Published in final edited form as:

Osteoporos Int. 2006 ; 17(5): 783–790. doi:10.1007/s00198-005-0058-x.

Relationship between insulin-like growth factor I, dehydroepiandrosterone sulfate and proresorptive cytokines and bone density in cystic fibrosis

C. M. Gordon,

Children's Hospital, Boston, MA, USA. Children's Hospital, Division of Endocrinology, 300 Longwood Avenue, Boston, MA 02115, USA, Tel.: +1-617-3558492, Fax: +1-617-7300195

E. Binello,

Children's Hospital, Boston, MA, USA

M. S. LeBoff,

Brigham and Women's Hospital, Boston, MA, USA

M. E. Wohl,

Children's Hospital, Boston, MA, USA

C. J. Rosen, and

St. Joseph Hospital, Bangor, ME, USA

A. A. Colin

Children's Hospital, Boston, MA, USA

C. M. Gordon: catherine.gordon@childrens.harvard.edu

Abstract

Introduction—Patients with cystic fibrosis (CF) are known to be at risk for early osteoporosis, and the mechanisms that mediate bone loss are still being delineated. The aim of the present investigation was to investigate if a correlation exists in these patients between skeletal measurements by dual-energy x-ray absorptiometry (DXA) and two anabolic factors, dehydroepiandrosterone (DHEA) and insulin-like growth factor I (IGF-I), and proresorptive factors such as the cytokines interleukin-1 β , tumor necrosis factor α , and interleukin-6.

Methods—We studied 32 outpatients (18 females; mean age: 26.2 $\pm$ 7.9 years) at a tertiary care medical center. The subjects had venous samples obtained, underwent anthropometric and bone mineral density (BMD) measurements, and completed a health survey. Serum IGF-I concentrations were below the age-adjusted mean in 78% of the participants, and DHEA sulfate (DHEAS) concentrations were low in 72%. Serum concentrations of all cytokines were on the low side of normal; nonetheless, there was a modest inverse correlation between IL-1 β and BMD at all sites.

Results—In univariate analyses, IGF-I and DHEAS were significant correlates of BMD or bone mineral content. In final multivariate models controlling for anthropometric and other variables of relevance to bone density, only IGF-I was identified as a significant independent skeletal predictor. While alterations in DHEAS, IGF-I, and specific cytokines may contribute to skeletal deficits in patients with CF, of these factors a low IGF-I concentration appears to be most strongly correlated with BMD.

Conclusions—These findings may have therapeutic implications for enhancing bone density in these patients.

Keywords

Bone mineral density; Cystic fibrosis; Dual-energy x-ray absorptiometry; Insulin-like growth factor I; Osteoporosis Pediatrics

Introduction

With the improvement of therapies and life expectancy for patients with cystic fibrosis (CF), complications such as osteoporosis have become increasingly common [1, 2]. Previous studies have documented a decreased bone mineral content (BMC) in both children [3–8] and adults [1, 6, 9, 10] with this disease as well as an increased fracture rate [10–12]. There is debate in the literature as to the mechanisms that mediate bone loss in patients with CF. Well-recognized skeletal predictors include malnutrition, vitamin D deficiency, delayed puberty, hypogonadism, reduced physical activity, chronic respiratory acidosis, and glucocorticoid therapy [1–4, 6, 7, 9, 11–15]. However, there remain gaps in our knowledge on hormonal and other circulating factors that contribute to bone loss and on anabolic and antiresorptive strategies that may be employed to prevent skeletal deficits in these patients.

To our knowledge, no previous studies have examined the role of the anabolic factors dehydroepiandrosterone (DHEA) or insulin-like growth factor I (IGF-I) on bone density in patients with CF. DHEA circulates primarily as a sulfated derivative, DHEAS, with its longer half life. Both steroids rise sharply during puberty to achieve peak levels during early adulthood. This pattern mirrors the increase in bone density that occurs during adolescence. DHEA [16] and IGF-I [16, 17] have been shown to be low in states of malnutrition such as anorexia nervosa. The skeletal effects of DHEA replacement therapy have been studied in two groups with known low concentrations of this hormone and osteoporosis: young women with anorexia nervosa and elderly women, respectively [18–20]. A positive correlation was noted between plasma DHEA and BMD in older women, suggesting antiosteoporotic effects [18]. Small skeletal gains and increased serum levels of bone formation markers have been noted following DHEA replacement in both postmenopausal women [19] and adolescents with anorexia [20], suggesting that this hormone possesses anabolic properties. In systemic lupus erythematosus, DHEA therapy has also been shown to prevent the skeletal losses seen in those patients receiving glucocorticoid therapy [21]. IGFs play an important role in the maintenance of bone mass [22], and together with osteoprotegerin and other molecules regulate the coupling of bone formation and resorption [23, 24]. Previous studies have shown that IGF-I concentrations are low in patients with CF [25, 26], but the relevance of this finding to skeletal physiology has not been examined to date. The anabolic effects of DHEA on the skeleton appear to be mediated through the skeletal IGF-I system [22, 27].

Chronic inflammation may directly contribute to skeletal deficits in patients with CF. The proresorptive cytokines, tumor necrosis factor α (TNF- α), interleukin 1 β (IL-1 β), and interleukin 6 (IL-6) [28], stimulate bone resorption and/or osteoclast formation. Two reports have shown that TNF- α production by lung macrophages was elevated in patients with CF [29, 30], and other studies have shown elevations of serum TNF- α [31], IL-1 β [30], and IL-6 [30, 32, 33] concentrations in these patients. Haworth et al. [34] found an inverse correlation between skeletal losses and serum IL-6 concentrations over a 1-year period in a study of young adults with CF. In studies using both rodent [35] and human in vitro [36] models, DHEA supplementation led to decreased IL-6 concentrations, suggesting antiresorptive properties. One study showed increased IL-6, IL-1 β , and TNF- α concentrations during acute

lung infections in patients with CF that were associated with increased bone resorption and decreased bone formation markers [37].

To gain new information about anabolic and proresorptive mechanisms that may influence bone turnover and bone mass in patients with CF, we examined a cohort of CF patients with the objective of determining whether a direct correlation exists between the anabolic factors IGF-I or DHEAS and measurements of bone formation and bone mass in patients with CF. We also examined if there was a direct association between proresorptive cytokine concentrations and levels of bone resorption markers, and an inverse correlation between these cytokines and bone density. We tested the hypothesis that IGF-I and DHEAS levels are positively correlated with bone mineral density (BMD) and that proresorptive cytokine concentrations are negatively correlated with the latter.

Materials and methods

Subjects

We studied 32 patients (18 females) who were recruited from the CF Program at Children's Hospital Boston, a program that follows both pediatric and adult patients. All patients had CF based on documentation of elevated sweat chloride levels or cystic fibrosis transmembrane regulator (CFTR) gene mutational analysis. Patients were studied as outpatients and were without acute interventions such as intravenous antibiotics for pulmonary exacerbations. Exclusion criteria included receipt of medications known to alter BMD, including sex steroid or daily oral glucocorticoid therapy. All patients gave written informed consent according to the guidelines of the hospital's Committee on Clinical Investigation. For minors under age 18 years, a parent or guardian also gave consent.

Study design, treatment, and measurements

Semi-structured interviews were conducted in the General Clinical Research Center, Children's Hospital Boston. Questions were posed regarding previous hospitalizations and surgeries, fractures sustained, medications, co-morbid conditions, and family history of osteoporosis.

During the study visit, weight (in kilograms) and height (in centimeters) of the subjects (wearing only a hospital gown) were determined after voiding. All weights were obtained on the same scale; height was obtained using the same stadiometer (Perspective Enterprises, Kalamazoo, Mich.). The body mass index (BMI) was calculated from these measurements in kilograms per square meter of surface area. Tanner pubertal staging was determined by a pediatric endocrinologist (CMG). Subjects had venous blood drawn from an antecubital vein, and a second morning void was collected before 10:00 am after an overnight fast.

Each participant underwent BMD (grams per square centimeter), BMC (grams) and bone area (square centimeters) measurements of the total body, lumbar spine, and total hip (encompassing femoral neck, trochanter, and intertrochanteric region), and body composition was determined by dual energy x-ray absorptiometry (DXA) using a Hologic 4500 machine (Hologic, Waltham, Mass.). A fan-beam in the array mode was used for all subjects. BMC and bone area were examined in addition to BMD to evaluate for differential effects of hormonal or anthropometric factors on BMC and bone size. Hip, spine, and total body measurements were compared with age- and gender-matched controls [38, 39]. For participants between the age of 15–20 years, pediatric normative data were used [40]. All participants were fully mature (Tanner stage 5 for pubic hair, genitalia, and/or breasts), and all females were postmenarchal. Given this fact and the mean age of the female participants, bone density was interpreted with respect to chronologic rather than gynecologic age. Because of the known variation in BMD that occurs with age and between gender groups,

BMD Z-scores were used as the primary outcome variable for both univariate and multivariate analyses.

Subjects had venous blood samples obtained for the determination of DHEAS measurements by double-antibody radioimmunoassay (RIA) in the Children's Hospital Boston Endocrine Laboratory, and bone-specific alkaline phosphatase (BSAP) levels were determined by immunoradiometric assay (Esoterix Endocrinology, Calabasas Hill, Calif.). Urinary levels of cross-linked N-telopeptides (NTx) were determined using an enzyme-linked immunosorbent assay (ELISA) on a second morning void (Esoterix Endocrinology). Serum levels of IL-6, TNF- α , and IL-1 β were also determined by high-sensitivity ELISA (R&D Systems, Minneapolis, Minn.). IGF-I was measured by RIA (Alpco Diagnostics, Windham, N.H.) after removal of residual IGF binding proteins by acid ethanol extraction, followed by the addition of IGF-II. Levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroxine, thyroid stimulating hormone (TSH), and prolactin were measured by the Bayer Immuno-1 System (Bayer Diagnostics Division, Tarrytown, N.Y.). Total estradiol and testosterone were measured by RIA (Esoterix Endocrinology). Serum 25OHD concentrations were measured by competitive binding assay, and intact parathyroid hormone (PTH) levels by a two-site chemiluminescence immunoassay (Nichols Institutes, San Clemente, Calif.). Samples for each test were run in duplicate. Interassay coefficients of variation (CV) were (with the higher %CV noted for the lowest concentrations): 4.5–5.4% for DHEAS; 9.0–15.0% for 25OHD; 5.4–7.0% for PTH; 2.19% for IGF-I; 3.5–3.9% for FSH; 3.4–4.6% for LH; 3.3–4.0% for thyroxine; 3.6–5.4% for TSH; 9.2–10.9% for estradiol; 4.0–4.3% for prolactin; 4.3–9.9% for total testosterone; 8.2–19.2% for IL-1 β ; 3.6–5.1% for TNF- α ; 6.5–9.6% for IL-6.

Statistical analysis

A one-sample *t* test was used to compare concentrations of specific hormones and bone turnover markers to established mean levels for age. The normal range and mean for age was obtained from the reference laboratory where each assay was performed. The normal range for a young adult was used, given the mean age of the participants (Table 1). Subgroup means for specific variables were compared using independent *t* tests. To examine univariate relationships between continuous variables, we performed Pearson correlation analyses. Significant univariate correlates were then entered into multivariate linear regression models to determine which variables were independent predictors of bone density and BMC, controlling for potential confounders. Weight, lean body mass, and gender were included as independent variables in all multivariate models as each is known to be a potential determinant of BMD; height was also included in this core model as patients with CF have been shown to have reduced final heights [41]. IGF-I, DHEAS, and IL-1 β were each added to the core model, then all three variables were entered simultaneously. Statistical analyses were performed using SPSS software (SPSS, Chicago, Ill.). A *p*-value < 0.05 was considered to be statistically significant.

Results

Description of study sample

Clinical and anthropometric data are summarized in Table 1. No subjects regularly consumed alcohol or used cigarettes. All participants were receiving inhaled glucocorticoids in the form of fluticasone propionate. Among the female participants, 4 (22%) reported having secondary amenorrhea.

Fracture history and family history of osteoporosis

Over 50% of the patients reported having sustained a fracture (Table 1). There was no difference in BMD or BMC for those reporting a fracture and those who did not. Ten participants (23%) reported a history of osteoporosis in a first-degree relative. Total body BMD was significantly lower in participants with a positive family history of osteoporosis: 1.068 ± 0.0619 g/cm² (mean $\pm$ SD) versus 1.136 ± 0.103 ($p=0.031$).

Biochemical data

In all participants, thyroid function tests and LH and FSH concentrations were within the normal range. Serum prolactin concentrations were normal except for a slight elevation in one adolescent girl who was receiving metoclopramide.

Serum DHEAS levels were significantly below the age-adjusted mean in 72% of the participants. The mean was below the reference mean only in females: 143 versus 233 μ g/dl, $p=0.025$; in males, the mean level approached the established mean for young adult males (210 versus 270 μ g/dl, p = non-significant). DHEAS was significantly correlated with weight ($r=0.42$, $p=0.017$); there was also a trend in a correlation between this hormone and lean body mass (LBM) ($r=0.35$, $p=0.051$). There was no significant correlation between DHEAS concentration and levels of BSAP or NTx.

Serum IGF-I levels were below the mean for age in 78% of participants: 216.3 versus 314 ng/ml in females ($p=0.002$); 270.5 versus 380 ng/ml in males ($p < 0.001$). The univariate analyses revealed a significant correlation between IGF-I and age ($r = -0.67$, $p < 0.001$) and LBM by DXA ($r = 0.42$, $p=0.018$). IGF-I was significantly correlated with both BSAP and NTx ($r = 0.41$, $p=0.022$ and $r = 0.40$, $p=0.027$, respectively).

Mean serum levels of BSAP were subnormal for age compared to the reference mean for age (20.7 ± 13.9 versus 28 ng/ml, $p=0.006$), whereas urinary NTx levels were elevated [77.9 ± 65.9 versus 39 nmol/mmol creatinine (Cr), $p=0.003$]. Bone turnover was significantly higher in males than in females, as reflected by higher urinary NTx and serum BSAP levels (109.2 versus 55.3 nmol/mmol Cr, $p < 0.001$ and 30.2 versus 12.7 ng/ml, $p=0.022$, respectively).

The mean serum IL-1 β level was 0.76 ± 0.42 pg/ml (range: 0.32–2.05; normal: <40); for TNF- α , the mean was 2.41 ± 0.42 pg/ml (range: 1.8–3.7; normal: 2–6.3); for IL-6, the mean was 2.1 ± 2.2 pg/ml (range: 0.3–11.0; normal: 0–5.7). For all cytokines, the serum concentrations were at the lower range of normal. There was no significant correlation between cytokine concentrations and either BSAP or NTx levels. There was a significant inverse correlation between serum IL-1 β and BMD at the spine (Fig. 1), hip, and total body (Table 2).

Defined as a 25OHD <15 ng/ml, 12 patients (38%) were vitamin D-deficient; defined as a 25OHD <20 ng/ml, 15 participants (47%) met criteria for vitamin D insufficiency. The mean PTH level was 27.4 ± 32.0 pg/ml, (range: 13.4–169.9). There was an inverse trend noted between 25OHD and PTH that approached significance ($r = -0.33$, $p=0.065$). There was no significant correlation noted between concentrations of 25OHD or PTH and either BMD or BMC at any site.

Serum estradiol and testosterone concentrations were measured for both the male and female participants (Table 3). There was no significant correlation noted between either hormone and either BMD or BMC at any site.

Bone mass (BMD, BMC, and bone area) data

Hip—Mean BMD of the total hip was 0.892 ± 0.149 g/cm², representing a median Z-score of -0.65 (range: -2.5 – 1.20). BMD was >1 SD below the mean in 38% ($n=11$) of the participants and >2 SD below the mean in 7% ($n=2$) of the cohort. The hip BMD Z-score was significantly correlated with IGF-I and IL-1 β (Table 2) as well as with weight ($r=0.41$, $p=0.022$) and lean body mass by DXA ($r=0.50$, $p=0.004$). In a multivariate model controlling for weight, height, lean body mass, and gender, IGF-I remained a significant independent predictor (Table 2). These five variables accounted for 43% of the variation in BMD at this site.

Hip BMC was positively correlated with DHEAS (Fig. 1) and IGF-I (Table 2) as well as with weight, height, and LBM ($p<0.001$). By multiple regression, IGF-I was again found to be a significant independent predictor (Table 2). Hip area was positively associated with DHEAS concentration (Table 2).

Lumbar spine—Mean lumbar spine (L1–L4) BMD was 0.936 ± 0.135 g/cm², representing a median lumbar Z-score of -1.05 (range: -4.0 – 1.5). In 53% of patients ($n=17$), lumbar BMD was >1 SD below the mean for age-matched controls; in 19% of the sample ($n=6$), it was >2 SD below the mean. Significant univariate correlates at this site included IGF-I and IL-1 β (Fig. 1, Table 2). In a multivariate model controlling for height, weight, LBM, and gender, IGF-I was identified as a significant predictor of spinal BMD Z-score. These variables accounted for 53% of the variation in spinal BMD.

Lumbar BMC was positively correlated with IGF-I, which was found to be a significant independent predictor of BMC (Table 2).

Total body—Mean total body BMD was 1.100 ± 0.101 g/cm². IL-1 β was a significant univariate correlate (Table 2), in addition to fat mass by DXA ($r = -0.38$, $p=0.032$). In a multivariate model, there were no significant independent predictors of BMD identified.

Total body BMC was significantly correlated only with IGF-I (Table 2), and weight ($r = 0.66$, $p<0.001$) and height ($r = 0.60$, $p<0.001$). In a final multivariate model, height ($\beta = -0.51$, $p=0.042$) was a significant independent predictor of BMC at this site. Total body bone area was positively correlated with DHEAS concentration (Table 2).

Discussion

To our knowledge, this is the first study to examine whether the anabolic factors IGF-I and DHEA are predictors of bone mass in patients with CF. DHEAS concentrations were particularly low among the female participants, and IGF-I levels were below the age-adjusted mean in all participants. We also found serum proresorptive cytokine concentrations to be in the lower range of normal. Nonetheless, serum IL-1 β concentration was inversely correlated with BMD at all sites, suggesting a role for this factor as a mediator of bone loss in these patients. In multivariate models controlling for weight, height, and other factors known to influence BMD, only IGF-I was found to be an independent skeletal predictor.

This study is the first to report a direct correlation between IGF-I and parameters of bone mass in patients with CF. IGF-I has been shown to fluctuate even with acute changes in nutritional status [42]. In the present study, serum IGF-I concentrations were significantly reduced compared to the reference standard for healthy counterparts, confirming previous reports suggesting that this factor reflects the underlying catabolic state of these patients [25, 26]. Furthermore, we found a significant direct correlation between IGF-I and LBM in this

study, thereby supporting the premise that nutritional status modulates circulating levels of this peptide. We also noted a direct correlation between IGF-I concentrations and markers of bone turnover, which supports the assumption that this protein serves as a coupling factor in the bone turnover cycle [23, 24]. In addition to their characteristic low bone mass, patients with CF are often shorter than age-matched controls [41]. Thus, low IGF-I concentrations may serve as a marker of compromised growth as well as of subnormal bone accretion in affected children and adults.

We found DHEAS concentrations to be positively correlated with weight, a finding which corroborates with results reported elsewhere, suggesting that this hormone is nutrition-dependent [16, 43]. There was also a positive correlation found between serum DHEAS and hip BMC, and bone area of both the hip and total body. The positive trend noted between DHEAS and LBM suggests that potential anabolic effects on bone mass and size may be mediated through hormone-induced changes in body composition. Within our gender groups, DHEAS levels were significantly below the threshold for age only in the female participants. DHEA replacement has been studied in young women with anorexia nervosa, a model illustrating the negative effects of malnutrition on bone [20, 43], but to our knowledge, its role in skeletal health in CF has not been previously examined. Short-term DHEA replacement has been shown to decrease markers of bone resorption and increase bone formation in young women with anorexia nervosa [43]. In a 1-year study examining the effects of DHEA versus estrogen replacement therapy in these patients, the young women in the group receiving DHEA had significantly higher levels of BSAP, osteocalcin, and IGF-I than those in the group receiving standard therapy. Given the current findings in the female participants, those therapeutic interventions in patients with anorexia nervosa may be relevant to patients with CF. There is also evidence to support the role of DHEA in the modulation of the skeletal IGF-I regulatory system that may have therapeutic implications for this group [27].

We found serum concentrations of proresorptive cytokines to be surprisingly low, accompanied by a modest, but significant inverse correlation between IL-1 β and BMD at all sites. The current results are similar to the low cytokine concentrations found in our study of young women with anorexia nervosa [16]. However, in that group, no significant correlations were found between cytokine levels and bone density. Our present findings differ from those reported previously showing elevated cytokines in patients with CF [29–34]. One explanation is the use of inhaled glucocorticoids by all study participants in the current sample. As these medications are widely prescribed as anti-inflammatory agents to patients with CF, it was not possible to exclude patients who were receiving this therapy. In adults with chronic obstructive pulmonary disease, inhaled glucocorticoids have been shown to have systemic anti-inflammatory activity [44]. We are not aware of previous serum cytokine measurements in patients with CF following treatment with these agents. However, a study by Wojtczak et al. [45] demonstrated a reduction in inflammatory markers in bronchoalveolar lavage of children with CF. Such a reduction in the airway is likely to be reflected systemically. Thus, these medications may have reduced proresorptive cytokine secretion in the participants in the current study. Also noteworthy is the fact that cytokine measurements in the current study were obtained at a time of relative health. A previous study that examined the relationship between serum cytokines and bone turnover markers was carried out at the time of an acute infection, a clinical setting that would enhance cytokine secretion [37]. Methodologic differences used in the measurement of cytokines could have also contributed to the differing results, including either the assay type or its sensitivity and whether serum or saliva were analyzed. The significant inverse correlation between IL-1 β and bone mass adds to accumulating evidence suggesting a role for cytokines in the mediation of bone loss in these patients [37, 46]. The unique role of IL-1 β with regard to skeletal health is unclear and deserves further study.

In the current study, despite the majority of participants reporting use of vitamin D supplements, many were found to be vitamin D-deficient. Previous studies have documented both low [34, 48–50] and normal [50, 51] serum 25OHD concentrations in patients with CF. A prevalence of vitamin D deficiency of 38% seen among the current participants is the exact prevalence noted by Haworth et al. [34], and participants in that study were also receiving vitamin D supplementation. While previous studies have shown that vitamin D status is a predictor of bone mass in these patients [12, 47], our data replicate those of two previous studies [6, 9] in showing that this variable was not predictive.

The results of the current study must be interpreted in light of acknowledged limitations. As this study utilized a cross-sectional design, the associations that are noted do not imply causality. It could be argued that low serum IGF-I is simply a marker of malnutrition in these patients. However, we controlled for both weight and lean body mass in our final multivariate models, and IGF-I was found to be a significant independent predictor of hip and spinal bone density. Our sample size was small, thereby limiting analyses of all variables of interest as well as our statistical power to detect potentially important differences. Skeletal measurements were determined by DXA, which measures areal bone density, but not volumetric BMD. Therefore, the BMD Z-scores reported could be underestimates of true volumetric bone density in our study subjects. However, our findings are similar to those of Gibbens et al. [3], who found that volumetric bone density measurements obtained by quantitative computed tomography were also low in patients with CF. We also found no correlation between height and bone density at any site, suggesting that our findings represent a true reduction in BMD rather than simply a reflection of small bone size. Data on fractures were obtained by self-report, with its inherent limitations, and no information was available as to whether fractures were pathologic in origin. Future studies should evaluate whether these patients have fractured in the absence of trauma and confirm patient reports with radiologic findings. Lastly, the low cytokine concentrations in this sample represented an unexpected finding, and our explanation for the data is speculative. Unfortunately, we did not obtain detailed information regarding the dose or duration of the fluticasone preparations used by the current participants. This information will be needed in future protocols to confirm the speculation that these agents lead to the suppression of cytokine secretion. Replication of results during and after an acute pulmonary exacerbation would also be informative.

In conclusion, novel mechanisms are proposed herein related to the role of low concentrations of IGF-I and DHEA in the mediation of bone loss in patients with CF. Unlike previous reports, we found low levels of proresorptive cytokines, but an inverse correlation between the cytokine IL-1 β and BMD. These results underscore the need for randomized trials to evaluate the efficacy and safety of both antiresorptive and anabolic agents in patients with CF, as we found both bone resorption and bone formation to be altered. In this context, as DHEA has been shown to exhibit anabolic effects through the skeletal IGF system, abnormalities in IGF-I and DHEAS as noted in this study may have therapeutic implications for adolescents and young adults with this disease. However, future longitudinal studies are needed to confirm this speculation, especially given the multifactorial etiology of bone loss in these patients.

Acknowledgments

We wish to thank our patients for their participation, and the skilled and dedicated nurses in the General Clinical Research Center for their excellent patient care. We also gratefully acknowledge Suzanne Muggeo, BA and Julie Burgess, BS, for technical support, Avery LeBoff Williams for editorial assistance, and Henry A. Feldman, PhD for biostatistical advice. This work was funded by NIH Grant MO1-RR-2172 and Project 5-T71-MC-0000-10-S1-RO from the Maternal and Child Health Bureau.

References

1. Bachrach LK, Loutit CW, Moss RB. Osteopenia in adults with cystic fibrosis. *Am J Med.* 1994; 96:27–34. [PubMed: 8304359]
2. Bhudhikanok GS, Wang MC, Marcus R, Harkins A, Moss RB, Bachrach LK. Bone acquisition and loss in children and adults with cystic fibrosis. *J Pediatr.* 1998; 133:18–27. [PubMed: 9672505]
3. Gibbens DT, Gilsanz V, Boechat MI, Dufer D, Carlson ME, Wang CI. Osteoporosis in cystic fibrosis. *J Pediatr.* 1988; 133:295–300. [PubMed: 3397792]
4. DeSchepper J, Smitz J, Dab I, Piepsz A, Jonckheer M, Bergmann P. Low serum bone gammacarboxyglutamic acid protein concentrations in patients with cystic fibrosis: correlation with hormonal parameters and bone mineral density. *Horm Res.* 1993; 39:197–201. [PubMed: 8314203]
5. Henderson RC, Madsen CD. Bone density in children and adolescents with cystic fibrosis. *J Pediatr.* 1996; 128:28–34. [PubMed: 8551418]
6. Bhudhikanok GS, Lim J, Marcus R, Harkins A, Moss RB, Bachrach LK. Correlates of osteopenia in patients with cystic fibrosis. *Pediatrics.* 1996; 97:103–111. [PubMed: 8545201]
7. Baroncelli GI, De Luca F, Maguzzu G, Arrigo T, Sferlazzas C, Catena C, Bertelloni S, Saggese G. Bone demineralization in cystic fibrosis: Evidence of imbalance between bone formation and degradation. *Pediatr Res.* 1997; 41:397–403. [PubMed: 9078542]
8. Buntain HM, Greer RM, Schluter PJ, Wong JC, Batch JA, Potter JM, Lewindon PJ, Powell E, Wainwright CE, Bell SC. Bone mineral density in Australian children, adolescents and adults with cystic fibrosis: a controlled cross sectional study. *Thorax.* 2004; 59:149–155. [PubMed: 14760157]
9. Grey AB, Ames RW, Matthews RD, Reid IR. Bone mineral density and body composition in adult patients with cystic fibrosis. *Thorax.* 1993; 48:589–593. [PubMed: 8346485]
10. Elkin SL, Fairney A, Burnett S, Kemp M, Kyd P, Burgess J, Compston JE, Hodson ME. Vertebral deformities and low bone density in adults with cystic fibrosis: a cross-sectional study. *Osteoporos Int.* 2001; 12:366–372. [PubMed: 11444084]
11. Henderson RC, Specter BB. Kyphosis and fractures in children and young adults with cystic fibrosis. *J Pediatr.* 1994; 125:208–212. [PubMed: 8040763]
12. Donovan DS Jr, Papadopoulos A, Staron RB, Addesso V, Schulman L, McGregor C, Cosman F, Lindsay RL, Shane E. Bone mass and vitamin D deficiency in adults with advanced cystic fibrosis lung disease. *Am J Respir Crit Care Med.* 1998; 157:1892–1899. [PubMed: 9620924]
13. Salamoni F, Roulet M, Gudinchet F, Pilet M, Thiébaud D, Burckhardt P. Bone mineral content in cystic fibrosis patients: correlation with fat-free mass. *Arch Dis Child.* 1996; 74:314–318. [PubMed: 8669931]
14. Haworth CS, Selby PL, Webb AK, et al. Low bone mineral density in adults with cystic fibrosis. *Thorax.* 1999; 54:961–967. [PubMed: 10525552]
15. Conway SP, Morton AM, Oldroyd B, et al. Osteoporosis and osteopenia in adults and adolescents with cystic fibrosis: prevalence and associated factors. *Thorax.* 2000; 55:798–804. [PubMed: 10950902]
16. Gordon CM, Goodman E, Emans SJ, Grace E, Becker KA, Rosen CJ, Gundberg CM, LeBoff MS. Physiologic regulators of bone turnover in young women with anorexia nervosa. *J Pediatr.* 2002; 141:64–70. [PubMed: 12091853]
17. Soyka LA, Grinspoon S, Levitsky LL, Herzog DB, Klibanski A. The effects of anorexia nervosa on bone metabolism in female adolescents. *J Clin Endocrinol Metab.* 1999; 84:4489–4496. [PubMed: 10599707]
18. Greendale GA, Edelstein S, Barrett-Connor E. Endogenous sex steroids and bone mineral density in older women and men: The Rancho Bernardo Study. *J Bone Miner Res.* 1997; 12:1833–1843. [PubMed: 9383688]
19. Labrie F, Diamond P, Cussan L, Gomez JL, Belanger A, Candas B. Effect of 12-month dehydroepiandrosterone replacement therapy on bone, vagina and endometrium in post-menopausal women. *J Clin Endocrinol Metab.* 1997; 82:3498–3505. [PubMed: 9329392]
20. Gordon CM, Goodman E, Emans SJ, Grace E, Becker KA, Rosen CJ, Gundberg CM, LeBoff MS. Effects of oral DHEA on bone density in young women with anorexia nervosa: a randomized trial. *J Clin Endocrinol Metab.* 2002; 87:4935–4941. [PubMed: 12414853]

21. Van Hollenhoven RF, Park JL, Genovese MC, West JP. A double-blind placebo-controlled clinical trial of dehydroepiandrosterone in severe systemic lupus erythematosus. *Lupus*. 1999; 8:181–187. [PubMed: 10342710]
22. Yakar S, Rosen CJ, Beamer WG, Ackert-Bicknell CL, Wu Y, Liu JL, Ooi GT, Setser J, Frystyk J, Boisclair YR, LeRoith D. Circulating levels of IGF-I directly regulate bone growth density. *J Clin Invest* 2002. 2002; 110:771–781.
23. Mohan S, Baylink DJ. Bone growth factors. *Clin Orthop*. 1991; 263:30–48. [PubMed: 1993386]
24. Ueland T. GH/IGF-I and bone resorption in vivo and in vitro. *Eur J Endocrinol*. 2005; 152:327–332. [PubMed: 15757846]
25. Laursen EM, Juul A, Lanng S, Hoiby N, Koch C, Muller J, Shakkebaek NE. Diminished concentrations of insulin-like growth factor I in cystic fibrosis. *Arch Dis Child*. 1995; 72:494–497. [PubMed: 7618932]
26. Sermet-Gaudelus I, Souberbielle JC, Azhar I, Ruiz JC, Magnine P, Colomb V, Le Bihan C, Folio D, Lenoir G. Insulin-like growth factor I correlates with lean body mass in cystic fibrosis patients. *Arch Dis Child*. 2003; 88:956–961. [PubMed: 14612353]
27. Casson PR, Santoro N, Elkind-Hirsch K, Carson SA, Hornsby PJ, Abraham G, Buster JE. Post menopausal dehydroepiandrosterone administration increases free insulin like growth factor-I and decreases high-density lipoprotein: a six-month trial. *Fertil Steril*. 1998; 70:107–110. [PubMed: 9660430]
28. Manolagas SC, Jilka RLL. Bone marrow, cytokines and bone remodeling. *N Engl J Med*. 1995; 332:305–311. [PubMed: 7816067]
29. Pffefer KD, Huecksteadt TP, Hoidal JR. Expression and resolution of tumor necrosis factor in macrophages from cystic fibrosis patients. *Am J Respir Cell Mol Biol*. 1993; 9:511–519. [PubMed: 8217192]
30. Bonfeld TL, Panuska JR, Konstan MW, Hillard KA, Hillard JB, Ghnaim H, Berger M. Inflammatory cytokines in cystic fibrosis lungs. *Am J Respir Crit Care Med*. 1995; 152:2111–2118. [PubMed: 8520783]
31. Teramoto S, Matsuse T, Ouchi Y. Increased production of TNF-alpha may play a role in osteoporosis in cystic fibrosis patients. *Chest*. 1997; 112:574. [PubMed: 9266915]
32. Noah TL, Black HR, Cheng PQ, Wood RE, Leigh MQ. Nasal and bronchoalveolar lavage fluid cytokines in early cystic fibrosis. *J Infect Dis*. 1997; 175:638–647. [PubMed: 9041336]
33. Nixon LS, Yung B, Bell SC, Elborn JS, Shale DJ. Circulating immunoreactive interleukin-6 in cystic fibrosis. *Am J Respir Crit Care Med*. 1998; 157:1764–1769. [PubMed: 9620903]
34. Haworth CS, Selby PI, Webb AK, et al. Inflammatory related changes in bone mineral content in adults with cystic fibrosis. *Thorax*. 2004; 59:613–617. [PubMed: 15223873]
35. Daynes RA, Araneo BA, Ershler WB, Maloney C, Li GZ, Ryu SY. Altered regulation of IL-6 production with normal aging: Possible linkage to the age associated decline in DHEA and its sulphated derivative. *J Immunol*. 1993; 150:5219–5230. [PubMed: 8515056]
36. Gordon CM, LeBoff MS, Glowacki J. Adrenal and gonadal steroids inhibit IL-6 secretion by human marrow cells. *Cytokine*. 2001; 16:178–186. [PubMed: 11814313]
37. Aris RM, Stephens AR, Ontjes DA, Denene Blackwood A, Lark RK, Hensler MB, Neuringer IP, Lester GE. Adverse alterations in bone metabolism are associated with lung infection in adults with cystic fibrosis. *Am J Respir Crit Care Med*. 2000; 162:1674–1678. [PubMed: 11069795]
38. Kelly TL. Bone mineral density reference databases for American men and women. *J Bone Miner Res*. 1990; 5:S249.
39. Looker AC, Wahner HW, Dunn WL, Calvo MS, Harris TB, Heyse SP, Johnston CC Jr, Lindsay RL. Proximal femur bone mineral levels of US adults. *Osteoporos Int*. 1995; 5:389–409. [PubMed: 8800790]
40. Zemel BS. Reference data for the whole body, lumbar spine and proximal femur for American children relative to age, gender, and body size. *J Bone Miner Res*. 2004; 1:S231.
41. Hardin DS. Growth problems and growth hormone treatment in children with cystic fibrosis. *J Pediatr Endocrinol Metab*. 2002; 15:731–735. [PubMed: 12092687]
42. Grinspoon SK, Baum HB, Peterson S, Klibanski A. Effects of rhIGF-I administration on bone turnover during short-term fasting. *J Clin Invest*. 1995; 96:900–906. [PubMed: 7543494]

43. Gordon CM, Grace E, Emans SJ, Goodman E, Crawford MH, LeBoff MS. Changes in bone turnover markers and menstrual function after short-term oral DHEA in young women with anorexia nervosa. *J Bone Miner Res.* 1999; 14:136–145. [PubMed: 9893076]
44. Sin DD, Lacy P, York E, Man SF. Effects of fluticasone on systemic markers of inflammation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2004; 170:721–722. [PubMed: 15447948]
45. Wojtczak HA, Kerby GS, Wagener JS, et al. Beclomethasone dipropionate reduced airway inflammation with adrenal suppression in young children with cystic fibrosis: a pilot study. *Pediatr Pulmonol.* 2001; 32:293–302. [PubMed: 11568990]
46. Aris RM, Merkel PA, Bachrach LK, et al. Guide to bone health and disease in cystic fibrosis (e-publication). *J Clin Endocrinol Metab.* 2005; 90:1888–1896. [PubMed: 15613415]
47. Hahn TJ, Squires AE, Halstead LR, Strominger DB. Reduced serum 25-hydroxyvitamin D concentration and disordered mineral metabolism in patients with cystic fibrosis. *J Pediatr.* 1979; 94:38–42. [PubMed: 758419]
48. Stead RJ, Houlender S, Agnew J, Thomas M, Hodson ME, Batten JC, Dadona P. Vitamin D and parathyroid hormone and bone mineralization in adults with cystic fibrosis. *Thorax.* 1998; 43:190–194. [PubMed: 3406903]
49. Reiter EO, Brugman SM, Pike JW, Pitt M, Dokoh S, Haussler MR, Gerstle RS, Taussig LM. Vitamin D metabolites in adolescents and young adults with cystic fibrosis: effects of sun and season. *J Pediatr.* 1985; 106:21–26. [PubMed: 3871230]
50. Thompson GN. Determinants of serum vitamin D levels in preadolescent cystic fibrosis children. *Acta Paediatr Scand.* 1987; 76:962–965. [PubMed: 3425314]
51. Hubbard VS, Farrell PM, di Sant'Agnese PA. 25-Hydroxycholecalciferol levels in patients with cystic fibrosis. *J Pediatr.* 1979; 94:84–86. [PubMed: 758431]

Fig. 1.

Significant simple correlates of bone mass: **a** IL-1 β , **b** IGF-I, **cCircle Female, *cross* male, *dotted line* normal female mean concentration, *dashed line* normal male mean concentration or mean for both genders**

Table 1

Clinical characteristics of the 32 participants

Age (years)	
Mean $\pm$ standard deviation (SD)	26.2 $\pm$ 7.9
Range	15–47
Body mass index (kg/m ²)	
Mean $\pm$ SD	21.4 $\pm$ 2.6
Range	16–28
Height (SD score)	
Median	−0.89
Range	−1.02 to −0.78
Fracture history [<i>n</i> (%)]	16 (50)
Multivitamin or ADEK use [<i>n</i> (%)]	25 (78)
Calcium supplement use [<i>n</i> (%)]	6 (19)
CF-related diabetes [<i>n</i> (%)]	11 (34)
Inhaled glucocorticoids [<i>n</i> (%)]	32 (100)

Table 2

Predictors of bone mass

Predictor	Measure	Analysis	Hip	Spinal (L1-L4)	Total body
DHEAS	BMD-Z	Simple ^a	0.21 (0.24)	0.20 (0.27)	-0.02 (0.99)
	BMC	Simple	0.44 (0.012)*	0.13 (0.47)	0.24 (0.19)
	Area	Simple	0.47 (0.007)*	0.14 (0.46)	0.36 (0.044)*
IGF-I	BMD-Z	Simple	0.52 (0.002)*	0.56 (0.001)*	0.22 (0.24)
		Core ^b	0.44 (0.017)*	0.54 (0.001)*	
		Multiple ^c	0.36 (0.055)	0.50 (0.004)*	
BMC		Simple	0.55 (0.001)*	0.52 (0.002)*	0.37 (0.037)*
		Core	0.27 (0.025)*	0.32 (0.046)*	
		Multiple	0.24 (0.052)	0.34 (0.046)*	
IL-1 β	Area	Simple	0.25 (0.17)	0.28 (0.12)	0.32 (0.076)
	BMD-Z	Simple	0.39 (0.028)*	-0.39 (0.026)*	-0.35 (0.048)*
		Core	-0.34 (0.052)	-0.30 (0.073)	
BMC		Simple	-0.11 (0.54)	-0.098 (0.59)	-0.20 (0.28)
	Area	Simple	0.16 (0.41)	0.25 (0.17)	0.01 (0.97)

* Findings are significant at $p < 0.05$ ^a Simple: Pearson r (p)^b Core: regression model adjusted for gender, weight, height, and LBM (lean body mass); β -coefficient (p)^c Multiple: regression model additionally adjusted for DHEAS, IGF-I, and IL-1 β ; β -coefficient (p)

Table 3

Bone turnover markers and hormonal variables by gender

Variable	Mean $\pm$ SD	Range	Normal range
Females (n=18)			
NTx (nmol/mmol Cr)	55.3 $\pm$ 27.6	26.0–127.0	10–65
BSAP (ng/ml)	12.7 $\pm$ 3.7	6.7–22.0	2–24
DHEA-S (μ g/dl)	142.6 $\pm$ 155.8	10.2–545.1	183–283
Total testosterone (ng/dl)	20.7 $\pm$ 7.9	3.0–31.0	10–55
Estradiol (pg/ml)	9.5 $\pm$ 9.0	0.50–27.0	30–100
IGF-I (ng/ml)	216.3 $\pm$ 69.6	142–399	217–589
Males (n=14)			
NTx (nmol/mmol Cr)	109.2 $\pm$ 89.2	14–304	10–65
BSAP (ng/ml)	30.2 $\pm$ 15.6	13–62	2–24
DHEA-S (μ g/dl)	209.9 $\pm$ 97.0	53–365	120–370
Testosterone (ng/dl)	423.4 $\pm$ 137.4	254–676	350–1030
Estradiol (pg/ml)	2.0 $\pm$ 0.76	0.8–3.0	0.8–3.5
IGF-I (ng/ml)	270.5 $\pm$ 106.1	116–440	257–601