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Calcium Kinetics Are Altered in Clinically Stable Girls with Cystic Fibrosis

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Reduced bone mass in individuals with cystic fibrosis (CF) may result from alterations in calcium metabolism. Bone calcium deposition and resorption rates, calcium balance, and markers of bone turnover were assessed using stable isotopes of calcium in 22 prepubertal and pubertal girls with CF. Bone calcium deposition was associated with the availability of dietary calcium, total serum osteocalcin, and leptin concentrations.

Reduced bone mass in individuals with CF may result from inadequate bone calcium (Ca) deposition, and excessive resorption, although these parameters have not been directly assessed in children with CF.

We used stable Ca isotopes to measure rates of bone Ca deposition (Vo+), resorption, and retention in 22 clinically stable girls with CF (aged 7–18 yr). Rates of bone Ca deposition were determined by mathematically modeling the disappearance of iv Ca stable isotope (^{42}Ca) for 6 d post dosing. Indirect markers of bone turnover and hormones associated with pubertal development were also assessed.

Rates of bone Ca deposition and retention were highest during early puberty (Tanner stages 2 and 3). Calcium deposition rates in prepubertal (Tanner 1) and postmenarchal girls (Tanner stages 4 and 5) did not support substantial bone Ca retention. Net absorption of dietary Ca and serum osteocalcin and leptin concentrations were positively associated with Vo+. Time post menarche and serum leptin concentrations explained 91% of the variability in Vo+ (*P* = 0.0007). Serum total osteocalcin was low (10.9 ± 5.4 ng/ml), and a substantial percentage of osteocalcin was undercarboxylated (54.3 ± 11.8%).

We concluded that increased calcium absorption and serum leptin concentrations were significantly associated with rates of bone Ca deposition, demonstrating an impact of nutritional status on this process. Rates of bone Ca deposition were lower than typically reported in healthy children, as were indirect markers of bone formation. These alterations in bone turnover contribute to reduced bone mass in girls with CF. (*J Clin Endocrinol Metab* 89: 3385–3391, 2004)

A VARIETY OF studies have documented a reduction in bone mineral density in children with CF (1–3). Characterizing the magnitude and determinants of bone mineral deposition and resorption in children with CF is of the utmost importance, because much of peak bone mass is accrued during puberty (4).

Most efforts to characterize the impact of cystic fibrosis (CF) on alterations in bone mineral turnover have occurred among adults with this disease. Reductions in osteoblast function and activity and decreases in osteoblast number have been observed in the ileac crest (5), and increased osteoclast numbers have been reported from postmortem histomorphometric studies in adults with CF (6). Moreover, adults with CF have higher concentrations of bone resorptive markers, compared with healthy individuals (7), and these markers become increasingly elevated as severity of lung disease increases (8, 9).

Previous studies of bone turnover markers in children with CF have found low circulating concentrations of osteocalcin, a marker of bone formation (10, 11) and elevations in markers of bone resorption (11, 12). These findings suggest that bone mineral accretion is reduced and resorption is enhanced in patients with CF, even at a time when rapid bone mineral accretion should be occurring.

Although some data exist on indirect markers of bone turnover, to date there have been no reports characterizing the actual turnover of bone calcium (Ca) in patients with CF. Because Ca is the major mineral of bone, comprising 32.2% of bone mineral content, (13), it is of particular interest to understand the dynamics of this nutrient in relation to bone development. Direct measures of the kinetics of bone Ca deposition and measures of bone Ca resorption and retention can be obtained using stable isotopes of Ca. Calcium kinetic studies in healthy adolescent girls have shown that bone Ca deposition is maximized during pubertal development in the months before menarche and declines thereafter (14–16).

To determine whether abnormalities in bone Ca deposition, resorption, and retention exist in patients with CF, we assessed these parameters in girls with CF at varying stages

Abbreviations: BMI, Body mass index; CF, cystic fibrosis; DXA, dual-energy x-ray absorptiometry; ln, natural logarithm; LS BMD, lumbar spine bone mineral density; NTX, *N*-telopeptide; OC, osteocalcin; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; 25(OH)D, serum 25-hydroxyvitamin D; TBBMC, total body bone mineral content; TBBMC%, percentage of TBBMC expected based on the prediction equation; ucOC, undercarboxylated OC; ucOC%, percentage of OC not bound to hydroxyapatite *in vitro*; Vbal, bone Ca balance; Vendo, endogenous fecal Ca loss; Vo+, bone Ca deposition; Vo−, bone Ca resorption.

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of pubertal development using stable Ca isotopes. We also assessed markers of bone turnover and examined predictors of Ca kinetic measures in an effort to characterize factors that influence bone Ca acquisition in girls with CF.

Subjects and Methods

Girls with CF from 7–18 yr of age were recruited from the Johns Hopkins Cystic Fibrosis Center and two other regional CF centers (Harrisburg, PA, and Wilmington, DE). Twenty-three girls were enrolled, but because of incomplete sample collections in one subject, bone Ca deposition and turnover could be determined in only 22 study subjects. The study was approved by the Johns Hopkins Joint Committee on Clinical Investigation, and assent and consent were obtained from each subject and her parent or guardian. Study volunteers were required to be clinically stable at the time of the study, generally compliant with their CF treatment regimen, and must not have received any oral glucocorticoid preparations for the month preceding the kinetic study. All prescribed medications were continued over the course of the study, including pancreatic enzymes (in 20 pancreatic insufficient girls) and ADEK vitamin tablets (one tablet per day before age 10 yr and two tablets per day in those aged 10 yr and older). The ADEK tablets contained 400 IU vitamin D and 150 μg vitamin K per tablet. Calcium absorption and endogenous fecal Ca losses in these subjects have recently been published (17, 18).

Fasted girls were admitted for a 24-h stay to the Johns Hopkins Pediatric Clinical Research Unit (PCRU) in the morning before breakfast. A fasting blood sample (15 ml) was obtained and girls were given ^{44}Ca (0.35 mg/kg body weight) in whole milk with breakfast. After breakfast, ^{42}Ca (0.2 mg/kg) was administered over 5 min through a heparin lock. Blood samples (3 ml) were obtained at 5, 15, 30, 60, 120, 360, and 480 min after administration of the iv tracer. Complete 24-h urine (in three 8-h aliquots) and stool collections were obtained after tracer administration. Girls collected three spot urine samples daily and complete stool output at home in Ca-free containers for the subsequent 5 d, for a total of 6 d of sample collection.

Girls self-selected their foods during the 24-h stay in the PCRU, and foods were pre- and postweighed to obtain exact intakes. Upon returning home, subjects recorded their dietary intake for 3 d so that information on typical nutrient intakes could be ascertained. Diet records were analyzed for Ca and energy content using the Minnesota Nutrition Data System (version 2.91), and nutrient intake was determined from the average of the 4 d of record collection.

Weight, height, and body mass index (BMI; kilograms per square meter) were expressed as Z-scores for age (19). Tanner stage of breast development was evaluated by a pediatric endocrinologist. Girls were characterized as prepubertal (Tanner 1), early pubertal (Tanner 2 and 3), or late pubertal (Tanner 4 and 5), as in other Ca kinetic studies of healthy girls (16, 20). All Tanner 1–3 girls were premenarcheal, and all Tanner 4–5 girls were postmenarchal.

Lumbar spine bone mineral density (LS BMD), total body bone mineral content (TBBMC), and body composition were obtained for each girl by dual-energy x-ray absorptiometry (DXA) using a Hologic QDR-4500A. Age- and sex-matched Z-scores were obtained for LS BMD (Hologic, Inc., Bedford, MA, software version 8.26a:3). TBBMC was compared with that expected based on a prediction equation that was developed from data from 483 healthy girls (21). This prediction equation accounts for height as well as age and race, thereby providing an appropriate reference for girls with CF who are expected to be small, compared with healthy girls of the same age. Actual TBBMC of the girls with CF was expressed as a percentage of that expected based on the prediction equation (TBBMC%). By these criteria, osteopenia was considered to exist when TBBMC% was 76–88% (−1 to −2 SD), and osteoporosis was considered to exist when TBBMC% was less than 76% (below −2 SD). Osteopenia and osteoporosis were also assessed using LS BMD Z-scores with cutoffs of −1 Z-score and −2 Z-score, respectively (21).

A clinical severity score was devised using a measure of the forced expiratory volume in one second obtained at a regular clinic visit within 2 months of the time of the study. Severity of lung disease was characterized according to the score for forced expiratory volume in one second relative to a healthy reference as follows: normal, 90% or greater of predicted; mild, 70–89% of predicted; moderate, 40–69% of predicted; or severe, less than 40% of predicted. Estimates of daily pancreatic enzyme intake were standardized based on the prescribed enzyme dose, assuming three meals and two snacks were eaten daily. Inhaled steroid use and history of oral glucocorticoid use were ascertained from verbal histories and a review of medical charts.

Isotopic analysis and multicompartmental modeling

Calcium was precipitated from serum and spot urine samples with ammonium oxalate (22), and dried samples reconstituted with 3% Ultrex nitric acid (JT Baker, Phillipsburg, NJ) were loaded onto a rhenium filament. The ratio of ^{42}Ca to ^{48}Ca or ^{43}Ca was measured using a quadrupole thermal ionization mass spectrometer (Finnigan THQ, Bremen, Germany) or magnetic sector thermal ionization mass spectrometer (Finnigan Triton, Bremen, Germany), respectively. Results were cross-validated between instruments. Ratios were corrected for isotopic fractionation. The enrichment of tracer in serum and spot urine samples was expressed as the delta percent excess, the degree to which the measured ratio was increased over the natural abundance ratio.

Rates of bone Ca deposition (Vo+) were determined based on the rate of disappearance of ^{42}Ca from serum and spot urine samples over the 6-d study period after accounting for urinary and endogenous fecal (Vendo) Ca losses using a multicompartmental model and the SAAM (simulation, analysis, and modeling) software (23). The compartmental model used to describe Ca transfer among body pools is based on the model first proposed by Neer *et al.* (24), and it has been extensively used in pediatric studies (14, 25). Due to incomplete fecal collections, actual measurements of Vendo were available for only 13 of the 22 girls (18). The mean value obtained in these 13 girls (2.7 mg/kg·d) was used in the kinetic measurements for the other nine subjects. Girls for whom endogenous fecal calcium data were available were evenly distributed across pubertal groups. Calculated geometric means (−1 SD, +1 SD) of Vo+ did not differ among girls for whom data were available and for whom values were estimated [measured: 1267 mg/d (988, 1618); estimated: 1466 mg/d (1017, 2126); $P = 0.25$], and predictors of Vo+ reported herein were consistent whether endogenous Ca losses were measured or imputed.

Rates of bone Ca resorption (Vo−) were calculated after accounting for other sources of Ca flux to and from the central exchangeable pool (diet, urinary Ca loss, and Vendo), and bone Ca balance (Vbal) was calculated as the difference between Vo+ and Vo−.

Hormones and bone markers

Serum IGF-I and estradiol (Diagnostic Systems Laboratories, Inc., Webster, TX), leptin (Linco Research Inc., St. Louis, MO), and serum and urinary *N*-telopeptide (NTX; Ostex International, Seattle, WA) were measured in baseline samples using ELISAs. Serum 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] were measured by immunoradiometric assays (Diasorin, Stillwater, MN), as was PTH (Nichols Institute, San Juan Capistrano, CA). Urinary creatinine was measured using a colorimetric assay (Metra Biosystems, Mountain View, CA).

Serum total osteocalcin (OC) and undercarboxylated OC (ucOC) were analyzed at the U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University by RIA using procedures described by Gundberg *et al.* (26) This assay uses human OC for standard and tracer and a polyclonal antibody directed to intact human OC (27) and recognizes both intact OC and the large N-terminal midmolecule fragment. The ucOC was expressed as the percentage of OC not bound to hydroxyapatite *in vitro* (ucOC%) (26). Results of OC and ucOC were unavailable for six study subjects (unrelated to those for whom endogenous fecal Ca data were unavailable) due to either insufficient sample volume or hemolyzed serum.

Data analysis

Student's *t* test, ANOVA with Scheffé's multiple comparisons test, and χ^2 analysis were used to look for differences in baseline characteristics and outcome measures by pubertal groups. A natural log transformation was used to normalize Vo+, which was skewed to high values, although this transformation was not necessary among the subset of postmenarchal girls when examining the relationship of Vo+ to

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time since menarche. Simple and multiple linear regressions were used to examine relationships of variables to the natural logarithm (ln) of Vo+. Pubertal state, clinical severity, and steroid use were coded as dummy variables. Data are reported as mean ± SD unless otherwise indicated. All statistical analyses were done using STATA version 7.0 (StataCorp, College Station, TX), and results were considered significant at $P < 0.05$.

Results

Subject characteristics

Characteristics of the study sample are summarized in Table 1. Average BMI Z-scores corresponded to approximately the 36th (prepubertal), 27th (early pubertal), and 44th (postmenarchal) percentiles. Among the pre- and early pubertal groups, these percentiles were similar to those reported nationally for girls with CF in the corresponding age ranges, whereas in the postmenarchal girls, this value was higher than that reported nationally (30th to 40th percentiles) (28). Although there was a tendency toward better age-adjusted nutritional status in the late pubertal girls, assessed as BMI Z-score, it was not statistically significant.

Average LS BMD Z-scores ($P = 0.05$) and TBBMC% ($P = 0.02$) were higher among the postmenarchal girls when compared with the premenarcheal girls combined, consistent with the better nutritional status found among the postmenarchal girls. Among all study subjects, 45% (10 of 22) were considered osteopenic or osteoporotic by LS BMD Z-scores, and 32% (7 of 22) were considered osteopenic or osteoporotic by TBBMC%. One early pubertal and one late pubertal girl were osteoporotic based on both LS BMD Z-score and TBBMC%. When defined by TBBMC%, rates of osteopenia or osteoporosis were highest among girls in early puberty (80% or four of five girls, $P < 0.02$).

TABLE 1. Anthropometric indices and BMC of study subjects

	Premenarcheal		Postmenarcheal
	Prepubertal (Tanner stage 1) (n = 7)	Early pubertal (Tanner stages 2, 3) (n = 5)	(Tanner stages 4, 5) (n = 10)
Age (yr)	9.2 ± 1.4	11.9 ± 1.4	15.3 ± 2.1
Weight (kg)	26.9 ± 4.0	36.2 ± 6.7	52.1 ± 10.0
Height (cm)	129.7 ± 6.0	145.9 ± 9.6	160.2 ± 6.1
BMI (kg/m^2)	15.9 ± 1.2	17.0 ± 2.2	20.2 ± 2.7
BMI/age Z-score	−0.35 ± 0.58	−0.60 ± 1.10	−0.16 ± 1.22
LS BMD Z-score	−0.86 ± 0.58	−1.18 ± 0.93	0.12 ± 1.43
TBBMC%	91.7 ± 6.0	86.8 ± 13.4	102.5 ± 12.1

Data are presented as mean ± SD.

Calcium intake averaged 1181 ± 443 mg/d (29.5 ± 11.1 mmol/d), ranging from 300 to 1943 mg/d (7.5–48.6 mmol/d) and did not differ by pubertal group. One girl took an oral Ca supplement (500 mg/d or 125 mmol/d), which was included in the measure of total Ca intake. The net amount of Ca absorbed averaged 276 ± 63, 512 ± 36, and 318 ± 142 mg/d (6.9 ± 1.6, 12.8 ± 0.9, 8.0 ± 3.6 mmol/d) among prepubertal, early pubertal, and late pubertal girls, respectively, and was highest among early pubertal girls, as previously reported (17).

Hormonal mediators of pubertal development were lower in the premenarcheal compared with postmenarchal girls, respectively (Table 2). Conversely, total OC and serum and urinary NTX were lower in the postmenarchal compared with premenarcheal girls. The ucOC% did not differ across pubertal groups and averaged 54.3 ± 11.8% (n = 15).

Leptin concentrations were significantly correlated with IGF-I concentrations ($r = 0.65$, $P = 0.001$). The correlation between estradiol and leptin approached significance ($r = 0.40$, $P = 0.07$), whereas an even weaker association was evident between estradiol and IGF-I ($r = 0.34$, $P = 0.12$). Total OC was strongly associated with IGF-I ($\beta = 0.020$, $P = 0.004$) within pubertal groups (multiple linear regression statistics: $F = 19.9$, $R^2 = 0.84$, $P = 0.0001$) when one outlying data point was removed.

Serum 25(OH)D averaged 32.1 ± 8.4 ng/ml (80.2 ± 20.9 nmol/liter), and 1,25(OH)$_2$D averaged 40.9 ± 9.7 pg/ml (98.1 ± 23.3 pmol/liter). Most girls were studied during the summer, when vitamin D status would be expected to be optimal, and vitamin D deficiency [25(OH)D < 20 ng/ml or ~50 nmol/liter] did not occur within this group of girls. Serum 1,25(OH)$_2$D was elevated (>45 pg/ml or ~120 pmol/liter) in three subjects. No girls had hyperparathyroidism (PTH > 65 pg/ml or ~7 pmol/liter) and PTH averaged 25.6 ± 12.3 pg/ml (2.7 ± 1.3 pmol/liter).

Fifteen subjects (68%) were taking inhaled steroid preparations at the time of the study. Past oral steroid use was reported in 10 girls, although only two girls reported repeated use of oral steroids beyond their preschool years. Only one girl had finished a course of oral glucocorticoids a month before the study for a condition not directly related to

TABLE 2. Hormones and bone markers in girls with CF

	Premenarcheal		Postmenarcheal
	Prepubertal (Tanner stage 1) (n = 7)	Early pubertal (Tanner stages 2, 3) (n = 5)	(Tanner stages 4, 5) (n = 10)
Leptin (ng/ml)[a]	3.6 ± 3.8	5.9 ± 1.9	10.1 ± 5.2
Estradiol (pg/ml)[a]	27.7 ± 18.0	37.4 ± 22.2	69.3 ± 33.2
IGF-I (ng/ml)[a]	226.9 ± 90.0	215.1 ± 84.8	382.0 ± 148.0
Total OC (ng/ml)[a,b]	15.1 ± 3.9	15.1 ± 1.0	7.3 ± 4.5
ucOC (%)[c]	55.5 ± 12.9	48.6 ± 7.8	56.0 ± 13.2
Urinary NTX (nm BCE/mM)[a]	542.1 ± 116.5	415.8 ± 111.6	227.4 ± 240.5
Serum NTX (nm BCE)[a]	55.3 ± 10.0	58.3 ± 18.2	24.2 ± 15.4

Mean ± SD; conversion to SI units as follows: leptin, nanomoles per liter = nanograms per milliliter × 0.0625; estradiol, picomoles per liter = picograms per milliliter × 3.671; IGF-I, nanomoles per liter = nanograms per milliliter × 0.131; OC, nanomoles per liter = nanograms per milliliter × 0.171; urinary NTX expressed per millimolar urinary creatinine.

[a] Values differ between premenarcheal and postmenarcheal girls by Student's *t* test, $P < 0.01$.

[b] Data available for five, three, and eight subjects among prepubertal, early pubertal, and postmenarcheal girls, respectively.

[c] Data available for five, three, and seven subjects among prepubertal, early pubertal, and postmenarcheal girls, respectively.

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her CF. Daily pancreatic enzyme use was 7.3 ± 2.2 (×1000) IU lipase units per kilogram body weight among the 20 girls who were pancreatic insufficient and did not differ among pubertal groups. Clinical severity score was normal in 50% of subjects, mild in 15% of subjects, and moderate or severe in 35% of subjects. Postmenarchal girls were more likely than pre- or early pubertal girls to have moderate or severe lung disease ($P < 0.05$). Nearly all girls (91%) reported being moderately or very active.

Bone calcium kinetics

Calcium kinetic measures are presented for each pubertal group in Table 3. The geometric mean of Vo+ was highest among the early pubertal girls, although the difference in Vo+ between the prepubertal and early pubertal girls was not statistically significant ($P = 0.11$). Although Vo− did not differ statistically among groups, Vbal was substantially higher in the girls in early puberty, compared with the prepubertal or late pubertal groups. In postmenarchal girls, Vbal did not significantly differ from zero (95% confidence interval −37 to 195 mg/d or −0.9 to 4.9 mmol/d).

Among all study subjects, the amount of Ca absorbed from the diet was positively associated with ln(Vo+) ($r = 0.6$, $P = 0.003$). Because both absorbed Ca and ln(Vo+) were elevated among the early pubertal girls, pubertal state was included in regression models to examine the influence of absorbed Ca within pubertal groups. In that regression model, the influence of absorbed Ca ($P = 0.07$) on ln(Vo+) was somewhat reduced.

Figure 1 shows the association of total OC, ucOC%, and Vo+ by pubertal group among the 15 girls for whom data for both OC and ucOC% were available. Similarly, ln(Vo+) was positively associated with total OC concentration ($r = 0.55$, $P = 0.034$), but the relationship of total OC to ln(Vo+) within pubertal groups failed to retain significance ($P = 0.33$). Therefore, the significant relationship of total OC with ln(Vo+) is explained by the fact that these variables changed in a similar fashion across pubertal groups, although total OC failed to capture the peak rates of bone Ca deposition that occurred during early puberty. The ucOC% did not differ across pubertal groups and was not associated with rates of bone Ca deposition.

Serum and urinary NTX concentrations were also positively associated with ln(Vo+) ($r = 0.60$, $P = 0.003$; $r = 0.63$, $P = 0.002$, respectively), reflecting the strong relationship between bone Ca deposition and bone Ca resorption ($r = 0.95$, $P < 0.0001$). As expected, serum and urinary NTX concentrations were also associated with Vo− ($r = 0.46$, $P = 0.03$; $r = 0.54$, $P = 0.009$, respectively).

In a multivariate analysis to control for differences in Vo+ among pubertal groups, leptin emerged as an important predictor of bone calcium deposition. Within each pubertal group, ln(Vo+) was positively associated with leptin, such that the influence of leptin concentration on ln(Vo+) appeared to operate at different thresholds, depending on pubertal state (Fig. 2). Leptin was associated with body fat content in kilograms ($r = 0.83$, $P < 0.001$), percentage body fat ($r = 0.72$, $P < 0.001$), and BMI Z-score ($r = 0.59$, $P = 0.003$), and each of those variables was positively associated with ln(Vo+) when substituted in the regression model for leptin. However, only leptin retained statistical significance when assessed simultaneously with those body composition variables, suggesting an independent effect of leptin on bone Ca deposition.

Calcium deposition declined after menarche, and among nine late pubertal girls (excluding one girl at 72 months post menarche),the time since menarche in months explained 65% of the variability in Vo+ [Vo+ (milligrams per day) = −848 × ln(months post menarche) + 3858], $F = 13.01$, $R^2 = 0.65$, $P = 0.009$). With the addition of serum leptin concen-

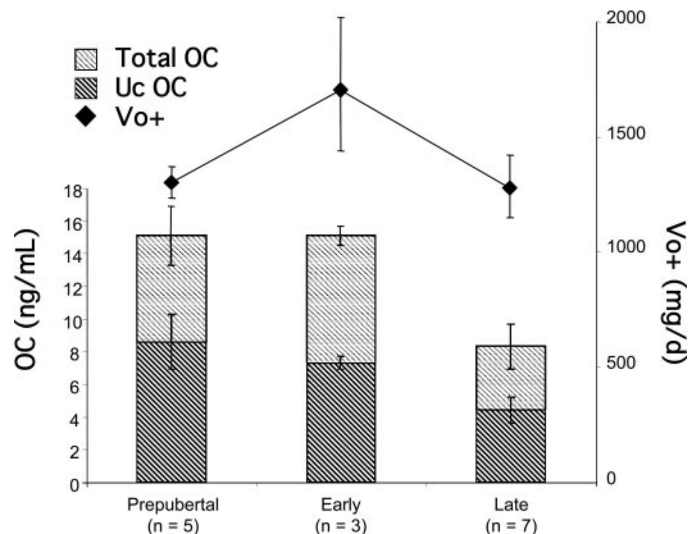

FIG. 1. The association of total and ucOC with rates of bone Ca deposition (Vo+, *right axis*) by pubertal group in 15 girls with CF. Mean ± SEM total serum OC (nanograms per milliliter; *left axis*) and the contribution of ucOC to total OC are shown by pubertal group. Total OC was significantly lower in the late pubertal group than in the prepubertal and early pubertal groups combined ($P < 0.05$). The percentage of total OC that was carboxylated did not differ by pubertal group. Total OC did not peak during early puberty when bone Ca deposition did. SI units for OC (nanomoles per liter) = nanograms per milliliter × 0.171; for Ca (micromoles per day) = milligrams per day × 0.025.

TABLE 3. Bone calcium deposition, resorption, and balance in girls with CF

	Premenarcheal		Postmenarcheal
	Prepubertal (Tanner stage 1) (n = 7)	Early pubertal (Tanner stages 2, 3) (n = 5)	(Tanner stages 4, 5) (n = 10)
Vo+ (mg/d)	1321 (1192, 1463)	1857 (1392, 2478)[a]	1185 (863, 1629)[b]
Vo− (mg/d)	1212 (1043, 1408)	1525 (1043, 2230)	1097 (780, 1541)
Vbal (mg/d)	103 ± 72[a]	305 ± 69[b]	79 ± 162[a]

Geometric mean (−1 SD, +1 SD) for Vo+ and Vo−; mean ± SD for Vbal. SI conversion to millimoles per day Ca = milligrams per day Ca × 0.25; *superscript letters* indicate where groups differ by ANOVA with Scheffe's multiple comparisons test, $P < 0.05$.

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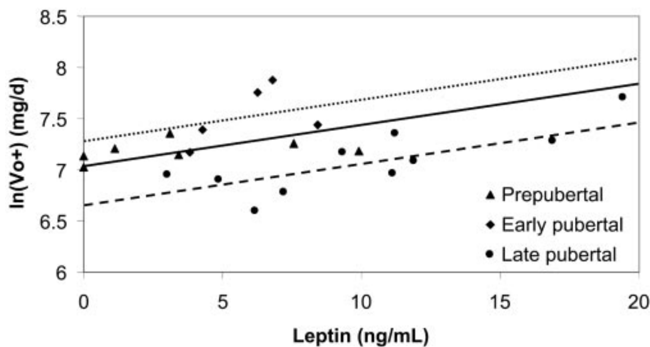

FIG. 2. Leptin concentration (nanograms per milliliter; $\beta = 0.041$, $P = 0.001$) was predictive of rates of bone Ca deposition (Vo+) within pubertal groups in 22 girls with CF. The y-intercept of leptin with ln(Vo+) for prepubertal girls (*solid line*) was 7.03. The y-intercept of the relationship of leptin with ln(Vo+) in early pubertal girls (*dotted line*) was 0.245 U higher ($P = 0.057$), and in late pubertal girls (*dashed line*) was 0.375 U lower ($P = 0.006$) than in the prepubertal girls. Regression statistics: $F = 10.34$, $R^2 = 0.63$, $P = 0.0004$. SI units for leptin (nanomoles per liter) = nanograms per milliliter × 0.0625.

tration into this model, 91% of the variability in Vo+ was explained in girls up to 30 months post menarche [Vo+ (milligrams per day) = −576 × ln(months post menarche) + 46.2 × leptin (nanograms per milliliter) + 2549; P values for ln(months post menarche) = 0.007, leptin = 0.006; $F = 30.6$; $R^2 = 0.91$, $P = 0.0007$]. The association of leptin with Vo+ was also stronger than the relationships of other body composition variables among this subset of girls.

Other biochemical markers (vitamin D, estradiol, IGF-I) and clinical measures such as clinical severity score, pancreatic enzyme dose, and history of oral or inhaled steroid use were not significantly related to rates of bone Ca deposition in either univariate or multivariate analyses.

Discussion

This is the first study to directly measure rates of bone Ca deposition and net Ca retention using stable isotopes of Ca in girls with CF. Our findings highlight the importance of nutritional status and the pubertal growth spurt in promoting bone Ca deposition in girls with CF. Our results show that bone Ca deposition and balance were greatest in early puberty, but by late puberty rates of bone Ca deposition were insufficient to support substantial Ca retention.

Several studies have characterized calcium retention in relation to pubertal state in healthy children. Abrams and Stuff (20) reported calcium retention rates of 131 ± 83, 161 ± 88, and 44 ± 91 mg/d, respectively, for prepubertal, early pubertal, and late pubertal healthy girls consuming approximately 950 mg/d Ca. Prepubertal and late pubertal girls with CF in this study retained Ca in amounts nearly identical with those observed among the healthy girls studied by Abrams and Stuff while consuming diets containing approximately 200 mg/d more calcium, suggesting that prepubertal and late pubertal girls with CF were less efficient than healthy girls at retaining dietary Ca. Conversely, girls with CF in early puberty retained more than 300 mg/d Ca on average, which is similar to Ca retention rates estimated for healthy girls consuming approximately 1100 mg/d Ca during peak bone mineralization (284 mg/d) using total body bone mineral data obtained from serial DXA scans (29) and which is considerably higher than Ca retention reported by Abrams and Stuff (20).

Bone calcium retention ultimately affects bone mineral content, which can be assessed using total body or site-specific DXA scans. In our study population, the highest kinetically derived rates of calcium retention were found in early pubertal girls, the group at highest risk for deficits in LS BMD Z-score and TBBMC%. Whereas this finding may appear contradictory, bone density measures represent the cumulative gain in bone over time and a lag between bone Ca deposition rates and accumulated bone mineral relative to reference norms is not unexpected. Also paradoxically, postmenarchal girls in our study had better bone mineral status than the premenarcheal groups despite lower net Ca retention. We feel this finding is due to the recruitment of adolescents in this age group that were particularly well nourished, compared with national averages, as evidenced by higher BMI Z-scores relative to those expected for their age. Several prior studies have supported a positive association of nutritional status with bone mineral status in both children and adults with CF (1, 30–32).

Based on evidence in healthy children using stable isotopic methods identical with those reported here, Vo+ is thought to be the primary determinant of bone calcium retention among children consuming and absorbing typical amounts of dietary Ca (33). We found a trend toward higher rates of bone Ca deposition during early puberty (Tanner stages 2 and 3), compared with those measured during prepuberty or late puberty (Tanner stages 4 and 5), a pattern of change that is consistent with that demonstrated in healthy girls in other stable isotope studies (14, 16). Furthermore, rates of bone Ca deposition found among girls with CF in early puberty were comparable with those reported in a recent study of Ca kinetics in healthy girls consuming approximately 1200 mg/d dietary Ca (1952 ± 407 mg/d in healthy Tanner 2 girls). In contrast, rates of Vo+ among the prepubertal girls with CF in this study were lower than those reported in healthy prepubertal girls, in whom Vo+ averaged more than 1500 mg/d (14). In late pubertal girls with CF, Vo+ was lower than predicted based on normative data. Of great interest, we found the decline in Vo+ in the months after menarche was 82% greater in girls with CF than that reported among healthy girls (slope for girls with CF: −848; slope for healthy girls: −466), (15), although a larger sample of girls with CF would be required to determine with certainty that this difference was statistically and biologically important. Because puberty is often delayed in CF (34) and because the decline in rates of calcium deposition may occur more rapidly after menarche in CF, early puberty may represent a particularly limited window of opportunity to optimize bone mineralization in adolescent girls with CF.

Bone calcium deposition was related to net calcium absorption among girls in this study, underscoring the importance of the provision of adequate dietary calcium. We previously found that these girls absorbed at least as much dietary Ca as that absorbed by healthy girls and that vitamin D status was generally adequate and unrelated to calcium absorption (17). Thus, encouraging consumption of adequate amounts of dietary Ca is an important strategy for improving

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Ca balance and ensuring optimal bone mineralization in patients with CF in this age group. This is particularly true because individuals with CF may be at risk of losing excessive body Ca through endogenous fecal losses (18), and the high dietary sodium intakes recommended for patients with this disease may increase urinary Ca excretion (17). These increased sources of Ca loss from the body may contribute to the inefficiency of dietary Ca retention in this group.

Bone turnover is often assessed using indirect markers of bone metabolism, including OC and NTX. In this study of girls with CF, OC concentrations were consistent with data previously reported for children with CF (10, 11) and lower than concentrations observed in healthy girls (11, 14). Furthermore, a peak in serum OC in early puberty was not observed among the girls with CF, as has been reported for healthy girls (11, 14), suggesting that osteocalcin may be inadequate as a marker of bone formation in detecting the increase in bone mineral deposition associated with the pubertal growth spurt.

Bone Ca deposition among our study subjects was not associated with the degree to which OC was carboxylated. Osteocalcin is a noncollagenous protein produced by osteoblasts that binds Ca and hydroxyapatite during bone mineralization and whose activity requires carboxylation of glutamic acid residues, a vitamin K-dependent process. A high ucOC% is a sensitive indicator of vitamin K deficiency (26), previously reported in patients with CF (35). In our subjects, ucOC% was more than double the value of 23% reported in healthy children using the same assay as that used in this study (26). Thus, vitamin K deficiency is a concern in this group, despite supplementary vitamin K ingestion, and the impact of vitamin K deficiency on bone development in this age group deserves further study.

Finally, among girls with CF in this study, urinary NTX was similar to age-appropriate norms in healthy children (36), in contrast to results by others (11) showing elevated NTX (~1000 nmol bone collagen equivalent per millimole creatinine) across pubertal groups in children and young adults with CF. Subjects in that study tended to have worse lung function than those that we studied, but age, pubertal state, and BMI were otherwise similar. Thus, measures of indirect markers of bone mineralization in this study are consistent with our Ca kinetic data in showing that compromised Vo+, rather than elevated Vo−, plays a larger role in the reduction in bone Ca balance found among these girls with CF. It is important to emphasize that our study subjects did not have many of the risk factors that are common to children with CF in that they were clinically stable, not taking oral glucocorticoid preparations, and generally active. It is likely that in children with the above risk factors, disease-related increases in bone Ca resorption might exaggerate the impact of reduced bone Ca deposition on bone development.

Puberty is a critical time for bone mineral acquisition, and a variety of physiological events occur during pubertal development to support the ability of the body to acquire and consolidate bone mineral. Serum leptin may have an influential role in mediating bone mineralization, although its role in bone health is controversial. In mice, intracerebroventricular administration of leptin decreases bone mineral density, demonstrating a centrally mediated negative effect of leptin on bone (37). Conversely, human studies have often found a positive association of circulating leptin with bone mass, and *in vitro* studies have found an impact of leptin on osteoblast differentiation and function, suggesting a positive systemic effect of leptin on bone (38). Khosla (38) reconciled these two disparate roles of leptin by theorizing that the central mechanism may serve to protect bone during energy deprivation when leptin concentrations are low, whereas a systemic role for leptin may become more influential as energy reserves improve. Average leptin concentrations among girls with CF in this study were similar to those reported for healthy girls during puberty (39) and are in agreement with normal concentrations of leptin reported elsewhere for individuals with CF (40). However, the association of serum leptin with bone Ca deposition rates may have particular significance for children with CF who, due to the fat malabsorption associated with this disease, tend to have lower body fat despite higher energy intakes than healthy individuals.

Leptin likely also plays a role in the promotion of pubertal development. Later age at menarche has been found in pubertal girls with low leptin concentrations (41), suggesting a link between leptin and gonadal hormone production. Among our study subjects, leptin was associated with estradiol concentrations, supporting the existence of such a relationship. However, estradiol concentrations among our study subjects were higher than those previously reported for girls with CF and similar to those observed in healthy girls at the same stage of pubertal development (34). Additionally, the lack of association between estradiol and Vo+ suggests that estradiol concentrations do not limit bone calcium retention in girls with CF.

GH and IGF-I are also important mediators of pubertal development, (42), and the effects of IGF-I on bone development during puberty may in part be mediated by osteoblast proliferation and OC production (43). A positive association of IGF-I with OC was observed in the girls with CF in this study, and IGF-I, as well as OC, did not reach concentrations reported for healthy girls during the pubertal growth spurt (500 ng/ml) (44, 45). IGF-I has been reported to be low in individuals with CF, particularly when nutritional status is compromised (40, 44, 45). Thus, the production of leptin, IGF-I, and ultimately OC may be adversely affected by compromised nutritional status among pubertal children with CF, thereby compromising their ability to deposit mineral into bone.

In this group of girls with CF, bone Ca deposition rates during pre- and late puberty were insufficient to allow girls to retain adequate amounts of bone Ca. Bone Ca deposition rates were related to dietary intake of Ca, serum leptin concentrations, and OC concentrations. Nutritional counseling of children with CF should therefore emphasize the importance of achieving optimal intakes of Ca to maximize bone Ca deposition in children with this disease. Optimizing body mass through the provision of adequate calories and other nutrients that support bone development is also essential. More research on the role of OC and leptin in promoting bone health during pubertal development in CF is required. Additional longitudinal studies in children across the pubertal growth spurt may assist in the identification of factors essential for maximal bone Ca deposition in this vulnerable

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pediatric group. It is particularly important to maximize bone Ca deposition and retention in children with CF during puberty, when the foundation for later bone health is established.

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