0021-7557/08/84-01/18
Jornal de Pediatria

Prevalence of bone mineral disease among adolescents with cystic fibrosis

Reinaldo José do Amaral Caldeira,[1] Vânia de Matos Fonseca,[2] Saint Clair dos Santos Gomes Junior,[3] Célia Regina Moutinho de Miranda Chaves[4]

Abstract

Objective: To evaluate the prevalence of bone mineral disease among adolescents with cystic fibrosis and to relate the findings with the variables studied.

Methods: The study enrolled 37 adolescents who were assessed for: nutritional status according to height/age and body mass/age ratios; bone mineral density of the lumbar spine and entire body by densitometry with dual emission X-ray; daily dietary intake according to a 3-day dietary recall; and pulmonary function by the forced expiratory volume in one second test.

Results: Mean age was 13.2 (±2.8) years. Nutritional status was adequate in 70.3 and 75.7% of patients according to the height/age and body mass/age indices, respectively; 54.1% of the patients exhibited reduced lumbar spine bone mineral density and 32.5% for the whole body. There was a positive correlation between bone mineral density and body mass index (p = 0.04). Lung disease and pancreatic insufficiency exhibited a correlation with altered bone mineral density. The dietary recall revealed adequate percentages of calcium, phosphorous and calories, according to the nutritional recommendations laid out in the European Cystic Fibrosis Consensus. The multivariate analysis indicated that these variables were not statistically significant.

Conclusions: There is a high prevalence of bone mineral disease among adolescents. Good nutritional status, pancreatic enzyme replacement and control of lung disease may have a protective effect on bone mass.

J Pediatr (Rio J). 2008;84(1):18-25: Bone mineral density, cystic fibrosis, adolescents.

Introduction

The creation of specialist cystic fibrosis (CF) centers of excellence and the advances achieved by the scientific community in molecule biology in genetics, ion transport, immunology, respiratory physiotherapy, nutritional supplementation, enzyme replacement, antibiotic therapy and lung transplantation have been enabling the life expectancy of these patients to increase for more than 30 years.[1,2] However, as a result of this increased opportunity, a new complication has emerged; bone mineral disease (BMD) was first described in 1979 by Mischler et al.[3]

Since the symptomology of brittle bones is uncommon at the end of childhood and adolescence, and due to the slow and progressive course of this disease, bone fractures may be the first manifestation of CF.[4]

While the pathogenesis of CF-linked BMD has not yet been completely elucidated, it is believed that there are multiple

1. Mestre. Instituto Fernandes Figueira, Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, RJ, Brazil.
2. Doutora. Instituto Fernandes Figueira, Fiocruz, Rio de Janeiro, RJ, Brazil. Instituto de Saúde da Comunidade, Universidade Federal Fluminense (UFF), Rio de Janeiro, RJ, Brazil.
3. Doutor. Instituto Fernandes Figueira, Fiocruz, Rio de Janeiro, RJ, Brazil.
4. Doutora. Instituto Fernandes Figueira, Fiocruz, Rio de Janeiro, RJ, Brazil.

This study was carried out as part of the Masters Program at Instituto Fernandes Figueira, Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, RJ, Brazil.

No conflicts of interest declared concerning the publication of this article.

Suggested citation: Caldeira RJ, Fonseca VM, Gomes Jr SC, Chaves CR. Prevalence of bone mineral disease among adolescents with cystic fibrosis. J Pediatr (Rio J). 2008;84(1):18-25.

Manuscript received May 08 2007, accepted for publication August 01 2007.

doi:10.2223/JPED.1737

factors involved:[5] malabsorption of vitamins D and K, observed in 40% of CF cases;[6] low intake and absorption of calcium and malnutrition, due to pancreatic insufficiency, present in 85-90% of CF patients;[7] delayed puberty/hypogonadism, found in 73 to 88% of CF cases;[7] the pulmonary inflammatory response, the main cause of 80% of deaths due to CF;[8] reduced physical activity and inactivity, being the primary consequence of the chronic suppurative lung disease; and the oral and inhaled corticoid therapy used in 82 and 31% of CF cases, respectively.[9]

The majority of adult patients seen at specialist CF centers exhibit significant bone mineralization deficiencies and a high risk of fractures in response to minimal traumas of vertebrae or ribs.[7,10]

Although studies with adolescent CF patients have shown that they also exhibit osteoporosis or osteopenia, treating BMD with medication can cause undesirable effects, such as gastroesophageal reflux and esophagitis, in addition to reduced absorption in the presence of food.[11,12] Prevention is therefore the most effective approach for maintaining healthy bones and improving the quality of life of these patients, and is achieved through nutritional and respiratory control, physical activity and exposure to sunshine.[7]

This study therefore attempts to investigate the prevalence of BMD among adolescents with CF, treated at the Instituto Fernandes Figueira (IFF), which is part of the Fundação Oswaldo Cruz and a Ministry of Health center of excellence, with the objective of contributing to its prevention.

Methods

Study

For this study we performed a survey of all patients aged 10 to 18 years with a diagnosis of CF who were seen at the IFF between May and June of 2006. Inclusion criteria were: have undergone bone densitometry (BD), not having a primary BMD and not having received a lung transplantation. Data were collected from the medical records of patients seen at the pulmonology, nutrition, pediatrics and physiotherapy clinics. During the data collection period, 37 patients met the inclusion criteria. These patients are part of a cohort that is being systematically followed-up by the IFF since the point at which the disease of each patient was diagnosed. The number of patients analyzed guarantees a reliability of 99% and power of 80% for the statistical analyses carried out, assuming a BMD prevalence among CF patients varying from 40 to 60%.

This study made use of a secondary database (medical records from standard medical consultations), which would normally be subject to information bias. This may possibly have been minimized by the fact that two of the authors of this paper are treating and monitoring these patients and are responsible for a large proportion of the records made, while quality control is further facilitated by the small number of individual sufferers. Memory bias may also have occurred, affecting the variables reported by the children's parents or guardians (physical activity, exposure to the sun, use of medications). However, CF patients, due to the characteristics of the disease itself, demand an extremely close doctor-patient-carer relationship involving great trust, and in which this information is very much present in the memories of carers.

Data collection

Data were collected on the adolescent's identity (name, medical record number, age and sex), age at diagnosis of CF, presence of pancreatic sufficiency or insufficiency, according to the fecal fat test by the quantitative method, presence of diabetes mellitus according to a positive oral glucose tolerance test result and use of vitamins D and K. Anthropometric assessment was based on the standard growth curves published by the National Center for Health Statistics (NCHS),[13] using the height/age (H/A) and body mass index/age (BMI/A) indices and adopting the cutoff points recommended by the Centers for Disease Control and Prevention (CDC), expressed as percentiles. Body composition was calculated using Slaughter's equation[14] (fat free mass and body fat by mass). Intakes of calories, calcium and phosphorous were estimated using the program NutWin version 1.5.[15] Calorie intakes were expressed as percentages of the recommended intakes for these patients and were considered sufficient when a point 20 to 50% above the recommended was achieved.[16,17] Physical activity was defined as the habit of performing daily exercise, for a minimum of 20 minutes, three times a week.[18] Exposure to sun was defined as a minimum of 30 minutes per day.[19]

Bone integrity was assessed according to the presence or absence of fractures and by type of trauma (mild or spontaneous and severe). Notes were made on use and route of administration of corticoids, whether oral or inhaled, and whether there was a family history of osteoporosis.

Pulmonary function was tested by spirometry, which was considered valid if performed up to a minimum of 6 months prior to data collection, considering that these patients underwent pulmonary function testing twice a year, using forced expiratory volume in one second (VEF_1), which classifies the severity of obstructive and restrictive ventilatory disorders as mild (≥ 60%), moderate (from 41 to 59%) or severe (≤ 40%).[20] Patients were considered colonized if they had had more than three positive sputum cultures within a six month interval indicating CF-specific bacteria, such as *Staphylococcus aureus, Pseudomonas aeruginosa* or *Burkholderia cepacia*.[1]

The BD for the bone mineral density measure was obtained by dual emission X-ray absorptiometry (DEXA) for the whole body and lumbar spine (L1 to L4), using Lunar/GE Healthcare brand Prodigy Advance equipment (software version 9.15).

World Health Organization criteria were used to diagnose BMD, where patients are defined as normal down to a z-score of -1; osteopenia is defined as -1 to -2.5; and osteoporosis as below -2.5.[21]

Statistical analyses

The chi-square and Fisher tests were used for categorical variables. Continuous variables were analyzed using Student's *t* test (for variables with normal distribution), and the Kruskal-Wallis non-parametric test for variables that did not exhibit normal distribution. Variables with statistical significance in the bivariate analysis or that were clinically related to the outcome were incorporated into a logistic regression model for multivariate analysis. The stepwise method was used to define the final model. Statistical tests were performed with reference to a significance level of $p \leq 0.05$ for the parameters estimated. The bivariate analysis was performed using Epi-Info version 3.3.2.[22] Logistic regression was performed using SPSS version 12 for Windows.

This research was approved by the Human Research Ethics Committee at the IFF (on 31st May, 2006).

Results

A total of 37 adolescents with CF were studied (Table 1), with a median age of 13 years. In this group, 59.5% were female and 40.5% male. The median age of patients at the time of diagnosis of CF and was 2 years.

The BD test revealed that osteopenia in the lumbar spine was more prevalent among females (53.8%) and osteoporosis among males (57.1%). For the whole body, osteopenia was also more prevalent among females (70%), but osteoporosis exhibited the same percentage prevalence in both sexes. Overall, 54.1% of the study sample exhibited abnormal BD and 45.9% were normal.

The groups proved similar in terms of the variables analyzed (Tables 2 and 3). Differences were observed in the distribution of: BMI ($p = 0.04$); pancreatic insufficiency ($p = 0.003$); bacterial colonization ($p = 0.03$); respiratory function test ($p = 0.03$); and use of vitamins D and K ($p = 0.03$). Nevertheless, these variables did not retain significance in the analysis of association using the logistic regression model.

Discussion

The median of 13 years' chronological age observed for the 37 adolescents in this study is not a sufficient indicator of the rhythm of growth or of nutritional requirements, since there is great individual variability between individuals in this age group, when two important events take place almost simultaneously: the growth spurt, characterized by substantial increase in height, and peak bone mass. In this study, delayed puberty was not observed, perhaps because of the low median age of the sample.

The median of 2 years of age at diagnosis of CF among these adolescents is considered late. This can be explained by the fact that the disease is still little known and is difficult to diagnose. According to Gibson et al.,[1] the majority of CF cases are identified based on clinical symptoms, 43.8% of which are respiratory, 24.4% related to developmental deficiencies and 18.5% due to meconium ileus. Diagnosis cannot always be confirmed by the sweat test, since 1 to 2% of CF patients exhibit normal electrolyte concentrations in their sweat. In 71% of cases, diagnosis is established at around 1 year, in 8% only after 10 years of age and in 4% of cases, not until adulthood.[23,24]

The method used to measure bone mineral density in this study was DEXA, which is considered the gold standard, measuring both axial and appendicular skeleton, and therefore capable of assessing both cortical and trabecular bone. One of the great advantages of the method is the low risk of exposure to radiation (30 times less than is employed in an X-ray). The test is capable of detecting losses of less than 5%, whereas X-rays detect bone mass losses from 30 to 50% onwards.[11]

The prevalence of patients with abnormal BD was 54.1%. A study carried out by Bhudhikanok et al.,[25] on 49 children, adolescents and adults with CF, also found osteopenia in 21 (42.8%) children and adolescents. In this study we observed greater compromise of the lumbar spine BD, with 35.1% osteopenia and 18.9% osteoporosis. This can be explained by the fact that approximately 65% of the vertebrae are made up of trabecular bone,[26] which is more vulnerable to corticoid use, inflammatory cytokines and reduced levels of the sex hormones estradiol and testosterone; all characteristics present in CF. Furthermore, altered BD in the entire body is less common, since a large part of the skeleton is made up of cortical bone with more resistant mineralization and compact bone tissue, predominating in the long bones, neck of the femur and part of the radius. Work carried out by Ujhelyi et al.,[27] with 44 patients, detected a high proportion of spinal deformities, affecting almost exclusively the structure of thoracic vertebrae. The clinical manifestations of osteoporosis can occur very early, during childhood and early adolescence, but have not received their full recognition.[7] For this reason, Kerem et al.[17] recommend that patients with CF should undergo BD tests every 2 or 3 years from age 6 onwards, or with greater frequency if there is lung disease and oral corticoid use. It should be emphasized that, after diagnosis, the medication used to treat osteoporosis, in addition to exhibiting undesirable effects, does not offer great efficacy, and the bone mass gained after 2 years is just five to 10% in the lumbar spine, which further increases the importance of controlling predisposing factors.

Another relevant fact is that, according to Shane et al.,[28] Osteoporosis is a limiting factor for lung transplantation, since

Table 1 - Distribution of the characteristics of adolescents treated for CF at the IFF, according to factors involved in bone disease, from May to June, 2006

Factor	n	%
Nutritional status		
Normal height/age (P > 5)	26	70.3
Low height/age (P ≤ 5)	11	29.7
Normal weight (BMI; P > 5)	28	75.7
Underweight (BMI; P ≤ 5)	9	24.3
Colonized		
Yes	29	78.4
No	8	21.6
Respiratory function test*		
VEF_1 ≤ 40	3	8.3
VEF_1 41 to 59	6	16.7
VEF_1 ≥ 60	27	75
Pancreatic insufficiency		
Yes	29	78.4
No	8	21.6
Diabetes mellitus		
Yes	4	10.8
No	33	89.2
Vitamin D and K replacement		
Yes	29	78.4
No	8	21.6
Calcium intake		
Adequate	20	54.1
Inadequate	17	45.9
Phosphorous intake		
Adequate	34	91.9
Inadequate	3	8.1
Calorie intake		
Adequate	34	91.9
Inadequate	3	8.1
Fractures*		
Yes	12	33.3
No	24	66.7
Type of trauma*		
None	24	66.7
Mild or spontaneous	9	25
Severe	3	8.3
Corticoid therapy†		
Inhaled	11	29.7
Not used	26	70.3
Family osteoporosis *		
Present	9	25
Absent	27	75
Physical activity		
Exercises	25	67.6
Does not exercise	12	32.4
Time of exposure to sun *		
> 30 min	22	61.1
< 30 min	14	38.9

BMI = body mass index; P = percentile; VEF_1 = forced expiratory volume in one second.
* Information on this variable was nor available for all patients.
† The variable oral corticoid therapy is not shown because none of the patients used it orally.

Table 2 - Distribution of continuous variables according to bone densitometry results of adolescents with cystic fibrosis seen at the Instituto Fernandes Figueira from May to June, 2006

	Bone densitometry				
	Normal (z score ≥ -1) n = 17		Abnormal (z score < -1) n = 20		
Variables	Mean (SD)	Median	Mean (SD)	Median	p
Age (years)	13.7 (2.8)	14.0	12.7 (2.7)	12.0	0.30
Age at diagnosis (years)	3.2 (4.7)	1.0	5.5 (4.9)	5.0	0.15
Weight (kg)	42.3 (13.0)	40.0	34.8 (13.1)	30.0	0.08
BMI (kg/m^2)	18.8 (3.4)	17.6	16.8 (2.4)	15.6	0.04
VEF_1 (%)	87.5 (20.1)	93.5	69.2 (33.5)	71.0	0.10*
Calorie intake (Kcal)	2,481.8 (650.8)	2,450.0	2,651.7 (875.2)	2,430.0	0.50
Calcium intake (mg)	1,062.1 (495.7)	953.7	951.6 (368.9)	951.1	0.44
Phosphorous intake (mg)	1,494 (560.2)	1,541.9	1,337.9 (639.4)	1,375.9	0.43
Exposure to sun (min/day)	52.3 (37.8)	40.0	46.6 (34.6)	45.5	0.62
Fat free mass (kg)	32.0 (8.7)	33.1	29.6 (10.5)	26.1	0.46
Body fat by weight (kg)	9.4	8.4	6.7 (4.7)	5.2	

BMI = body mass index; SD = standard deviation; VEF_1 = forced expiratory volume in one second.
* Kruskal-Wallis test.

the large doses of immunosuppressors, including corticosteroids and cyclosporins, and the long periods of rest and inactivity after transplantation can all contribute to BMD.

Mean BMI/A was lower among the adolescents with abnormal BD, which is in agreement with results published by Mischler,[3] who studied 27 patients with CF aged 5 to 24 years, finding low weight in the majority of patients with bone demineralization. Frangolias et al.[18] linked BMI/A with BD and also found evidence of BMD in the hips and vertebrae of 85% of those with reduced BMI/A. Among the adolescents studied here, 24.3% of cases also exhibited low weight, according to BMI/A. This demonstrates that nutrition is critical for adolescents' development and that inadequate dietary intake can alter somatic growth, particularly of the skeleton, which attains 90% of its maturity by 18 years, and little can be done after this age. These findings are evidence that nutritional status is associated with BMD.

Pulmonary involvement, demonstrated by the presence of bacterial colonization in 65% of cases with abnormal BD, exhibited a positive association with BMD. This result is in agreement with Gronowitz et al.,[29] who studied BMD in patients with normal weight and stature and observed that it was directly related to loss of respiratory function. The respiratory function test categories, based on VEF_1 values, were reduced in conjunction with abnormal BD. Pulmonary injury secondary to post-colonization tissue inflammation is accompanied by an increase in the concentration of cytokines in circulation and precedes changes to pulmonary function, and these cytokines stimulate bone reabsorption and inhibit its formation, which explains why lung disease is associated with BMD.

The majority of the study population exhibited adequate calorie, calcium and phosphorous intakes, above the European Cystic Fibrosis Consensus recommendations.[30] However, the mean calcium (951.6 mg) and phosphorous (1,337.9 mg) intake were lower among patients with abnormal BD. This means that the attention of carers should always be focused on the adolescent CF patient's great demand for nutrients and calories, resulting from accelerated growth, disease severity and physical activity.

Sixty percent of the patients with pancreatic insufficiency also had abnormal BD. This result should be given due consideration, since more than 90% of CF patients exhibit exocrinal pancreatic insufficiency.[29] Another factor that should not be forgotten is that the symptoms of pancreatic disease only manifest once 98% of the pancreas has been destroyed. For this reason the diagnostic tests for pancreatic disease should always be carried out in order that enzyme replacement and vitamin and mineral supplementation can be initiated.

The presence of fractures from mild trauma was more frequent among patients with abnormal BD. This fact should be given its due weight since the occurrence of this type of fracture during day-to-day activities may be the first sign of osteoporosis. Buntain et al.[26] also observed a high prevalence of fractures among adolescents with CF, primarily of vertebrae. Fractures debilitate CF patients, often irreversibly, and

Table 3 - Distribution of categorical variables according to bone densitometry results of adolescents with cystic fibrosis seen at the Instituto Fernandes Figueira from May to June, 2006

	Bone densitometry				
	Normal (z score ≥ -1) n = 17		Abnormal (z score < -1) n = 20		
Variables	**n**	**%**	**n**	**%**	**p**
Sex					
Female	12	70.6	10	50.0	0.17
Male	5	29.4	10	50.0	
Nutritional status					
Normal height/age (P > 5)	14	82.4	12	60.0	0.13*
Low stature/age (P ≤ 5)	3	17.6	8	40.0	
Normal weight (BMI; P > 5)	15	88.2	13	65.0	0.10*
Underweight (BMI; P ≤ 5)	2	11.8	7	35.0	
Pancreatic insufficiency					
Yes	17	100.0	12	60.0	0.003†
No	0	0.0	8	40.0	
Diabetes mellitus					
Yes	2	11.8	2	10.0	0.63*
No	15	88.2	18	90.0	
Bacterial colonization					
Yes	16	94.1	13	65.0	0.03*
No	1	5.9	7	35.0	
Respiratory function test*					
VEF_1 ≤ 40	16	94.1	11	57.9	0.03†
VEF_1 41 to 59	1	5.9	5	26.3	
VEF_1 ≥ 60	0	0.0	3	15.8	
Vitamin D and K					
Yes	17	100.0	12	60.0	0.03†
No	0	0.0	8	40.0	
Fractures					
Yes	4	25.0	7	35.0	0.39*
No	12	75.0	13	65.0	
Type of trauma					
None	11	68.8	13	65.0	0.58*
Mild or spontaneous	3	18.8	6	30.0	
Severe	2	12.5	1	5.0	
Family osteoporosis*					
Yes	5	31.2	4	20.0	0.34*
No	11	68.8	16	80.0	
Exposure to sun					
Yes	15	93.8	18	90.0	0.58*
No	1	6.2	2	10.0	
Physical activity					
Exercises	11	64.7	14	70.0	0.37*
Does not exercise	6	35.3	6	30.0	

BMI = body mass index; P = percentile; VEF_1 = forced expiratory volume in one second.
* Fisher's exact test.
† The statistical test could not be applied because of the figure zero.

when the ribs are fractured this can alter the clinical course by causing chest pain, inhibiting the cough reflex and removal of secretions from the airways, accelerating the decline in pulmonary function.

Around 10% of the patients with abnormal BD were not being exposed to sunlight, and, among those who were, mean time of exposure was lower than among those with normal BD. Work by Reiter et al.[19] correlated the seasons of the year

in the Northern hemisphere with levels of vitamin D in the blood and demonstrated the increased risk of osteopenia with reduced serum vitamin D, resultant on the lower levels of sun during the winter, demonstrating its importance in vitamin D production.

None of the patients in this study were using oral corticoid therapy. Inhaled corticoid was being used both by patients with normal and abnormal BD, without statistical difference. Balfour-Lynn[31] also observed that inhaled corticoid therapy, even in high doses, has little effect on bone mineral density and fractures. In contrast, Bhudhikanok et al.[25] studied 21 adolescents less than 18 years old on oral corticoids and concluded that systemic corticoid is a risk factor for BMD.

The chosen risk factors were analyzed using a regression model, but were not significantly associated with BMD. Most probably, this result reflects the fact that the study population was less compromised in terms of these variables; one example of this is demonstrated by the BMI of this group which was compatible with well-nourished adolescents. The same was true with relation to VEF_1, which exhibited the high values typical of mild lung disease or normality.

Despite the high prevalence of BMD in this study population, since this is a cross-sectional study, certain limitations should be taken into account. The most important of these is the temporal question, to show whether the exposure factor precedes the outcome studied. Longitudinal studies with systematic follow-up of these patients should help with understanding the natural history of CF-linked BMD and, consequently, strategies for prevention and treatment. Such studies should include measures like calorie and calcium intake, exposure to the sun, physical activity, regular use of enzymes and liposoluble D and K vitamins, adequate control of pulmonary infection and periodical BD scans from 10 years of age onwards. Therefore, due to the scarcity of information on the subject of populations of CF patients treated in Brazil, we recommend that prospective studies be carried out.

We conclude that there is a high prevalence of BMD in the study population of adolescents with CF, with the lumbar spine the most frequent. Lung disease, pancreatic insufficiency and malnutrition were the risks most closely associated with BMD. All preventative efforts should be directed at the end of childhood and start of adolescence, since, if osteoporosis is not diagnosed in adolescents with CF, little can be done later on to increase bone mass.

References

1. Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. Am J Respir Crit Care Med. 2003;168:918-51.
2. Botton E, Saraux A, Laselve H, Jousse S, Le Goff. Musculoskeletal manifestations in cystic fibrosis. Joint Bone Spine. 2003;70:327-35.
3. Mischler EH, Chesney PJ, Chesney RW, Mazess RB. Demineralization in cystic fibrosis detected by direct photon absorptiometry. Am J Dis Child. 1979;133:632-5.
4. Brousse C, Nguyen-Plantin S, Friard S, Grenet D, Stern M. Étude de la densité minérale osseuse chez des patients insuffisants respiratoires chroniques. Rev Mal Respir. 2001;18(4 Pt 1):411-5.
5. Aris RM, Lester GE, Caminiti M, Blackwood AP, Hensler M, Lark RK, et al. Efficacy of alendronate in adults with cystic fibrosis with low bone density. Am J Respir Crit Care Med. 2004;169:77-82.
6. Conway SP. Impact of lung inflammation on bone metabolism in adolescents with cystic fibrosis. Paediatr Respir Rev. 2001; 2:324-31.
7. Hecker TM, Aris RM. Management of osteoporosis in adults with cystic fibrosis. Drugs. 2004;64:133-47.
8. Schulze KJ, O'Brien KO, Germain-Lee EL, Baer DJ, Leonard AL, Rosenstein BJ. Endogenous fecal losses of calcium compromise calcium balance in pancreatic-insufficient girls with cystic fibrosis. J Pediatr. 2003;143:765-71.
9. Buntain HM, Greer RM, Schluter PJ, Wong JC, Batch JA, Potter JM, et al. Bone mineral density in Australian children, adolescents, and adults with cystic fibrosis: a controlled cross sectional study. Thorax. 2004;59:149-55.
10. Conway SP, Morton AM, Oldroyd B, Truscott JG, White H, Smith AH, et al. Osteoporosis and osteopenia in adults and adolescents with cystic fibrosis: prevalence and associated factors. Thorax. 2000;55:798-804.
11. Campos LM, Liphaus BL, Silva CA, Pereira RM. Osteporose na infância e na adolescência. J Pediatr (Rio J). 2003;79:481-8.
12. Allgrove J. Use of biphosphonates in children and adolescents. J Pediatr Endocrinol Metab. 2002;15 Suppl 3:921-8.
13. U.S. Department of Health and Human Services, Center for Disease Control and Prevention, National Center for Health Statistics, CDC/NCHS/2000. Growth charts. Hyattsville. Available at: http://www.cdc.gov/growthcharts/. Access: 20/08/2006.
14. Slaughter MH, Lohman TG, Boilean RA, Horswill CA, Stillman RJ, Van Loan MD, et al. Skinfold equations for estimation of body fatness in children and youth. Hum Biol. 1988;60:709-23.
15. Anção MS, Cuppari L, Draibe AS, Sigulem D. Programa de apoio à Nutrição- NutWin-Versão 1,5 [CD- ROM]. São Paulo: Departamento de Informática em Saúde, SPDM- UNIFESP/EPM; 2002.
16. Adde FV, Dolce P, Tanikawa CE, Uehara DY, Cardoso AL, Rosov T, et al. Suplementação dietética em pacientes com fibrose cística. J Ped 1997; 73 (5): 317-23.
17. Kerem E, Conway S, Elborn S, Heigerman H; Consensus Committee. Standards of care for patients with cystic fibrosis: a European Consensus 2005. J Cyst Fibros. 2005;4:7-26.
18. Frangolias DD, Paré PD, Kendle DL, Davidson AG, Wong L, Raboud J, et al. Role of exercise and nutrition status on bone mineral density in cystic fibrosis. J Cyst Fibros. 2003;2:163-70.
19. Reiter EO, Brugman SM, Pike JW, Pitt M, Dokoh S, Haussler MR, et al. Vitamin D metabolites in adolescents and young adults with cystic fibrosis: effects of sun and season. J Pediatr. 1985; 106:21-6.
20. Dia RM, Chauvet PR, Siqueira HR, Rufino R. Testes de função respiratória: do laboratório à aplicação com 100 exercícios para diagnósticos. São Paulo: Atheneu, 2000.

21. World Health Organization (WHO). Assessment of fracture risk and its application to screening for post-menopausal osteoporosis. Geneva: WHO; 1994. (WHO Technical Report Series n. 843).

22. EpiInfo 2000: a database and statistics program for public health professionals for use on Windows 95, 98, NT, and 2000 computers. Atlanta:Centers for Disease Control and Prevention; 2000.

23. Boucher RC. Human airway ion transport. Part one. Am J Resp Crit Care Med. 1994;150:271-81.

24. Boucher RC. Human airway ion transport. Part two. Am J Resp Crit Care Med. 1994;150:581-93.

25. Bhudhikanok GS, Lim J, Marcus R, Harkins A, Moss RB, Bachrach LK. Correlates of osteopenia in patients with cystic fibrosis. Pediatrics. 1996;97:103-11.

26. Buntain HM, Schluter PJ, Bell SC, Greer RM, Wong JC, Batch J, et al. Controlled longitudinal study of bone mass accrual in children and adolescents with cystic fibrosis. Thorax. 2006; 61:146-54.

27. Ujhelyi R, Treszl A, Vásárhelyi B, Holics K, Tóth M, Arató A, et al. Bone mineral density and bone acquisition in children and young adults with cystic fibrosis: a follow-up study. J Pediatr Gastroenterol Nutr. 2004;38:401-6.

28. Shane E, Silverberg SJ, Donovan D, Papadopoulos A, Staron RB, Adesso V, et al. Osteoporosis in lung transplantation candidates with end-stage pulmonary disease. Am J Med. 1996; 101:262-9.

29. Gronowitz E, Garemo M, Lindblad A, Mllström D, Strandvik B. Decreased bone mineral density in normal growing patients with cystic fibrosis. Acta Paediatr. 2003;92:688-93.

30. Littlewood JM. European cystic fibrosis society consensus on standards- a roadmap to "best care". J Cyst Fibros. 2005; 4:1-5.

31. Balfour-Lynn IM. Asthma in CF: corticosteroids. Pediatr Pulmonol. 2002; 34 Suppl 24:101-2.

Correspondence:
Reinaldo José do Amaral Caldeira
Avenida Rui Barbosa, 716
CEP 22250-020 – Rio de Janeiro, RJ – Brazil
Tel.: +55 (21) 2554.1719
E-mail: reinaldojacaldeira@yahoo.com.br