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ORIGINAL ARTICLE

Iliac bone histomorphometry in children with newly diagnosed inflammatory bowel disease

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Abstract
Summary Children with inflammatory bowel disease (IBD) manifest low bone mass; the cause remains unclear. We performed transilial bone biopsies in 20 IBD children at diagnosis and found a mild cortical bone deficit and slow bone turnover. It is possible that low mechanical stimulation due to inadequate muscle mass contributes to the bone deficit.
Introduction Children with newly diagnosed IBD can have low bone mineral density and disturbed bone metabolism, but the tissue level characteristics of the bone involvement in pediatric IBD have not been elucidated.
Methods In the present study, we evaluated the skeletal status, including static histomorphometry on transiliac bone samples, in 20 patients (age range 8.4 to 17.7 years, 12 boys) with newly diagnosed IBD and compared results to published normative data.
Results Despite normal height (mean Z-score 0.04, SD 1.2), areal bone mineral density at the lumbar spine was moderately low (mean age- and sex-specific Z-score −0.8, SD 1.1). Total body bone mineral content and lean mass were low for age and sex as well (mean Z-scores −1.2, SD 0.9 and −2.0, SD 0.9, respectively). Biochemical bone markers indicated low bone formation and resorption activity. Bone histomorphometry revealed a slightly low cortical width (mean 23%, SD 25%, below the result expected for age) but a normal amount of trabecular bone. The percentage of trabecular bone surface covered by osteoid or osteoclasts was low, suggesting that both bone formation and bone resorption were suppressed.
Conclusions Our results indicate that young patients manifest a mild cortical bone deficit at the iliac crest and slow trabecular bone turnover even at diagnosis, in the setting of IBD.

Keywords Bone formation · Bone resorption · Histomorphometry · Inflammatory bowel disease · Osteoporosis

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Introduction

Inflammatory bowel disease (IBD) is an idiopathic destructive chronic inflammatory condition of the gastrointestinal tract. The prevalence of IBD in children below 15 years of age is 20/100,000 [1]. About 80% of pediatric IBD patients have Crohn's disease; the remainder has ulcerative colitis. Apart from the gastrointestinal tract, IBD affects many organ systems, including the skeleton. The effect on the skeleton is often difficult to distinguish from the bone effect of medications that are used to control the inflammatory process, such as glucocorticoids.

Nevertheless, it is clear that IBD by itself has an effect on the skeleton even before any medication is given. Several studies have found that children with IBD have low

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bone mass and density even in newly diagnosed patients [2, 3]. In a few cases, severe osteoporosis with vertebral compression fractures was observed at diagnosis [4]. The etiology of the bone mass deficit in this setting remains incompletely understood. However, several in vitro studies have found that the serum of children with IBD contains factors that inhibit osteoblasts [5, 6]. In addition, young IBD patients often suffer from compromised nutritional intake, malabsorption, delayed puberty, growth retardation, and vitamin D deficiency, all of which may have a detrimental effect on bone development [7, 8].

A number of studies have examined the skeletal effects of IBD on the pediatric skeleton by using noninvasive techniques, such as dual-energy X-ray absorptiometry (DXA) and biochemical markers of bone metabolism [2, 3]. However, the interpretation of such results is often difficult. DXA-derived areal bone mineral density (BMD) is influenced by bone size and does not distinguish between osteomalacia (i.e., insufficient mineralization of bone matrix) and osteoporosis (insufficient amount of normally mineralized bone matrix). Biochemical parameters of bone turnover are influenced by many factors such as the speed of longitudinal growth, bone mass, and the speed of bone mass accrual [9].

Many of the uncertainties surrounding these noninvasive methods can be circumvented by examining bone tissue from the ilium using quantitative histomorphometry [10]. This technique allows examining the bone with unsurpassed resolution, provides information on the amount and structure of both cortical and trabecular bone, and quantifies bone metabolism independent of growth rate and bone mass. At present, there is little information on the bone tissue level characteristics of IBD. Histomorphometric studies on adult IBD patients have shown that both osteomalacia and osteoporosis can occur following small bowel resection [11, 12]. In adults who suffered from osteoporosis in the context of long-standing IBD, a markedly low trabecular bone volume and a negative remodeling balance on trabecular bone surfaces were found [13]. However, to our knowledge, histomorphometric data are not available for pediatric IBD or for newly diagnosed IBD in either children or adults. In the present study, we therefore aimed to assess the bone abnormalities in newly diagnosed pediatric IBD patients at the tissue level.

Methods

Patients and study design

This prospective cross-sectional study was conducted through the pediatric IBD clinic at the Children's Hospital of Eastern Ontario (Ottawa, Canada), a tertiary care academic children's hospital. The study protocol was approved by the hospital's Institutional Review Board. Informed consent (and assent, where appropriate) was obtained from all participants prior to enrollment in the study. Between January 1, 2004 and December 31, 2005, all children who were suspected of having IBD and underwent diagnostic endoscopy to confirm the diagnosis were invited to participate in this study. If the macroscopic appearance during endoscopy was compatible with a diagnosis of IBD, one of the investigators (LMW) performed a bone biopsy immediately following endoscopy. Both the bone biopsy and the endoscopy were carried out under general anesthesia. The other study procedures were performed either on the day of the endoscopy or within the following 2 weeks, before treatment of IBD was instituted. Of the 52 patients who were approached for enrolment in the study, 20 consented to participate. In all patients who declined participation, the stated reason was that they did not want to undergo the bone biopsy procedure. No participant withdrew from the study. Histologic evaluation of mucosal biopsies obtained during endoscopy confirmed the diagnosis of IBD in each of the study participants.

Clinical evaluation

Disease severity ratings were determined according to the Pediatric Crohn's Disease Activity Index or Pediatric Ulcerative Colitis Activity Index, as appropriate [14, 15]. Information regarding the duration of IBD symptoms prior to diagnosis was based on parent and/or participant recall. Pubertal development was assessed by physical examination according to Tanner staging, using published photographs as a normal reference [16–18]. Calcium and vitamin D intake (both dietary and supplemental) were quantified according to a 3-day recall under the supervision of a certified pediatric IBD dietician. Height was determined on a Harpenden stadiometer. Weight was measured using a digital weight scale. Height and weight Z-scores were calculated using the National Center for Health Statistics Growth Charts [19].

Biochemical analyses

Serum and urine concentrations of calcium, phosphorus, and creatinine as well as serum alkaline phosphatase activity were measured using standard methods. Serum intact parathyroid hormone was quantified by immunoradiometric assay (N-tact*; Incstar Corp., Stillwater, MN, USA). Serum 25-OH vitamin D levels were measured by radioimmunoassay (Osteo SP; Incstar Corp., Stillwater, MN, USA). Serum levels of the carboxy-terminal propeptide of type I collagen (C1CP), a marker of bone formation,

were measured by enzyme immunoassay (Metra CICP, Quidel Corporation, San Diego, CA, USA). Results were compared to pediatric reference data supplied by the manufacturer. The bone resorption marker urinary cross-linked N-telopeptide of type I collagen (NTX) was quantified by enzyme-linked immunoabsorbent assay (Osteomark®; Ostex, Seattle, WA, USA) on the second void sample of the morning. Results for urinary NTX to creatinine ratios were compared with published reference data [20]. Patients were fasting at the time of blood and urine sampling.

Radiological studies

A lateral radiographic view of the thoracolumbar spine was obtained in each patient to assess vertebral morphology. In addition, a radiograph of the left hand and wrist was obtained to screen for the presence of rickets and to evaluate bone age [21]. The radiographs were assessed independently by two pediatric radiologists. Discrepancies between readers were resolved by consensus.

Lumbar spine (L2 to L4) areal BMD, total body bone mineral content (BMC), and total body lean mass were measured in the anteroposterior direction by DXA (Lunar Prodigy; General Electric; Madison, WI, USA). To account for bone size, bone mineral apparent density (BMAD) was derived using the method proposed by Kroger et al. [22]. Areal BMD and BMAD of the lumbar spine, total body BMC, and total body lean mass results were transformed to age-and sex-specific Z-scores using published reference data [23]. Total body lean mass results were further converted to gender- and height-specific Z-scores [24]. The relationship between total body BMC and total body lean mass was also explored by comparing the ratio between these two measures to gender- and age-matched reference data [24].

Iliac bone histomorphometry

Transiliac bone samples were obtained at a site 2 cm posterior of the superior anterior iliac spine. The procedure was well tolerated in all patients. To obtain the maximum information from histomorphometry, tetracycline double labeling should be performed prior to biopsy [10]. However, a tetracycline labeling course takes close to 3 weeks to complete. When, as in the present study, the bone biopsy is performed at the same time as the endoscopy that establishes the diagnosis of IBD, this length of time is not available for ethical reasons because the endoscopy procedure cannot be delayed for the purpose of completing tetracycline labeling. Consequently, dynamic measures of bone formation could not be performed in the present study.

Sample preparation and histomorphometric analyses were performed using previously described procedures [25]. Measurements were carried out using a digitizing table with Osteomeasure® software (Osteometrics Inc., Atlanta, GA, USA). Nomenclature and abbreviations follow the recommendations of the American Society for Bone and Mineral Research [26]. Results were expressed as percentages of the average value of the age-specific reference range using reference data established in our laboratory [25].

Statistical analyses

Age- and gender-matched Z-scores arising from anthropometry and densitometry were tested for significant deviation from 0 using the one-sample *t* test. Results for bone resorption and formation markers as well as bone histomorphometry parameters were expressed as a percentage of the average of the age-specific reference range. The difference of these data from 100 was tested for significance using the one-sample *t* test. Statistical analyses were performed using SPSS software (version 15.0).

Results

Twenty patients (age range 8.4 to 17.7 years, eight girls, 12 boys) participated in the study (Table 1). Seventeen patients had a diagnosis of Crohn's disease and three were diagnosed with ulcerative colitis. IBD symptoms had been present for a median of 5.4 months (range 3.1 to 12.4 months). Six of the Crohn's disease patients had mild disease (a Pediatric Crohn's Disease Activity Index between 12.5 and 30); the remaining patients had moderate to severe involvement (a Pediatric Crohn's Disease Activity Index above 30). Crohn's disease location was ileal in three, colonic in two, and ileocolonic in 12 patients. Among the ulcerative colitis group, one patient each had mild, moderate, and severe involvement. Ulcerative colitis was left-sided in one patient and pancolitis was present in two patients. Bone age was similar to chronological age in this cohort of patients. Half of the patients were in early to midpuberty. Calcium and vitamin D intakes were at or above the Dietary Reference Intake in the majority of patients [27].

The clinical characteristics of the 32 patients who declined participation in the study were as follows. Firstly, 13 of the children who declined participation went on to show no evidence of Crohn's disease or ulcerative colitis on gastrointestinal biopsy. Of the remaining 19 patients who declined participation, 14 had Crohn's disease (five girls, nine boys) and five had ulcerative colitis (four girls). Four of the Crohn's disease patients who declined participation in the study had mild disease, the remaining patients had moderate to severe involvement. Crohn's disease location

Table 1 Clinical characteristics of the study population (N=20 unless otherwise indicated)

Outcome measure	Results, N=20
Gender (N, male/female)	12/8
Age in years at time of diagnosis	
Median	14.7
IQR (25%, 75%)	(11.6, 16.4)
Bone age in years (N=19)	
Median	15.0
IQR (25%, 75%)	(13, 16.5)
Bone age difference to chronological age (months; N=19)	
Median	1.6
IQR (25%, 75%)	(−4.1, 4.1)
Pubertal status (number of patients with Tanner I, II–IV, V)	3, 10, 7
Daily calcium intake (mg, N=19)	1,657 (1,392, 2,689)
Daily vitamin D intake (IU, N=19)	392 (147, 616)
Serum 25-OH vitamin D (nmol/L)	61 (19)

Results are given as mean (SD) or median (interquartile range 25th percentile, 75th percentile)

IU international units

was ileal in six children, colonic in two, ileocolonic in three, and ileocolonic plus upper intestinal in the remaining three patients. Among the ulcerative colitis group, one patient had mild involvement; three were moderately affected and one child had severe involvement. Ulcerative colitis was left-sided in one patient and pancolitis was present in the remaining children. For those with Crohn's disease, the Pediatric Crohn's Disease Activity Index was similar between those who participated in the study (mean (SD) 34.2 (15.2)) compared to those who declined (mean (SD), 43.2 (15.9), p=0.12). Similarly, the Pediatric Ulcerative Colitis Activity Index for those with ulcerative colitis was no different between those who participated (mean (SD), 37.5 (7.1)) compared to those who did not (mean (SD), 56.3 (14.4), p=0.16).

No radiographic evidence of vertebral compression fractures or rickets was found in any of the patients. Even though average height was normal in the study cohort, mean weight was slightly low (Table 2). Lumbar spine areal BMD and BMAD, total body BMC, and total body lean mass were significantly below the results expected in healthy subjects of the same age and gender (Table 2). Similarly, the height-specific total body lean mass Z-score was significantly below the healthy average result. On the other hand, the ratio between total body BMC and lean mass was normal for age.

Serum levels of calcium, phosphorus, parathyroid hormone, and creatinine were within the reference range for all participants. The 25-OH vitamin D status of the cohort was as follows: three patients had levels between 26 and 40 nmol/L; 12 children had levels greater than 40 nmol/L but less than or equal to 75 nmol/L, and only five patients presented with levels greater than 75 nmol/L. By multivariable linear regression with total body lean mass Z-score as the dependent variable (controlling for height Z-score), there was a nonsignificant relationship between 25-OH vitamin D level and total body lean mass Z-score (unstandardized coefficient beta (95% confidence interval) 0.005 (−0.011, 0.021), p=0.492). Urinary NTX to creatinine ratios and serum concentrations of C1CP were 28% (SD 33) and 63% (SD 28), respectively, below the result expected for healthy subjects of the same age and gender (p<0.01 in both cases for the difference to the mean value of the reference range), indicating low bone metabolism.

As to iliac bone histomorphometry, the size of the biopsy sample core was appropriate for age (Table 3), with a

Table 2 Anthropometry and DXA results in the study population (N=20)

	Mean (SD)	Range	p
Height	0.04 (1.2)	(−1.7, 2.2)	0.89
Weight	−0.5 (1.0)	(−1.9, 2.1)	0.05
Lumbar spine areal BMD	−0.8 (1.1)	(−3.1, 0.6)	0.002
Lumbar spine BMAD	−0.4 (0.8)	(−2.6, 0.8)	0.04
Total body BMC	−1.2 (0.9)	(−3.0, 0.1)	<0.001
Total body lean mass	−2.0 (0.9)	(−3.4, −0.3)	<0.001
Total body lean mass for height	−1.0 (0.9)	(−2.8, 0.3)	<0.001
Total body BMC/lean mass for age	0.0 (0.9)	(−2.0, 1.9)	0.97

Results are given as age- and gender-specific Z-scores. p values indicate the significance level for the difference of mean Z-scores from 0 (one-sample t test)

Table 3 Histomorphometric results in transiliac bone samples

	Raw results, N=20	Average percent relative to the healthy age- and gender-matched mean	p[a]
Structural parameters			
Core width (μm)	8.5 (1.9)	+5 (24)	0.35
Trabecular number (/mm)	1.8 (0.2)	+10 (12)	0.001
Trabecular thickness (μm)	146 (23)	−3 (16)	0.34
Bone volume/tissue volume (%)	26.8 (4.5)	+6 (18)	0.16
Cortical width (μm)	802 (255)	−23 (25)	<0.001
Formation parameters			
Osteoid thickness (μm)	7.0 (1.4)	+9 (21)	0.09
Osteoid surface/bone surface (%)	16.2 (5.7)	−31 (22)	<0.001
Osteoid volume/bone volume (%)	1.6 (0.8)	−23 (35)	0.008
Osteoblast surface/bone surface (%)	6.1 (2.9)	−16 (37)	0.07
Resorption parameters			
Osteoclast surface/bone surface (%)	0.89 (0.4)	−18 (38)	0.04
Eroded surface/bone surface (%)	21.6 (4.9)	28 (33)	0.001

Results are given in mean (SD)

[a] p values were calculated by the one-sample t test

normal amount of trabecular bone. However, average cortical width was significantly reduced at 23% below the mean value of the age-specific reference range (p<0.001). Osteoid thickness was below 9 μm in all patients, which is the cutoff value above which a mineralization defect is diagnosed in pediatric histomorphometry, as proposed earlier [28]. Osteoid surface and volume as well as bone resorption parameters were low compared to age-specific norms. However, resorption parameters yielded conflicting results. Whereas osteoclast surface was low, eroded surface was elevated.

Discussion

The present study confirms earlier reports that newly diagnosed pediatric IBD patients on average have a somewhat low bone mass and low biochemical marker of bone metabolism when compared to age-matched peers [2]. Our study extends these earlier observations by examining bone structure and metabolism on the tissue level. Iliac bone histomorphometry showed that the main structural abnormality in young IBD patients was a mildly reduced cortical thickness, whereas the amount of trabecular bone was preserved. As to bone metabolism, we found histomorphometric evidence that both bone formation and resorption activities were slightly low for age but there was no sign of a mineralization defect in any patient. Importantly, none of the patients had signs of vertebral compression fractures.

Our DXA results are very similar to those of earlier studies in newly diagnosed pediatric IBD patients [2, 3]. They also replicate the observation made by Burnham et al. who observed that lean mass tends to be low in young IBD patients [29, 30]. However, whereas the patients of Burnham et al. had already received treatment for Crohn's disease, our results demonstrate that low lean mass is already present before medication with potential muscle toxicity is given.

Lean mass is a surrogate measure of muscle mass, which presumably reflects muscle force and therefore provides an estimate of the mechanical forces to which the skeleton is exposed [31]. It is thus possible that the low bone mass in young IBD patients is a consequence of low muscle force. In line with this hypothesis, the ratio between total body BMC and total body lean mass was normal in our study. Nevertheless, it is also possible that muscle and bone findings do not have a cause–effect relationship but are independent results of the underlying inflammatory process. In any case, more studies into the muscle involvement in IBD are warranted.

Our histomorphometric data suggest that the bone mass deficit was related to cortical thinning in our newly diagnosed children and adolescents with IBD, whereas the amount of trabecular bone was normal. This is somewhat surprising, as histomorphometric studies in adults who suffered from osteoporosis in the context of IBD have revealed markedly low trabecular bone volume [13]. Similarly, animal models of IBD are characterized by a decrease in the amount of trabecular bone after induction of IBD, at least in mature animals [32, 33]. However, the developing skeleton may respond differently to IBD at the iliac crest than the mature skeleton.

We found that biochemical markers of both bone formation and resorption were low in newly diagnosed

pediatric IBD when compared to age- and sex-specific reference ranges. This closely mirrors results published by Sylvester et al. [2]. Such bone marker data are not easy to interpret in children, as they are influenced by a large number of confounders, such as growth rate, maturational delay, low bone mass, and nutritional deficits, which may all be different between IBD patients and a healthy population [9]. After adjusting for a multitude of such confounders, Tuchman et al. found that young IBD patients had low bone formation and elevated bone resorption markers [9]. However, their patients had already received treatment for IBD, which makes direct comparison with our data difficult.

Our histomorphometric data clearly show that bone formation activity is low on trabecular bone surfaces, as osteoid surface and volume were both low for age. The picture was somewhat more complicated with regard to bone resorption, as the two "resorption markers" pointed in opposite directions. The osteoclast-covered surface was low, whereas eroded surface was elevated. However, even though eroded surface is usually regarded as an indicator of bone resorption, it is also influenced by bone formation. A bone surface has an eroded aspect when osteoclast resorption has taken place at a location and osteoblasts have not yet arrived to smooth out the surface irregularities [34]. Thus, eroded surface will not only be elevated when bone resorption activity is high but also when osteoblast activity is acutely suppressed. Given that bone formation parameters and osteoclast surface were low, it therefore seems more likely that in our study the elevated eroded surface was indicative of suppressed bone formation. The simultaneous suppression of bone formation and resorption on trabecular surfaces are also in accordance with the finding that the amount of trabecular bone at the iliac crest was normal in our patient cohort.

Although our study was not powered to assess the relationship between vitamin D status and muscle or bone measures, it is interesting to note that three of our 20 patients (15%) had serum levels of 25-OH vitamin D below 40 nmol/L and that only five children presented with levels greater than 75 nmol/L. None of the patients, including those with 25-OH vitamin D levels less than 40 nmol/L, had signs of a mineralization defect, either at the level of the growth plate (as assessed by X-ray of the hand and wrist) or on the level of the bone tissue (as assessed by iliac bone histomorphometry).

We found no evidence for vertebral compression in our cohort of newly diagnosed patients. There are nevertheless case reports of children with both newly diagnosed [4] and established [35] IBD manifesting vertebral deformity. The lack of vertebral compression in our study may have been due to a shorter duration of IBD symptoms, resulting in a less severely affected cohort. The prevalence of vertebral compression has been shown to range from 14% to 22% among adult IBD patients [36, 37]. Similar prevalence studies of vertebral compression in pediatric IBD patients, where both the underlying inflammatory condition and the effect of glucocorticoids have the potential to impact bone development, merit attention in the future.

Taken together, in this study, none of our newly diagnosed pediatric IBD patients had signs of vertebral pathology or of a mineralization disorder. However, our results indicate that young IBD patients at diagnosis have a mild cortical bone deficit at the iliac crest and slow turnover of trabecular bone. It is possible that low mechanical stimulation due to inadequate muscle mass contributes to the bone deficit. More mechanistic studies are warranted that investigate the effect of the inflammatory process on both muscle and bone tissue.

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Conflicts of interest None of the authors has a conflict of interest

References

1. Kim SC, Ferry GD (2004) Inflammatory bowel diseases in pediatric and adolescent patients: clinical, therapeutic, and psychosocial considerations. Gastroenterology 126:1550–1560
2. Sylvester FA, Wyzga N, Hyams JS, Davis PM, Lerer T, Vance K, Hawker G, Griffiths AM (2007) Natural history of bone metabolism and bone mineral density in children with inflammatory bowel disease. Inflamm Bowel Dis 13:42–50
3. Harpavat M, Greenspan SL, O'Brien C, Chang CC, Bowen A, Keljo DJ (2005) Altered bone mass in children at diagnosis of Crohn disease: a pilot study. J Pediatr Gastroenterol Nutr 40:295–300
4. Thearle M, Horlick M, Bilezikian JP, Levy J, Gertner JM, Levine LS, Harbison M, Berdon W, Oberfield SE (2000) Osteoporosis: an unusual presentation of childhood Crohn's disease. J Clin Endocrinol Metab 85:2122–2126
5. Sylvester FA, Wyzga N, Hyams JS, Gronowicz GA (2002) Effect of Crohn's disease on bone metabolism in vitro: a role for interleukin-6. J Bone Miner Res 17:695–702
6. Varghese S, Wyzga N, Griffiths AM, Sylvester FA (2002) Effects of serum from children with newly diagnosed Crohn disease on primary cultures of rat osteoblasts. J Pediatr Gastroenterol Nutr 35:641–648

7. Pappa HM, Gordon CM, Saslowsky TM, Zholudev A, Horr B, Shih MC, Grand RJ (2006) Vitamin D status in children and young adults with inflammatory bowel disease. Pediatrics 118:1950–1961
8. Gupta A, Paski S, Issenman R, Webber C (2004) Lumbar spine bone mineral density at diagnosis and during follow-up in children with IBD. J Clin Densitom 7:290–295
9. Tuchman S, Thayu M, Shults J, Zemel BS, Burnham JM, Leonard MB (2008) Interpretation of biomarkers of bone metabolism in children: impact of growth velocity and body size in healthy children and chronic disease. J Pediatr 153:484–490
10. Rauch F (2006) Watching bone cells at work: what we can see from bone biopsies. Pediatr Nephrol 21:457–462
11. Compston JE, Ayers AB, Horton LW, Tighe JR, Creamer B (1978) Osteomalacia after small-intestinal resection. Lancet 1:9–12
12. Hessov I, Mosekilde L, Melsen F, Fasth S, Hulten L, Lund B, Sorensen OH (1984) Osteopenia with normal vitamin D metabolites after small-bowel resection for Crohn's disease. Scand J Gastroenterol 19:691–696
13. Croucher PI, Vedi S, Motley RJ, Garrahan NJ, Stanton MR, Compston JE (1993) Reduced bone formation in patients with osteoporosis associated with inflammatory bowel disease. Osteoporos Int 3:236–241
14. Hyams J, Markowitz J, Otley A, Rosh J, Mack D, Bousvaros A, Kugathasan S, Pfefferkorn M, Tolia V, Evans J, Treem W, Wyllie R, Rothbaum R, del Rosario J, Katz A, Mezoff A, Oliva-Hemker M, Lerer T, Griffiths A (2005) Evaluation of the pediatric Crohn disease activity index: a prospective multicenter experience. J Pediatr Gastroenterol Nutr 41:416–421
15. Turner D, Otley AR, Mack D, Hyams J, de Bruijne J, Uusoue K, Walters TD, Zachos M, Mamula P, Beaton DE, Steinhart AH, Griffiths AM (2007) Development, validation, and evaluation of a pediatric ulcerative colitis activity index: a prospective multicenter study. Gastroenterology 133:423–432
16. Marshall WA, Tanner JM (1969) Variations in pattern of pubertal changes in girls. Arch Dis Child 44:291–303
17. Marshall WA, Tanner JM (1970) Variations in the pattern of pubertal changes in boys. Arch Dis Child 45:13–23
18. van Wieringen JC (1971) Growth diagrams. Netherlands Wooplter-Noorhoff, Groningen
19. Ogden CL, Kuczmarski RJ, Flegal KM, Mei Z, Guo S, Wei R, Grummer-Strawn LM, Curtin LR, Roche AF, Johnson CL (2002) Centers for disease control and prevention 2000 growth charts for the United States: improvements to the 1977 national center for health statistics version. Pediatrics 109:45–60
20. Bollen AM, Eyre DR (1994) Bone resorption rates in children monitored by the urinary assay of collagen type I cross-linked peptides. Bone 15:31–34
21. Greulich WW, Pyle SI (1959) Radiographic atlas of skeletal development of the hand and wrist, 2nd edn. Stanford University Press, Palo Alto
22. Kroger H, Kotaniemi A, Vainio P, Alhava E (1992) Bone densitometry of the spine and femur in children by dual-energy X-ray absorptiometry. Bone Miner 17:75–85
23. van der Sluis IM, de Ridder MA, Boot AM, Krenning EP, de Muinck Keizer-Schrama SM (2002) Reference data for bone density and body composition measured with dual energy X ray absorptiometry in white children and young adults. Arch Dis Child 87:341–347 discussion 341–347
24. Hogler W, Briody J, Woodhead HJ, Chan A, Cowell CT (2003) Importance of lean mass in the interpretation of total body densitometry in children and adolescents. J Pediatr 143:81–88
25. Glorieux FH, Travers R, Taylor A, Bowen JR, Rauch F, Norman M, Parfitt AM (2000) Normative data for iliac bone histomorphometry in growing children. Bone 26:103–109
26. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR (1987) Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR histomorphometry nomenclature committee. J Bone Miner Res 2:595–610
27. Institute of Medicine (1997) Dietary reference intakes for calcium, phosphorus, magnesium, Vitamin D, fluoride. National Academy Press, Washington, D.C
28. Terpstra L, Rauch F, Plotkin H, Travers R, Glorieux FH (2002) Bone mineralization in polyostotic fibrous dysplasia: histomorphometric analysis. J Bone Miner Res 17:1949–1953
29. Burnham JM, Shults J, Semeao E, Foster B, Zemel BS, Stallings VA, Leonard MB (2004) Whole body BMC in pediatric Crohn disease: independent effects of altered growth, maturation, and body composition. J Bone Miner Res 19:1961–1968
30. Burnham JM, Shults J, Semeao E, Foster BJ, Zemel BS, Stallings VA, Leonard MB (2005) Body-composition alterations consistent with cachexia in children and young adults with Crohn disease. Am J Clin Nutr 82:413–420
31. Binkley TL, Specker BL (2008) Muscle–bone relationships in the lower leg of healthy pre-pubertal females and males. J Musculoskelet Neuronal Interact 8:239–243
32. Lin CL, Moniz C, Chambers TJ, Chow JW (1996) Colitis causes bone loss in rats through suppression of bone formation. Gastroenterology 111:1263–1271
33. Hamdani G, Gabet Y, Rachmilewitz D, Karmeli F, Bab I, Dresner-Pollak R (2008) Dextran sodium sulfate-induced colitis causes rapid bone loss in mice. Bone 43:945–950
34. Parfitt AM (1993) Morphometry of bone resorption: introduction and overview. Bone 14:435–441
35. Semeao EJ, Stallings VA, Peck SN, Piccoli DA (1997) Vertebral compression fractures in pediatric patients with Crohn's disease. Gastroenterology 112:1710–1713
36. Klaus J, Armbrecht G, Steinkamp M, Bruckel J, Rieber A, Adler G, Reinshagen M, Felsenberg D, von Tirpitz C (2002) High prevalence of osteoporotic vertebral fractures in patients with Crohn's disease. Gut 51:654–658
37. Stockbrugger RW, Schoon EJ, Bollani S, Mills PR, Israeli E, Landgraf L, Felsenberg D, Ljunghall S, Nygard G, Persson T, Graffner H, Bianchi Porro G, Ferguson A (2002) Discordance between the degree of osteopenia and the prevalence of spontaneous vertebral fractures in Crohn's disease. Aliment Pharmacol Ther 16:1519–1527