

Calcium acquisition rates do not support age-appropriate gains in total body bone mineral content in prepuberty and late puberty in girls with cystic fibrosis

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Abstract *Introduction:* Few longitudinal data are available characterizing bone development in adolescents with cystic fibrosis (CF) although this is a critical time for bone mineralization. *Materials and methods:* Dual energy X-ray absorptiometry (DXA) scans were obtained at 1- to 4-year intervals in 18 prepubertal and pubertal girls (age 7–18 years) with CF to determine calcium (Ca) accretion rates and changes (Δ) in total body bone mineral content (TBBMC) and lumbar spine bone mineral density (LS BMD) Z-scores. Daily Ca acquisition rates were calculated assuming TBBMC

was composed of 32.2% Ca. *Results:* Bone Ca accretion averaged 82 mg/day (2.05 mmol/day) [(range:–38 to +197 mg/day (–0.95 to 4.9 mmol/day)] on ~1,200 mg/day (30 mmol/day) Ca intakes. Estimated mean peak Ca accretion was 160 mg/day (4 mmol/day) at age 13 years; losses of bone Ca occurred in late puberty. Gains in insulin-like growth factor 1 (IGF-1) predicted Ca accretion ($p<0.06$). *Discussion:* Body mass index (BMI) Z-score predicted LS BMD and TBBMC Z-score cross-sectionally but did not predict Δ TBBMC Z-score. Changes in TBBMC Z-score paralleled Ca accretion rates with age. Bone Ca accretion in girls with CF fell below rates in healthy girls during prepuberty and late puberty despite Ca intakes approaching recommendations. IGF-1 and BMI Z-scores may identify children with CF at risk of compromised bone accretion, and more data are required to elucidate roles of lung function and glucocorticoid use in compromised bone health.

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Introduction

Over the past two decades, considerable attention has been focused on issues related to bone health in children and adults with cystic fibrosis (CF). While recognized as a severe problem in adults, the degree to which bone mass is compromised in children is less well characterized, and at present, little is known about determinants of bone gain across the pubertal growth spurt in children with CF despite the fact that over 40% of adult peak bone mass is acquired at this time in healthy children [1]. Nearly all studies examining reduced bone mass in children with CF are cross-sectional, and while many studies have reported compromised bone mass in children with CF [2, 3], some data suggest that reduced bone mass in children with CF is proportional to their smaller body size [4, 5]. Longitudinal assessments of bone mass during periods of growth are required to better explain the natural history of osteoporosis in individuals with CF, yet few studies are available

examining changes in bone mass in children and adolescents with CF over time.

Assessing changes in bone mass over time potentially allows one to determine whether gains in bone mass during growth are adequate when compared with gains observed in healthy populations. Some difficulties with this approach, however, are the lack of reference databases for skeletal sites other than the lumbar spine and femoral neck. Of the three longitudinal studies of bone health in children with CF that we were able to find published to date, two found no changes in bone mineral density (BMD) Z-scores of the lumbar spine and femoral neck over time [6, 7]. The third study showed declining whole-body BMD Z-scores over a 1- to 2-year period in adolescents (<20 years) in comparison to a locally recruited reference group matched for age and gender [8].

Serial total body dual energy X-ray absorptiometry DXA scans also provide an opportunity to easily derive measures of bone calcium (Ca) accretion rates since Ca comprises 32.2% of bone mineral [9]. Using serial total body DXA scans in healthy children, peak rates of bone Ca accretion in girls were determined to occur at a mean age of 12.5 years and averaged 284 mg/day (7.1 mmol/day) on Ca intakes of 1,100 mg/day (27.5 mmol/day) [10].

We recently reported data from stable isotope studies of Ca kinetics that adolescent girls with CF absorb adequate amounts of Ca from their diet [11] but have compromised Ca retention due to excessive endogenous fecal Ca losses [12]. We have also reported that rates of bone Ca deposition are particularly low among prepubertal and postmenarcheal girls, which may limit the window of opportunity for bone mineral consolidation in this group [13]. In the study reported here, we obtained follow-up DXA scans in 18 girls with CF who had participated in our previous Ca kinetic studies in order to assess Ca accretion rates and changes in lumbar spine BMD (LS BMD) and total body bone mineral content (TBBMC) relative to appropriate reference populations. Additionally, we examined predictors of these parameters in order to better understand effectors of bone health over time in children with CF.

Materials and methods

Study population

Girls with CF who had previously participated in a Ca kinetic and absorption study between July 1999 and October 2001 were invited to return for a follow-up assessment of their bone health 1–4 years following the original study. Of the 23 original study subjects, one was deceased, two were too ill to fully participate, and two others declined participation. Therefore, data on the 18 girls who completed both baseline and follow-up assessments of bone parameters are presented here. The baseline and follow-up studies were undertaken in the Pediatric Clinical Research Unit (PCRU) at Johns Hopkins Hospital. The Johns Hopkins School of Medicine Institutional Review Board and School of Public Health Committees

on Human Research approved all studies. Assent and consent were obtained from each subject and her guardian. Girls were required to be compliant with their usual treatment regimens and in generally good health during the baseline study, with no evidence of recent pulmonary exacerbations or oral steroid use.

Study design

During the baseline Ca kinetic study, weight and height were measured, blood (15 ml) was collected, Tanner stage of breast development was assessed by a pediatric endocrinologist, a medical history was taken, and total body and lumbar spine DXA scans were completed as previously described [11–13]. During the follow-up examination, the clinical nursing staff recorded weight and height and collected 20 ml of blood for subsequent biochemical analyses. A brief medical survey was administered, which included a history of oral steroid use, drug use, fracture history, physical exercise patterns, and hospitalizations over the interval between the baseline and follow-up visits. Tanner stage was evaluated by self report with a guardian's assistance as necessary [14], and menstrual history was tracked over the study interval. Tanner stage was categorized as prepubertal (Tanner 1), early pubertal (Tanner 2, 3), or late pubertal (Tanner 4, 5). At both baseline and follow-up, all prepubertal and early pubertal girls were premenarcheal while all late pubertal girls were postmenarcheal. All postmenarcheal girls menstruated by age 16, a standard cutoff for defining delayed puberty. However, three girls who menstruated at 15 years of age were considered to have had delayed puberty for the purposes of this study; all other postmenarcheal girls had their first menses before turning 14 years of age. Anthropometric measures of weight, height, and body mass index (BMI) were converted to Z-scores using the National Center for Health Statistics/Centers for Disease Control and Prevention (NCHS/CDC) growth charts [15].

Clinical severity of disease at baseline and follow-up was determined according to the forced expiratory volume in a 1-s [forced expiratory volume (FEV1)] measure taken during the regular clinic visit most closely timed (within 2 months) to the date of study participation. This value is expressed as a percent of predicted FEV1 based on age and height. Girls with an FEV1 score >90% were classified as having normal lung function; an FEV1 of 70–89% was classified as mild lung disease; and an FEV1 of 40–69% was classified as moderate lung disease. Oral and inhaled steroid use was determined through a retrospective review of each patient's medical chart and patient report.

Bone density

TBBMC, LS BMD, and body composition were determined in each subject by DXA using a Hologic QDR-4500A. Gender- and age-matched Z-scores were determined for the LS BMD from the Hologic database (Hologic, Inc.,

Bedford, MA, USA; software version 8.26a:3). Z-scores for TBBMC given the age, height, and race (all Caucasian) of each girl with CF were determined relative to reference data obtained on a Hologic instrument on over 1,000 children [16–18]. The addition of height to the Z-score calculation helps ensure that girls with CF were appropriately compared to healthy girls of similar stature and degree of pubertal development. By definition, Z-scores beyond -1 and -2 indicate bone mass below that observed in 84% and 97.7% of the reference population, respectively, and are the cutoffs currently recommended for screening the CF population for bone disease [19]. Therefore, we used these cutoffs as a guide to indicate the degree to which bone mass was compromised in girls with CF in cross-sectional measures at baseline and follow-up. Using this approach, if girls with CF maintained a consistent trajectory in bone acquisition relative to the reference population over time, expected changes in Z-scores for LS BMD and TBBMC would be zero, with positive changes representing gains and negative changes representing losses relative to the reference group.

Determination of Ca accretion

To determine the Ca content of bone at each study time point, TBBMC was multiplied by 32.2%, the percent of bone mineral that is composed of Ca [9]. The change in total body Ca content between each time point was determined as the difference between the follow-up and baseline visits for each subject. Net daily Ca accretion rate in units of milligrams per day were obtained by dividing the net change in total body Ca (after conversion to milligrams) by the number of days that had elapsed between studies.

Hormones and biomarkers

Laboratory tests were completed on all girls at baseline and on 16 of the 18 girls at follow-up. Serum 25-hydroxy vitamin D [25(OH)D] was measured at both time points using a commercially available radioimmunoassay (Diasorin, Inc, Stillwater, MN, USA). The intra-assay coefficient of variation (CV) was within 10%. Parathyroid hormone (PTH) was measured at baseline using an immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA, USA) and at follow-up by the Johns Hopkins Clinical Core Laboratory using an automated immunoassay (Nichols Institute). Insulin-like growth factor 1 (IGF-1) was measured at baseline and follow-up using an Immulite 1000 (DPC, Los Angeles, CA, USA). Osteocalcin (OC) was analyzed in baseline samples at the USDA Human Nutrition Research Center on Aging at Tufts University by radioimmunoassay.

Nutritional assessment

Nutritional assessment of dietary intake at baseline was determined using a 24-h weighed food intake (during an

inpatient stay) and a 3-day food record. At follow-up, nutrient intakes were determined using a food frequency questionnaire administered by the study staff (Block Dietary Data System, CA, USA). At follow-up, two girls did not fill out the survey, and two surveys could not be analyzed because of abnormally low or high nutrient intake due to difficulties in estimating portion sizes. One girl was taking a Ca supplement at the time of the baseline study, which was included in her estimate of daily Ca intake. All study participants were prescribed daily multivitamin and mineral supplements (exclusive of Ca but including 400 or 800 IU of vitamin D) as part of their usual treatment regimen.

Statistical analysis

Paired *t* tests were used to assess significant differences in subject characteristics between baseline and follow-up measurements. *T* tests within pubertal groups were used to determine whether measures of LS BMD Z-score, TBBMC Z-score, and their changes over time differed from zero. Analysis of variance (ANOVA) with Scheffe's multiple comparisons test was used to determine whether measures of bone mass differed by pubertal groups at baseline or follow-up, and analysis of covariance (ANCOVA) was used to assess changes in these measures by pubertal groups. Regression analysis was used to identify determinants of LS BMD Z-score, TBBMC Z-score, their changes over time, and daily Ca accretion rates. Results of statistical analyses were considered significant if $p < 0.05$ and, because of small sample size, were considered noteworthy at $p < 0.10$. All statistical analyses were done using STATA, v 8.0 (College Station, TX, USA).

Results

Subject characteristics

Subject characteristics at baseline and follow-up are presented in Table 1. Girls ranged in age from 7.6 to 17.9 years at the baseline study and from 8.7 to 19.8 years at the follow-up. The mean time elapsed between the first visit and the second visit was 2.13 ± 1.14 years and ranged from 1.06 to 4.10 years. Although significant increases in absolute weight, height, and BMI occurred over time as expected, the average weight-for-age, height-for-age, and BMI Z-scores did not change significantly and remained below average at follow-up. BMI Z-scores ranged widely among study subjects at both baseline (-2.83 to 1.21) and follow-up (-2.86 to 1.28). Although there were no differences in BMI Z-scores across pubertal groups, mean baseline BMI Z-scores were -0.31 ± 0.62 , -0.60 ± 1.09 , and 0.00 ± 1.30 for pre-, early, and late pubertal girls, respectively, indicating that on average the late pubertal girls were better nourished than the national average as collected and reported by the CF Foundation Registry [20].

Table 1 Characteristics of the 18 study subjects at baseline and follow-up^a

	Baseline	Follow-up
Age (years) ^b	12.1±3.2	14.1±3.5
Weight (kg) ^b	39.6±13.9	45.7±13.0
Height (cm) ^b	146±15	152±13
BMI (kg/m ²) ^b	18.0±3.0	19.4±3.0
Weight-for-age Z-score	-0.36±1.02	-0.29±0.94
Height-for-age Z-score	-0.24±0.77	-0.30±0.75
BMI-for-age Z-score	-0.27±1.05	-0.13±1.02
Caloric intake (kcal)	2,720±869	2,680±974 ^c
Calcium intake (mg/day)	1,196±486	1,152±564 ^c
Calcium intake (mmol/d)	(30±12)	(29±14)
IGF-1 (ng/ml)	314±184	366±160 ^d
IGF-1 (nmol/l)	(41±24)	(48±21)
25(OH)D (ng/ml)	33.3±8.6	29.7±13.0 ^e
25(OH)D (nmol/l)	(83±21)	(74±32)
PTH (pg/ml)	25.2±11.6	40.3±18.5 ^e
PTH (pmol/l)	(2.7±1.2)	(4.3±2.5)
OC (ng/ml)	13.7±3.34 ^f	-
OC (nmol/l)	(2.3±0.6)	-

BMI body mass index, IGF-1 insulin-like growth factor 1, 25(OH)D 25-hydroxy vitamin D, PTH parathyroid hormone, OC osteocalcin

^aMean±SD, (range)

^bStatistically significant for changes between baseline and follow-up, $p < 0.05$ using paired t test

^cData available for $n=14$ girls

^dData available for $n=16$ girls

^eData available for $n=15$ girls

^fData available for $n=13$ girls

Little difference in dietary patterns was evident between baseline and follow-up using the two different assessment methods. At both time points, girls were consuming close to the recommended caloric intake for this population, and dietary Ca intake approached the current recommendations of 1,300 mg/day (32.5 mmol/day) for this age group [21].

Mean biochemical measures did not change significantly between the baseline and follow-up assessments. Serum 25(OH)D concentrations were not indicative of overt vitamin D deficiency (<15 ng/ml or <37 nmol/l) in any subject at either baseline or follow-up. However, in spite of daily vitamin A, D, E, and K supplementation, 44% and 67% of girls had 25(OH)D concentrations below 30 ng/ml (75 nmol/l) at baseline and follow-up, respectively. At follow-up, three subjects (20%) had elevated PTH concentrations (>65 pg/ml or >6.9 pmol/l), which were not associated with suboptimal vitamin D status. Baseline measures of IGF-1 and OC were lower than those observed in healthy populations [22, 23]. As expected, IGF-1 values were significantly higher ($p=0.001$) and OC values were lower ($p=0.09$) in late pubertal versus prepubertal girls at baseline. However, differences in IGF-1 by pubertal group did not persist at follow-up when less-well-nourished girls matriculated into the late pubertal group.

Mean percent predicted FEV1 was 86±19% (range: 47–107%) at the time of the baseline study in 17 girls for whom data were available. FEV1 score was not related to age in

this sample. Ten girls (59%) were considered to have normal lung function (FEV1 >90% of predicted) while three (18%) and four (24%) girls were considered to have mild (FEV1 70–89% of predicted) or moderate (FEV1 40–69%) lung disease, respectively. Seven girls (39%) reported using oral steroids in the interval between baseline and follow-up.

Ca accretion and bone mineral content

Bone mass and Ca accretion rates, stratified by baseline and follow-up pubertal status, are presented in Table 2. Of note is the extended time interval (3.90 and 3.95 years) of the two girls who matured from early pubertal to late pubertal status over the course of the study. Total body bone mineral content increased across pubertal groups, reflecting pubertal growth. The greatest gains in TBBMC occurred in the girls with the greatest time interval between studies. However, when Ca accretion was averaged over that time interval, daily gains of approximately 100 mg/day (2.5 mmol/day) were observed in the two girls with the longest elapsed time interval, a value slightly greater than the average of 82 mg/day (2.1 mmol/day) for the entire group. Calcium accretion rates ranged from an average daily loss of 38 mg/day (0.95 mmol/day) to an average daily gain of 197 mg/day (4.9 mmol/day). There was a tendency toward increased Ca accretion rates associated with early puberty (girls who remained in Tanner stages 2–3 throughout the study period) although differences among pubertal groups in Ca accretion rates were not statistically significant when assessed by pubertal status at baseline, follow-up, or both.

Mean TBBMC Z-scores at baseline and follow-up did not differ significantly from zero, nor did changes in TBBMC Z-score differ from zero (Table 2). At baseline, TBBMC Z-scores were higher among late pubertal than early pubertal girls although this difference did not exist at follow-up. Mean LS BMD Z-scores at baseline and follow-up were substantially below zero among girls who started the study in prepuberty. Changes in LS BMD Z-score did not differ from zero among any of the pubertal groups. As was the case for TBBMC Z-scores, LS BMD Z-scores were highest among late pubertal girls at baseline, but this difference did not exist at follow-up. The highest LS BMD and TBBMC Z-scores among girls who began the study in late puberty likely reflects the unusually good nutritional status among those girls since the association of late pubertal status with improved bone health was diminished when early pubertal girls advanced into the late pubertal group over the course of the study.

Changes in TBBMC and LS BMD Z-scores over the course of the study are shown in Fig. 1. We compared the percent of children in our cohort who were less than -1 Z-scores with the expected rate of 16% assuming a normal reference distribution. At the baseline assessment, 4/18 girls (22.2%) had TBBMC Z-scores below -1, which is comparable to that expected. At the time of the follow-up study, one of these girl's Z-scores had declined to below -2,

Table 2 Measures of bone mineral content and calcium (Ca) accretion by pubertal group at baseline and follow-up^a

Number	All Subjects 18	Baseline:	Prepubertal	Prepubertal	Early pubertal	Early pubertal	Late pubertal
		Follow-up:	Prepubertal	Early pubertal	Early pubertal	Late pubertal	Late pubertal
			2	4	3	2	7
Age at midpoint (years)	13.1±3.3		8.1±0.0	10.4±0.6	11.7±0.8	15.2±1.2	16.1±2.0
Time interval (years)	2.02±1.07		1.15±0.11	1.92±0.70	1.17±0.06	3.93±0.03	2.14±1.05
Baseline TBBMC (g) ^b	1,316±539		637±35	879±68	1,096±312	1,166±205	1,897±275
Follow-up TBBMC (g) ^c	1,498±527		696±30	1,092±134	1,280±350	1,616±80	2,019±259
ΔTBBMC (g) ^d	182±160		60±6	212±98	183±75	450±285	122±136
Ca accretion (mg/dday) (mmol/day)	82±65 (2.1±1.6)		45.7±0.07 (1.1±0.002)	105.2±57.5 (2.6±1.4)	139.1±61.0 (3.5±1.5)	100.6±62.9 (2.5±1.6)	49.8±67.6 (1.2±1.7)
Baseline TBBMC Z-score ^e	-0.29±1.01		-1.04±0.85	-0.31±0.45	-0.71±1.41	-1.47±0.95	0.46±0.66
Follow-up TBBMC Z-score	-0.45±1.16		-1.65±0.61	-0.40±0.45	-0.64±1.21	-1.58±1.58	0.10±0.92
ΔTBBMC Z-score	-0.17±0.60		0.61±0.24	-0.09±0.64	0.07±0.31	-0.12±0.63	-0.35±0.56
Baseline LS BMD Z-score ^{b,f}	-0.40±1.13		-1.39±0.29 ^h	-0.53±0.55	-1.10±0.96	-1.29±1.24	0.52±1.04
Follow-up LS BMD Z-score ^c	-0.46±0.94		-1.40±0.14 ^g	-0.58±0.46 ^h	-1.10±0.87	-1.15±1.20	0.34±0.69
ΔLS BMD Z-score	-0.07±0.42		-0.02±0.43	-0.04±0.40	0.0±0.73	0.14±0.04	-0.18±0.43

TBBMC total body bone mineral content, LS BMD lumbar spine bone mineral density, Δ change between baseline and follow-up, ANOVA analysis of variance

^aMean±SD

^bSignificant differences by pubertal state at baseline: late > early and late > pre, $p < 0.05$ by ANOVA with Scheffe's multiple comparisons test

^cSignificant differences by pubertal state at follow-up: late > early and late > pre, $p < 0.05$ by ANOVA with Scheffe's multiple comparisons test

^d $p = 0.06$ for ANOVA comparing five pubertal groups; no significant differences among groups by Scheffe's multiple comparisons test

^eSignificant differences by pubertal state at baseline: late > early, $p < 0.05$ by ANOVA with Scheffe's multiple comparisons test

^fValues indicated with superscript g are different than 0 Z-score units, $p < 0.10$; values indicated with superscript h are significantly different than 0 Z-score units, $p < 0.05$

and one girl's Z-score improved. Two additional girls had Z-scores that declined to below -1 such that at follow-up, 5/18 girls (31.1%) had TBBMC Z-scores below -1 ($p = 0.08$). Overall, 11 girls (61%) experienced declining TBBMC Z-scores, with a maximum loss of -1.49 Z-score units, while seven (39%) demonstrated improved TBBMC Z-scores, with a maximum gain of 0.64 Z-score units.

Lumbar spine BMD Z-scores indicated a more substantial bone deficit than that detected from the whole-body measures that accounted for height. At the baseline assessment, nearly twice as many as expected, or 7/18 (38.8%), of these girls had LS BMD Z-scores below -1 ($p = 0.01$). Of those seven, one girl's Z-score declined to below -2 while one girl's Z-score improved substantially. Nine (50%) girls experienced declining LS BMD Z-scores, with a maximum loss of -0.95 Z-score units, while 9 (50%) demonstrated improved LS BMD Z-scores, with a maximum gain of 0.84 Z-score units.

In cross-sectional analyses of baseline bone mineral measures, the strongest determinant of both TBBMC Z-score and LS BMD Z-score after controlling for pubertal group was BMI Z-score. (For TBBMC Z-score: $\beta_{\text{BMI Z-score}} = 0.51$, $p = 0.005$, $n = 18$, $R^2 = 0.67$, $p < 0.002$; for LS BMD Z-score: $\beta_{\text{BMI Z-score}} = 0.56$, $p = 0.004$, $n = 18$, $R^2 = 0.71$, $p = 0.0005$). These associations persisted at follow-up. (For TBBMC Z-score: $\beta_{\text{BMI Z-score}} = 0.60$, $p = 0.02$, $n = 18$, $R^2 = 0.44$, $p = 0.04$; for LS BMD Z-score: $\beta_{\text{BMI Z-score}} = 0.45$, $p = 0.031$, $n = 18$, $R^2 = 0.51$, $p = 0.016$). Additionally, among postmenarcheal girls, mean TBBMC

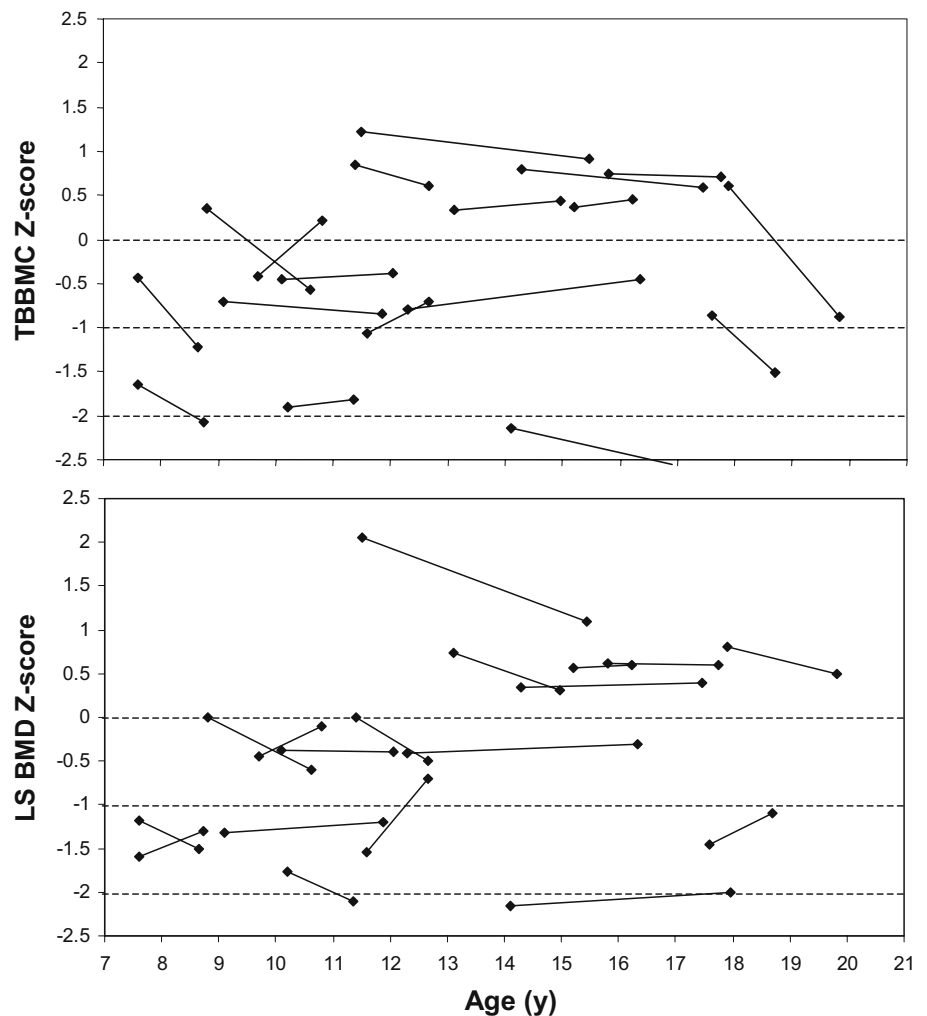
and LS BMD Z-scores were approximately 2 units lower among the three girls in whom puberty had been delayed (age 15) compared with the rest of the group at both the baseline (TBBMC Z-scores -1.27 ± 0.76 vs. 0.68 ± 0.33 , $p = 0.001$; LS BMD Z-scores -1.34 ± 0.88 vs. 0.85 ± 0.61 , $p = 0.003$) and follow-up (TBBMC Z-scores -1.56 ± 1.12 vs. 0.37 ± 0.64 , $p = 0.01$; LS BMD Z-scores -1.13 ± 0.85 vs. 0.58 ± 0.28 , $p = 0.002$) assessments.

Determinants of Ca accretion and changes in TBBMC and LS BMD Z-scores

Ca accretion rates determined from longitudinal DXA measures were associated with bone Ca deposition rates (expressed as natural logs) assessed at baseline using stable isotopes of Ca ($r = 0.54$, $p = 0.02$) [13]. Ca deposition rates peaked in midpuberty [13] as did Ca accretion rates, which peaked at an estimated 13 years of age (Fig. 2).

To further explore the relation of Ca accretion rates with changes in TBBMC Z-scores, these variables were plotted against the midinterval age of each individual in the study (Fig. 2). Among girls who were premenarcheal at the study follow-up (Tanner stages 1-3), increasing age was associated with increasing Ca accretion and TBBMC Z-scores that improved with age. Among girls who were postmenarcheal at the study follow-up (Tanner stages 4-5), increasing age was associated with decreasing Ca accretion and declining TBBMC Z-scores. The peak gains in both Ca

Fig. 1 Changes in total body bone mineral content (TBBMC) and lumbar spine bone mineral density (LS BMD) Z-scores with age in 18 girls with cystic fibrosis (CF). Two measures of TBBMC and LS BMD were obtained in each of 18 girls with CF across intervals ranging from 1 to 4 years. Z-scores for each measure based on age (LS BMD) or age and height (TBBMC) were determined, and the change in Z-score units over time is presented for each of the 18 study subjects. The average change in the TBBMC Z-score was -0.23 ± 0.52 (range -1.49 to 0.64) while the average change in the LS BMD Z-score was -0.07 ± 0.42 (range -0.95 to 0.84)



accretion and TBBMC Z-scores occurred at an estimated 13 years of age, at which time predicted average Ca accretion was 160 mg/day (4 mmol/day), and predicted gains in TBBMC Z-scores were 0.43 relative to an expectation of zero gain. However, among girls $< \sim 10.5$ years and $> \sim 15$ years, predicted changes in TBBMC Z-scores were negative, indicative of net bone mineral losses relative to expected values.

When controlling for the relationship of Ca accretion to age in a multiple linear regression model, the only additional variable that predicted Ca accretion rates to an appreciable extent was the degree to which IGF-1 changed over the study interval ($\beta_{\Delta\text{IGF-1}} = 0.13$, $P = 0.06$; $R^2 = 0.33$, $n = 16$, $P < 0.002$). The change in IGF-1 ranged from a decline of 334 ng/ml (43.8 nmol/l) to an increase of 432 ng/ml (56.6 nmol/l), with values increasing on average by 65.5 ± 193.5 ng/ml (8.6 ± 25.3 nmol/l). Baseline concentrations of 25(OH)D, PTH, OC, and longitudinal changes in 25(OH)D concentrations over time did not significantly predict rates of Ca accretion.

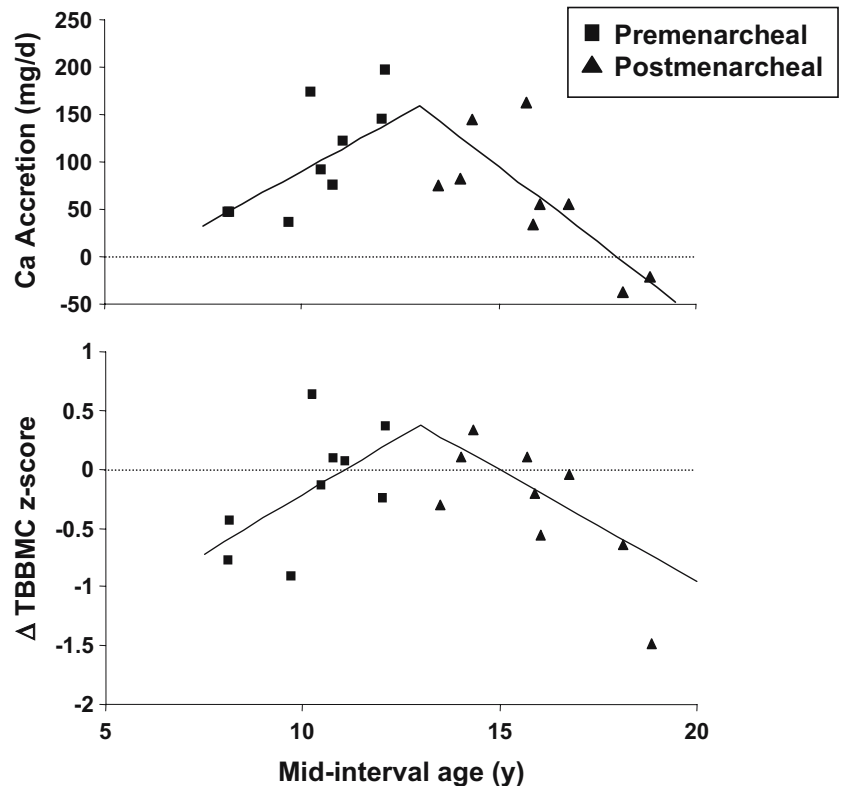
Although predictive of TBBMC Z-scores in cross-sectional analyses, neither baseline BMI Z-score nor the change in BMI Z-score was associated with the degree to

which TBBMC changed over the course of the study. Further, clinical and other biochemical variables were not significantly associated with changes in TBBMC Z-score observed over the study interval after adjusting for the relationship of $\Delta\text{TBBMC Z-score}$ with age, likely due to the small sample size.

A weak tendency toward increasing $\Delta\text{TBBMC Z-score}$ with increasing baseline FEV1 was noticed after controlling for age ($\beta_{\text{FEV1}} = 0.008$, $p = 0.15$, $n = 17$, $R^2 = 0.41$, $p = 0.07$). In turn, FEV1 was predictive of subsequent oral steroid use (baseline FEV1 percent predicted was $73.7 \pm 17.0\%$ among subsequent steroid users vs. $94.0 \pm 14.6\%$ among nonusers, $p = 0.02$), and OC concentrations were positively correlated with FEV1 scores ($r = 0.56$, $p = 0.06$).

Unlike the change in TBBMC Z-score, the change in LS BMD Z-score was unrelated to age. The greatest gains in LS BMD Z-score occurred among girls who had the poorest LS BMD Z-scores at baseline, and the least gains in LS BMD Z-score occurred among those with the best baseline LS BMD Z-scores ($r = 0.61$, $p = 0.007$). Other biochemical and anthropometric variables were not associated with the change in LS BMD Z-score.

Fig. 2 Relationship of calcium (Ca) accretion rate (mg/day) and the change in total body bone mineral content (TBBMC) Z-score with midinterval age in 18 girls with cystic fibrosis (CF) according to their menarcheal status at follow-up. The peak Ca accretion rate was estimated to occur at 13 years of age, such that the relationship between age and Ca accretion was described by the regression equation: $\Delta\text{Ca accretion (mg/d)} = 23 * (\text{age in y}) - 55 * (\text{time beyond 13 y}) - 141$; $p_{\text{age}} = 0.01$, $p_{\text{age} > 13} = 0.001$, $F = 9.8$, $\text{prob} > F = 0.002$, $R^2 = 0.57$. The relationship of the change in TBBMC Z-score with age demonstrated a parallel relationship and was described by the equation: $\Delta\text{TBBMC Z-score} = 0.20 * (\text{age in y}) - 0.43 * (\text{time beyond 13 y}) - 2.21$; $p_{\text{age}} = 0.02$, $p_{\text{age} > 13} = 0.004$, $F = 6.5$, $\text{prob} > F = 0.09$, $R^2 = 0.46$. Peak gains of 0.42 TBBMC Z-score units were predicted at 13 years of age



Discussion

We found that low bone mass was common among this otherwise healthy cohort of adolescent girls with CF. At baseline, nearly 40% had deficits in expected bone mineral content at the lumbar spine (Z-scores greater than -1) and over 20% had deficits in expected TBBMC even after accounting for their stature. These deficits did not appear to be solely related to delays in rates of bone acquisition in children with CF compared with a healthy population, as they persisted 1–4 years later in over 30% of this cohort using either measure.

Patterns of bone Ca acquisition across puberty

In healthy females, rates of bone mineral acquisition during puberty are maximized in the months surrounding the onset of menses [24]. Females continue to accrue bone mass at slower rates until peak bone mass is achieved by age 20–30 years, after which bone mass typically remains fairly stable until the onset of menopause [25]. Our data in girls with CF are consistent with that observed in healthy girls showing the highest rates of bone mineral acquisition at midpuberty. However, both our kinetic study of bone Ca deposition and the longitudinal study of Ca acquisition suggest that bone Ca deposition rates in prepuberty and late puberty in girls with CF are insufficient to support appropriate gains in bone mineral.

In our present study, we found rates of daily bone Ca accretion that averaged 82 mg/day (2.1 mmol/day) and ranged from losses of nearly 40 mg/day (1 mmol/day) to gains of nearly 200 mg/day (5 mmol/day). This cohort of girls with CF began the study with good clinical status and consumed diets containing calcium intakes approaching 1,200 mg/day (30 mmol/day). Average peak rates of Ca accretion were estimated to be ~ 160 mg/day (4 mmol/day) at 13 years of age, which is well below the mean peak Ca accretion rates of 284 mg/day (7.1 mmol/day) reported by Bailey et al. [10] in healthy girls consuming 1,100 mg Ca/day (27.5 mmol/day). That study also used Ca acquisition rates obtained from serial DXA scans although they were able to capture data in children at regular 12-month intervals specifically timed around the time of peak bone mineral acquisition. An estimate of peak bone Ca acquisition of 212 mg/day (5.3 mmol/day) was reported in that same population of healthy girls based on cross-sectional assessment of single DXA scans [26], an approach known to underestimate peak rates [10]. Although the maximum Ca acquisition rate observed among the girls with CF approached that value, on average, Ca acquisition rates fell well below the value of 212 mg/day obtained from cross-sectional assessments of healthy girls.

Our estimated peak Ca acquisition rate also falls below the average rate of bone Ca balance of 305 mg/day (7.6 mmol/day) reported in our original cohort at baseline using stable Ca isotopes. However, Ca acquisition rates obtained for early and late-pubertal girls were similar using either the Ca kinetic or serial DXA approach [13]. It is

likely that these differences between serial total body DXA measures in midpuberty and daily estimates based on isotopic measures occur in part as a result of differences in the methodological assumptions or techniques utilized. For example, our kinetic data may have overestimated Ca retention if endogenous Ca losses were underestimated [12]. Moreover, girls were recruited for the baseline kinetic study when they were in good clinical condition and when they were not taking oral glucocorticoids. Longer-term measures of net skeletal gain over time take into account average Ca accretion over intervals in which children may have had infections, required steroids, or experienced hospitalization for pulmonary exacerbations. Thus, this longitudinal approach is likely to be more reflective of true average Ca accretion rates over time.

By late adolescence (age 18 years), measured losses of bone Ca content of 40 mg/day (1 mmol/day) were evident, and predicted losses reached 50 mg/day (1.25 mmol/day) by age 20 years. This degree of Ca loss is comparable with that reported in postmenopausal women who are not receiving hormone replacement therapy [27] and would translate to a net loss of 14.6 g of Ca per year at an age during which bone mass should be stable or slightly increasing. Although more data would be required to establish that the losses of Ca we observed in our study group are generalizable, it is interesting to note that they do occur in girls whose bone health was unusually good at the baseline study. Thus, it could be argued either that we are observing regression to the mean, or, conversely, that more extreme losses might be expected in girls of poorer nutritional and clinical status. Nonetheless, the joint effects of a failure to attain peak bone mass coupled with an earlier onset of loss of bone mineral are consistent with the numerous reports of both osteopenia and osteoporosis in adults with CF [28, 29].

Changes in bone mineral content

Changes in TBBMC Z-score over the study interval paralleled Ca accretion rates. Declines in the change in TBBMC Z-scores among the youngest and oldest girls in this study demonstrate that Ca acquisition in early and late puberty was insufficient to allow girls to maintain a consistent trajectory in total body bone mineral acquisition over time relative to healthy peers. Although girls in midpuberty demonstrated gains in TBBMC Z-scores, maximum predicted gains of less than 0.42 Z-score units would have little long-term benefit when average TBBMC Z-scores were predicted to decline by 0.43 Z-score units per year among girls beyond the age of 13 years. Our data demonstrating relative losses in TBBMC Z-scores is consistent with the propensity toward whole-body losses of BMD reported by Bhudhikanok et al. in 11 girls and nine boys (age 8–18) with CF [8]. In that study, the greatest losses in BMD relative to healthy peers occurred among premenarcheal girls receiving glucocorticoid therapy.

Total body measures of bone mineral content are advocated for use in growing children as opposed to site-

specific measures of BMD [30, 31] although reference data are limited for children. We used an online database that is accessible to clinicians with which to determine changes in bone mineral status in our subjects with CF relative to those expected in a healthy population. This database allowed us to adjust for height as well as age so that girls with CF were compared with healthy girls of similar stature, thus avoiding a criticism of references based on age alone. With this approach, TBBMC proved more useful for assessing changes in bone status than LS BMD. The lack of change in LS BMD was consistent with that observed in two other longitudinal studies of children with CF which demonstrated low initial LS BMD Z-scores did not improve or decline over a 2-year interval [6, 7] in groups of 19 or 16 preadolescent and adolescent girls, respectively. These findings suggest that site-specific measures are not sufficiently sensitive to changes in bone mineral content over periods of growth and further emphasize the need to standardize the use of whole-body measures of bone mineral content in children.

Predictors of bone status

Because not all children with CF currently have regular DXA scans, it is important to identify risk factors for low bone mass and inadequate Ca accretion in children with CF. Current recommendations are to obtain DXA scans in children >8 years who are underweight (<90% ideal body weight), have FEV1 scores <50%, ingest >5 mg/day glucocorticoids for over 90 days per year, or experience delayed puberty and fractures [19]. Most participants in this study would not have met these criteria, yet reduced BMD was not uncommon. Thus, further examination of the criteria for screening for poor bone health are required in this age group to refine these recommendations.

Our study supports the use of body weight for screening for low bone mass. In cross-sectional analyses, BMI Z-score was positively associated with LS BMD and TBBMC Z-scores, with a 1-unit increase in BMI Z-score associated with an approximate 0.5 unit benefit in either bone measure. Relationships of BMI or other measures of nutritional status have been demonstrated to be predictive of bone status in a variety of studies in individuals with CF [2, 29, 32, 33].

Among the girls in this study, FEV1 was only weakly predictive of Δ TBBMC although only one girl in our study had an FEV1 below 50% at baseline. An association of lung function with bone mass has been demonstrated in other studies [2, 29, 32–34], and individuals with lower FEV1 scores are likely to experience pulmonary exacerbations and more frequently require oral glucocorticoid treatment, both of which may independently contribute to compromised bone mineralization [35–37]. However, among the girls in this study, use of glucocorticoids in the interval between DXA scans did not predict declines in TBBMC Z-scores.

Finally, delayed puberty as defined by menarche at >15 years of age was predictive of reduced bone mass in

postmenarcheal girls. While a potentially useful screening tool, its use is more complex in premenarcheal girls and in boys. Thus, biomarkers related to pubertal development could be useful for assessing current or future risk of low bone mass. Although not related to bone mineral Z-scores per se, among our study cohort, changes in IGF-1 were associated with Ca accretion rates, such that every 10 ng/ml (1.31 nmol/l) improvement in IGF-1 concentration was associated with a 1.3 mg/day (0.0325 mmol/day) gain in accumulated Ca. Given the huge range in the degree to which IGF-1 values changed over the course of the study, a better understanding of effectors of IGF-1 concentrations and their specific role in bone development in children with CF is warranted. Other baseline biochemical indicators with Ca-regulating functions, such as vitamin D and PTH, were not associated with changes in bone mass over time. Although seasonal variability in vitamin D status was not captured in this study, there was no evidence of frank vitamin D deficiency among our subjects. Furthermore, baseline PTH was within the normal range, thereby suggesting that perturbations in the vitamin D/PTH axis were not paramount in determining bone mass in this group of girls.

Summary

Although puberty is a critical time of life for bone acquisition, few studies in patients with CF have examined predictors of bone health during this period. Children with CF have a reduced window of opportunity for bone acquisition, and because many aspects of this disease further limit Ca retention, children at risk need to be identified and targeted for intervention during this formative period. Lung function (FEV1) and BMI Z-scores are routinely assessed in pediatric patients with CF and may be useful markers for more aggressive monitoring of bone health in this group. Serum IGF-1 concentrations are not routinely assessed in this population but may provide additional insight into the determinants of bone health in this age group. Greater availability of longitudinal data on bone mineral acquisition during puberty in both boys and girls with CF across the spectrum of nutritional and clinical status is essential for elucidating typical patterns of bone gain and significant risk factors for suboptimal mineral acquisition during this critical period of bone development.

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