

# Efficiency of calcium absorption is not compromised in clinically stable prepubertal and pubertal girls with cystic fibrosis<sup>1-4</sup>

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## ABSTRACT

**Background:** Reduced bone mass is common in both children and adults with cystic fibrosis (CF) and may be a consequence of inadequate calcium absorption. The effect of CF on intestinal calcium absorption and retention has not been described in children.

**Objective:** Calcium absorption and urinary losses were characterized in clinically stable girls with CF consuming self-selected diets and following usual pancreatic enzyme regimens.

**Design:** The percentage of calcium absorption was assessed in 23 girls (aged 7–18 y) with CF by using oral (<sup>44</sup>Ca) and intravenous (<sup>42</sup>Ca) stable isotopes. Girls were grouped according to Tanner stage of breast development. True calcium absorption ( $V_a$ ) was determined as the product of percentage calcium absorption and average 4-d daily calcium intake. Calcium balance was estimated by subtracting urinary calcium and estimated endogenous fecal losses from the measure of  $V_a$ . Analysis of variance was used to compare outcomes among pubertal groups, and regression analysis was used to describe the relations of percentage and total calcium absorption to calcium intake and of urinary calcium to sodium excretion.

**Results:** Percentage calcium absorption was inversely related to calcium intake. Percentage absorption and  $V_a$  were similar to values observed in healthy girls in other studies. Total calcium absorption and estimated calcium balance were significantly greater among girls in early puberty (Tanner stages 2–3) than in prepubertal or late-pubertal girls ( $P < 0.05$ ). Urinary calcium was positively related to urinary sodium excretion ( $P = 0.02$ ).

**Conclusion:** The efficiency of calcium absorption was not compromised in clinically stable girls with CF. *Am J Clin Nutr* 2003;78:110–6.

**KEY WORDS** Cystic fibrosis, bone, calcium, stable isotopes, puberty, girls

## INTRODUCTION

Medical advances since the 1950s have lengthened the median survival age of persons with cystic fibrosis (CF) from childhood to >30 y of age (1). As the life expectancy of patients with CF increases, secondary sequelae of the disease have a greater effect on quality of life. Osteopenia is recognized as a problem in both adults (2–5) and children (2, 3, 6, 7) with CF, a topic that is reviewed elsewhere (8, 9). The etiology of osteopenia in patients with CF is likely multifactorial, and causal factors may include poor nutritional status, repeated episodes of infection and inflammation, glucocorticoid use, altered hormonal status, and a

reduction in weight-bearing activities, which are necessary to promote bone strength. A better understanding of the mechanisms responsible for the development of osteopenia in persons with CF will help physicians to prevent its consequences, including osteoporosis, kyphosis, and fracture (10).

Although CF has traditionally been considered a pediatric disease, most information on the effect of CF on bone mass has been acquired in adults with the disease. However, it is particularly important to characterize the factors associated with bone development in children with CF because the optimal acquisition of bone mass during the pubertal growth spurt can help to protect against losses later in life. About 40% of peak bone mass is achieved during puberty in girls (11), and peak rates of bone mineralization occur at 12.5 y of age in girls and at 14.0 y of age in boys (12).

Calcium is the major mineral of bone, comprising 32.2% of bone mineral (13), and >99% of the body's calcium is stored in bone (14). In recognition of the need for sufficient calcium as a substrate for optimal bone mineralization, the adequate intake recommendation for dietary calcium was recently increased to 32.5 mmol/d (1300 mg/d) for children aged 9–18 y (15).

Although adequate dietary calcium intake is recognized as an important component of promoting bone health, impaired calcium absorption may limit the availability of calcium for bone formation. In particular, the bioavailability of calcium may be low in CF as a result of malabsorption caused by pancreatic insufficiency. Calcium may form insoluble micelles with dietary fats (16), and compromised vitamin D status (3, 17) may limit calcium absorption in persons with CF. Despite speculation that calcium absorption is compromised in patients with CF, this issue has not been studied in children with the disease.

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To determine whether calcium absorption is impaired in children with CF, we measured percentage and true calcium absorption in prepubertal and pubertal girls with CF by using dual calcium stable isotopes. We estimated calcium retention, explored the predictors of calcium absorption, and studied the relation between calcium absorption and bone mineral density.

## SUBJECTS AND METHODS

### Protocol

A total of 23 girls with CF (aged 7–18 y) were recruited: 21 from the Johns Hopkins Cystic Fibrosis Center and 2 from other regional CF centers (in Harrisburg, PA, and Wilmington, DE). 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.

Subjects were clinically stable and generally compliant with their treatment regimen. They had not taken oral glucocorticoid preparations for  $\geq 1$  mo before the study. All prescribed medications were continued during the course of the study, including supplements containing vitamins A, D, E, and K (ADEK tablets, which contained 400 IU vitamin D per tablet) and pancreatic enzymes in 21 of the girls (excluding 2 pancreatic-sufficient, late-pubertal girls). For comparison purposes, pancreatic enzyme doses were standardized by assuming that the girls consumed 3 meals and 2 snacks per day, and were also expressed per kg body wt.

On the day of the study, the girls were admitted to the Johns Hopkins Pediatric Clinical Research Unit in the morning before they had consumed breakfast. Anthropometric measures were obtained and compared with current US reference data to obtain  $z$  scores (18). A fasting blood sample (15 mL) was obtained from a heparin lock that was inserted into the girls' forearms. With breakfast, the girls consumed  $\approx 60$  mL whole-fat milk containing  $^{44}\text{Ca}$  (0.35 mg/kg body wt), which had been added to the milk the night before the study. After the oral tracer administration, a 24-h urine collection was begun, with aliquots collected in 8-h time intervals. After breakfast,  $^{42}\text{Ca}$  (0.2 mg/kg) was administered through the heparin lock.

The girls self-selected their foods during the 24-h study period, and a weighed-food record for the 24-h period was obtained. On returning home, the subjects were asked to carefully record their dietary intake and supplement use for the subsequent 3 d to obtain a better estimate of usual dietary calcium intake. Diet records were assessed for nutrient intakes by using the Minnesota NUTRITION DATA SYSTEM (version 2.91; University of Minnesota, Minneapolis) and included intake from supplements if taken.

On the day of the isotope study, total-body and lumbar spine bone mineral content and body composition were obtained by dual-energy X-ray absorptiometry with a Hologic QDR-4500A scanner (Hologic Inc, Bedford, MA). Lumbar spine  $z$  scores were generated by using age- and sex-matched data from Hologic software (version 8.26a:3).

The Tanner stage of breast development was evaluated by a pediatric endocrinologist on the day of the study. Girls were characterized by pubertal group on the basis of categories previously used in a similar study of calcium absorption and retention in healthy girls (19), with Tanner stage 1 considered prepubertal, Tanner stages 2–3 considered early pubertal, and Tanner stages 4–5 considered late pubertal. The clinical severity of CF was scored as normal ( $\geq 90\%$  of predicted), mild

(70–90% of predicted), moderate (40–69% of predicted), or severe ( $< 40\%$  of predicted) on the basis of measurements of forced expiratory volume in 1 s.

### Isotope analysis and calculations

Calcium was extracted from urine by using an ammonium oxalate precipitation method and was loaded onto a rhenium filament (20). Isotopic ratios ( $^{44}\text{Ca}/^{48}\text{Ca}$  and  $^{42}\text{Ca}/^{48}\text{Ca}$ ) in each urine sample were measured by using a quadrupole thermal ionization mass spectrometer (Finnigan THQ, Bremen, Germany). Ratios were corrected for isotopic fractionation by normalizing the data to the  $^{43/48}\text{Ca}$  ratio. With the use of this instrumentation, relative SDs  $< 1.0\%$  are typically achieved for the isotope ratios (21). The enrichment of tracer in each sample was expressed as the delta percent excess, the degree to which the measured ratio was increased over the natural abundance ratio.

Percentage calcium absorption was calculated as the ratio of the cumulative oral tracer recovery to the cumulative intravenous tracer recovery in the 24-h urine collection obtained postdosing. The equations for this calculation were previously reported (22).

True calcium absorption ( $V_a$ ) was determined as the product of percentage calcium absorption and average calcium intake (measured as the mean of the 24-h weighed-food record and the 3-d home diet record). An estimate of calcium balance was obtained by subtracting calcium losses, which comprised 24-h urinary calcium excretion and assumed endogenous fecal calcium losses ( $V_{\text{endo}}$ ), from  $V_a$ .  $V_{\text{endo}}$  was assumed to be 0.04 mmol/d ( $1.6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ), as found in 25 children of similar ages consuming comparable calcium intakes of 29.5–37 mmol/d (1180–1480 mg/d) (23).

### Urine analysis

Total urinary calcium and sodium excretion were measured in each 24-h urine collection by using atomic absorption spectrophotometry (model 3300; Perkin Elmer, Norwalk, CT).

### Fecal fat analysis

Fecal fat was measured at the US Department of Agriculture Human Nutrition Research Center. Stool samples were homogenized in blenders with weighed quantities of deionized water. Blended samples were freeze-dried, and the fat analyses were performed in duplicate with a CEM Corp (Matthews, NC) FES 80 fat extractor by using methylene chloride as the extraction solvent. The variation in values for replicates was  $< 2\%$ . Data were expressed as the percentage of fat in dry fecal matter and wet weight in all samples averaged across the 6-d collection period for each subject.

### Hormone analyses

Parathyroid hormone was measured in baseline serum samples by using a commercial immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). All samples were completed in one run. Intraassay variation was within 5%. Serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D (from cholecalciferol and ergocalciferol combined) were measured by using commercially available radioimmunoassays (Diasorin, Inc, Stillwater, MN). The intraassay CV was 10% for both assays.

### Data analysis

Differences in baseline characteristics and outcome measures among pubertal groups were tested by using analysis of variance

**TABLE 1**  
Subject characteristics<sup>1</sup>

	Prepubertal girls (n = 7)	Early-pubertal girls (n = 5)	Late-pubertal girls (n = 11)
Age (y)	9.2 ± 1.4 <sup>a</sup>	11.9 ± 1.4 <sup>a</sup>	15.5 ± 2.0 <sup>b</sup>
Weight (kg)	26.9 ± 4.0 <sup>a</sup>	36.2 ± 6.7 <sup>a</sup>	51.9 ± 9.5 <sup>b</sup>
Weight-for-age z score	-0.67 ± 0.60	-0.75 ± 1.07	-0.26 ± 1.20
Height (cm)	129.7 ± 6.0 <sup>a</sup>	145.9 ± 9.6 <sup>b</sup>	160.2 ± 5.8 <sup>c</sup>
Height-for-age z score	-0.68 ± 0.62	-0.50 ± 1.0	-0.08 ± 0.86
BMI (kg/m <sup>2</sup> )	15.9 ± 1.2 <sup>a</sup>	17.0 ± 2.2 <sup>a</sup>	20.1 ± 2.5 <sup>b</sup>
BMI-for-age z score	-0.35 ± 0.58	-0.60 ± 1.10	-0.19 ± 1.16
Lumbar spine z score	-0.86 ± 0.58	-1.18 ± 0.93	0.00 ± 1.42

<sup>1</sup> $\bar{x} \pm \text{SD}$ . Values in a row with different superscript letters are significantly different,  $P < 0.05$  (ANOVA with Scheffé's multiple comparisons test).

with Scheffé's multiple comparison test or with a chi-square test for discrete variables. Percentage calcium absorption was skewed to high values, so a natural logarithmic transformation was used; data for percentage absorption are therefore presented as geometric means. Multiple linear regression was used to characterize the relations of percentage and total calcium absorption with calcium intake and pubertal state; pubertal state was coded as a dummy variable with the prepubertal group serving as the reference. Relations of additional clinical and biochemical variables with percentage calcium absorption were explored by adding them to this model. Simple linear regression was used to assess relations among clinical and hormonal variables, between urinary calcium excretion and sodium excretion, and between calcium intake and total calcium absorption, for which a square root transformation of calcium intake was used to obtain the best fit for the curvilinear relation.

We assumed that the same nonlinear relation between fractional absorption and calcium intake described by Heaney et al (24), which was based on >500 dual radioisotope studies of fractional calcium absorption in women, would be applicable to the relation of fractional calcium absorption to calcium intake in our data. That relation was described by the following equation:

$$\text{Fractional absorption} = 0.2195 \times [\text{Ca intake (g)}]^{-0.43915} \quad (1)$$

Therefore, as in that study, we transformed calcium intake (in g) to the -0.44 power and related this transformed variable to fractional absorption by using linear regression.  $\beta$  coefficients for the transformed calcium intake variable for the girls with CF and the women observed by Heaney et al (24) were expressed as a ratio to

determine the factor by which calcium absorption differed between the study groups. Relations of calcium balance parameters and other variables with lumbar spine z scores were explored by using regression analysis and Student's *t* test.

Our sample size was selected to enable us detect a 7% difference in percentage calcium absorption between girls with CF and values reported for healthy girls (19), with a power of 0.8 and a level of significance of 0.05, assuming an SD of 10%. All analyses were done by using STATA statistical software (version 7.0; StataCorp, College Station, TX), and differences were considered significant at  $P < 0.05$ .

## RESULTS

Characteristics of the subjects are presented in **Table 1**. All pre- and early-pubertal girls (Tanner stages 1–3) were premenarcheal, and all late-pubertal girls (Tanner 4–5) were postmenarcheal. Weight-for-age z scores and height-for-age z scores corresponded roughly to the 30th and 35th percentiles, respectively. According to data summarized by the Cystic Fibrosis Foundation from the year 1999, girls with CF in this age range average between the 20th and 30th percentiles nationwide for these measures, indicating that our sample had somewhat better-than-average nutritional status (25). Nine girls (39%) had osteopenia (lumbar spine z scores between -1 and -2), and 2 (9%) had osteoporosis (lumbar spine z scores < -2). Eight girls (35%) had a history of fracture. Lumbar spine z scores did not differ significantly between the girls with a history of fracture and those without ( $-0.83 \pm 1.19$  compared with  $-0.35 \pm 1.21$ ;  $P = 0.40$ ).

Sixteen girls (70%) were taking inhaled steroid preparations, although none had taken oral steroids within 1 mo of participating in the study. Four girls routinely received supplementary feeds through a gastrostomy tube, one had CF-related diabetes mellitus, and 2 were pancreatic sufficient. Most girls (91%) reported being at least moderately active. Girls took either 1 or 2 ADEK vitamin tablets daily, depending on their age, thereby ingesting either 400 or 800 IU vitamin D/d.

The parameters associated with calcium balance are detailed in **Table 2**. Calcium intake from dietary sources averaged  $29 \pm 11.1$  mmol/d ( $1161 \pm 444$  mg/d; range: 7.5–48.6 mmol/d, or 300–1943 mg/d) and did not differ significantly across pubertal groups. Nineteen of the 23 girls (83%) had average dietary calcium intakes >20 mmol/d (800 mg/d), which approximates typical intakes for this age group in the United States (15). Ten of the 23 girls (43%) had calcium intakes above

**TABLE 2**  
Calcium absorption and estimated balance in girls with cystic fibrosis<sup>1</sup>

	Prepubertal girls (n = 7)	Early-pubertal girls (n = 5)	Late-pubertal girls (n = 11)
Calcium intake (mmol/d)	28.03 ± 10.72 <sup>2</sup>	32.94 ± 8.59	27.86 ± 12.78
Calcium absorption (%)	26.7 (16.7, 42.9) <sup>3</sup>	39.9 (31.3, 50.9)	29.8 (18.6, 47.7)
Calcium absorbed (mmol/d)	6.89 ± 1.58 <sup>a</sup>	12.81 ± 0.89 <sup>b</sup>	8.21 ± 3.48 <sup>a</sup>
Urinary calcium (mmol/d)	2.38 ± 0.72	2.26 ± 2.07	3.16 ± 1.38
Estimated balance (mmol/d) <sup>4</sup>	3.43 ± 2.10 <sup>a</sup>	9.11 ± 2.14 <sup>b</sup>	2.97 ± 4.17 <sup>a</sup>

<sup>1</sup>Values in a row with different superscript letters are significantly different,  $P < 0.05$  (ANOVA with Scheffé's multiple comparisons test).

<sup>2</sup> $\bar{x} \pm \text{SD}$ .

<sup>3</sup>Geometric  $\bar{x}$  (-1 SD, +1 SD).

<sup>4</sup>Assumes endogenous fecal losses of  $1.6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ . Estimated balance = true calcium absorption - (urinary calcium excretion + endogenous fecal losses).

TABLE 3

Clinical characteristics and their relation to percentage calcium absorption in 23 girls with cystic fibrosis<sup>1</sup>

	$\bar{x} \pm \text{SD}$	Range	$\beta$ coefficient	<i>P</i>
Fecal fat				
(% of dry wt)	20.5 ± 12.1	2.6–48	0.0106	0.068
(% of wet wt)	5.6 ± 4.0	0.7–15.1	0.0348	0.044
Pancreatic enzyme use				
(1000 IU lipase/d) <sup>2</sup>	285 ± 94	130–520	–0.000106	0.908
(1000 IU lipase · kg <sup>-1</sup> · d <sup>-1</sup> ) <sup>3</sup>	7.5 ± 2.3	3.5–11.1	–0.00745	0.838
25-Hydroxyvitamin D (nmol/L)	78.4 ± 22.2	37.7–126.3	0.000568	0.901
1,25-Dihydroxyvitamin D (pmol/L)	97.8 ± 22.8	61.5–154.8	–0.000202	0.951
Parathyroid hormone (pmol/L)	2.74 ± 1.27	1.00–5.27	0.0657	0.298
Clinical severity <sup>2,3</sup>				
Mild	—	—	–0.0436	0.864
Moderate to severe	—	—	–0.129	0.525

<sup>1</sup>Relation of individual variables to ln[calcium absorption (%)] after adjustment for pubertal group and calcium intake (mmol/d).<sup>2</sup> $\beta$  coefficients represent comparisons with girls with normal scores for forced expiratory volume in 1 s.<sup>3</sup>*n* = 21.

the current recommendation of 32.5 mmol/d (1300 mg/d). One girl obtained 12.5 mmol Ca/d (500 mg/d) from oral supplements.

Geometric means of percentage calcium absorption did not differ significantly by pubertal group (Table 2). Because percentage calcium absorption was strongly inversely related to calcium intake ( $P = 0.001$ ), an analysis adjusted for calcium intake showed that percentage absorption was significantly higher in the early-pubertal girls ( $P = 0.013$ ) than in the prepubertal girls (regression statistics:  $F = 7.33$ ,  $R^2 = 0.54$ ,  $P = 0.002$ ).

Clinical characteristics and hormonal data and their relations to percentage calcium absorption, adjusted for pubertal state and calcium intake, are presented in Table 3. Fecal fat (%) was moderately positively associated with percentage calcium absorption, an association that was not substantially ameliorated when the analysis was controlled for total energy or fat content of the diet to account for the possibility that fecal fat was associated with better diet quality. Five girls (22%) had fecal fat outputs >7.6% of fecal wet weight, which is the upper limit of an established reference interval for stool fat content in healthy children (26), and the fat content of dry fecal matter was associated with total enzyme intake ( $P = 0.03$ ).

Clinical and hormonal variables did not differ significantly between pubertal groups, with the exception of total pancreatic enzyme intake (which increased across pubertal groups;  $F = 4.0$ ,  $P = 0.04$ ) and 25-hydroxyvitamin D status (which decreased across pubertal groups;  $F = 9.4$ ,  $P = 0.001$ ). Clinical severity scores were normal in 10 of 21 (48%) girls, mild in 3 of 21 (14%) girls, moderate in 7 of 21 (33%) girls, and severe in 1 of 21 (5%) girls. Postmenarcheal girls (60%) were more likely than premenarcheal girls (18%) to have moderate or severe pulmonary disease ( $P < 0.05$ ).

The regression of (calcium intake)<sup>-0.44</sup> on fractional absorption adequately described the inverse association of these variables (Figure 1). Fractional absorption among the girls with CF averaged  $\approx 1.6$  times that of the women described by Heaney et al (24), as determined by comparing the  $\beta$  coefficients of the calcium intake variable (0.3595/0.2195). When pubertal state was included in the regression model, fractional absorption was  $\approx 16\%$  higher in the early-pubertal girls than in the prepubertal reference group.

Total calcium absorption and the estimate for calcium balance were both significantly higher in girls in early puberty than in prepubertal and late-pubertal girls (Table 2). Total calcium

absorption increased as dietary calcium intake increased, although the incremental gains associated with improved calcium intake decreased at higher intakes (Figure 2). In multivariate analyses that were adjusted for calcium intake, early-pubertal girls absorbed an estimated 5.4 mmol/d more calcium than did the prepubertal reference group, consistent with the unadjusted means shown in Table 2.

Urinary calcium excretion did not vary by pubertal group (Table 2), and average 24-h urinary calcium excretion was 2.73 ± 1.40 mmol/d, or 0.0688 ± 0.0305 mmol · kg<sup>-1</sup> · d<sup>-1</sup>. Urinary calcium excretion was not related to calcium intake or to total absorption, but was positively related to urinary sodium excretion (Figure 3).

Lumbar spine *z* scores were not related to calcium balance estimates among all girls or within pubertal groups. Among 3 girls with 1,25-dihydroxyvitamin D concentrations > 108 pmol/L,

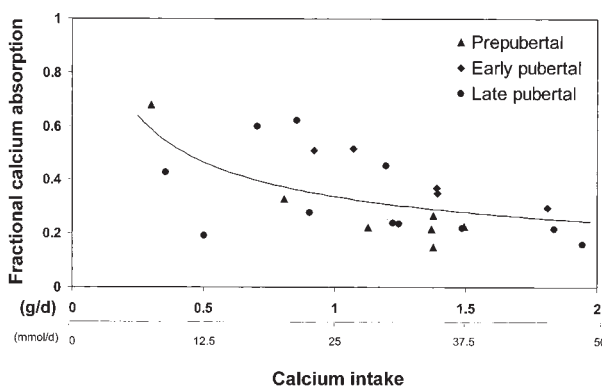
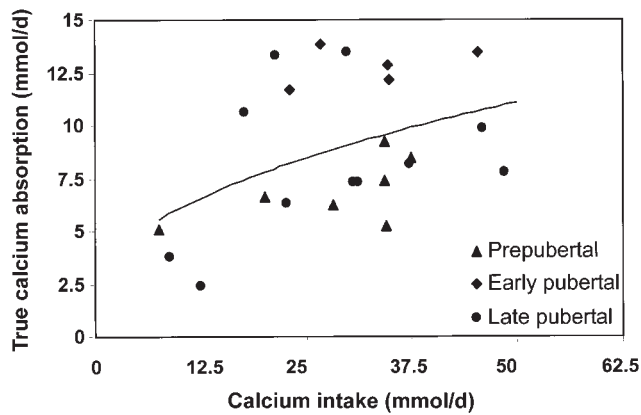


FIGURE 1. The relation of fractional calcium absorption to daily calcium intake in 23 girls with cystic fibrosis. Calcium intake (g/d) was raised to the  $-0.44$  power by using a transformation determined from >500 calcium absorption studies conducted in women (24). The inverse association of fractional absorption and calcium intake was described by the following equation: fractional absorption =  $0.3595 \times (\text{calcium intake})^{-0.44} - 0.021$  ( $F = 9.84$ ,  $R^2 = 0.32$ ,  $P = 0.005$ ). When pubertal groups were included in the regression model, fractional absorption was significantly higher in early-pubertal girls ( $P = 0.04$ ) than in prepubertal girls across the range of calcium intakes (multiple linear regression statistics:  $F = 5.58$ ,  $R^2 = 0.47$ ,  $P = 0.006$ ).



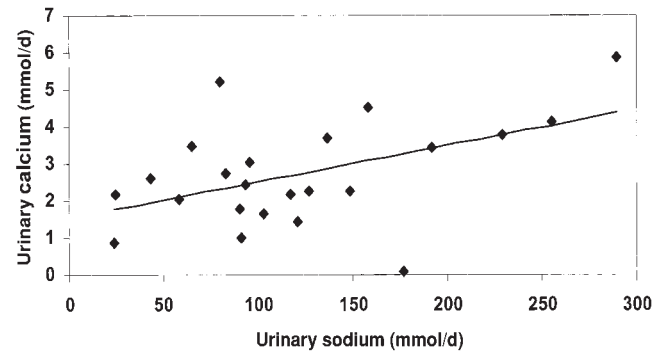
**FIGURE 2.** True calcium absorption ( $V_a$ ) in relation to daily calcium intake in 23 girls with cystic fibrosis:  $V_a = 1.29 \times (\text{calcium intake})^{0.50} + 2.02$  ( $F = 4.80$ ,  $R^2 = 0.19$ ,  $P = 0.04$ ). When pubertal groups were included in the regression model,  $V_a$  was significantly higher in early-pubertal girls ( $P = 0.001$ ) than in prepubertal girls across the range of calcium intakes (multiple linear regression statistics:  $F = 7.35$ ,  $R^2 = 0.54$ ,  $P = 0.002$ ).

possibly indicating chronic calcium insufficiency, lumbar spine  $z$  scores were lower than in the remainder of the group ( $-1.84 \pm 0.29$  compared with  $-0.32 \pm 1.15$ ;  $P = 0.0002$ ). However, 25-hydroxyvitamin D, parathyroid hormone, and dietary calcium intake and absorption in these 3 girls were not significantly different from values in the rest of the study group.

## DISCUSSION

Our findings suggest that calcium balance among clinically stable girls with CF was not limited by poor calcium absorption or excessive urinary calcium losses, although bone mineral density was reduced in this group of girls. The efficiency of calcium absorption in these girls with CF was similar to that of healthy girls assessed with the use of comparable methods at similar stages of puberty. Abrams and Stuff (19) reported mean percentage calcium absorption of 27.7%, 34.4%, and 25.0% among prepubertal, early-pubertal, and late-pubertal girls, respectively, consuming  $\approx 24$  mmol Ca/d (960 mg/d), or  $\approx 5$  mmol/d less than the girls in the present study. A more recent report found percentage calcium absorption to be 36.6% in early-pubertal girls consuming  $\approx 30$  mmol Ca/d (1200 mg/d) (27). In the study by Abrams and Stuff, average total calcium absorption was 6.15, 7.56, and 5.85 mmol/d, and estimated calcium retention was 3.28, 4.03, and 1.1 mmol/d across pubertal groups, respectively (19). Therefore, mean total calcium absorption and estimated calcium retention among girls with CF were comparable to or greater than the values observed in healthy girls studied under similar circumstances. These estimates of retention would be sufficient to support reported rates of peak bone calcium accretion (7.1 mmol/d) during early puberty (12).

Furthermore, the inverse relation between fractional absorption and dietary calcium intake was similar to that initially described by Heaney et al (24) in women. On average, the girls in the present study absorbed calcium  $\approx 1.6$  times more efficiently than did the women studied by Heaney et al. On the basis of a cross-over study of girls consuming 7.05 and 35.3 mmol Ca/d, O'Brien et al



**FIGURE 3.** A significant positive relation was found between 24-h urinary calcium excretion ( $V_u$ ) and urinary sodium excretion in 23 girls with cystic fibrosis:  $V_u = 0.0098 \times (\text{sodium}) + 1.53$  ( $F = 6.56$ ,  $R^2 = 0.24$ ,  $P = 0.02$ ).

(28) estimated that healthy adolescent girls absorb calcium  $\approx 1.45$  times more efficiently than do women.

Although the girls with CF in the present study were able to adapt to low calcium intakes by increasing absorption efficiency, higher intakes were associated with improved total calcium absorption. Although our subjects ingested more calcium than typical adolescents do, more than one-half did not meet current recommendations (15). With an upper tolerable intake of 62.5 mmol/d (2500 mg/d) (15), calcium intakes could be safely increased in this group, ideally from dietary rather than supplemental sources. High intakes of supplemental calcium have been linked to renal stones (29), which may occur in excess in persons with CF (30, 31). Additionally, the bioavailability of calcium from supplementary feeds in persons with CF has not been examined and may not be similar to that of calcium in orally ingested foods.

Urinary calcium losses were not excessive in these girls with CF and did not appreciably compromise calcium balance. Urinary calcium excretion was similar in the present study to values reported in healthy girls consuming  $\approx 22$  mmol/d (870 mg/d) and was similarly unrelated to dietary calcium intake (32). Urinary calcium losses were related to sodium excretion, as observed among healthy girls across a similar range of sodium outputs (32). Because persons with CF are encouraged to ingest liberal amounts of salt to prevent hyponatremia (33), more research is required to identify optimal salt intake to prevent excessive urinary calcium losses.


Although calcium malabsorption has long been assumed to be a cause of compromised calcium balance, contributing to reduced bone mass in persons with CF, few studies have directly examined this issue. An inverse relation between fractional calcium absorption and fecal fat excretion was found in patients with steatorrhea (16). Hahn et al (3) speculated that calcium malabsorption occurred in adolescents and young adults with CF with poor carotene status and low urinary calcium excretion, although calcium absorption itself was not measured. An early abstract reported no difference in the absorption of calcium (determined by forearm radioactivity after oral and intravenous administration of a calcium radiotracer) in 5 patients with CF (34). More recently, Aris et al (35) studied calcium absorption in adults with CF and in control subjects and found that adults with CF had a 3% reduction in oral calcium radiotracer in serum 5 h after a test meal when pancreatic enzymes were not taken. No significant differences were reported between the control subjects and the CF patients when pancreatic enzymes were taken.

Previous studies that measured calcium absorption in patients with CF used a single calcium radioisotopic tracer (16), a technique that does not control for potential differences in the miscible calcium pool among individuals (2, 35). The size of this pool varies markedly with pubertal status (36) and may also be altered in disease states (37). Furthermore, radiotracers cannot safely and ethically be used in pediatric groups, in whom information on calcium acquisition and bone development is especially important.

Our findings challenge long-held assumptions about the effect of CF on calcium absorption; thus, the potential mechanisms that may act to preserve normal calcium absorption in this disease warrant consideration. Passive calcium absorption is responsible for most calcium absorption when calcium intake is relatively high (>20 mmol/d, or >800 mg/d), and this process is less affected by vitamin D status than is the active absorption that predominates at lower calcium intakes (38). Thus, the lack of a relation between 25-hydroxyvitamin D status and calcium absorption in this study was not entirely surprising. We do not conclude, however, that vitamin D status in this group of girls was necessarily adequate. Although appropriate cutoffs for vitamin D sufficiency are not well defined (39), higher concentrations of 25-hydroxyvitamin D would be expected in girls ingesting as much as 800 IU supplementary vitamin D/d. Furthermore, because elevated 1,25-dihydroxyvitamin D was found to be related to reduced bone mass in 13% of our sample, the parathyroid hormone-vitamin D axis may be an important factor in the development of osteopenia and osteoporosis among some persons with CF, despite apparently adequate calcium intake and absorption.

A lower pH in the gut of persons with CF than in healthy persons (40) may enhance the bioavailability of calcium by improving its solubility and perhaps by increasing intestinal permeability. Paracellular nutrient transport is increased in persons with CF (41), an effect that is reduced when luminal acidity is controlled with proton pump inhibitors (42). Increased permeability of the gastrointestinal tract could facilitate passive calcium absorption, which occurs through a paracellular mechanism. An intriguing positive association between fecal fat (%) and percentage absorption in our study suggests a relation between the degree of intestinal pathology and percentage calcium absorption and is also counter to the relation expected between these variables if calcium was being bound by fat and precipitated in the gut.

If intestinal permeability enhances calcium absorption by allowing increased paracellular transport, endogenous losses of calcium into the gastrointestinal tract might also be increased by this mechanism, thereby ultimately compromising calcium balance. Normal calcium absorption associated with elevated endogenous fecal losses of calcium was shown in dual radioisotope tracer studies of adults with untreated celiac disease (43) and protein-losing enteropathy associated with intestinal lymphangiectasia (44). Although we did not measure intestinal permeability in our subjects, we will be able to assess endogenous fecal losses of calcium in a subset of our study subjects.

The results of the present study show that calcium absorption was not compromised in clinically stable girls with CF who were receiving usual pancreatic enzyme therapy and was greatest in early puberty, illustrating the importance of the adolescent years for optimizing dietary calcium intake to promote calcium retention. Further study is required to determine to what degree calcium absorption and balance are influenced by specific gut abnormalities in subjects with CF. These issues are particularly important to address in children with CF, who are at increased risk of osteoporosis and fracture later in life. 

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