured by HR-pQCT in children and adults with CF.
References

Trends in Bone Mineral Density in Young Adults with Cystic Fibrosis over a 15 Year Period

Melissa S. Putman, MD;1,2 Joshua F. Baker, MD, MSCE;3 Ahmet Uluer, DO;4 Karen Herlyn MD, MPH;5 Allen Lapey, MD;6 Leonard Sicilian, MD;7 Angela Pizzo Tillotson, FNP-BC;8 Catherine M. Gordon, MD, MSc;2,9 Peter A. Merkel, MD,MPH;3 Joel S. Finkelstein, MD1

1Endocrine Unit, Department of Medicine, Massachusetts General Hospital, Boston, MA; 2Division of Endocrinology, Boston Children’s Hospital, Boston, MA; 3Division of Rheumatology, University of Pennsylvania, Philadelphia, PA; 4Division of Respiratory Diseases, Boston Children’s Hospital, Boston, MA; 5Poliklinik fuer Rheumatologie, University Hospital Schleswig-Holstein, Campus Luebeck, Germany; 6Pulmonary Division, Department of Pediatrics, Massachusetts General Hospital, Boston, MA; 7Pulmonary Division, Department of Medicine, Massachusetts General Hospital, Boston, MA; 8Mattina R. Proctor Diabetes Center, Mercy Hospital, Portland Maine; 9Divisions of Adolescent Medicine and Endocrinology, Hasbro Children’s Hospital, Providence, RI

Word Count: 3251

Address for Correspondence: msputman@partners.org; Endocrine Unit, Massachusetts General Hospital, 50 Blossom Street, THR-1051, Boston, MA 02114; phone 617-726-6723; fax 617-726-1703

Grant Support: This work was supported in part by a research grant from The Cystic Fibrosis Foundation, The Boomer Esaison Heroes Foundation, and the Mallinckrodt General Clinical Research Center Massachusetts General Hospital, NIH Grant M01 RR 01066. This project was also supported by the Harvard Clinical and Translational Science Center (Grant Numbers 8 UL1 TR000170-05, 1 UL1TR001102-01, and 1 UL1RR025758-04). A Vertex Pharmaceuticals Investigator Initiated Studies Grant provided partial support for procedures in this study. Dr. Putman was supported by an NIH T32 award and the Endocrine Society Amgen Fellowship Award. Dr. Baker is supported by a VA Clinical Science Research and Development Career Development Award. Dr. Merkel was supported by grants from the NIH including a Clinical Associate Physician Award from the National Center for Research Resources (NCRR) and a Mid-Career Development Award in Clinical Investigation (K24-AR-02224) from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS).

Disclosures: All authors state that they have no conflicts of interest.

Abbreviations: DXA, dual energy X-ray absorptiometry; BMD, bone mineral density; CF, cystic fibrosis; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; NTX, N-telopeptide; BSAP, bone specific alkaline phosphatase
Abstract

Background: Improvements in clinical care have led to increased life expectancy in patients with cystic fibrosis (CF) over the past several decades. Whether these improvements have had significant effects on bone health in patients with CF is unclear.

Methods: This is a cross-sectional study comparing clinical characteristics and bone mineral density (BMD) measured by dual energy X-ray absorptiometry (DXA) in adults with CF evaluated in 1995-1999 to age-, race-, and gender matched patients with CF evaluated in 2011-2013 at the same center on calibrated DXA machines.

Results: The cohorts were similar in terms of age, BMI, pancreatic insufficiency, presence of F508del mutation, and reproductive history. In the most recent cohort, pulmonary function was superior, and fewer patients had vitamin D deficiency or secondary hyperparathyroidism. Areal BMD measures of the PA spine, lateral spine, and distal radius were similarly low in the two cohorts.

Conclusions: Although pulmonary function and vitamin D status were better in patients in the present-day cohort, areal BMD of the spine was reduced in a significant number of patients and was no different in patients with CF today than in the late 1990s. Further attention to optimizing bone health may be necessary to prevent CF-related bone disease.

Keywords: Cystic fibrosis, osteoporosis, dual energy X-ray absorptiometry, bone turnover markers, vitamin D
Introduction

Over the past several decades, patients with cystic fibrosis (CF) have been living longer, largely due to improvements in the treatment of the underlying pulmonary and gastrointestinal manifestations of their disease. With this increasing life expectancy, other complications of CF have become more prevalent, including low bone density and fractures.

Multiple studies have confirmed that adolescents and young adults with CF have lower areal bone mineral density (BMD) as measured by dual energy X-ray absorptiometry (DXA) and are at higher risk for fracture (1-5). In particular, patients with CF are at risk for rib and vertebral fractures, which can lead to significant morbidity and mortality (6, 7). An unusual pattern of bone turnover has also been described in this patient population, with higher than expected markers of bone resorption and lower than expected markers of bone formation (8, 9). Although the exact pathogenesis of CF-related bone disease is unclear, patients with CF possess multiple risk factors for low bone density, including pancreatic insufficiency, malabsorption, poor nutritional status, vitamin D deficiency, delayed puberty, glucocorticoid use, inflammation, and physical inactivity (10).

Since the late 1990s, awareness of CF-related bone disease has led to increased attention to bone density screening, optimization of nutritional status, and reduction of modifiable risk factors for low bone density. The Cystic Fibrosis Foundation and the European Cystic Fibrosis Society published guidelines in 2005 and 2011, respectively, addressing the importance of bone health in this population (10, 11). At the same time, establishment of CF Centers has occurred that focus on standardizing the care of CF patients and the development of new medications and interventions to improve
respiratory function (12). Whether these improvements in clinical care have led to significant changes in bone health in patients with CF over the past 15 years is unclear. The purpose of this study was to compare DXA-derived areal BMD in patients with CF evaluated 15 years ago with age-, race- and gender-matched patients with CF in the present day. We hypothesized that patients in the present-day cohort would exhibit improved BMD compared to the historic cohort, due to advances in clinical care and increased attention to bone and mineral status in these patients.

Materials and Methods

Subjects and Eligibility Criteria

Two cohorts of ambulatory patients with CF were evaluated in this study. In the historic cohort, adults with cystic fibrosis ages 18-50 years of age were recruited from the Massachusetts General Hospital (MGH) Cystic Fibrosis Center between November 1995 and June 1999. Exclusion criteria included history of solid organ transplantation and current pregnancy. This protocol was approved by the Institutional Review Board (IRB) at MGH. In the present-day cohort, subjects were recruited from the MGH and Boston Children’s Hospital (BCH) Cystic Fibrosis Centers between December 2011 and November 2013. Exclusion criteria were the same as for the historic cohort. This protocol was approved by the MGH IRB with ceded review by the BCH IRB. Written informed consent was obtained from all participants. A total of 52 subjects were enrolled in the historic cohort and 38 in the present day cohort. Thirty-two subjects in each cohort were able to be matched by age, race, and gender and were included in this study.

Clinical Assessments
In both the historic and present-day cohorts, study procedures were performed in the MGH Clinical Research Center. Subjects were surveyed regarding medical history, current medication use, history of fractures, pubertal and reproductive history, and lifetime glucocorticoid exposure. A registered bionutritionist assessed calcium and vitamin D intake using the validated NDS-R (13). The NDS-R version was updated in the years between the recruitment of the two study cohorts to reflect the most updated nutrient data available at the time. Medical records were reviewed to obtain pulmonary function tests and CFTR genotype. In all subjects, height was measured on a wall-mounted stadiometer and weight on a standardized scale.

**Laboratory Assessment**

After fasting overnight, morning serum and urine samples were obtained in all subjects. In the historic cohort, the labs were processed by the MGH General Clinical Research Center, and in the present-day cohort, labs were processed through the MGH Clinical Laboratory Research Core. Urine N-telopeptide (NTX) was measured by competitive-inhibition enzyme-linked immunosorbent assay (Osteomark) in both cohorts. Serum 25-hydroxyvitamin D [25(OH)D] levels were measured by radioimmunoassay (DiaSorin in the historic cohort; Abbott Diagnostics in the present-day cohort). Bone specific alkaline phosphatase (BSAP) was measured using enzyme immunoassay (Metra Biosystems in the historic cohort; Quidel Corporation in the present-day cohort). Intact parathyroid hormone (PTH) was measured by immunoradiometric assay (Nichols Institute) in the historic cohort and by electrochemiluminescence immunoassay (Roche Diagnostics) in the present-day cohort. The reference ranges established by the manufacturers were the same for both cohorts.
**Assessment of areal BMD**

In both cohorts, DXA areal BMD of the lumbar spine in the posterior-anterior (PA) and lateral projections and the distal radius were measured in the MGH Bone Density Center. Scans were performed on a QDR4500A model in the historic cohort and a Discovery A model in the current cohort (Hologic Inc, Bedford, MA). When the QDR4500A model was replaced by the more recent model, calibration studies were performed to allow for direct comparisons.

**Statistical Analysis**

Statistical analysis was performed using SAS 9.3 software (SAS Institute Inc., Cary, NC). Subjects in the historic and present-day cohorts were matched by age (within 5 years), race, and gender to minimize effects of these covariates on bone outcomes. Characteristics between subjects in the two cohorts were compared using independent samples two-sided t-tests for normally distributed data or Wilcoxon rank sum for non-normally distributed data. Categorical variables were compared using Chi Square or Fisher Exact tests.

Because the laboratory manufacturers differed between the historic cohort and the present cohort for all assays with the exception urine NTX, we did not perform direct comparisons between lab values between the two cohorts. Instead, using manufacturer references ranges, BSAP and PTH results in each cohort were categorized normal or abnormal. Subjects were also categorized as vitamin D sufficient or deficient, as defined by a 25(OH)D level below 20 ng/mL (14). The vitamin D assays (DiaSorin and Abbott) have good correlation at 25(OH)D levels of less than 50 ng/mL, but the Abbott assay has systemic positive bias compared to DiaSorin at levels above than 50 ng/mL (15).
Therefore, this bias was avoided by introducing the above categories. These categorical variables were then compared between cohorts using Chi Square or Fisher Exact test.

The urine NTX method and manufacturer was the same in both cohorts, and these results were compared between cohorts using Wilcoxon rank sum for non-normally distributed data.

The primary outcome was PA spine areal BMD. General linear regression was used to compare BMD between groups before and after multivariable adjustment for pulmonary function as measured by percent predicted forced expiratory volume in one second (FEV1), presence of vitamin D deficiency, presence of secondary hyperparathyroidism, and prior steroid use. Covariates in the multivariable model were chosen based on clinical relevance as well as significance on the univariate screen.

To evaluate associations between clinical characteristics and areal BMD among all CF subjects (n=64), correlations were performed using Pearson correlation coefficient, or Spearman correlation coefficient in the case of non-normally distributed data. Clinical covariates included FEV1, BMI, glucocorticoid use, age at menarche in females, age at pubarche in males, calcium and vitamin D intake, urine NTX, and other laboratory values recoded into normal or abnormal categories. In addition, ANOVA was used to determine if areal BMD differed significantly between patients with CFTR genotypes containing zero, one, or two copies of the F508del mutation.

Based on the standard deviation of the primary outcome in the current cohort, this sample size has 80% power to detect at least a 7% difference in absolute BMD between cohorts. A smaller difference was not considered clinically meaningful. Data are reported
as mean ± standard deviation (SD) unless otherwise noted, and p values ≤0.05 are considered statistically significant.

Results

Cohort Characteristics

The mean time difference between measurements in the historic cohort (evaluated between 1995 to 1999) and the present-day cohort (evaluated between 2011 to 2013) was 15.0 ± 1.3 years. Clinical characteristics of subjects in both cohorts are presented in Table 1. Age, height, weight, and BMI were similar between cohorts. Nine patients (28%) in the historic cohort and seven (22%) in the present-day cohort were underweight with a BMI <18.5 (p=0.56). A similar, small number of patients, 10 (31%) in the historic cohort and 8 (25%) in the current cohort (p=0.58), had a BMI at or above that recommended by the CF Foundation (16).

Pulmonary function was moderately impaired in both cohorts, and the historic cohort had a significantly lower percent predicted FEV1 than the present-day cohort (p=0.03). There were no differences between cohorts in reported fractures, pubertal and reproductive history, the prevalence of pancreatic insufficiency, or the presence of the F508del mutation.

The average daily dietary and supplemental calcium and vitamin D intake was above the national Recommended Daily Allowance (RDA) (14) in both cohorts. Although not significant, there was a trend toward higher vitamin D intake in the present-day cohort than the historic cohort (Table 1). Although a similar small number of patients in both cohorts reported current use of oral glucocorticoids, fewer patients in the historic cohort reported ever using oral glucocorticoids (p=0.029), and the median
duration of prior oral glucocorticoid use was significantly lower in the historic cohort (p=0.012). Current use of inhaled glucocorticoids was also significantly greater in patients in the present-day cohort (p=<0.001).

Laboratory Results

Laboratory results are presented in Table 2. Urine NTX levels were similar in both cohorts (p=0.83). A greater number of patients in the historic cohort had 25(OH)D levels less than 20 ng/ml than in the current cohort (48% vs. 22%, respectively, p=0.039). The seasons during which the 25(OH)D levels were drawn did not differ between the two cohorts (p=0.18). Serum PTH levels were above the normal range in 19% of patients in the historic cohort, whereas no patients in the present-day cohort had an elevated PTH level (p=0.026). No differences in normal or abnormal BSAP levels between cohorts were noted. No patients in either cohort had renal failure (data not shown).

Areal BMD Results

There were no significant differences in unadjusted areal BMD measurements of the PA spine, lateral spine, or distal radius in the historic cohort compared to the present-day cohort. Multivariable adjustment accounting for percent predicted FEV1, vitamin D sufficiency, secondary hyperparathyroidism, and glucocorticoid use did not change the significance of these findings.

Z-scores were reported using the Hologic reference databases available at the time that each DXA scan was obtained. Twelve patients in the historic cohort and 11 patients in the present-day cohort had a Z-score of at least 1.0 standard deviation below the mean, and five and three patients in the historic and present-day cohorts, respectively, had a Z-
score at least 2.0 standard deviations below the mean (Figure 1). These proportions were 179 similar between cohorts (p=0.74).

Areal BMD of the spine was positively associated with BMI and negatively 181 associated with urine NTX in all subjects (n=64) (Figure 2). Reported duration of prior 182 glucocorticoid use, current use of inhaled steroids, pubertal history, calcium and vitamin 183 D intake, and FEV1 were not significantly correlated with BMD. PA spine BMD was not 184 associated with the presence of F508del.

Discussion

Despite improvements in clinical care and life expectancy in patients with CF 187 over the past 15-20 years, areal BMD as measured by DXA is no better in young adults 188 with CF today than in an age-, race-, and gender matched cohort of young adults with CF 189 evaluated in 1995-1999. Areal BMD was similar between these cohorts even after 190 adjusting for the significantly improved pulmonary status and lower rate of vitamin D 191 deficiency and secondary hyperparathyroidism of the present-day patients. These data 192 suggest that further attention to optimizing bone health in CF patients, possibly by 193 focusing on modifiable factors such as the avoidance glucocorticoids and improvement in 194 nutritional status and BMI, will be important to prevent skeletal consequences related to 195 CF.

Over the past 15-20 years, treatments aimed at improving nutritional status and 197 slowing pulmonary decline in patients with CF have led to greater life expectancy and 198 improved quality of life (17). The development of health care teams and CF Centers 199 dedicated to the multidisciplinary treatment of these patients has allowed for 200 standardization of care and utilization of best practice protocols. In addition, aggressive
management of respiratory infections, improvements in therapies targeted at mucociliary clearance, and a push for early identification and management of non-pulmonary complications have also contributed to improved clinical care of patients with CF (12).

Despite these clinical improvements, CF-related bone disease remains a concerning clinical problem leading to significant morbidity. In a recent meta-analysis reviewing studies published prior to 2008, the pooled prevalence of osteoporosis and osteopenia in adults with CF were 24% and 38%, respectively, and the incidence increased with age (18). Guidelines published in 2005 (10) and 2011 (11) recommend routine DXA screening, optimization of calcium and vitamin D intake, and aggressive nutritional interventions to maintain adequate body weight.

To our knowledge, this is the first study directly comparing BMD in two cohorts of age-, gender- and race matched cohorts of ambulatory patients measured over a decade and a half apart on calibrated DXA machines. Pulmonary function was significantly better in the present-day cohort, and fewer patients in the current cohort had vitamin D deficiency or secondary hyperparathyroidism. For these reasons, we expected that bone density in the current cohort would be better than in the historic cohort. However, areal BMD in young adults with CF today is identical to matched patients measured 15 years ago. A significant and similar number of patients had reduced bone density Z-scores below -1.0, and almost half of patients in each cohort reported a prior fracture.

There are several possible explanations for this lack of improvement in BMD. First, as in previous studies (19-22), we found a significant correlation between BMI and BMD in patients with CF. Despite increased attention to nutritional status over recent years, BMI was similar between the two cohorts. Although the CF Foundation
recommends that women attain a BMI of at least 22 kg/m2 or greater and men at least 23 kg/m2 (16), only a minority of patients in either cohort met that goal. Difficulty achieving nutritional goals remain as significant a problem today as 15 years ago, and this issue may help to explain the similar BMD findings between cohorts.

In addition, more patients in the present-day cohort reported prior oral glucocorticoid use and current inhaled steroid use, likely related to differences in treatment practices between the two cohorts. Systemic and inhaled glucocorticoids can be detrimental to bone (23-25). Although the duration of oral glucocorticoid use and current use of inhaled steroids were not correlated with BMD, the greater exposure to glucocorticoids in the current cohort may help to explain the lack of improvement in BMD compared to the historic cohort. These data support the recommendations to minimize glucocorticoid use whenever possible (10, 11).

Other potential factors that may affect bone health in patients with CF include delayed puberty, hypogonadism, and pancreatic insufficiency. Patients in both cohorts reported an overall normal age at menarche and pubarche, and menstrual and reproductive history in females was not significantly different. Similar numbers of patients also reported pancreatic insufficiency treated with pancreatic enzyme replacement. The similarity of these characteristics may have contributed to the equivalent BMD between cohorts.

Abnormalities in bone turnover have been described in patients with CF, with high bone resorption and low bone formation (8-10). In both the historic and current cohorts, urine NTX, a marker of bone resorption, was similar and exceeded the normal range in 42% of patients. Urine NTX was also significantly and negatively correlated
with areal BMD of the spine. These results are consistent with previous studies (8, 9) and suggest that increased bone resorption plays a prominent role in CF-related bone disease, past and present.

The cause of this striking uncoupling of bone turnover has not been fully elucidated and raises the intriguing possibility that CF-related bone disease may be in part due to CFTR dysfunction itself (26-28). CFTR is expressed in human bone cells (29) and may affect osteoclast activation by altering osteoprotegerin production (27, 30).

Other potential factors that may increase bone resorption could include underlying inflammation, chronic respiratory acidosis, hyperglycemia, and reduced physical activity. The fact that average BMD in patients with CF has not changed in 15 years despite significant improvements in clinical care and life expectancy could support the hypothesis that these underlying factors may be playing an independent role in CF-related bone disease. This also raises the possibility that improving CFTR function with new CFTR modulators may also improve bone health in CF patients in the future.

Strengths and limitations of this study merit consideration. Our study has several important strengths, including the detailed clinical information regarding factors affecting skeletal health in patients with CF prospectively collected in each cohort. In addition, both cohorts of patients were relatively homogenous, limited to young, ambulatory adults prior to onset of severe lung disease and transplantation. Importantly, the use of calibrated DXA machines at a single center allowed for direct comparisons of BMD between the two cohorts despite measurements occurring 15 years apart. Our study also has several important limitations. The sample size was relatively small, and it is possible that smaller, albeit less clinically meaningful, differences in BMD could be uncovered if
a larger number of patients were evaluated. In addition, BMD of the proximal femur was not assessed in the historic cohort. Laboratory methods and manufacturers have also changed over the past 15 years, and this limited our ability to make direct comparisons between laboratory results between the two cohorts. In addition, serum 25(OH)D was measured by RIA rather than LCMS, which was not available in the historic cohort.

Finally, the cross-sectional design of this study cannot capture the dynamic changes that occur in the skeleton nor account for the effects of clinical variables that may have occurred in the years prior to this evaluation.

In conclusion, young adults with CF evaluated in late 1990s had similar areal BMD of the spine and distal radius as age-, gender- and race-matched patients measured 15 years later. Despite improved pulmonary function and lower prevalence of vitamin D deficiency and secondary hyperparathyroidism in the present-day cohort, a similar and significant number of patients had low BMD Z-scores and reported fractures. These results suggest that attention to bone health, including aggressive nutritional interventions to optimize BMI and attempts to minimize glucocorticoid use, may be important in preventing this complication. Further studies will be required to improve our understanding of the prevention and treatment of CF-related bone disease.

Acknowledgements

The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the NIH, NCRR, NIAMS, Department of Veterans Affairs or the United States Government. The authors gratefully acknowledge
the support of the dedicated staff of the MGH Clinical Research Center, the Research Groups of the MGH and BCH CF Centers, and the MGH Bone Density Center.

References


Table 1: Clinical and Demographic features of Subjects with CF in the historic and present-day cohorts. BMI, body mass index; FEV1, % predicted forced expiration in 1 second.

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD), Median (range), or n (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Historic Cohort (n=32)</td>
<td>Present-Day Cohort (n=32)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>27.6 (7.0)</td>
<td>27.5 (7.7)</td>
</tr>
<tr>
<td>Female</td>
<td>19 (59%)</td>
<td>19 (59%)</td>
</tr>
<tr>
<td>Caucasian Race</td>
<td>32 (100%)</td>
<td>32 (100%)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.0 (6.2)</td>
<td>166.3 (8.3)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.7 (10.1)</td>
<td>58.5 (11.3)</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>20.6 (2.7)</td>
<td>21.1 (3.7)</td>
</tr>
<tr>
<td>Percent predicted FEV1 (%)</td>
<td>53.9 (21.8)</td>
<td>67.5 (28.2)</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>1927 (426-4762)</td>
<td>1836 (539-9795)</td>
</tr>
<tr>
<td>Vitamin D intake (IU/day)</td>
<td>910 (77-1501)</td>
<td>1397 (36-8279)</td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>26 (81%)</td>
<td>27 (84%)</td>
</tr>
<tr>
<td>CFTR genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F508del/F508del</td>
<td>15 (47%)</td>
<td>11 (34%)</td>
</tr>
<tr>
<td>F508del/Other</td>
<td>12 (37.5%)</td>
<td>13 (41%)</td>
</tr>
<tr>
<td>Other/Other</td>
<td>5 (15.5%)</td>
<td>8 (25%)</td>
</tr>
<tr>
<td>Previous fracture</td>
<td>14 (44%)</td>
<td>13 (41%)</td>
</tr>
<tr>
<td>Age menarche (y)</td>
<td>13.6 (2.6)</td>
<td>12.7 (1.4)</td>
</tr>
<tr>
<td>Amenorrhea &gt;6 months</td>
<td>5 (26%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>7 (22%)</td>
<td>5 (16%)</td>
</tr>
<tr>
<td>Age pubarche (males)</td>
<td>12.5 (11-19)</td>
<td>13 (11-15)</td>
</tr>
<tr>
<td>Use of oral glucorticoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current use</td>
<td>2 (6%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Previous use</td>
<td>14 (44%)</td>
<td>22 (69%)</td>
</tr>
<tr>
<td>Lifetime exposure (mo)</td>
<td>4.0 (8.7)</td>
<td>8.4 (10.4)</td>
</tr>
<tr>
<td>Current use of inhaled glucocorticoids</td>
<td>8 (25%)</td>
<td>22 (69%)</td>
</tr>
</tbody>
</table>
Table 2: Laboratory Results. Data expressed as median (range).

PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; NTX, N-telopeptide; BSAP, bone specific alkaline phosphatase

<table>
<thead>
<tr>
<th></th>
<th>Historic Cohort</th>
<th>Present-Day Cohort</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH (pg/mL)</td>
<td>29 (11-95)</td>
<td>33 (16-60)</td>
<td>10-60</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>20 (1-68)</td>
<td>27 (12-91)</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Urine NTX (nM BCE/mM Cre)</td>
<td>67 (14-473)</td>
<td>54 (19-236)</td>
<td>3-65</td>
</tr>
<tr>
<td>BSAP (U/L)</td>
<td>21.3 (11-110)</td>
<td>27.9 (13-56)</td>
<td>Female 11.6-30.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male 15.0 - 41.3</td>
</tr>
</tbody>
</table>

Table 3. DXA Areal BMD Results. Data are expressed as mean ± SE. Multivariable adjusted model includes the following covariates: FEV1, vitamin D sufficiency (defined as 25[OH]D>20ng/mL), secondary hyperparathyroidism (defined as elevated PTH level), and glucocorticoid use.

FEV1, % predicted forced expiration in 1 second; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; PA, posterior-anterior; BMD, bone mineral density.

<table>
<thead>
<tr>
<th>DXA areal BMD</th>
<th>Unadjusted</th>
<th>Multivariable-Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Historic Cohort</td>
<td>Present-Day Cohort</td>
</tr>
<tr>
<td>PA Spine (g/cm²)</td>
<td>0.983 ± 0.034</td>
<td>0.981 ± 0.034</td>
</tr>
<tr>
<td>Lateral Spine (g/cm²)</td>
<td>0.780 ± 0.017</td>
<td>0.803 ± 0.019</td>
</tr>
<tr>
<td>1/3 Distal Radius (g/cm²)</td>
<td>0.723 ± 0.016</td>
<td>0.730 ± 0.012</td>
</tr>
</tbody>
</table>
Figure 1: Percent of patients with normal or reduced PA spine BMD Z-scores in the historic cohort and present-day cohort (p=0.74).
PA, posterior-anterior; BMD, bone mineral density.

Figure 2. Correlations between PA spine BMD and BMI (2a) and urine NTX (2b) among all subjects with CF.
PA, posterior-anterior; BMD, bone mineral density; BMI, body mass index; NTX, N-telopeptide; CF, cystic fibrosis
Figure 2

Left graph: BMI (kg/m²) vs. PA Spine BMD (g/cm²)
- Correlation coefficient: r = 0.32, p = 0.01

Right graph: Urine NTX (nMBCE/mMCre) vs. PA Spine BMD (g/cm²)
- Correlation coefficient: r = -0.52, p = 0.01
Compromised Bone Microarchitecture and Estimated Bone Strength in Young Adults with Cystic Fibrosis

Melissa S. Putman, MD;1,2 Carly E. Milliren, MPH;3 Nicholas Derrico, BA;1 Ahmet Uluer, DO;5 Leonard Sicilian, MD;4 Allen Lapey, MD;6 Gregory Sawicki, MD, MPH;5 Catherine M. Gordon, MD, MSc;2,7 Mary L. Bouxsein, PhD;1 Joel S. Finkelstein, MD1

1Endocrine Unit, Department of Medicine, Massachusetts General Hospital, Boston, MA; 2Division of Endocrinology, Boston Children’s Hospital; 3Clinical Research Center, Boston Children’s Hospital; 4Pulmonary Division, Department of Medicine, Massachusetts General Hospital; 5Division of Respiratory Diseases, Boston Children’s Hospital; 6Pulmonary Division, Department of Pediatrics, Massachusetts General Hospital; 7Divisions of Adolescent Medicine and Endocrinology, Hasbro Children’s Hospital

Abbreviated Title: Compromised bone microarchitecture in CF

Keywords: Cystic fibrosis, high resolution peripheral quantitative computed tomography, dual energy X-ray absorptiometry, bone microarchitecture, microfinite element analysis

Word Count (excluding abstract, figure captions, and references): 3597

Address for Correspondence: mspputman@partners.org; Endocrine Unit, Massachusetts General Hospital, 50 Blossom Street, THR-1051, Boston, MA 02114; phone 617-726-6723; fax 617-726-1703

Grant Support: This study was supported in part by an NIH T32 award and the Endocrine Society Amgen Fellowship Award. A Vertex Pharmaceuticals Investigator Initiated Studies Grant provided partial support for procedures in this study. The HR-pQCT measurements were made possible by an NCRR Shared Equipment Grant (1S10RR023405-01).

Disclosures: All authors state that they have no conflicts of interest.
Abstract

Context: Young adults with cystic fibrosis (CF) are at risk for low bone density and fractures, but the underlying alterations in bone microarchitecture that may contribute to their increased fracture risk are currently unknown.

Objective: The main goal of this study was to utilize high resolution peripheral quantitative computed tomography (HR-pQCT) to characterize the bone micro-architecture, volumetric bone mineral density (vBMD), and estimated strength of the radius and tibia in young adults with CF compared to healthy volunteers.

Design: Cross-sectional study.

Setting: Outpatient clinical research center within a tertiary academic medical center.

Participants: Thirty young adults with CF ages 18-40 years were evaluated and compared to 60 healthy volunteers matched by age (± 2 years), gender, and race.

Intervention: N/A

Main Outcome Measures: The primary outcomes were HR-pQCT-derived cortical and trabecular vBMD, bone microarchitecture, and estimates of bone strength.

Results: At the radius and tibia, young adults with CF had smaller bone cross-sectional area and lower vBMD. Cortical and trabecular microarchitecture were compromised at both sites, most notably involving the trabecular bone of the tibia. These differences translated into lower estimated bone strength both at the radius and tibia. After accounting for BMI differences, young adults with CF had lower bone area and estimated bone strength at the radius and had compromised trabecular microarchitecture and lower total and trabecular vBMD and estimated bone strength at the tibia. Alterations in trabecular bone density and microarchitecture and estimated strength measures of the tibia were also greater than expected based on DXA-derived areal BMD differences.

Conclusions: Young adults with CF have compromised bone microarchitecture and lower estimated bone strength at both the radius and tibia, even after accounting for their smaller body size. These skeletal
deficits likely explain the higher fracture risk observed in young adults with CF.
Introduction

Over the past several decades, life expectancy for patients with cystic fibrosis (CF) has increased significantly. As patients live longer, other comorbidities related to this disease have emerged, including CF-related bone disease (1). The pathogenesis of the low bone mineral density (BMD) and increased fracture risk in patients with CF is currently not well understood. Obtaining new information about the underlying skeletal composition of these patients may be an important step in the monitoring, prevention, and the potential treatment of this complication.

Fracture rates in patients with CF are higher than in healthy individuals, particularly rib and vertebral fractures (2-6). In patients with CF, these fractures can have severe repercussions, leading to pain, kyphosis, chest wall deformities, reduced lung volumes, ineffective cough and airway clearance, and ultimately compromised lung function. In addition, severe bone disease may obviate lung transplantation. Due to this high morbidity, CF related bone disease can significantly affect the health, well-being, and longevity of these patients.

Areal BMD, as measured by dual-energy X-ray absorptiometry (DXA), is low in patients with CF (7-10). DXA has important limitations, however, particularly in patients with CF. First, alteration in bone size introduces an artifact in DXA measurements such that values are reduced in smaller bones. Thus, in patients with CF, small bone size could account for their lower DXA values. Second, DXA cannot distinguish between trabecular and cortical bone (11, 12). Third, DXA may not predict fracture risk accurately in patients with CF, such that their risk of fracture is increased even with normal DXA areal BMD measurements (5).

High resolution peripheral quantitative computed tomography (HR-pQCT) is a new imaging modality that has a resolution (voxel size) of 82 um and allows for the non-invasive assessment of volumetric bone mineral density (vBMD) and microstructure at peripheral skeletal sites (13-15). Furthermore, unlike DXA, HR-pQCT may be less affected by projection artifacts caused by bone size and adipose tissue. Finally, bone geometry and material characteristics can be integrated to provide an
estimation of bone strength by applying microfinite element analyses (μFEA) to HR-pQCT images (16,
17).

The goals of this study were to utilize HR-pQCT to (1) characterize the bone micro-architecture,
vBMD, and estimated strength of the radius and tibia in young adults with CF compared to healthy
volunteers, (2) determine whether these HR-pQCT derived parameters provide information independent
of areal BMD that may help to explain the increased fracture risk in patients with CF, and (3) evaluate
clinical characteristics of young adults with CF that may be associated with worsening bone structure and
strength.

Materials and Methods

Subjects and Eligibility Criteria

Young adults aged 18–40 years with CF were recruited from the Massachusetts General Hospital
and Boston Children’s Hospital Cystic Fibrosis Centers. Exclusion criteria included history of solid
organ transplantation, current pregnancy, and *Burkholderia dolosa* infection (due to infection control
issues). Data from healthy volunteers were obtained from studies enrolling subjects from the community
to establish a normal database for HRpQCT measures. Two healthy volunteers were matched to each
subject with CF by age (within two years), race, and gender. Exclusion criteria included current
pregnancy or a history of disorders known to affect bone metabolism. The protocol was approved by the
Partners Healthcare Human Research Committee with ceded review by the Boston Children’s Hospital
Committee on Clinical Investigation. Written informed consent was obtained from all participants.

Clinical Assessments

Subjects with CF were surveyed regarding medical history, medication use, history of fractures,
pubertal and reproductive history, and lifetime glucocorticoid exposure. A registered bionutritionist
assessed calcium and vitamin D intake using the validated NDS-R (18) and physical activity using the
Modified Activity Questionnaire (19). Medical records were reviewed to obtain the most recent
pulmonary function tests and CF genotype. When available, reported fractures were confirmed with Xray
reports. In all subjects, height was measured on a wall-mounted standiometer and weight on an electronic scale. Race was self-reported.

**Laboratory Assessment**

After fasting overnight, morning serum and urine samples were obtained in subjects with CF. Serum creatinine, calcium and phosphorus levels were performed by Labcorp (Raritan, New Jersey). The remaining labs were processed through the Massachusetts General Hospital Clinical Laboratory Research Core. Serum 25-hydroxyvitamin D (25[OH]D) levels were measured by chemiluminescent microparticle immunoassay (Abbott Diagnostics, Abbott Park, IL). Bone specific alkaline phosphatase (BSAP) levels were obtained by enzyme immunoassay (Quidel Corporation, San Diego, CA). Parathyroid hormone (PTH) and C-telopeptide (CTX) levels were measured by electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN). Urine N-telopeptide (NTX) levels were measured by competitive-inhibition enzyme-linked immunosorbent assay (Alere Scarborough Inc, Scarborough ME) and amino-terminal propeptide of type I collagen (P1NP) by quantitative radioimmunoassay (Orion Diagnostica, Espoo, Finland). References ranges for each assay were obtained from the manufacturer.

**Assessment of areal BMD**

Areal BMD of the lumbar spine in the posterior-anterior (PA) projection, total hip, femoral neck, and whole body (excluding the head) along with body composition were measured using DXA (Discovery A, Hologic Inc, Bedford, MA). A standard quality control program was employed that included daily measurement of a Hologic DXA anthropomorphic spine phantom and visual review of all images by an experienced investigator.

**Assessment of vBMD, bone microarchitecture, and bone strength**

vBMD and microarchitecture of the distal radius and tibia were assessed by HR-pQCT (Xtreme CT, Scanco Medical AG, Basserdorf, Switzerland) at the standard regions of interest using previously described methods (13-15). Quality control was maintained with daily scanning of the manufacturer’s
phantom. All HR-pQCT scans were reviewed for motion artifact and repeated if significant motion artifact was noted.

HR-pQCT scans were analyzed using Scanco software version V6.0 to provide total and trabecular vBMD (mgHA/cm$^3$), trabecular thickness (mm), trabecular number (mm$^{-1}$), trabecular separation (mm), and trabecular distribution (mm). Cortical microarchitecture was characterized by processing HR-pQCT images with a semi-automated technique implemented in Scanco software (20-22). After image segmentation of cortical bone, the following measures were obtained: cortical vBMD (mgHA/cm$^3$), cortical thickness (mm), cortical and trabecular area (mm$^2$), and cortical porosity (%).

µFEA was used to estimate radius and tibia biomechanical properties under uniaxial compression as previously described (23, 24), providing estimated stiffness (N/mm) and failure load (N).

In our laboratory, reproducibility for HR-pQCT measurements at the radius and tibia range from 0.2-1.4% for vBMD parameters, 0.3-8.6% for trabecular microarchitecture parameters, 0.6-2.4% for cortical microarchitecture parameters, 7.3-20.2% for cortical porosity measurements, and 2.1-3.0% for µFEA measures.

**Statistical Analysis**

Statistical analysis was performed using SAS 9.3 software (SAS Institute Inc., Cary, NC). Clinical characteristics of subjects with CF and healthy volunteers were compared using independent samples two-sided t-tests for normally distributed data or Wilcoxon rank sum for non-normally distributed data. Primary outcomes were HR-pQCT-derived microarchitectural and vBMD measures and µFEA results at the radius and tibia. The study was designed to match two healthy volunteers to each subject with CF to minimize effects of age, race and gender on bone outcomes. To account for this matching, generalized linear models adjusting for each matched group were used to compare differences in means of areal BMD and HR-pQCT parameters between CF subjects and healthy volunteers. Multivariable linear regression was then performed adjusting for BMI, followed by an additional analysis adjusting for total hip areal BMD in addition to BMI. BMI was chosen as a covariate of interest based on
clinical relevance and correlation with outcome variables (Spearman r=0.05-0.52). Total hip areal BMD was chosen because this site represents a balance of both cortical and trabecular bone.

To evaluate associations between clinical characteristics and bone microarchitecture and strength among CF subjects, correlations were performed using Pearson correlation coefficient, or Spearman correlation coefficient in the case of non-normally distributed data, between clinical covariates and bone outcomes (total vBMD and estimated failure load) among subjects with CF. Clinical covariates included 25OHD level, PTH level, bone formation and resorption markers, physical activity level, prior oral glucocorticoid use, and most recent pulmonary function as measured by percent predicted forced expiratory volume in one second (FEV1). Linear regression was used to determine if the presence of DELF508 mutation was a significant predictor of bone strength in subjects with CF as well as to determine if FEV1 effects on bone outcomes were independent of physical activity level.

Data are reported as mean ± standard deviation (SD) unless otherwise noted, and p values <0.05 are considered statistically significant.

Results

Cohort Characteristics

Clinical characteristics of the healthy volunteers and the subjects with CF are presented in Table 1. The mean age of all subjects was 25 years, and 60% were female. Because a majority of patients with CF are Caucasian, all subjects with CF enrolled in this study were of Caucasian race. Young adults with CF were shorter, weighed less, and had a lower BMI than healthy volunteers. All women enrolled in this study were premenopausal.

Additional characteristics specific to subjects with CF are presented in Table 2. A broad range of CFTR genotypes were represented in this study, though most subjects had at least one copy of the most common mutation, delF508. A majority of patients had pancreatic insufficiency requiring pancreatic enzyme replacement, and 17% had CF-related diabetes. Subjects reported excellent dietary and supplemental calcium and vitamin D intake. FEV1 was on average 75% predicted, indicating mild-to-
moderate pulmonary impairment. Twenty-three percent of subjects had experienced at least one non-
digital, non-facial fracture, involving rib (3 patients), wrist (2 patients), or ankle (1 patient). One patient
experienced vertebral compression fractures at T3 and T5 at age 23 years.

Laboratory results in patients with CF are also presented in Table 2. Serum creatinine, calcium,
and phosphorus levels were normal (data not shown). Six patients had a 25OHD level less than 20 ng/mL,
and no patients had a level below 10 ng/mL. Median urine NTX/Cr level was above the normal range.
Serum PTH, CTX, P1NP, and bone specific alkaline phosphatase were within the expected reference
range.

Areal BMD

Areal BMD measurements of the total hip, femoral neck, PA spine, and whole body were
significantly lower in young adults with CF than age-, race-, and gender-matched healthy volunteers
(Table 3). Seventy-three percent of young adults with CF had a Z-score of less than -1.0 at one or more
sites, and 23% had a Z-score of less than -2.0. Although somewhat attenuated, differences remained
significant after adjustment for BMI at all measured sites with the exception of areal BMD at the PA
spine.

HR-pQCT Findings

Radius

In unadjusted analyses, subjects with CF had smaller bones, as indicated by the lower trabecular,
cortical, and total bone cross-sectional areas (Table 3). Trabecular and total vBMD were also lower in
patients with CF than healthy controls. Both cortical and trabecular thickness were lower in subjects with
CF, but the remainder of cortical and trabecular micro-architecture, including cortical porosity and
trabecular separation and number, were similar between the two groups. Subjects with CF also had lower
stiffness and failure load than their healthy peers.

After adjustment for BMI, CF patients continued to have lower total, cortical, and trabecular area,
though volumetric BMD differences were no longer significant. Trabecular thickness, estimated stiffness,
and failure load also remained significantly lower in subjects with CF than healthy controls after BMI adjustment. The addition of total hip areal BMD to the multivariable model eliminated all differences between cohorts. **Figure 2** illustrates µFEA results before and after multivariable adjustment.

**Tibia**

Compromises in micro-architecture and vBMD were most notable at the tibia, and trabecular bone was predominately affected (**Figure 1** and **Table 3**). In unadjusted analyses, subjects with CF had smaller bones and lower total, cortical, and trabecular vBMD. The lower trabecular vBMD was associated with fewer and thinner trabeculae along with wider separation and greater heterogeneity. Cortical microarchitecture was similar between groups. As in the radius, CF patients had significantly lower strength estimates of the tibia as measured by µFEA than their healthy peers (**Figure 2**).

Adjustment for BMI attenuated some but not all of these differences. Bone size and cortical vBMD were no longer significantly different between groups; however, young adults with CF continued to demonstrate inferior trabecular bone characteristics, including lower vBMD, trabecular thickness, and trabecular number associated with greater trabecular separation and heterogeneity. Estimated tibial stiffness and failure load also remained lower in subjects with CF after adjusting for BMI differences.

Unlike the radius, deficits in trabecular bone of the tibia remained significant after accounting for total hip areal BMD, and patients with CF had lower estimated bone strength at this weight bearing site independent of BMI and areal BMD (**Figure 2**).

**Clinical predictors of vBMD and estimated bone strength**

Within the CF cohort, total vBMD and estimated failure load of both the radius and tibia were moderately correlated with pulmonary status as measured by percent predicted FEV1 (**Figure 3**). Physical activity levels also were directly correlated with total vBMD of the radius (r=0.39, p=0.04) and tibia (r=0.38, p=0.04) and with failure load of the radius (r=0.39, p=0.04) and tibia (r=0.41, p=0.03). Percent predicted FEV1 remained a significant predictor of total vBMD and estimates of bone strength at the tibia even after adjustment for physical activity level (data not shown). Estimated failure load at the
radius was inversely associated with PTH levels ($r=-0.44$, $p=0.01$) with a similar but non-significant trend noted at the tibia ($r=-0.33$, $p=0.06$). Failure load was positively associated with BSAP at the radius ($r=0.43$, $p=0.01$) and tibia ($r=0.40$, $p=0.03$). Vitamin D levels, prior oral glucocorticoid use, other bone turnover markers, and the presence of the delF508 CFTR mutation were not significantly associated with these outcomes (data not shown). Due to the small number of patients with fractures ($n=7$), this study was underpowered to detect differences in DXA or HR-pQCT outcomes in fracture vs. non-fracture patients with CF.

**Discussion**

Bone microarchitecture and volumetric BMD are compromised in young adults with CF and are associated with lower estimated bone strength at both the radius and tibia. These skeletal alterations are greater than expected from the differences in BMI between patients with CF and their healthy peers, and differences in trabecular vBMD and trabecular microarchitecture at the tibia persisted even after adjustment for DXA-derived areal BMD of the hip, suggesting that DXA does not capture all of these bone structural and strength deficits in patients with CF. These findings may explain the increased fracture risk observed in patients with CF.

To our knowledge, this is the first study to characterize bone microarchitecture and predicted strength using HR-pQCT in young adults with CF. Histomorphometric data from iliac crest bone biopsies are limited, but have suggested that patients with CF have decreased cortical and trabecular bone mass, with evidence of lower bone formation rates and increased osteoclastic activity (25-27). In one study assessing vBMD measured by standard pQCT (28), adolescents and young adults with CF had lower cortical thickness of the radius, consistent with our findings; however, unlike our study, trabecular and total vBMD were normal. This discrepancy may be explained by different study designs because comparisons were made to a reference database rather than the matched healthy controls that were utilized in our study. In addition, the lower resolution of pQCT (0.4 mm) is not ideal for assessing
microarchitectural differences, particularly the detailed trabecular bone characteristics described in our study.

HR-pQCT studies involving other patient populations with secondary osteoporosis, such as acromegaly (29), have demonstrated that this technology is capable of providing important information about alterations in bone structure and strength. Deficits in HR-pQCT-derived cortical and trabecular parameters and in μFEA-derived strength measures described in the patients with CF in this study were of similar magnitude as those noted in patients with osteoporosis, chronic kidney disease, and incident fracture, suggesting that these differences may likely be of clinical significance (24, 30-32).

Young adults with CF in this study were shorter, weighed less, and had a lower BMI than matched healthy volunteers, as is commonly observed in this patient population. This size difference was reflected in the lower bone area at both the radius and tibia in these subjects. Lower weight applied to weight-bearing bones may also contribute to suboptimal bone structure and density, particularly if these patients are less physically active than their peers. The prominent deficits in trabecular and cortical bone structure and vBMD at the tibia observed in this study supports the hypothesis that weight bearing plays a role in these bone alterations.

To explore the confounding impact of body size on measures of bone density, size, and microarchitecture in patients with CF, we adjusted DXA and HR-pQCT values for BMI. Not only do adults with CF have smaller bones, but they also have inferior microarchitecture out of proportion to their lower BMI, particularly at the tibia, so that their estimated bone strength is lower than expected for their different body size. These factors may explain the increased fragility observed in this patient population.

Previous studies have demonstrated lower areal BMD in children and adults with CF, but these findings are difficult to interpret in the setting of their smaller body size. To determine whether HR-pQCT provides information independent of DXA, multivariable adjustment for areal BMD in addition to BMI was performed. In this analysis, differences in bone structure and strength at the radius were eliminated;
however, subjects with CF continued to have compromised trabecular bone characteristics and lower estimated bone strength at the tibia even after normalizing for areal BMD differences. This finding suggests that alterations in the trabecular bone and estimated bone strength in the weight bearing bones of young adults with CF are greater than reflected in areal BMD findings, which may help explain the propensity to fracture trabecular-rich bones such as vertebrae. Further prospective studies will be required to determine if HR-pQCT can potentially improve fracture prediction in this population.

There are many possible explanations for the bone alterations observed in patients with CF, including vitamin D deficiency, malabsorption, physical inactivity, malnutrition, glucocorticoid use, hormonal deficiencies, delayed puberty, inflammation, possibly CF-related diabetes and CFTR dysfunction itself (1, 33, 34). The differences in vBMD and bone area observed in the patients with CF in our study, despite their young age, may provide support to the theory that they may fail to attain peak bone mass, predisposing to more fragile bones later in life. In this study, physical activity levels were positively associated with bone strength estimates at the radius and tibia, suggesting that a reduction in physical activity may play a role in CF-related bone disease. In addition, failure load at the radius and tibia were directly associated with measures of pulmonary function in patients with CF, a finding that has also been noted in other studies evaluating areal BMD and pulmonary function (2, 35, 36). Interestingly, in our study, FEV1 remained a predictor of tibial bone strength independent of physical activity level, implying that other factors associated with declining lung function, such as inflammation, poor nutrition, or chronic respiratory acidosis, may be contributing. Lastly, bone specific CFTR genotype-phenotype correlations have not yet been fully elucidated, and in this cohort DelF508 mutation was not a predictor of estimated bone strength, although the power to detect such differences was limited.

In this cohort of patients with CF, vitamin D deficiency did not appear to be a significant issue. Most patients had a serum 25(OH)D level greater than 20 ng/mL. Serum 25(OH)D levels were not significantly associated with vBMD or bone strength measures, possibly because reported vitamin D intake was high enough to produce adequate levels in this ambulatory, relatively healthy cohort. Vitamin
D deficiency and calcium malabsorption may be more common and have a greater influence on bone health in more severely ill patients. Interestingly, PTH levels were inversely associated with failure load at the radius, with a similar though non-significant trend at the tibia. Higher PTH values may reflect inadequate calcium intake or calcium malabsorption that is independent of vitamin D. Lower BSAP levels were also directly associated with lower failure load in subjects with CF. Urine NTX levels were elevated, suggesting increased bone resorption in these subjects, which is consistent with previous reports (37, 38). Further studies investigating the underlying cause of these bone turnover abnormalities and associations with bone structure will be required to understand the significance of these alterations.

The strengths of this study include the relatively homogeneous cohort of subjects with CF, limited to an ambulatory group of young adults evaluated prior to lung transplantation. In addition, by assigning each subject with CF to two healthy volunteers matched by age, race, and gender, the effects of these covariates on the outcomes were minimized. Our study has also limitations. The sample size was relatively small, and it is possible that additional bone abnormalities would be uncovered if a larger number of patients were evaluated; however, the significant findings that were noted suggest that there was adequate power to identify several important differences. In addition, only peripheral skeletal sites were evaluated in this study, and further studies are needed to determine if findings are also representative of other skeletal regions. Finally, the cross-sectional design of this study cannot capture the dynamic changes that occur in the skeleton across childhood and the young adult years nor account for the effects of clinical variables occurring in the years prior to this evaluation.

In conclusion, several measures of bone microarchitecture are compromised in young adults with CF, and these alterations appear to contribute to lower estimated bone strength compared to age-, race-, and gender-matched healthy volunteers. These findings may explain the increased fracture risk observed in this patient population. Although some of these differences are eliminated by adjustment for BMI and areal BMD, many persist after these adjustments, suggesting that HR-pQCT provides additional unique
information on bone integrity in patients with CF. Further studies are needed to understand the underlying pathophysiology and clinical implications of these bone alterations.

Acknowledgements

This study was supported in part by an NIH T32 award and the Endocrine Society Amgen Fellowship Award. A Vertex Pharmaceuticals Investigator Initiated Studies Grant provided partial support for procedures in this study. The HR-pQCT measurements were made possible by an NCRR Shared Equipment Grant (1S10RR023405-01). The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the NIA, NINR, ORWH or the NIH. The authors gratefully acknowledge the support of the dedicated staff of the MGH Clinical Research Center, the Research Groups of the MGH and BCH CF Centers, and Anita St. John, RN.

REFERENCES


16. Macneil JA, Boyd SK 2008 Bone strength at the distal radius can be estimated from high-resolution peripheral quantitative computed tomography and the finite element method. Bone 42:1203-13


22. Nishiyama KK, Macdonald HM, Buie HR, Hanley DA, Boyd SK 2010 Postmenopausal women with osteopenia have higher cortical porosity and thinner cortices at the distal radius and tibia than women with normal aBMD: an in vivo HR-pQCT study. J Bone Miner Res 25:882-90


29. **Madeira M, Neto LV, de Paula Paranhos Neto F, Barbosa Lima IC, Carvalho de Mendonca LM, Gadelha MR, Fleiuss de Farias ML** 2013 Acromegaly has a negative influence on trabecular bone, but not on cortical bone, as assessed by high-resolution peripheral quantitative computed tomography. J Clin Endocrinol Metab 98:1734-41


### Table 1. Clinical and demographic characteristics of all subjects

<table>
<thead>
<tr>
<th>Clinical or Demographic Factor</th>
<th>Mean (SD) or n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects with CF (n=30)</td>
<td>Healthy volunteers (n=60)</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>25.0 (5.0)</td>
<td>25.3 (4.3)</td>
</tr>
<tr>
<td>Female</td>
<td>18 (60%)</td>
<td>36 (60%)</td>
</tr>
<tr>
<td>Caucasian Race</td>
<td>30 (100%)</td>
<td>60 (100%)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165 (8)</td>
<td>170 (9)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.0 (10.6)</td>
<td>71.6 (14.8)</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>20.8 (3.2)</td>
<td>24.5 (3.7)</td>
</tr>
</tbody>
</table>

### Table 2. Clinical characteristics of subjects with CF.

PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; CTX, C-telopeptide; NTX, N-telopeptide; P1NP, amino-terminal propeptide of type I collagen; BSAP, bone specific alkaline phosphatase

<table>
<thead>
<tr>
<th>Clinical Parameter</th>
<th>Mean (SD) or n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFTR Genotypes</td>
<td></td>
</tr>
<tr>
<td>DelF508/DelF508</td>
<td>11 (37%)</td>
</tr>
<tr>
<td>DelF508/Other</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (23%)</td>
</tr>
<tr>
<td>Most recent FEV1 (% predicted)</td>
<td>73.2 (28.2)</td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>27 (90%)</td>
</tr>
<tr>
<td>CF-related diabetes</td>
<td>5 (17%)</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>2240 (1750)</td>
</tr>
<tr>
<td>Vitamin D intake (IU/day)</td>
<td>2380 (2490)</td>
</tr>
<tr>
<td>History of fracture</td>
<td>7 (23%)</td>
</tr>
<tr>
<td>Medication use in past year</td>
<td></td>
</tr>
<tr>
<td>Oral glucocorticoids</td>
<td>9 (30%)</td>
</tr>
<tr>
<td>Inhaled glucocorticoids</td>
<td>20 (67%)</td>
</tr>
<tr>
<td>Proton pump inhibitors</td>
<td>21 (70%)</td>
</tr>
<tr>
<td>Lifetime glucocorticoid exposure</td>
<td>7 (0-36)</td>
</tr>
<tr>
<td>Laboratory results</td>
<td></td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>36 (14)</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>28 (12-90)</td>
</tr>
<tr>
<td>CTX (ng/mL)</td>
<td>0.51 (0.22)</td>
</tr>
<tr>
<td>Urine NTX (nM BCE/mM Cre)</td>
<td>76 (19-236)</td>
</tr>
<tr>
<td>P1NP (ug/L)</td>
<td>50 (19)</td>
</tr>
<tr>
<td>BSAP (U/L)</td>
<td>29.1 (10.8)</td>
</tr>
</tbody>
</table>
Table 3. HR-pQCT measurements of the radius and tibia in subjects with CF and healthy volunteers. Data are expressed as mean ± SE. Unadjusted group means are presented along with p-values for the unadjusted analyses, BMI-adjusted analyses, and BMI and total hip areal BMD-adjusted analyses. aBMD, areal bone mineral density; vBMD, volumetric BMD; trab, trabecular; cort, cortical. *Healthy volunteers as reference.

<table>
<thead>
<tr>
<th></th>
<th>Cystic Fibrosis</th>
<th>Healthy Volunteers</th>
<th>Mean Percent Difference*</th>
<th>Unadjusted p value</th>
<th>BMI Adjusted p value</th>
<th>BMI and aBMD Adjusted p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DXA aBMD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Hip (g/cm²)</td>
<td>0.870 ± 0.024</td>
<td>1.034 ± 0.017</td>
<td>-15.9%</td>
<td>&lt;0.001</td>
<td>0.007</td>
<td>-</td>
</tr>
<tr>
<td>Femoral neck (g/cm²)</td>
<td>0.770 ± 0.024</td>
<td>0.936 ± 0.017</td>
<td>-17.7%</td>
<td>&lt;0.001</td>
<td>0.004</td>
<td>-</td>
</tr>
<tr>
<td>PA spine (g/cm²)</td>
<td>0.973 ± 0.022</td>
<td>1.069 ± 0.016</td>
<td>-9.0%</td>
<td>0.001</td>
<td>0.31</td>
<td>-</td>
</tr>
<tr>
<td>Whole body (g/cm²)</td>
<td>0.970 ± 0.018</td>
<td>1.071 ± 0.014</td>
<td>-9.4%</td>
<td>&lt;0.001</td>
<td>0.042</td>
<td>-</td>
</tr>
<tr>
<td>Percent fat mass (g/cm²)</td>
<td>25.9 ± 0.9</td>
<td>28.8 ± 0.7</td>
<td>-10.3%</td>
<td>0.011</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>RADIUS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Density</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total vBMD (mgHA/cm³)</td>
<td>303.2 ± 8.4</td>
<td>332 ± 5.9</td>
<td>-8.7%</td>
<td>0.007</td>
<td>0.27</td>
<td>0.98</td>
</tr>
<tr>
<td>Cort vBMD (mgHA/cm³)</td>
<td>941.3 ± 5.0</td>
<td>952.6 ± 3.5</td>
<td>-1.2%</td>
<td>0.068</td>
<td>0.68</td>
<td>0.86</td>
</tr>
<tr>
<td>Trab vBMD (mgHA/cm³)</td>
<td>165.7 ± 5.5</td>
<td>186.7 ± 3.9</td>
<td>-11.3%</td>
<td>0.003</td>
<td>0.08</td>
<td>0.66</td>
</tr>
<tr>
<td><strong>Area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Area (mm²)</td>
<td>263.9 ± 9.1</td>
<td>300.4 ± 6.4</td>
<td>-12.1%</td>
<td>0.002</td>
<td>0.021</td>
<td>0.21</td>
</tr>
<tr>
<td>Cort Area (mm²)</td>
<td>52.0 ± 1.7</td>
<td>61.6 ± 1.2</td>
<td>-15.6%</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>0.43</td>
</tr>
<tr>
<td>Trab Area (mm²)</td>
<td>214.6 ± 8.9</td>
<td>241.7 ± 6.3</td>
<td>-11.2%</td>
<td>0.015</td>
<td>0.046</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Cort Structure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cort Thickness (mm)</td>
<td>0.823 ± 0.027</td>
<td>0.903 ± 0.019</td>
<td>-8.8%</td>
<td>0.021</td>
<td>0.50</td>
<td>0.81</td>
</tr>
<tr>
<td>Cort Porosity (%)</td>
<td>1.07 ± 0.13</td>
<td>1.31 ± 0.09</td>
<td>-18.0%</td>
<td>0.14</td>
<td>0.06</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Trab Structure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trab Thickness (mm)</td>
<td>0.068 ± 0.001</td>
<td>0.076 ± 0.001</td>
<td>-9.8%</td>
<td>0.001</td>
<td>0.011</td>
<td>0.15</td>
</tr>
<tr>
<td>Trab Number (mm⁻¹)</td>
<td>2.00 ± 0.04</td>
<td>2.06 ± 0.03</td>
<td>-2.7%</td>
<td>0.30</td>
<td>0.83</td>
<td>0.39</td>
</tr>
<tr>
<td>Trab Separation (mm)</td>
<td>0.44 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>+5.2%</td>
<td>0.14</td>
<td>0.81</td>
<td>0.31</td>
</tr>
<tr>
<td>St Dev Trab Distribution (µm)</td>
<td>0.18 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>+5.6%</td>
<td>0.33</td>
<td>0.87</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>TIBIA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Density</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total vBMD (mgHA/cm³)</td>
<td>286.3 ± 7.6</td>
<td>334.1 ± 5.3</td>
<td>-14.3%</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.022</td>
</tr>
<tr>
<td>Cort vBMD (mgHA/cm³)</td>
<td>934.9 ± 6.9</td>
<td>958.6 ± 4.9</td>
<td>-2.5%</td>
<td>0.007</td>
<td>0.13</td>
<td>0.16</td>
</tr>
<tr>
<td>Trab vBMD (mgHA/cm³)</td>
<td>158.7 ± 5.0</td>
<td>202.9 ± 3.6</td>
<td>-21.8%</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Area (mm²)</td>
<td>650.2 ± 23.8</td>
<td>739.3 ± 16.9</td>
<td>-12.1%</td>
<td>0.003</td>
<td>0.08</td>
<td>0.55</td>
</tr>
<tr>
<td>Cort Area (mm²)</td>
<td>109.3 ± 3.7</td>
<td>126.5 ± 2.6</td>
<td>-13.6%</td>
<td>&lt;0.001</td>
<td>0.15</td>
<td>0.65</td>
</tr>
<tr>
<td>Trab Area (mm²)</td>
<td>544.7 ± 22.8</td>
<td>616.6 ± 17.2</td>
<td>-11.7%</td>
<td>0.013</td>
<td>0.10</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Cort Structure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cort Thickness (mm)</td>
<td>1.19 ± 0.04</td>
<td>1.26 ± 0.03</td>
<td>-5.9%</td>
<td>0.10</td>
<td>0.88</td>
<td>0.62</td>
</tr>
<tr>
<td>Cort Porosity (%)</td>
<td>3.61 ± 0.33</td>
<td>2.96 ± 0.23</td>
<td>+21.9%</td>
<td>0.11</td>
<td>0.22</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Trab Structure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trab Thickness (mm)</td>
<td>0.076 ± 0.002</td>
<td>0.0825 ± 0.002</td>
<td>-7.3%</td>
<td>0.025</td>
<td>0.017</td>
<td>0.07</td>
</tr>
<tr>
<td>Trab Number (mm⁻¹)</td>
<td>1.71 ± 0.05</td>
<td>2.06 ± 0.034</td>
<td>-16.7%</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.024</td>
</tr>
<tr>
<td>Trab Separation (mm)</td>
<td>0.52 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>+24.4%</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.010</td>
</tr>
<tr>
<td>St Dev Trab Distribution (µm)</td>
<td>0.23 ± 0.01</td>
<td>0.18 ± 0.01</td>
<td>+25.9%</td>
<td>&lt;0.001</td>
<td>0.040</td>
<td>0.35</td>
</tr>
</tbody>
</table>
**Figure 1:** HR-pQCT scans of the tibia in a representative young woman with CF (a) and female healthy volunteer (b).

**Figure 2. µFEA results.** Stiffness (a) and failure load (b) of the radius and tibia in young adults with CF (black bars) and age-, gender-, and race-matched healthy volunteers (gray bars). Data are expressed as mean ± SE and are presented for the unadjusted analysis, the BMI-adjusted analysis, and the BMI- and total hip areal BMD-adjusted analysis. * indicates p<0.05.

**Figure 3. Correlations with FEV1.** Scatter plots correlating percent predicted FEV1 with total vBMD of the radius (a) and tibia (b) and failure load of the radius (c) and tibia (d). FEV1, percent predicted forced expiratory volume in one second; vBMD, volumetric bone mineral density.
Figure 3

(a) Total vBMD Radius (mgHA/cm³) vs. % predicted FEV₁, r 0.46, p 0.01

(b) Total vBMD Tibia (mgHA/cm³) vs. % predicted FEV₁, r 0.65, p < 0.01

(c) Failure Load Radius (N) vs. % predicted FEV₁, r 0.46, p < 0.01

(d) Failure Load Tibia (N) vs. % predicted FEV₁, r 0.49, p < 0.01