Bone status in cystic fibrosis

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Abstract

Aim—To investigate bone mineral status of children with cystic fibrosis (CF).

Methods—In 29 children with CF and 49 matched controls, bone mineral content (BMC), projected bone area (BA), and areal bone mineral density (BMD) of the whole body, total hip, and lumbar spine (L1–L4) were measured using dual energy α ray absorptiometry. The BMC values at each site were adjusted for BA, height, and weight. At the lumbar spine, the bone mineral apparent density (BMAD) was calculated by dividing the BMC by the estimated volume, derived from BA. Vertebral (T12–L3) trabecular bone mineral density (vTBMD) was measured using quantitative computed tomography in children with CF. Calcaneal broadband ultrasound attenuation (BUA) was measured in CF patients and controls using quantitative ultrasound. The disease severity of CF children was evaluated by the Shwachman–Kulczycki (SK) score.

Results—The mean BUA, whole body and regional BA, adjusted BMC, and areal BMD of children with CF were not different from those of controls. The mean BMAD of the lumbar spine was reduced in CF patients compared with controls, whereas the mean vTBMD standard deviation scores were significantly higher in CF patients. The median SK score of the CF group was 81 (range 42–100), indicating that as a group our CF patient population had relatively mild disease.

Conclusion—The normal vertebral BMC, decreased BMAD, and higher vTBMD suggests that the vertebral cortical thickness or density might be reduced in CF subjects. The overall bone mineral status of CF children with relatively mild disease was not different from size matched controls.

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and puberty,⁷ and excessive bone loss.⁸ Epidemiological studies in adults have shown that bone mineral content (BMC) and density (BMD) are important predictors of an individual's fracture risk.⁹ Reduced BMC and BMD have been reported both in children and adults with CF. Linear BMD of the forearm bones measured using single photon absorptiometry was found to be decreased in adolescents and adults.^{10–11} However, this technique did not permit assessment of BMC or BMD at the spine or the total hip. Recent studies using dual energy α ray absorptiometry (DXA) to assess bone mass and areal BMD (g/cm²) in the spine and proximal femur in children have yielded conflicting results. Henderson and Madsen¹² and Bhudhikanok and colleagues^{7–13} found significant reduction in BMD at the lumbar spine and proximal femur in CF patients. However, using gender, age, and nutritional status matched controls, Salamoni and colleagues¹⁴ reported normal whole body and lumbar spine BMD in children with CF, whereas Laursen and colleagues⁶ found shorter and narrower bones, but increased size corrected BMC, in children and teenagers with CF. Bone mineral density (g/cm³) of the trabecular component of the vertebral bodies measured by quantitative computed tomography (QCT) has been reported to be decreased in children and adults with CF.^{15–17}

None of these techniques measure the bone structure, which is an important determinant of bone strength.¹⁸ Broadband ultrasound attenuation (BUA) at the calcaneum measured by quantitative ultrasound (QUS) has been shown to predict fracture risk in elderly women independently of bone mineral density.¹⁹ It has been suggested that ultrasonic attenuation through bone reflects both density and structural properties.¹⁸ In this cross sectional study, we measured the calcaneal BUA and BMC in grams, projected bone area (BA) in cm², and areal bone mineral density (BMD) in g/cm² of the whole body, total hip, and lumbar spine (L1–L4) in children with CF and intentionally age, gender, pubertal stage, height, and weight matched controls. In CF children, we selectively measured the vertebral (T12–L3) trabecular bone mineral density (vTBMD) using QCT. We also measured the nutritional parameters relevant to bone mineralisation and estimated weight bearing physical activity. We hypothesised that as a group children with CF would have reduced:

- vTBMD standard deviation scores
- calcaneal BUA, and whole body, total hip, and lumbar spine BMC and BMD compared to intentionally age, gender, pubertal stage, height, and weight matched controls.

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Improvements in nutritional and pulmonary management of cystic fibrosis (CF) have resulted in improved survival,¹ with many patients surviving into adulthood.² A traumatic fractures of the axial and appendicular skeleton are becoming an important health problem in adolescents and adults with CF.^{3–5} Thoracic kyphosis resulting from wedge fractures of vertebral bodies has also been reported in this age group.^{3–4} Factors responsible for bone fragility in CF subjects include small bone size,⁶ inadequate gain in bone mass during growth

Subjects and methods

Eighty five children between 5 and 16 years of age attending the regional paediatric CF clinic were invited to participate in the study. Twenty nine children (12 girls and 17 boys) agreed to participate in the study. Children with spinal deformities, immobilising disease, or major surgery in the previous 12 months were excluded. The diagnosis of CF had been confirmed by sweat test and by CF gene mutation analysis. Nineteen patients were homozygous for delta F508 mutation and eight had one delta F508 and one other known CF mutation; gene status in two patients was unknown. All patients were on unrestricted diet and taking Ketovite multivitamin liquid and tablets (Paines & Byrne, West Byfleet, Surrey, UK) and pancreatic enzyme supplements. Fifteen children were receiving oral calorie supplements: Polycal (Nutricia Clinical Care, Trowbridge, Wiltshire, UK) in five; Fresubin (Fresenius Ltd, Warrington, UK) in three; Forti juice (Nutricia Clinical Care, Trowbridge, Wiltshire, UK) in two; and Scandishake (Scientific Hospital Supplies, Liverpool, UK) in five.

Parents of 385 local school children who agreed to allow their children to participate in this study were asked to complete a questionnaire with details of previous medical history. Children with a history of medical problems known to effect bone mineralisation, and those who had been treated with oral or inhaled corticosteroids were excluded. For each CF patient we aimed to recruit two control subjects matched for age, gender, pubertal stage, height, and weight. Matching criteria for age was ± 12 months, height ± 10 cm, and weight ± 5 kg. However, one of the controls was 6.1 kg heavier than the CF subject. Twenty one CF patients had two control subjects each, and in eight patients only one matched control could be recruited. Written consent was obtained from the parents or guardians of all the children. The study was approved by the Salford Research Ethics Committee.

Weight was measured to the nearest 0.25 kg using a digital electronic scale. Height was measured to the nearest millimetre using a portable stadiometer (Karrimetre Mark III, Raven Equipment Ltd). Body mass index ($\text{BMI} = \text{weight}/\text{height}^2$) was calculated from height and weight. Weight, height, and BMI standard deviation (SD) score were calculated using the 1990 UK normative data supplied by the Child Growth Foundation.²⁰ Children with CF underwent full pubertal assessment.²¹ In controls puberty was assessed by direct assessment²¹ or by using the previously validated self assessment method.²²

Disease severity was evaluated by the Shwachman-Kulczycki (SK) score.²³ Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were measured in the CF subjects and expressed as percentage of expected for height.²⁴ Three patients had received oral corticosteroid therapy for less than three weeks and 18 had been treated with inhaled corticosteroids.

Three day food diaries were completed and returned by 21 children with CF. Daily calcium intake was estimated using McCance and Widdowson's composition of food tables²⁵ and Microdiet software (Salford University System, Salford, UK). A previously validated questionnaire²⁶ was used to estimate the number of hours of weight bearing physical activity per week in CF children and controls.

BMC in grams and the projected postero-anterior bone area (BA) in cm^2 were measured using the Hologic QDR 4500 Acclaim fan beam DXA scanner (Hologic Inc., Bedford, Massachusetts, USA), using the V8.24a:3 software for the whole body, the total hip, and the lumbar spine (L1–L4). The areal BMD in g/cm^2 (BMD) was calculated by dividing BMC in grams by the projected BA in cm^2 . The bone mineral apparent density (BMAD) of the lumbar spine in g/cm^3 was calculated by dividing the total vertebral BMC in grams by the estimated volume in cm^3 , derived from $\text{BA}^{1.5}$, as described by Carter and colleagues.²⁷ The whole body scans were performed with subjects lying supine and wearing T-shirt and shorts. The in vivo coefficient of variation (CV) for whole body BMC for adults is 0.92%; and for BMD of lumbar spine 1.09% and for total hip 1.26%. The total effective ionising radiation dose for all the DXA scans ranged between 5.6 and 10.8 μSv , depending on the size of the subject, and whether the ovaries were included in the scans.

The vTBMD in mg/cm^3 was measured through the mid plane of T12–L3 vertebral bodies by QCT using a Philips Tomoscan SR 4000 scanner (Philips Medical Imaging, Best, Holland), by single energy, low dose scanning technique (120 Kv, 50 mA, 2 second scan time). The mean vTBMD in mg/cm^3 was expressed as mineral equivalents of K_2HPO_4 in water. The effective ionising radiation dose for this measurement is 100 μSv and the precision in our unit for healthy adults is 1.3%. The vTBMD values were transformed into SD score using the published values in children from the USA.²⁸

The BUA, which reflects the frequency dependence of ultrasound attenuation in the frequency range 200–600 kHz, was measured in triplicate at the left calcaneum using the Paediatric Contact Ultrasound Bone Analyser (McCue Ultrasonic Limited, Winchester, UK) system as described previously.²⁹ BUA values of CF patients were converted to SD scores using published normative values for UK children.³⁰

Non-fasting blood samples were obtained only from CF subjects, between 9 and 11 am, for analysis of serum calcium, phosphate, alkaline phosphate, alanine transaminase, and albumin using routine biochemical methods. Serum parathyroid hormone (PTH) concentration was measured using a two site chemiluminance method (Chiron Diagnostic Ltd, Halstead, UK). The normal range in adults for this assay is 11–54 pg/ml, the minimal detectable concentration 1.4 pg/ml, with inter- and intra-assay coefficient of variation of less than 10%.

Table 1 Age, anthropometry, and clinical characteristics of children with cystic fibrosis and controls

	Patient (n = 29)	Controls (n = 21 pairs and 8 singletons)
Age (y)	10.08 (3.22)	10.00 (3.19)
Height (cm)	136.78 (18.11)	136.81 (18.47)
Height SDS	-0.55 (0.93)	-0.47 (0.91)
Weight	30.10 (14.46)	33.12 (12.39)
Weight SDS	-0.478 (1.08)	-0.386 (0.84)
BMI	15.20 (4.15)	16.99 (2.02)
BMI SDS	-0.185 (1.08)	-0.142 (0.714)

The data are expressed as mean (± 1 SD).

Serum 25-hydroxyvitamin D (25-OHD) concentration was estimated using the ^{125}I radioimmunoassay kit (Diasorin Corp., Minnesota, USA). The minimum detectable concentration with this method is 5 nmol/l, inter- and intra-assay coefficient of variation less than 10%, and adult reference range 22–117 nmol/l. Serum 1,25-dihydroxyvitamin D (1,25(OH) $_2$ D) concentration was estimated using the IDS gamma-B 1,25D kit (Diasorin Corp., Minnesota, USA); the inter- and intra-assay coefficient of variation for this technique is less than 10%,³¹ and adult reference range 54–140 pmol/l.

STATISTICAL ANALYSIS

The statistical analyses were performed using the SPSS software, version 6.1 for Windows (SPSS Inc., Chicago, Illinois, USA). The symmetrically distributed data are expressed as mean (± 1 SD) where appropriate. Those variables that showed evidence of being asymmetric are presented as median (range). Where there were two controls, average value of the variable was calculated and paired Student's *t* test used to compare the values of the variables in the two groups. Variables which were notably asymmetric were compared using the Wilcoxon test for two related samples. A single

group Student's *t* test was used to determine whether vTBMD SD score data of the CF patients was significantly different from zero. Calcaneal BUA and BMC values at the lumbar spine, whole body, and total hip were compared with those of the control group, after correcting for differences in age, weight, and height by means of covariant analysis. Linear regression analysis was used to study the relation between measures of BMC, BMD, vTBMD BUA, and indices of disease severity.

Results

Table 1 summarises the clinical characteristics of patients with cystic fibrosis and controls. Among the CF subjects, 11 boys and five girls were prepubertal, one boy and two girls were in Tanner stage 2, two boys and three girls in stage 3, and two boys and one girl in stage 4 of pubertal development. The median SK score of the CF group was 81 (range 42–100) indicating that, as a group, our CF patient population had relatively mild disease. The mean (SD) FEV $_1$ and FVC expressed as percentage predicted for height were 77.00% (20.27) and 88.93% (19.66) respectively.²⁴

The dietary calcium intake for all but four CF patients was above the recommended nutrient intake for age and gender (data not shown). Exercise questionnaires were returned by 24 children with CF and 34 controls. The median (range) weekly duration of weight bearing physical activity in CF subjects was 8.7 hours (1.5–38.5 hours) compared to 4.75 hours (0–28 hours) in controls (*p* = 0.07).

Serum calcium concentration, corrected for albumin, was below the lower end of the normal range of 2.25 mmol/l in four patients. Serum phosphate, alkaline phosphatase, alanine transferase, and albumin concentrations were within the normal range for age in all patients. The median serum concentration of 25-OHD (marker of individual's vitamin D status) was 37.0 nmol/l (18.0–68.0); only one patient had serum 25-OHD concentration below the normal range (22–117 nmol/l). The median serum 1,25(OH) $_2$ D concentration (the biologically active form of vitamin D) for the CF patients was 94.5 pmol/l (54.0–140.0); the concentrations were within the normal adult range (48–110 pmol/l) for all patients. Serum PTH concentration was within the normal range in all patients. Table 2 shows mean calcaneal BUA, vTBMD, and DXA measured whole body and regional bone mineral content, size, and density data. The BA, areal BMD, and adjusted BMC of whole body, lumbar spine, and total hip were not different between the two groups. The CF patients had similar vertebral BMC, non-significantly larger bone volume, and lower BMAD, compared to size matched controls. In contrast, the mean vTBMD SD score was significantly higher in the CF patients compared to the North American normative group.²⁹ The calcaneal BUA adjusted for height and weight and calcaneal BUA SD were not different between the CF patients and matched controls (table 2).

As shown in table 3, age, weight, height, and BMI correlated with BMC, areal BMD, BUA,

Table 2 Mean (± 1 SD) values for DXA measured whole body, total hip, and lumbar spine BMC, BA, BMD, BMC adjusted for bone area, height, and weight; lumbar spine BMAD; calcaneal BUA, BUA adjusted for height and weight, and BUA SD score. Mean values for vTBMD and vTBMD SD scores for children with CF are also shown

	CF patients	Controls	95% CI of the difference
Lumbar spine	n = 29	n = 21 pairs and 8 singletons	
BA (cm ^{2})	40.67 (10.27)	38.07 (11.22)	-0.499 to 5.651
BMC (g)	28.37 (12.26)	28.39 (13.04)	-2.430 to 2.389
Adjusted BMC (SE)	27.05 (1.01)	28.82 (0.76)	-4.33 to 0.791
BMD (g/cm ^{3})	0.68 (0.14)	0.71 (0.12)	-0.071 to 0.002
Bone volume (cm ^{3})	265.15 (98.21)	242.70 (109.67)	-6.667 to 51.575
BMAD (g/cm ^{3})	0.11 (0.02)	0.12 (0.01)*	-0.018 to -0.003
vTBMD (mg/cm ^{3})	179.42 (20.75)	ND	
vTBMD SD score	0.30 (0.51)*	ND	
Whole body	n = 28	n = 20 pairs and 8 singletons	
BMC (kg)	1.25 (0.52)	1.31 (0.51)	-0.137 to 0.018
Adjusted BMC (SE)	1.26 (0.03)	1.30 (0.18)	-0.10 to -0.002
BA (cm ^{2})	1559.11 (403.87)	1772.84 (858.27)	-516.32 to 88.85
BMD (g/cm ^{3})	0.78 (0.13)	0.79 (0.10)	-0.047 to 0.020
Total hip	n = 29	n = 21 pairs and 8 singletons	
BMC (g)	16.69 (8.24)	18.91 (9.09)*	-3.849 to 0.604
Adjusted BMC (SE)	18.16 (0.45)	18.17 (0.33)	-1.15 to 1.13
BA (cm ^{2})	24.14 (8.01)	24.34 (8.10)	-0.870 to 0.476
BMD (g/cm ^{3})	0.72 (0.13)	0.74 (0.12)	-0.058 to 0.006
Calcaneal	n = 29	n = 18 pairs and 11 singletons	
BUA (dB/MHz)	57.69 (25.74)	54.18 (23.30)	-5.696 to 12.724
BUA SD score	-0.002 (1.63)	-0.02 (1.27)	-0.649 to 0.677
Adjusted BUA	57.87 (3.22)	58.08 (2.48)	-8.32 to 7.89

*p < 0.01.

Table 3 Correlation coefficients for DXA measured whole body, total hip, and lumbar spine BMC, BMD, and calculated BMAD; QUS measured calcaneal BUA; and QCT measured vTBMD with age, nutritional status (height, weight, and BMI), disease severity (SK score), and lung function (FVC and FEV₁) parameters

	Whole body BMC	Whole body BMD	Total hip BMC	Total hip BMD	L1-L4 BMC	L1-L4 BMD	vTBMD	BUA
Age	0.89**	0.80**	0.82**	0.77**	0.87**	0.81*	0.47*	0.47*
Height	0.94**	0.83**	0.82**	0.77**	0.89**	0.82**	0.39*	0.61**
Weight	0.94**	0.81**	0.90**	0.84**	0.86**	0.90**	0.44*	0.60**
BMI	0.70**	0.53**	0.69**	0.61**	0.66**	0.65**	0.24	0.42*
SK score	-0.24	-0.28	-0.14	-0.18	-0.27	-0.16	-0.50**	0.00
FEV ₁	-0.19	-0.27	-0.18	-0.25	-0.25	-0.14	-0.31	-0.02
FVC	-0.08	-0.10	-0.05	-0.10	-0.15	-0.03	-0.18	-0.01

*p < 0.05, **p < 0.01.

and vTBMD. The significant inverse relation between SK scores and vTBMD was unexpected.

Discussion

To the best of our knowledge, this is the first study in which DXA, QCT, and QUS have measured the bone status of CF children. We believe that such an approach permits a more comprehensive evaluation of factors that determine bone strength, namely the bone size (envelope), bone mass contained within the envelope, volumetric trabecular bone density, and bone structure, than if only one of these techniques was used. The majority of studies of bone mass and density measurements in CF children have been performed using DXA, which provides a measure of areal BMD that represents the integral (cortical and trabecular) amount of bone mineral within the bone envelope of the region of skeleton scanned divided by its projected bone area. As the depth, and therefore the volume of the scanned bone is not measured, areal BMD fails to distinguish between changes in mineral density and bone size in growing children. This is an important consideration, as chronic childhood illness, such as CF, might impair both growth and mineralisation of the skeleton. In order to minimise the influence of bone size on DXA measured BMC and BMD, we recruited intentionally age, gender, pubertal stage, height, and weight matched controls for each CF subject¹⁴ from the local population. As it is not possible to recruit perfect size matched controls, we further adjusted the BMC at each site by taking into account the influence of bone area, height, and weight.³² The size adjusted whole body, lumbar spine, and total total hip BMC were not different in the CF and control groups.

At the lumbar spine, we also calculated the BMAD, by dividing the vertebral BMC by estimated volume (BA^{1.5}). The BMAD represents the estimated cortical and trabecular volumetric (g/cm³) bone density within the estimated envelope of the vertebral body. In the CF group, the mean estimated lumbar spine bone volume was non-significantly higher (9.1%), whereas the BMAD was significantly lower (8.3 %) than in the control group. This suggests that in CF subjects there is an expansion of vertebral bone envelope that is not fully mineralised. However, the vTBMD, which was selectively estimated using QCT, was significantly higher in CF patients than in North American children.²⁸ As about half of the total human vertebral body bone mineral content

consists of cortical bone,³³ our finding of normal total vertebral BMC, decreased BMAD, and higher vTBMD suggest that vertebral cortical thickness or density might be reduced in CF subjects. The mean calcaneal BUA, which reflects both density and structural properties of the bone, was not different in the two groups.

Taken together, our results suggest that the overall bone mineral content, density, and possibly bone structure of our CF patients was essentially normal, although the cortical thickness or density of the vertebrae might be decreased. Thus, contrary to our hypothesis, the main finding of our study was that bone mineralisation of our CF patients was essentially normal. These findings are similar to those of Salamoni and colleagues,¹⁴ who found that BMC was normal in well nourished children with CF, compared with age, gender, and BMI matched controls. As the mean height SD scores of CF patients and controls were both about -0.5 (table 1), their bones are likely to be smaller relative to those of unselected population controls. Thus, it is likely that our CF patients have smaller, but normally mineralised, bones. This thesis is in keeping with the findings of Laursen and colleagues,⁶ who reported that the whole body BMC, adjusted for size using the method of Mølgaard and colleagues,³⁴ was significantly increased in patients with CF under the age of 19 years, compared with controls. However, these subjects had significantly smaller and narrower bones, leading the authors to conclude that reduced bone size was an important factor for decreased bone mass in younger patients with CF.⁶

The recommended nutrient intake for dietary calcium was above the normal value for age in all but four CF subjects. This, together with normal serum concentration of PTH, 25-OHD, and 1,25(OH)₂D, suggests adequate calcium intake and absorption in the CF subjects. Inadequate intake or malabsorption of calcium and vitamin D deficiency would have resulted in secondary hyperparathyroidism. Our patients were more active compared to controls, although the median activity between the two groups was not significantly different. The lung function and median SK score of 81 (the higher the score the milder the disease, and vice versa) indicate that as a group, the CF patients in our study had relatively mild disease. Only three CF patients had been treated with a short course (less than four weeks) of oral corticosteroids. Thus, we believe that adequate intake of calcium, and normal

vitamin D status in our physically active and less severely affected cohort of young CF children explains their normal overall bone mineral status. We are unable however to explain the negative relation between SK score and vTBMD SD score.

In summary, results of our study suggest that bone mineralisation, and possibly structure, were normal in our CF children. However, our results and conclusions only apply to relatively healthy and well nourished CF children. Our results also suggest that the maintenance of overall good health and nutritional status of CF subjects might allow normal bone mass acquisition and development of normal bone size and shape during childhood and adolescence. As fractures are an important secondary healthy problem in adolescents and adults with CF, longitudinal measurements of bone size, shape, mass, and structural parameters during childhood, puberty, and advancing age are needed to understand the pathogenesis of bone fragility in subjects with this disease.

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