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Bone Vol. 26, No. 2
February 2000:103–109

ORIGINAL ARTICLES

Normative Data for Iliac Bone Histomorphometry in Growing Children

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Many insights into normal and pathologic bone development can only be gained by bone histomorphometry. However, the use of this technique in pediatrics has so far been hampered by the lack of reference data. Therefore, we obtained transfixing iliac bone samples from 58 individuals between 1.5 and 22.9 years of age (25 male; tetracycline labeling performed in 48 subjects), who underwent surgery for reasons independent of abnormalities in bone development and metabolism. The results of histomorphometric analyses of cancellous parameters and cortical width are presented as means and standard deviations, as well as medians and ranges in five age groups. In addition, the original data are available from the authors. There were significant age-dependent increases in both cortical width and cancellous bone volume, the latter being due to an increase in trabecular thickness. Osteoid thickness did not vary significantly with age. Bone surface-based indicators of bone formation showed an age-dependent decline, reflecting similar changes in activation frequency. Mineral apposition rate decreased continuously with age. Parameters of bone resorption did not vary significantly between age groups. Paired biopsies from adjacent sites, obtained in eight subjects, were used to examine the reproducibility of histomorphometric parameters in children. The lowest coefficients of variation (<10%) were found for structural measures, as well as mineral apposition rate and wall thickness. The highest variability was found for cellular parameters. The availability of reference material will greatly facilitate the use of histomorphometry in pediatrics. (Bone 26: 103–109; 2000)

Key Words: Bone metabolism; Children; Growth; Histology; Histomorphometry; Reference data.

Introduction

Despite the invasive character of the procedure, the inclusion of a bone biopsy in protocols aiming at the diagnosis and therapeutic follow-up of pediatric metabolic bone disease is progressively gaining ground. Because in the growing skeleton modeling and remodeling activities are high and likely change with age, it is critical to compare quantitative data obtained in a given patient with those from a proper control group. A number of detailed histomorphometric normative data pertaining to the mature and aging skeleton have been published during the last 20 years,[6,7,10,14,19,21] but data in children are scarce.

Several studies have presented some histomorphometric values of children without metabolic bone disease.[1,18,20] Yet, in most cases, prior tetracycline labeling was not performed and therefore only static parameters could be measured. A larger collection of about 30 tetracycline labeled biopsies from healthy children between 2.5 and 18 years has served as a control group in various studies on pediatric bone diseases.[5,15–17] However, no reports on age changes in individual histomorphometric parameters during growth are available at present.

We thus undertook to harvest tetracycline-labeled transfixing iliac bone samples from a large group of children, who underwent surgery for reasons independent of abnormalities in bone development and metabolism. Our aim was to establish normative data for static and dynamic parameters of bone histomorphometry in discrete age groups spanning the whole growth period. The present report presents these normative data for use by others in the field. A detailed analysis of the implications of our results for bone physiology during growth will be published elsewhere.

Materials and Methods

Subjects

The study population comprised 58 healthy white subjects (25 males; age 1.5–22.9 years), in whom bone biopsies were obtained during surgery for various orthopedic conditions. In eight of these individuals, two adjacent bone cores were obtained. All subjects were ambulatory, had normal renal function as assessed by measurement of serum creatinine, and had no evidence of any metabolic bone disease. Orthopedic conditions included lower limb deformities (n = 14), scoliosis (n = 24), clubfeet (n = 4), and other problems that require corrective surgery (exostoses, cubitus valgus, equinovarus of the foot) (n = 16). None was immobilized prior to surgery or received medications known to affect bone metabolism. Originally, 62 subjects had been studied, 4 of whom had to be excluded from the present analysis for the following reasons (n = 1 each): unexplained low serum calcium level at the time of biopsy; lumbar spine bone mineral density below the reference range; crushed biopsy core; presence of growth plate cartilage (as bone

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8756-3282/00/$20.00
PII S8756-3282(99)00257-4

turnover is focally increased in bone adjacent to the growth plate). The study cohort was selected so that the ages were reasonably evenly distributed. It was not a major goal of this study to assess gender-specific differences. Therefore, there was an uneven gender distribution among age groups.

Informed consent was obtained in each instance from the subject and/or a legal guardian. The study protocol was approved by the relevant ethics committees (Shriners Hospital and DuPont Institute).

Bone Biopsy

Full-thickness transiliac bone biopsies were obtained with a Bordier trephine (5–7 mm core diameter) under general anesthesia, from a site located 2 cm below and behind the anterior superior iliac spine. In 48 subjects, biopsies were collected on days 4 or 5 after dual labeling with demeclocycline (15–20 mg/kg per day taken orally during two 2 day periods separated by a 10-day-free interval). Complete biopsy cores containing both cortices could be obtained in all but one case. No side effects of this procedure were noted other than transient local discomfort.

Biopsy specimens were fixed in 10% phosphate-buffered formalin (pH 7.1) and kept at room temperature for 48–72 h. They were then dehydrated in increasing concentrations of ethanol, cleared with xylene, and embedded in methylmethacrylate. After polymerization, the blocks were trimmed with an Isomet diamond saw (Buehler, Lake Bluff, IL) to remove excess plastic. Undecalcified 6 μm-thick sections were cut with a Polycut E microtome (Reichert-Jung, Heidelberg, Germany), placed on chromium-alum gelatine-coated slides and flattened to dry at 50°C for 18 h. For each specimen, two to five series of consecutive sections were cut at least 150 μm apart. The sections were deplastified with ethylene glycol monoethylacetate to allow for optimal staining. In each series, three consecutive sections were selected. Two were stained with either toluidine blue (pH 3.7) or Masson Goldner Trichrome, and the third mounted unstained for fluorescence microscopy.

Histomorphometry

All parameters were determined in at least two sections of a biopsy to obtain a measurable tissue area of 40–50 mm^2. Cortical width was directly measured at 500 μm intervals as the distance between the periosteal surface and the endocortical surface of each cortex. For the determination of cancellous parameters, the entire cancellous tissue area was analyzed, including the transitional zone.[4] However, the endocortical perimeter was excluded, because, in growing individuals, cellular activity clearly differs between endocortical and cancellous surfaces, due to the presence of modeling drifts (unpublished observation).

Mineralized bone was defined as a green (with Goldner stain) structure containing osteocytes. Osteoid was counted as red-staining seams of at least 1.5 μm in width at the bone-bone marrow interface. Eroded perimeter was quantified in toluidine-stained sections and was defined as scalloped or ragged appearance of the bone-bone marrow interface with or without the presence of osteoclasts. This included also shallow excavations, which were identified by the presence of eroded lamellae at the bone surface. Wall width was measured on Goldner-stained sections under polarized light as the distance from quiescent bone surfaces to the abrupt change in collagen fiber orientation. Measurements of cells were performed using toluidine-stained sections. Active osteoblasts were defined as cells directly apposed to osteoid and exhibiting a definite Golgi apparatus. Osteoclasts were multinucleated cells in close vicinity to an eroded surface. No specific osteoclast staining was performed.

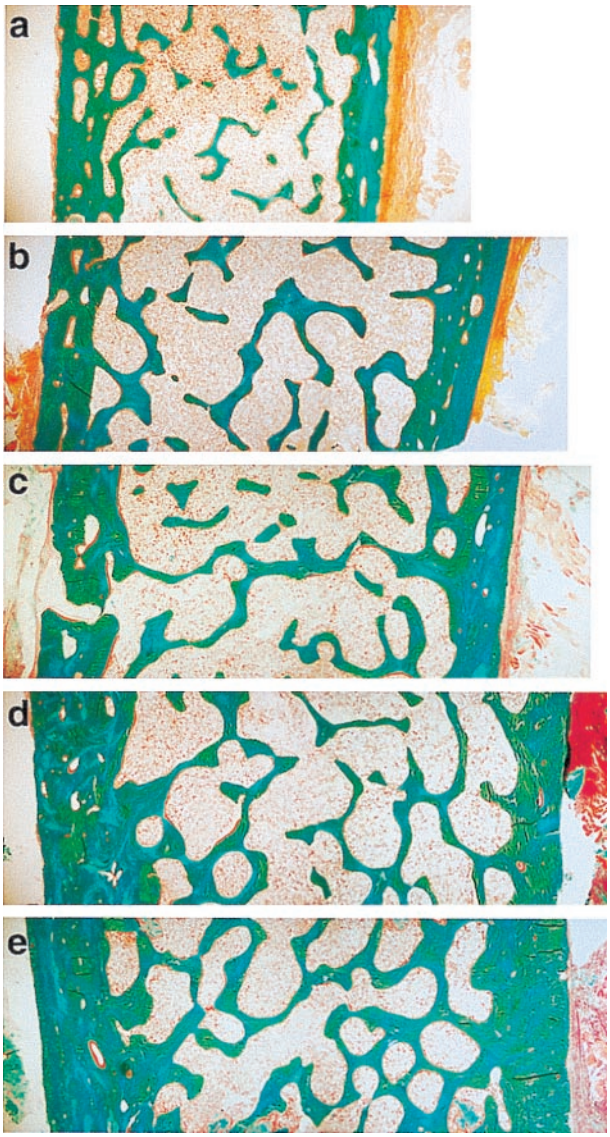

Figure 1. Representative sections of transfixing iliac biopsies from each age group. (A) Female, 2 years; (B) male, 9 years; (C) female, 13 years; (D) female, 16 years; (E) female, 21 years.

The following primary measures were obtained in cancellous bone: tissue area; bone area; bone perimeter; osteoid area; osteoid perimeter; eroded perimeter; wall width (measured at 50 μm intervals); osteoblast perimeter; osteoclast perimeter; osteoclast number; double-label perimeter; interlabel distance (taken at 50 μm intervals along the entire extent of the double label); and single-label perimeter. All parameters were derived from these primary measures using standard formulas (see **Table A1** in Appendix).[10,12]

Cortical width was determined at a magnification of ×32. All other measurements were carried out at a magnification of ×200. All analyses were performed using a digitizing table with OsTEOMEASURE software (Osteometrics, Atlanta, GA). Nomenclature and abbreviations followed the recommendations of the American Society for Bone and Mineral Research.[10]

Statistical Analyses

The 58 study participants were separated into five age groups: 1.5–6.9 years (n = 10, male = 6, labeled = 10); 7.0–10.9 years

Table 1. Structural histomorphometric parameters from 1.5 to 23 years in five age groups (n = 58)

	All	1.5–6.9 yr	7.0–10.9 yr	11.0–13.9 yr	14.0–16.9 yr	17.0–22.9 yr	*p*
n (M/F)	58 (25/33)	10 (6/4)	10 (8/2)	14 (2/12)	12 (4/8)	12 (5/7)	
Ct.Wi	0.96 ± 0.35	0.70 ± 0.28	0.97 ± 0.37	0.90 ± 0.33	1.18 ± 0.35	1.01 ± 0.20	0.01
(mm)	0.91 (0.35–1.81)	0.65 (0.35–1.33)	0.88 (0.61–1.81)	0.85 (0.45–1.57)	1.15 (0.76–1.81)	1.08 (0.63–1.30)	0.01
BV/TV	23.9 ± 5.3	17.7 ± 2.6	22.4 ± 4.2	24.4 ± 4.3	25.7 ± 5.3	27.8 ± 4.5	<0.0001
(%)	24.0 (13.5–35.5)	18.2 (13.5–22.9)	22.6 (16.5–29.7)	24.0 (18.0–32.0)	24.9 (19.5–35.5)	27.4 (18.9–34.7)	0.0001
Md.V/TV	23.3 ± 5.4	17.0 ± 2.6	21.8 ± 4.0	23.9 ± 4.2	25.2 ± 5.4	27.4 ± 4.6	<0.0001
(%)	23.3 (12.9–35.1)	17.4 (12.9–22.4)	21.9 (16.2–38.4)	23.3 (17.5–31.5)	24.4 (18.8–35.1)	27.1 (18.3–34.4)	<0.0001
Tb.Th	139 ± 28	101 ± 11	129 ± 17	148 ± 23	157 ± 22	153 ± 24	<0.0001
(μm)	141 (87–193)	101 (87–115)	129 (96–154)	148 (102–193)	151 (129–188)	156 (111–192)	<0.0001
Tb.N	1.72 ± 0.23	1.77 ± 0.31	1.73 ± 0.17	1.66 ± 0.22	1.63 ± 0.16	1.83 ± 0.27	0.22
(/mm)	1.72 (1.17–2.40)	1.83 (1.17–2.13)	1.74 (1.49–2.11)	1.64 (1.26–2.14)	1.62 (1.39–1.93)	1.84 (1.43–2.40)	0.20
Tb.Sp	452 ± 82	481 ± 112	453 ± 62	464 ± 78	461 ± 70	404 ± 77	0.21
(μm)	450 (306–737)	458 (379–737)	441 (334–544)	462 (318–651)	467 (335–545)	384 (306–566)	0.22
BS/BV	15.9 ± 3.5	21.2 ± 2.3	16.8 ± 2.5	14.7 ± 2.4	13.8 ± 1.9	14.2 ± 2.5	<0.0001
(mm^2/mm^3)	15.1 (11.0–24.3)	21.1 (18.5–24.3)	16.5 (13.7–22.1)	14.4 (11.0–20.8)	14.0 (11.3–16.6)	13.6 (11.0–19.2)	<0.0001
BS/TV	3.65 ± 0.50	3.75 ± 0.65	3.67 ± 0.36	3.52 ± 0.46	3.47 ± 0.35	3.88 ± 0.58	0.24
(mm^2/mm^3)	3.63 (2.49–5.09)	3.87 (2.49–4.51)	3.68 (3.16–4.47)	3.48 (2.67–4.55)	3.44 (2.96–4.13)	3.90 (3.03–5.09)	0.20

Values are mean ± SD in the upper row of each parameter and median (range) in the lower row. *p* values calculated by ANOVA for data in upper row and by Kruskal–Wallis test in lower row. Refer to Appendix for abbreviations.

(n = 10, male = 8, labeled = 8); 11.0–13.9 years (n = 14, male = 2, labeled = 12); 14.0–16.9 years (n = 12, male = 4, labeled = 10); and 17.0–22.9 years (n = 12, male = 5, labeled = 8). In subjects with two biopsies, the mean of the two measurements was used for subsequent calculations.

Means and standard deviations were calculated in each subgroup. For each parameter differences between subgroups were tested for significance using analysis of variance (ANOVA). Because not all histomorphometric parameters were normally distributed, median and ranges are also given. Age group differences for

Table 2. Static histomorphometric parameters of bone formation from 1.5 to 23 years in five age groups (n = 58)

	All	1.5–6.9 yr	7.0–10.9 yr	11.0–13.9 yr	14.0–16.9 yr	17.0–22.9 yr	*p*
O.Th	6.4 ± 1.4	5.8 ± 1.4	5.9 ± 1.1	6.7 ± 1.7	6.3 ± 1.0	6.9 ± 1.2	0.25
(μm)	6.1 (3.9–10.0)	5.8 (3.9–7.9)	5.9 (3.9–7.5)	6.2 (4.4–10.0)	6.0 (4.4–8.4)	6.6 (5.4–8.9)	0.38
OS/BS	24.9 ± 10.0	34.0 ± 6.7	29.1 ± 12.9	22.1 ± 7.8	25.7 ± 8.0	16.5 ± 5.4	0.0001
(%)	22.5 (4.9–54.3)	34.2 (23.6–46.7)	25.7 (12.8–54.3)	20.5 (12.8–36.5)	26.2 (17.6–46.3)	16.9 (4.9–23.9)	0.0002
OS/TV	0.91 ± 0.39	1.26 ± 0.28	1.08 ± 0.57	0.77 ± 0.29	0.89 ± 0.25	0.63 ± 0.20	0.0004
(mm^2/mm^3)	0.80 (0.19–2.43)	1.26 (0.91–1.89)	0.91 (0.46–2.43)	0.73 (0.47–1.44)	0.83 (0.54–1.45)	0.65 (0.19–1.03)	0.0002
OV/BV	2.42 ± 1.22	3.97 ± 1.19	2.64 ± 1.04	2.12 ± 1.00	2.18 ± 0.93	1.57 ± 0.67	<0.0001
(%)	2.26 (0.41–4.15)	4.09 (2.25–6.15)	2.51 (1.55–4.40)	2.04 (0.79–4.13)	2.14 (0.96–3.97)	1.54 (0.41–3.05)	0.0004
OV/TV	0.55 ± 0.25	0.70 ± 0.22	0.61 ± 0.32	0.52 ± 0.28	0.53 ± 0.17	0.42 ± 0.13	0.09
(%)	0.51 (0.12–1.31)	0.67 (0.45–1.14)	0.53 (0.27–1.31)	0.48 (0.21–1.10)	0.52 (0.25–0.88)	0.41 (0.12–0.60)	0.07
Ob.S/BS	7.2 ± 4.1	8.5 ± 4.1	8.2 ± 4.4	6.7 ± 4.5	7.9 ± 4.1	5.3 ± 2.7	0.30
(%)	6.4 (1.0–17.0)	8.4 (2.4–17.0)	9.0 (1.2–13.4)	5.8 (1.8–16.3)	7.0 (2.3–14.8)	5.0 (1.0–11.1)	0.28
Ob.S/OS	29.3 ± 12.7	25.7 ± 13.7	28.8 ± 15.1	28.9 ± 13.0	30.5 ± 11.6	32.0 ± 11.8	0.84
(%)	29.6 (5.9–55.6)	23.3 (8.8–54.5)	24.5 (5.9–55.6)	32.4 (11.0–48.4)	29.4 (12.5–48.4)	31.2 (14.5–48.9)	0.80
Ob.S/TV	0.26 ± 0.15	0.32 ± 0.15	0.31 ± 0.18	0.24 ± 0.17	0.27 ± 0.14	0.20 ± 0.08	0.32
(mm^2/mm^3)	0.24 (0.04–0.60)	0.33 (0.10–0.59)	0.32 (0.05–0.60)	0.21 (0.06–0.57)	0.24 (0.08–0.50)	0.21 (0.04–0.34)	0.34
W.Th	41.4 ± 5.7	33.9 ± 3.8	40.6 ± 3.0	45.1 ± 6.9	44.4 ± 3.2	41.1 ± 2.5	<0.0001
(μm)	41.5 (29.1–59.8)	33.1 (29.1–41.9)	41.2 (36.3–46.0)	45.2 (33.4–59.8)	43.4 (40.2–49.8)	40.9 (37.1–45.2)	<0.0001

Values are mean ± SD in the upper row of each parameter and median (range) in the lower row. *p* values calculated by ANOVA for data in upper row and by Kruskal–Wallis test in lower row.

Table 3. Dynamic histomorphometric parameters of bone formation from 1.5 to 23 years in five age groups (n = 48)

	All	1.5–6.9 yr	7.0–10.9 yr	11.0–13.9 yr	14.0–16.9 yr	17.0–22.9 yr	*p*
n (M/F)	48 (18/30)	10 (6/4)	8 (6/2)	12 (1/11)	10 (2/8)	8 (3/5)	
MS/BS	11.9 ± 4.5	12.5 ± 4.5	14.9 ± 4.5	11.7 ± 5.0	12.5 ± 3.4	7.9 ± 2.7	0.03
(%)	11.9 (3.3–22.1)	12.6 (6.3–20.2)	13.6 (8.1–20.7)	11.0 (5.2–22.1)	12.0 (7.9–19.2)	8.3 (3.3–12.4)	0.02
MS/OS	50.0 ± 15.6	37.7 ± 12.7	50.2 ± 21.6	53.3 ± 11.7	51.9 ± 13.7	57.9 ± 13.8	0.05
(%)	48.0 (15.2–98.1)	40.1 (15.2–57.7)	45.0 (27.7–98.1)	52.9 (36.8–78.0)	51.5 (35.9–68.2)	59.2 (39.9–74.9)	0.05
MAR	0.89 ± 0.14	1.04 ± 0.17	0.95 ± 0.07	0.87 ± 0.09	0.81 ± 0.09	0.75 ± 0.09	<0.0001
(μm/d)	0.88 (0.57–1.44)	1.02 (0.85–1.44)	0.97 (0.83–1.02)	0.90 (0.73–1.03)	0.83 (0.62–0.93)	0.77 (0.57–0.86)	<0.0001
Aj.AR	0.44 ± 0.13	0.40 ± 0.16	0.47 ± 0.18	0.46 ± 0.10	0.42 ± 0.11	0.43 ± 0.12	0.72
(μm/d)	0.42 (0.13–0.86)	0.42 (0.13–0.61)	0.42 (0.27–0.86)	0.49 (0.28–0.59)	0.39 (0.26–0.62)	0.39 (0.31–0.59)	0.84
Mlt	15.5 ± 4.8	16.7 ± 6.4	14.1 ± 4.3	14.5 ± 3.0	15.3 ± 3.6	17.3 ± 6.5	0.58
(d)	15.1 (8.7–29.1)	13.4 (11.7–29.1)	14.4 (8.7–22.2)	15.5 (10.0–17.9)	14.9 (10.5–22.6)	17.9 (9.3–28.6)	0.75
Omt	7.3 ± 1.9	5.7 ± 1.3	6.5 ± 1.0	7.6 ± 1.8	7.6 ± 1.2	9.4 ± 2.3	0.0002
(d)	6.9 (4.4–11.9)	5.1 (4.4–8.3)	6.3 (5.4–8.6)	7.1 (5.3–10.7)	7.9 (5.3–9.3)	9.3 (6.6–11.9)	0.001
BFR/BS	39.3 ± 17.5	48.1 ± 19.4	51.8 ± 16.1	37.3 ± 16.7	36.7 ± 10.4	22.2 ± 9.2	0.002
($\mu m^3/\mu m^2/y$)	35.5 (6.8–78.4)	48.6 (19.7–78.4)	49.3 (29.3–71.2)	33.8 (17.3–66.3)	34.7 (24.0–57.9)	23.2 (6.8–38.8)	0.004
BFR/BV	60.5 ± 34.8	97.1 ± 42.4	77.6 ± 26.6	49.9 ± 21.4	48.2 ± 18.5	28.9 ± 12.5	<0.0001
(%/y)	52.5 (8.2–161)	97.4 (35.5–161)	75.2 (38.0–111)	46.8 (25.3–96.0)	44.5 (26.8–87.4)	29.5 (8.2–51.4)	0.0002
BFR/TV	13.6 ± 6.7	17.3 ± 8.2	18.2 ± 6.7	12.4 ± 6.2	12.1 ± 3.5	8.2 ± 3.1	0.006
(%/y)	12.6 (2.4–30.0)	15.9 (5.8–29.9)	17.1 (10.3–30.0)	10.1 (6.4–25.4)	12.6 (6.8–17.1)	8.1 (2.4–12.7)	0.01
Ac.f	0.97 ± 0.47	1.40 ± 0.53	1.25 ± 0.37	0.83 ± 0.35	0.83 ± 0.27	0.54 ± 0.23	<0.0001
(/y)	0.86 (0.18–2.19)	1.37 (0.64–2.19)	1.24 (0.71–1.70)	0.75 (0.44–1.50)	0.81 (0.54–1.39)	0.53 (0.18–0.99)	0.0004
FP	105 ± 37	105 ± 18	99 ± 34	103 ± 28	114 ± 32	102 ± 27	0.92
(d)	98 (45–237)	87 (54–237)	98 (45–158)	100 (63–153)	116 (76–181)	106 (69–141)	0.67

Values are mean ± SD in the upper row of each parameter and median (range) in the lower row. *p* values calculated by ANOVA for data in upper row and by Kruskal–Wallis test in lower row.

these nonparametric data were evaluated using the Kruskal–Wallis test. Gender differences were evaluated by unpaired *t*-tests in each age group. The paired biopsies from eight individuals were used to calculate intrapair coefficients of variation (SD of the two measurements divided by the mean value) for each parameter.

Results

Figure 1 shows typical sections of biopsies from each age group. Tables 1–4 present mean and SD, as well as median and ranges of each parameter in the entire study population and in each age group.

Table 4. Static histomorphometric parameters of bone resorption from 1.5 to 23 years in five age groups (n = 58)

	All	1.5–6.9 yr	7.0–10.9 yr	11.0–13.9 yr	14.0–16.9 yr	17.0–22.9 yr	*p*
ES/BS	16.6 ± 5.6	14.8 ± 4.4	17.0 ± 6.0	14.9 ± 5.6	18.0 ± 5.7	18.0 ± 6.1	0.45
(%)	15.6 (8.5–32.5)	15.7 (9.2–22.3)	16.3 (8.6–28.7)	13.3 (8.5–30.1)	15.1 (12.6–32.5)	16.8 (10.1–31.2)	0.45
ES/TV	0.61 ± 0.23	0.57 ± 0.24	0.63 ± 0.24	0.52 ± 0.18	0.63 ± 0.23	0.71 ± 0.28	0.33
(mm^2/mm^3)	0.57 (0.29–1.19)	0.54 (0.32–1.01)	0.58 (0.31–1.07)	0.46 (0.29–0.95)	0.54 (0.38–1.16)	0.68 (0.31–1.19)	0.38
Oc.S/BS	1.09 ± 0.58	1.11 ± 0.75	1.29 ± 0.62	0.94 ± 0.38	1.14 ± 0.74	1.04 ± 0.41	0.68
(%)	0.96 (0.27–2.94)	0.81 (0.44–2.83)	1.50 (0.27–1.96)	0.93 (0.40–1.79)	1.03 (0.42–2.94)	0.94 (0.51–1.89)	0.65
Oc.S/TV	0.04 ± 0.02	0.04 ± 0.04	0.05 ± 0.03	0.03 ± 0.01	0.04 ± 0.03	0.04 ± 0.02	0.78
(mm^2/mm^3)	0.04 (0.01–0.13)	0.03 (0.02–0.13)	0.05 (0.01–0.09)	0.04 (0.01–0.06)	0.04 (0.01–0.11)	0.04 (0.02–0.08)	0.70
N.Oc/B.Pm	0.32 ± 0.17	0.35 ± 0.23	0.36 ± 0.16	0.29 ± 0.14	0.34 ± 0.22	0.31 ± 0.14	0.96
(mm/mm)	0.35 (0.09–0.83)	0.30 (0.14–0.83)	0.38 (0.09–0.57)	0.24 (0.13–0.65)	0.32 (0.13–0.87)	0.25 (0.15–0.60)	0.86
N.Oc/T.Ar	0.93 ± 0.54	1.02 ± 0.81	1.04 ± 0.50	0.80 ± 0.36	0.94 ± 0.63	0.92 ± 0.45	0.83
($/mm^2$)	0.85 (0.25–2.94)	0.73 (0.37–2.94)	1.09 (0.25–2.00)	0.79 (0.35–1.58)	0.83 (0.32–2.46)	0.81 (0.43–1.99)	0.79

Values are mean ± SD in the upper row of each parameter and median (range) in the lower row. *p* values calculated by ANOVA for data in upper row and by Kruskal–Wallis test in lower row.

Cortical width (Ct.Wi) was lowest in the youngest group and then appeared to remain stable (**Table 1**). In contrast, cancellous bone volume (BV/TV) and mineralized bone volume (Md.V/BV) showed a steady increase, which was mostly due to an increase in trabecular thickness (Tb.Th). The second determinant of bone volume, trabecular number (Tb.N), did not vary with age. The increase in trabecular thickness was also reflected by a decrease in the bone surface: volume ratio (BS/BV), whereas the bone surface:tissue volume ratio (BS/TV) remained unchanged.

Static parameters of bone formation are shown in **Table 2**. Osteoid thickness (O.Th) did not vary significantly with age, whereas the surface extent of osteoid (OS/BS) decreased steadily. Consequently, there was a decrease in osteoid volume relative to bone volume (OV/BV). Osteoblast parameters were very variable and therefore the differences between age groups did not reach statistical significance. Wall thickness (W.Th) depended on age, with the highest values between 11 and 16.9 years.

Dynamic parameters of bone formation are given in **Table 3**. The surface extent of mineralization (MS/BS) appeared to remain fairly constant, until a drop occurred in the oldest age group. Mineral apposition rate (MAR) decreased with age, but adjusted apposition rate (Aj.AR) remained constant. Bone formation rate (BFR) exhibited a significant age dependency regardless of the referent (Table 3b). Activation frequency (Ac.f) varied with age, but formation period (FP) was similar in all age groups. None of the parameters of bone resorption showed a significant age dependency (**Table 4**).

Significant differences between the genders were found in the 14.0–16.9 year group for osteoid surface extent (OS/BS; boys 33.3 ± 6.8%, girls 21.8 ± 4.1%; $p = 0.01$), relative osteoid volume (OV/BV; boys 3.09 ± 0.82%, girls 1.72 ± 0.60%; $p = 0.008$), and osteoblast surface extent (Ob.S/BS; boys 12.5 ± 3.5%, girls 5.6 ± 1.8%; $p = 0.0009$). In the 17.0–22.9 year group, a significant gender difference was detected only for Ob.S/BS (boys 7.4 ± 2.3%, girls 3.8 ± 1.7%; $p = 0.01$) and Ob.S/OS (boys 39.7 ± 8.5%, girls 26.4 ± 11.0%; $p = 0.048$). All other comparisons between the genders in each age group yielded nonsignificant differences.

To give an indication of the variability of histomorphometric data, we separately evaluated paired biopsies of adjacent locations, which were obtained in eight individuals (aged 11.7–20.2 years; male = 3; labeled = 6). The mean intrapair coefficients of variation of the individual parameters are shown in **Table 5**. Structural parameters showed a relatively small variability, whereas variations were highest for cellular parameters. Reproducibility was best for two primary parameters of osteoblast team function, mineral apposition rate, and wall thickness.

Table 5. Variability of histomorphometric parameters—intrapair coefficients of variation (cv) calculated from results in two adjacent biopsies from eight individuals (after previous tetracycline labeling in six subjects)

Parameter	cv (%)	Parameter	cv (%)
Structural		Dynamic formation	
Ct.Wi	9.8	MS/BS	22.8
BV/TV	8.4	MS/OS	10.9
Md.V/TV	8.4	MAR	4.2
Tb.Th	7.2	Aj.AR	7.9
Tb.N	5.6	Mlt	8.5
BS/BV	7.2	Omt	10.1
BS/TV	5.6	BFR/BS	19.6
		BFR/BV	22.1
Static formation		BFR/TV	21.7
O.Th	11.5	Ac.f	20.5
OS/BS	13.5	FP	8.5
OS/TV	17.1		
OV/BV	20.6	Resorption	
OV/TV	17.7	ES/BS	12.6
Ob.S/BS	21.5	ES/TV	15.2
Ob.S/OS	20.3	Oc.S/BS	22.7
Ob.S/TV	23.0	Oc.S/TV	29.7
W.Th	5.1	N.Oc/B.Pm	19.1
		N.Oc/T.Ar	22.8

Discussion

Although indirect methods to evaluate bone mass and metabolism have gained widespread popularity in recent years, many insights into normal and pathologic bone development can only be gained by bone histomorphometry. However, the use of this technique in children has so far been hampered by the lack of reference data. In the present study, we try to fill this gap by providing histomorphometric results of iliac crest biopsies that were obtained from children and adolescents without metabolic bone disease.

It is important to realize that these results can only be used for comparisons if the same methods are used as in the present study. Some parameters are more likely to depend on methodology than others. Measures of bone structure are probably least influenced by different staining and handling procedures. Indeed, our results on structural parameters are broadly similar to reference material used by others.[5,15–17]

Osteoid indices are generally higher in Goldner-stained sections (as used in this study) than in toluidine-stained sections.[7] The results for osteoid thickness and osteoid surface extent additionally depend on the cutoff threshold for osteoid width. In the present study, all parts of an osteoid seam with a width of >1.5 μm were measured. If a higher cutoff is used, then higher results for osteoid thickness and lower values for osteoid surface extent will be obtained.[13] This is exactly the pattern of differences between the present study and reference data contained in other reports, where the cutoff value for osteoid thickness was not specified.[5,15–17]

Wall thickness depends on the type of staining, and whether the cement line or the abrupt change in collagen fiber orientation is used to define a wall.[8] Cellular parameters depend on the degree of cellular preservation, which is influenced by sample fixation and staining technique. Mineralizing surface and mineral apposition rate vary with the labeling substance, labeling schedule, and dosage used.[11] This is highlighted by the observation that the extent of tetracycline-labeled surfaces is higher in our study than the values reported by others, who used a lower tetracycline dosage.[5,15–17]

What is measured as erosion surface depends on what the observer feels is a scalloped surface, which is a rather subjective interpretation.[9] In the present study, "erosion surface" included very shallow excavations, which were detected by identifying eroded lamellae under polarized light. This may explain why our results for erosion surface are much higher than in other studies.[5,15–17]

The need for histomorphometric reference data may arise in different situations. To give this report maximum versatility, we chose to present the data in different ways: as mean and standard deviation, as median and range, and as raw data, which are available from the authors on request. These original data might be most useful for researchers who want to select age-matched controls for a given study population.

The primary aim of the study was to describe the variation with age rather than the elucidation of gender differences. Therefore, there was an uneven distribution of male and female individuals among the age groups, which may explain why few differences were detected. Gender differences were only found in the last two

age groups and might be the result of differences in the timing of puberty. However, larger sample numbers are necessary to evaluate these effects. We are unable to provide any data on pubertal changes, as, unfortunately, pubertal stages were not evaluated in the study participants.

Our results in paired biopsies from eight subjects may provide an approximate impression of the reproducibility of histomorphometric analyses in our study population. The observed values are the integrated variations due to differences in biopsy site, sample processing, and sample analysis. Overall, the variability of repeated measurements appears to be smaller in children than in adults.[2,3] This may be explained by the higher bone turnover in children, which reduces the sampling error for parameters of bone formation and resorption.

In summary, the present report establishes the first age-dependent histomorphometric reference data for children and adolescents. The availability of reference material will greatly facilitate the use of this important technique in pediatrics.

Acknowledgments: The authors thank Dr. R. Weinstein and Dr. R. Recker for their helpful comments in the earlier stages of this project. We also thank Guy Charette for technical assistance with sample processing and Mark Lepik for artwork. This study was supported by the Shriners of North America and by the Deutsche Forschungsgemeinschaft (Grant Ra 803/1-1).

Appendix

Table A1. Formulae used to calculate histomorphometric values from primary measurements

Abbreviation	Parameter	Unit	Formula
Structural			
Ct.Wi	Cortical width	mm	Direct measurement
BV/TV	Bone volume/tissue volume	%	(bone area/tissue area) × 100
Md.V/TV	Mineralized volume/tissue volume	%	(BV/TV) − (OV/TV)
Tb.Th	Trabecular thickness	μm	(bone area/bone perimeter) × 2/1.2
Tb.N	Trabecular number	/mm	(BV/TV)/Tb.Th × 10
Tb.Sp	Trabecular separation	μm	(1000/Tb.N) − Tb.Th
BS/BV	Bone surface/bone volume	mm^2/mm^3	(bone perimeter/bone area) × 1.2
BS/TV	Bone surface/tissue volume	mm^2/mm^3	(bone perimeter/tissue area) × 1.2
Static formation			
O.Th	Osteoid thickness	μm	(osteoid area/osteoid perimeter) × 2/1.2
OS/BS	Osteoid surface/bone surface	%	(osteoid perimeter/bone perimeter) × 100
OS/TV	Osteoid surface/tissue volume	mm^2/mm^3	(osteoid perimeter/tissue area) × 1.2
OV/BV	Osteoid volume/bone volume	%	(osteoid area/bone area) × 100
OV/TV	Osteoid volume/tissue volume	%	(osteoid area/tissue area) × 100
Ob.S/BS	Osteoblast surface/bone surface	%	(osteoblast perimeter/bone perimeter) × 100
Ob.S/OS	Osteoblast surface/osteoid surface	%	(osteoblast perimeter/osteoid perimeter) × 100
Ob.S/TV	Osteoblast surface/tissue volume	mm^2/mm^3	(osteoblast perimeter/tissue area) × 1.2
W.Th	Wall thickness	μm	(distance between quiescent bone surface and change in lamellar direction)/1.2
Dynamic formation			
MS/BS	Mineralizing surface/bone surface	%	(perimeter double label + 1/2 perimeter single label)/bone perimeter × 100
MS/OS	Mineralizing surface/osteoid surface	%	(MS/BS)/(OS/BS)
MAR	Mineral apposition rate	μm/d	(distance between labels/marker interval)/1.2
Aj.AR	Adjusted apposition rate	μm/d	MAR × (MS/OS)/100
Mlt	Mineralization lag time	d	O.Th/Aj.AR
Omt	Osteoid maturation time	d	O.Th/MAR
BFR/BS	Bone formation rate/bone surface	$\mu m^3/\mu m^2/y$	MAR × (MS/BS) × 3.65
BFR/BV	Bone formation rate/bone volume	%/y	(BFR/BS) × (BS/BV)
BFR/TV	Bone formation rate/tissue volume	%/y	(BFR/BS) × (BS/TV)
Ac.F	Activation frequency	/y	(BFR/BS)/W.Th
FP	Formation period	d	W.Th/Aj.AR
Resorption			
ES/BS	Eroded surface/bone surface	%	(erosive perimeter/bone perimeter) × 100
ES/TV	Eroded surface/tissue volume	mm^2/mm^3	(erosive perimeter/tissue area) × 1.2
Oc.S/BS	Osteoclast surface/bone surface	%	(osteoclast perimeter/bone perimeter) × 100
Oc.S/TV	Osteoclast surface/tissue volume	mm^2/mm^3	(osteoclast perimeter/tissue area) × 1.2
N.Oc/B.Pm	Number of osteoclasts/bone perimeter	/mm	osteoclast number/bone perimeter
N.Oc/T.Ar	Number of osteoclasts/tissue area	$/mm^2$	osteoclast number/tissue area

References

1. Baron, R., Gertner, J. M., Lang, R., and Vignery, A. Increased bone turnover with decreased bone formation by osteoblasts in children with osteogenesis imperfecta tarda. Pediatr Res 17:204–207; 1983.
2. Chavassieux, P. M., Arlot, M. E., and Meunier, P. J. Intersample variation in bone histomorphometry: Comparison between parameter values measured on two contiguous transiliac bone biopsies. Calcif Tissue Int 37:345–350; 1985.
3. de Vernejoul, M. C., Kuntz, D., Miravet, L., Goutallier, D., and Ryckewaert, A. Bone histomorphometric reproducibility in normal patients. Calcif Tissue Int 33:369–374; 1981.
4. Keshawarz, N. M. and Recker, R. R. Expansion of the medullary cavity at the expense of cortex in postmenopausal osteoporosis. Metab Bone Dis Rel Res 5:223–228; 1984.
5. Klein, G. L., Herndon, D. N., Goodman, W. G., Langman, C. B., Phillipps, W. A., Dickson, I. R., Eastell, R., Naylor, K. E., Maloney, N. A., Desai, M., Benjamin, D., and Alfrey, A. C. Histomorphometric and biochemical characterization of bone following acute severe burns in children. Bone 17:455–460; 1995.
6. Malluche, H. H., Meyer, W., Sherman, D., and Massry, S. G. Quantitative bone histology in 84 normal American subjects. Micromorphometric analysis and evaluation of variance in iliac bone. Calcif Tissue Int 34:449–455; 1982.
7. Melsen, F., Melsen, B., Mosekilde, L., and Bergmann, S. Histomorphometric analysis of normal bone from the iliac crest. Acta Pathol Microbiol Scand A 86:70–81; 1978.
8. Parfitt, A. M. Bone-forming cells in clinical conditions. In: Hall, B. K., Ed. The Osteoblast and Osteocyte. Vol. 1. Caldwell, NJ: Telford; 1990; 351–429.
9. Parfitt, A. M. Morphometry of bone resorption: Introduction and overview. Bone 14:435–441; 1993.
10. Parfitt, A. M., Drezner, M. K., Glorieux, F. H., Kanis, J. A., Malluche, H., Meunier, P. J., Ott, S. M., and Recker, R. R. Bone histomorphometry: Standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 2:595–610; 1987.
11. Parfitt, A. M., Foldes, J., Villanueva, A. R., and Shih, M. S. Difference in label length between demethylchlortetracycline and oxytetracycline: Implications for the interpretation of bone histomorphometric data. Calcif Tissue Int 48:74–77; 1991.
12. Parfitt, A. M., Mathews, C. H., Villanueva, A. R., Kleerekoper, M., Frame, B., and Rao, D. S. Relationships between surface, volume, and thickness of iliac trabecular bone in aging and in osteoporosis. Implications for the microanatomic and cellular mechanisms of bone loss. J Clin Invest 72:1396–1409; 1983.
13. Recker, R. R., Kimmel, D. B., Parfitt, A. M., Davies, K. M., Keshawarz, N., and Hinders, S. Static and tetracycline-based bone histomorphometric data from 34 normal postmenopausal females. J Bone Miner Res 3:133–144; 1988.
14. Rehman, M. T., Hoyland, J. A., Denton, J., and Freemont, A. J. Age related histomorphometric changes in bone in normal British men and women. J Clin Pathol 47:529–534; 1994.
15. Salusky, I. B., Coburn, J. W., Brill, J., Foley, J., Slatopolsky, E., Fine, R. N., and Goodman, W. G. Bone disease in pediatric patients undergoing dialysis with CAPD or CCPD. Kidney Int 33:975–982; 1988.
16. Salusky, I. B., Ramirez, J. A., Oppenheim, W., Gales, B., Segre, G. V., and Goodman, W. G. Biochemical markers of renal osteodystrophy in pediatric patients undergoing CAPD/CCPD. Kidney Int 45:253–258; 1994.
17. Sanchez, C. P., Salusky, I. B., Kuizon, B. D., Ramirez, J. A., Gales, B., Ettenger, R. B., and Goodman, W. G. Bone disease in children and adolescents undergoing successful renal transplantation. Kidney Int 53:1358–1364; 1998.
18. Schnitzler, C. M., Daniels, E. D., Mesquita, J. M., Moodley, G. P., Zachen, D., Cakic, J., and Pettifor, J. M. Bone disease in African children with slipped capital femoral epiphysis: Histomorphometry of iliac crest biopsies. Bone 22:259–265; 1998.
19. Schnitzler, C. M., Pettifor, J. M., Mesquita, J. M., Bird, M. D., Schnaid, E., and Smyth, A. E. Histomorphometry of iliac crest bone in 346 normal black and white South African adults. Bone Miner 10:183–199; 1990.
20. Schnitzler, C. M., Pettifor, J. M., Patel, D., Mesquita, J. M., Moodley, G. P., and Zachen, D. Metabolic bone disease in black teenagers with genu valgum or varum without radiologic rickets: A bone histomorphometric study. J Bone Miner Res 9:479–486; 1994.
21. Vedi, S., Compston, J. E., Webb, A., and Tighe, J. R. Histomorphometric analysis of bone biopsies from the iliac crest of normal British subjects. Metab Bone Dis Rel Res 4:231–236; 1982.

Date Received: June 24, 1999
Date Revised: October 25, 1999
Date Accepted: October 26, 1999