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Sex- and Age-Specific Reference Curves for Serum Markers of Bone Turnover in Healthy Children from 2 Months to 18 Years

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Introduction: This study aimed to establish sex- and age-specific reference curves enabling the calculation of z-scores and to examine correlations between bone markers and anthropometric data.

Methods: Morning blood samples were obtained from 572 healthy children and adolescents (300 boys) aged 2 months to 18 yr. Height, weight, and pubertal stage were recorded. Serum osteocalcin (OC), bone-specific alkaline phosphatase (BALP), type-1 collagen degradation markers [carboxyterminal telopeptide region of type I collagen (ICTP), carboxyterminal telopeptide α1 chain of type I collagen (CTX)], and tartrate-resistant acid phosphatase (TRAP5b) were measured. Cross-sectional centile charts were created for the 3rd, 50th, and 97th centiles.

Results: Apart from TRAP5b, all bone markers were nonnormally distributed, requiring logarithmic (BALP, OC, ICTP) or square root (CTX) transformation. Back-transformed centile curves for age and sex are presented for practical use. All bone markers varied with age and pubertal stage ($P < 0.001$). Significant correlations were found between SD score (SDS) for bone formation markers BALP and OC ($r = 0.13$; $P = 0.004$), SDS for collagen degradation markers ICTP and CTX ($r = 0.14$; $P = 0.002$), and SDS for the phosphatases ($r = 0.34$, $P < 0.001$). Height and weight SDS correlated weakly with some bone marker SDS, particularly with lnBALP SDS ($r = 0.20$ and 0.24, respectively; both $P < 0.001$).

Conclusion: This study provides reference curves for OC, BALP, CTX, ICTP, and TRAP5b in healthy children. Taller and heavier individuals for age had greater bone marker concentrations, likely reflecting greater growth velocity. SDS for markers of bone formation, collagen degradation, and phosphatases were each independently correlated, suggesting they derive from the same biological processes. The possibility of calculating SDS will facilitate monitoring of antiresorptive therapy or disease progression in children with metabolic bone disease. **(*J Clin Endocrinol Metab* 92: 443–449, 2007)**

NORMAL PEDIATRIC REFERENCE ranges for serum markers of bone formation and resorption are a prerequisite for the assessment of metabolic bone disorders and for the monitoring of antiresorptive therapy or disease progression. In adults, bone turnover markers mainly represent bone remodeling and are commonly used as independent predictors of the risk of osteoporosis and fractures (1, 2), to monitor antiresorptive therapy (3–5) and also have a promising role in metastatic bone disease (6). In children, these markers are released into the circulation during the processes of bone remodeling, modeling, and growth in length. Aside from the remodeling process, osteoclast and osteoblast actions are not coupled during modeling or epiphyseal growth, thus introducing additional variability and reducing specificity. Skeletal growth and puberty lead to considerable changes in raw levels of bone formation and resorption markers with age, demonstrated by their correlation with growth velocity (7–11). Thus, any longitudinal measurement in a patient necessitates comparison relative to the physiologically changing reference curves.

Traditionally, markers of bone turnover have been measured in urine, which is useful and accurate in children old and healthy enough to carry out the instructions for obtaining a second void fasting urine (12, 13). In infants and children, the practical difficulties associated with serial urine collection are compounded by marked circadian and intraindividual variation in urinary markers (9, 14, 15) and by the necessity of expressing their concentration relative to creatinine (10, 16), itself subject to considerable biological variation and change with age as muscle mass increases (10, 17). Hence, the measurement of bone markers in serum is preferred (7, 9, 10, 18). Commonly used serum markers of bone formation are osteocalcin (OC) and bone-specific alkaline phosphatase (BALP), which are released at different stages of osteoblast proliferation and differentiation (19). Among others, commonly used markers of bone resorption are the carboxyterminal telopeptide region of type I collagen (ICTP), the carboxyterminal telopeptide α1 chain of type I collagen (CTX), and serum tartrate-resistant acid phosphatase 5b (TRAP5b).

Establishing pediatric reference ranges for bone markers and assessing their relation to sex, age, and anthropometric data requires a large population of healthy children. Previous normative studies partly suffered from low subject numbers. In addition, the routine use of these data has also been hindered by the lack of applying appropriate curve-fitting pro-

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Abbreviations: BALP, Bone-specific alkaline phosphatase; BMI, body mass index; CTX, carboxyterminal telopeptide α1 chain of type I collagen; CV, coefficient of variation; ICTP, carboxyterminal telopeptide region of type I collagen; OC, osteocalcin; SDS, SD score; TRAP5b, serum tartrate-resistant acid phosphatase 5b.

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cedures. Curve-fitting is essential because of the frequently skewed distribution of bone marker data, the age-related changes that occur within individual age groups and the different variation between age groups. These changes complicate the interpretation of longitudinal results and the monitoring of children in intervention studies.

The aims of this study were: 1) to establish sex- and age-specific reference equations for OC, BALP, ICTP, CTX, and TRAP5b in healthy children and adolescents, enabling calculation of SD-scores; 2) to present back-transformed normative curves for easy practical use; and 3) to test the correlation among markers of bone formation and resorption and their relation to anthropometric data.

Subjects and Methods

Between 2001 and 2005, healthy Caucasian children and adolescents (aged 2 months to 18 yr) seen as inpatients or outpatients for routine or preoperative investigations or for minor conditions or infections requiring blood sampling were recruited for this study. Results of neonates have been published previously (20). Children with any disease likely to affect bone metabolism, including disorders of vitamin D, parathyroid, growth or thyroid hormone, renal impairment, a history of recent fracture or burns, malnutrition, or dehydration, diabetes mellitus, or any chronic disease were not included in the study. In addition, children requiring treatment with corticosteroids or anticonvulsants, with immobility, muscular or severe neurological diseases, with identifiable genetic syndromes, major congenital malformations, or cancer were not included. The study protocol was approved by the local ethics committee and informed consent was obtained from children or their parents.

Blood samples were obtained in all children fulfilling the inclusion criteria between 0800 and 1000 h to avoid any bias from diurnal variation (14, 15, 21). Serum markers of bone formation (intact OC and BALP as exclusive markers of osteoblasts) and bone resorption (ICTP, CTX, and TRAP5b) were measured. Each sample of whole blood (1 ml) was centrifuged to obtain serum, which was aliquoted and immediately frozen at −80 C within 1 h of sampling, and then stored until the assays were run. All samples were analyzed in duplicate concurrently.

Anthropometry

Anthropometric data obtained from the record were height and weight, which were measured using a wall-mounted stadiometer and a calibrated weight scale, respectively, wearing underwear only. Body mass index (BMI) was calculated by using the formula: BMI = weight (kg)/height $(m)^2$. Age- and sex-specific SD scores (SDS) for height, weight, and BMI were calculated according to German reference data (22). Pubertal stages were assessed according to Tanner (23).

Biochemical markers of bone formation

Intact OC assay. OC is a noncollagenous protein produced by osteoblasts during the matrix mineralization phase. Found exclusively in mineralizing tissues, OC provides a close reflection of bone formation. OC was measured by a two-site immunoradiometric assay (Active Human Osteocalcin IRMA; Diagnostic Systems Laboratories, Sinsheim, Germany). Intraassay and interassay coefficients of variation (CVs) were 1.4–3.4% and 3.3–5.3%, respectively.

BALP assay. BALP is a synthetic product of osteoblasts involved in the process of osteoid mineralization. Bone and liver isoenzymes of alkaline phosphatase are products of a single gene and differ only as a result of posttranslational glycosylation. Current immunoassays for BALP possess a low cross-reactivity with the circulating liver isoenzyme. Serum BALP levels were measured by a solid-phase, two-site immunoradiometric assay (Tandem-R-Ostase; Hybritech Inc., San Diego, CA) based on two monoclonal antibodies. The intraassay and interassay CVs were 3.7–6.7% and 7.0–8.1%, respectively.

Biochemical markers of bone resorption

ICTP assay. ICTP was measured by RIA (Type I Telopeptide ICTP RIA kit; Orion Diagnostica, Espoo, Finland). The assay detects the C-terminal telopeptides of two α_1(I) chains in a type I collagen molecule cross-linked with the helical domain of another collagen chain (24). The ICTP molecule is released during collagen degradation by matrix metallopeptidases. The intraassay CV was 2.8–6.2% and the interassay CV was 4.1–7.9%. Samples were diluted 1:2 with 154 mmol/liter sodium chloride to achieve concentrations within the calibration curve.

CTX assay. Serum CTX was measured by ELISA (CrossLaps One Step ELISA; Osteometer Biotech, Herlev, Denmark). This assay uses a polyclonal antiserum raised against an immobilized synthetic peptide with an amino acid sequence (EKAHDGGR) specific for part of the C-terminal telopeptide of the α_1(I) collagen chain, where the aspartic acid residue (D) is β-isomerized (βCTX). The peptide sequence for CTX is shorter than that of ICTP. Intra- and interassay CVs were 5.0–5.4% and 5.4–8.1%, respectively.

TRAP5b assay. As an enzyme of osteoclasts, TRAP5b is involved in bone matrix degradation. The enzyme is released into the circulation during the resorption process itself or after detachment of the osteoclast from the bone surface and later degraded to fragments. In this study, TRAP5b activity was measured in surplus serum of 147 children (83 males and 64 females) using the Bone TRAP Assay (Medac, Hamburg, Germany). Intraassay and interassay CVs were 4.7–13.9% and 5.8–13.9%, respectively.

Data analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (version 12.0; SPSS Inc., Chicago, IL). Bone marker concentrations were tested for their normal distribution. Logarithmic or square root transformations were applied as needed to achieve a distribution as close as possible to normal. To create cross-sectional centile curves, we applied a model that uses the absolute residuals of the dependent variable because the SD varies with growth-related data (25). As an example, the procedure for the lnBALP/age centile curve involved the following: First, the mean curve (50th centile) was modeled by regression analysis. Thereafter, absolute residuals of lnBALP were regressed against age and the statistically best-fitting equation was obtained. The specific SD was obtained by multiplying this equation by $\sqrt{(\pi/2)}$. Finally, the 3rd and 97th centiles were derived using this SD estimate (50th centile ± 1.88 SD) (25). For easy practical use, we performed back transformation of the logarithmic/square root centile charts. To test the variation with sex and age, 2-yr age groups were derived from the original data set. Two-way ANOVA was performed with age groups and sex as categorical variables using post-hoc Bonferroni tests and *t* test for sex comparisons. Additional ANOVAs were run with Tanner stages and sex, with the rapidly growing infants (<3 yr) as a separate stage. Spearman's correlation was used to test correlations among SDS of bone markers and between SDS of bone markers and anthropometric data. The correlation among SDS was preferred over raw data to rule out age effects on the analysis. Data are presented as mean (SD) and changes at $P < 0.05$ were considered significant.

Results

A total of 572 children (300 boys) fulfilled the inclusion criteria for the study. Characteristics of the study population are presented in Table 1. In girls, mean SDS for height, weight, and BMI were 0.006 (1.016), −0.172 (1.026), and −0.293 (1.196), respectively. Corresponding values for boys were 0.056 (0.980), −0.101 (0.988) and −0.232 (1.116), respectively. The SDS were symmetrically distributed when plotted against age in both sexes.

Creation of centile charts

Mean and SD equations for all bone markers are given in Table 2. Apart from TRAP5b, all other bone markers showed

TABLE 1. Sex-specific height, weight, and BMI of all subjects, stratified by age group

Age (yr)	Boys				Girls			
	n	Height (cm)	Weight (kg)	BMI (kg/m²)	n	Height (cm)	Weight (kg)	BMI (kg/m²)
<1	11	70.91 ± 5.09	7.52 ± 1.19	14.88 ± 1.07	16	70.67 ± 5.61	7.76 ± 1.44	15.58 ± 1.41
1–2.99	51	87.50 ± 6.39	12.53 ± 2.21	16.31 ± 1.53	37	87.28 ± 5.91	11.60 ± 1.94	15.10 ± 1.67
3–4.99	46	105.22 ± 4.95	16.86 ± 2.25	15.20 ± 1.50	41	102.73 ± 5.67	16.10 ± 2.50	15.22 ± 1.78
5–6.99	40	118.02 ± 5.09	20.96 ± 2.85	14.86 ± 1.26	33	118.22 ± 5.79	20.88 ± 3.94	14.84 ± 1.90
7–8.99	32	131.08 ± 7.09	28.38 ± 5.64	16.43 ± 2.51	23	128.70 ± 5.98	26.81 ± 5.02	16.15 ± 2.70
9–10.99	19	139.74 ± 5.16	32.49 ± 6.97	16.56 ± 3.01	25	140.58 ± 7.27	34.61 ± 7.21	17.38 ± 2.65
11–12.99	23	150.07 ± 6.86	40.75 ± 9.60	17.97 ± 3.35	24	152.71 ± 9.15	42.36 ± 8.51	17.92 ± 2.68
13–14.99	33	164.69 ± 11.35	53.60 ± 12.01	19.54 ± 2.69	32	160.21 ± 7.82	52.26 ± 10.12	20.42 ± 3.63
15–16.99	31	174.69 ± 8.05	63.57 ± 11.60	20.74 ± 2.80	29	165.07 ± 5.33	56.46 ± 8.92	20.72 ± 3.06
>17	14	177.04 ± 6.25	69.13 ± 9.70	22.09 ± 3.32	12	162.58 ± 5.88	55.12 ± 7.86	21.00 ± 3.92

Values are expressed as means ± SD.

a skewed distribution with age and required logarithmic (OC, BALP, and ICTP) or square root (CTX) transformations. Sex- and age-specific reference curves showing the 3rd, 50th, and 97th centiles were created for lnOC, lnBALP, lnICTP, $\sqrt{}$CTX, and TRAP5b. The best fit for the 3rd, 50th, and 97th centiles was obtained by cubic equations for all bone markers. Equations for lnOC, lnBALP, lnICTP, and $\sqrt{}$CTX were then back-transformed to create smooth sex- and age-specific centile charts for use in clinical practice (Fig. 1, A and B).

Bone marker variation with age, sex, and Tanner stage

Significant variation with age was observed for all bone markers ($P < 0.001$). Sex was significant only for lnBALP ($P < 0.001$) and TRAP5b ($P = 0.002$), with higher concentrations observed in boys. In addition, there was a significant interaction between age and sex for lnICTP ($P < 0.001$). Serum levels of lnBALP were lower in children older than 15 yr compared with children younger than 15 yr ($P < 0.001$) and significantly greater in boys than girls over 13 yr ($P < 0.001$). LnICTP levels were lower in children older than 17 yr compared with all age groups younger than 15 yr ($P \leq 0.05$). Girls had greater lnICTP values than boys at age 11–13 yr ($P = 0.014$), while boys had greater values at age 13–15 yr ($P < 0.001$). In addition, infants less than 1 yr of age had significantly higher lnICTP values compared with children aged 1–13 yr and older than 15 yr ($P < 0.04$). Serum $\sqrt{}$CTX levels were lower in children older than 17 yr compared with age 2–15 yr ($P \leq 0.003$). A tendency for greater $\sqrt{}$CTX values in children from 11–15 yr was observed. Serum TRAP5b levels were lower in children older than 15 yr compared with children younger than 3 yr ($P < 0.02$). Boys aged 13–17 yr had greater TRAP5b values compared with girls of the same age groups ($P < 0.05$). As expected, bone marker concentrations also varied with Tanner stages ($P < 0.001$). Results by Tanner stage resembled those by age groups with concentrations declining for all markers but OC in Tanner stages 4 and 5.

Correlations among SDS of bone markers (Table 3)

Significant correlations were found between SDS for bone formation markers BALP and OC (r = 0.13; $P = 0.004$), as well as SDS for collagen degradation markers ICTP and CTX (r = 0.14; $P = 0.002$). The greatest correlation coefficient was observed between SDS for the two phosphatases (r = 0.34; $P < 0.001$). TRAP5b SDS also correlated with ICTP SDS (r = 0.22; $P = 0.008$).

Correlations between bone marker SDS and anthropometric SDS (Table 4)

Weak positive correlations between height SDS and lnBALP SDS (r = 0.20; $P < 0.001$) as well as $\sqrt{}$CTX SDS (r = 0.11; $P = 0.018$) were observed. In addition, weight SDS correlated positively with lnBALP SDS (r = 0.24, $P < 0.001$) and TRAP5b SDS (r = 0.20; $P = 0.019$). BMI SDS correlated weakly with lnBALP, lnICTP and TRAP5b SDS (r values ranging from 0.10–0.20; $P < 0.03$) and negatively with $\sqrt{}$CTX SDS (r = −0.13; $P = 0.004$).

Discussion

In this study we derived reference curves for five bone markers in a large cohort of healthy children aged 2 months

TABLE 2. Sex-specific equations for lnOC, lnBALP, lnICTP, $\sqrt{}$CTX, and TRAP5b

	Percentile	Boys	Girls
lnOC	50th	2.4249+0.1335*age-0.01*age²+0.0003*age³	2.4885+0.1161*age-0.0066*age²+0.000047*age³
	SD	0.4343+0.0004*age+0.0011*age²-0.00007*age³	0.4675+0.0026*age+0.0005*age²-0.00005*age³
lnBALP	50th	4.2152−0.2601*age+0.0398*age²-0.0016*age³	4.1199−0.1816*age+0.0315*age²-0.0015*age³
	SD	0.2199+0.0443*age-0.0054*age²+0.0002*age³	0.2322+0.0297*age-0.0028*age²+0.0001*age³
lnICTP	50th	3.2521−0.3189*age+0.042*age²-0.0015*age³	3.3723−0.3376*age+0.0463*age²-0.0018*age³
	SD	0.3570−0.0621*age+0.0085*age²-0.0003*age³	0.3997−0.0788*age+0.0096*age²-0.0003*age³
√CTX	50th	39.4029−1.9068*age+0.4234*age²-0.0185*age³	37.5947+0.041*age+0.1904*age²-0.0127*age³
	SD	6.9663−0.5886*age+0.0983*age²-0.0031*age³	6.7376−0.4718*age+0.1001*age²-0.0043*age³
TRAP5b	50th	8.1052−1.0423*age+0.1338*age²-0.0051*age³	6.8461−0.4430*age+0.0471*age²-0.0021*age³
	SD	1.7923−0.1366*age+0.0027*age²+0.0003*age³	1.8159−0.0589*age+0.0023*age²-0.0001*age³

Equations for the 50th centile (predicted mean) and SD are given; 3rd and 97th centiles are created by predicted mean ±1.88*SD equation * $\sqrt{}(\pi/2)$. To calculate an individual's SDS, the patient's age has to be entered in the sex-specific mean and SD equations. Then the SDS = (measured concentration − mean)/[SD*$\sqrt{}(\pi/2)$].

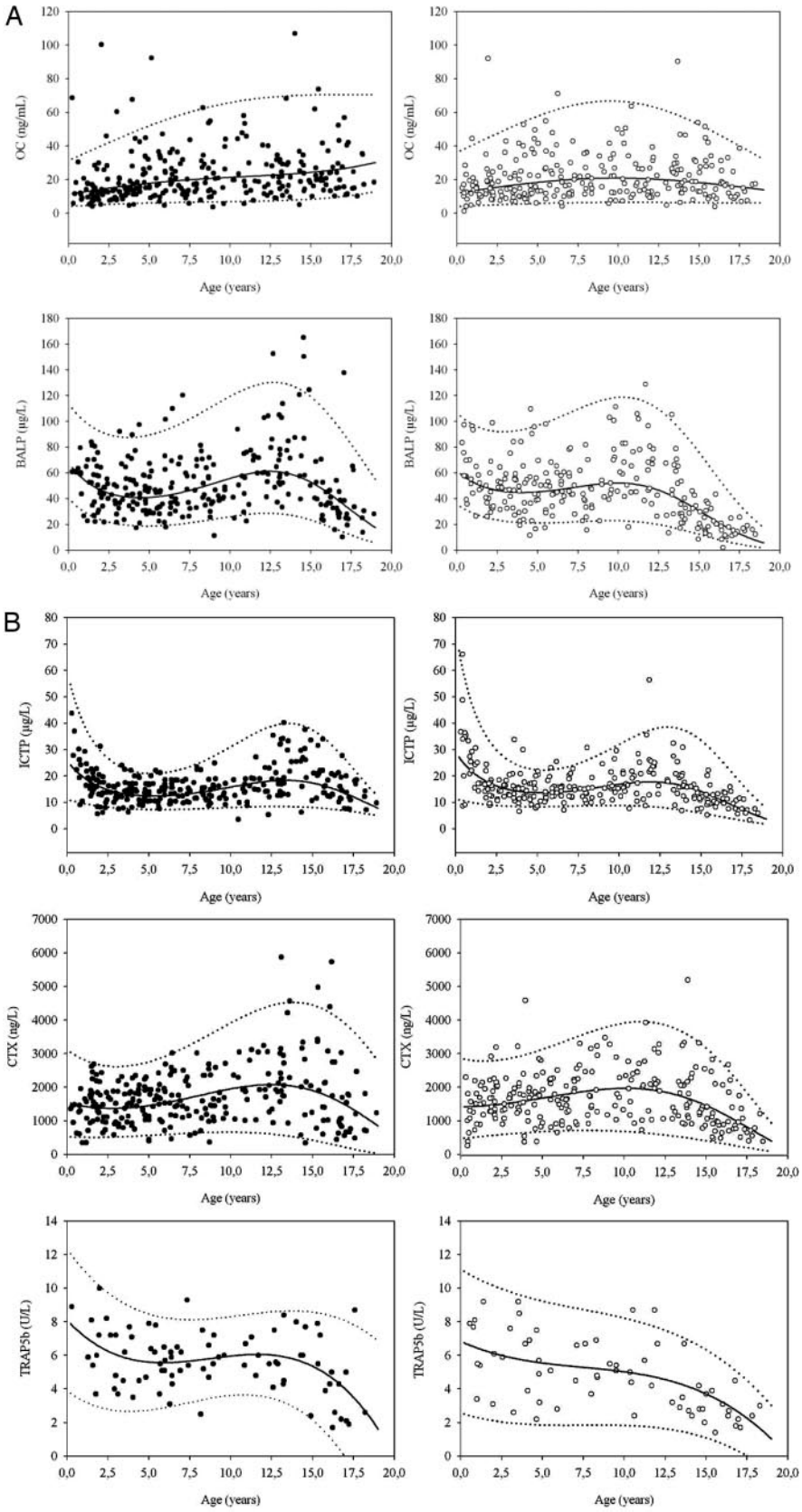

FIG. 1. A, Backtransformed reference curves for bone formation markers OC and BALP in boys (*filled circles*) and girls (*open circles*). Curves represent the 50th centile (*straight lines*) and 3rd/97th centile (*dotted lines*). B, Backtransformed reference curves for bone resorption markers ICTP, CTX, and TRAP5b in boys (*filled circles*) and girls (*open circles*). Curves represent the 50th centile (*straight lines*) and 3rd/97th centile (*dotted lines*).

TABLE 3. Correlations among SDS of lnOC, lnBALP, lnICTP, √CTX, and TRAP5b

	lnBALP SDS	lnICTP SDS	√CTX SDS	TRAP5b SDS
lnOC SDS				
r	0.13	0.08	−0.01	−0.06
P	0.004	0.063	0.837	0.500
lnBALP SDS				
r		0.32	0.08	0.34
P		<0.001	0.070	<0.001
lnICTP SDS				
r			0.14	0.22
P			0.002	0.008
√CTX SDS				
r				0.05
P				0.557

to 18 yr, enabling the calculation of sex- and age-specific SDS. Apart from OC, all markers decreased in late puberty with BALP, ICTP, and TRAP5b concentrations of boys decreasing later than girls, suggesting a strong relation of these markers to the later male pubertal growth spurt. SDS for markers of bone formation, collagen degradation, and phosphatases were each independently correlated, suggesting they derive from and reflect the same biological processes. Taller and heavier individuals for age had greater bone marker concentrations, particularly BALP, likely reflecting greater growth velocity and bone formation.

Childhood growth involves an orderly process of soft tissue synthesis, epiphyseal bone growth, and extensive bone modeling in addition to remodeling. In the assessment of changes in these processes, the clinician is bound to the two-dimensional measurement of bone mass using dual energy x-ray absorptiometry or the three-dimensional measurement of bone geometry, mass, and density using quantitative computed tomography. The use of biochemical bone markers complements these physical measures by providing a dynamic picture of whole-body bone turnover that can be repeated at much shorter intervals. This dynamic assessment allows early detection of effects of disease or treatment long before changes in bone mass or progression in bone disease can be accurately ascertained. Normative curves are thus a prerequisite tool for evaluating children with metabolic bone diseases.

TABLE 4. Correlations between SDS of bone markers and anthropometric measures

	Height SDS	Weight SDS	BMI SDS
lnOC SDS			
r	0.06	−0.03	−0.08
P	0.179	0.442	0.065
lnBALP SDS			
r	0.20	0.24	0.17
P	<0.001	<0.001	<0.001
lnICTP SDS			
r	0.07	0.11	0.10
P	0.105	0.008	0.026
√CTX SDS			
r	0.11	−0.05	−0.13
P	0.018	0.234	0.004
TRAP5b SDS			
r	0.11	0.18	0.20
P	0.198	0.028	0.019

The known high intraindividual variation in bone marker concentrations and their release during different anabolic and catabolic processes preclude their use for one-off diagnostic purposes (7, 8). A considerable number of markers of bone and collagen turnover have been designed but no single test fulfils all the criteria for an ideal marker. In addition, no marker in children is specific for any of the three different biological processes of remodeling, modeling and epiphyseal growth (9). Bone marker concentrations can be similar in a child with high bone remodeling and low growth rate and in a normally growing child. Therefore, knowledge of growth velocity and pubertal development is necessary in the correct interpretation of markers. We would recommend using a set of different formation and resorption markers as the preferred approach in the longitudinal assessment of bone diseases and in the monitoring of antiresorptive or growth modulating therapies.

The early change in bone marker concentrations following GH treatment in children with GH deficiency or idiopathic short stature gives a useful prediction of growth velocity response to treatment after 1 yr (7). Compared with OC, ICTP, and CTX, the change in BALP values after 3 months of GH therapy gave the best prediction of growth velocity response (8, 26). In general, however, the prediction of one individual marker may be too imprecise to serve as a basis for clinical decisions (27). Using the SDS change of a set of bone markers like ours, including IGF-1 (28, 29), for these purposes, may better help differentiate a true response to GH treatment from nonresponders, a group which itself needs to be defined first in terms of growth velocity (30). Such an approach could allow early GH dose adjustments or even GH withdrawal in nonresponders. Future studies will need to address whether such an approach could help reduce unnecessary treatment and its social and economic burdens.

Bisphosphonates are used in children as therapy for primary bone diseases such as osteogenesis imperfecta (31–33) and increasingly for secondary osteoporosis (34–36) caused by a variety of chronic diseases, cancer, or treatments, often associated with an increased fracture risk (37–43). In the treatment of these disorders, bisphosphonates act by inhibiting osteoclasts and thus bone resorption. In growing children, resorption occurs as part of the remodeling cycle, at the endocortical surface during modeling, during metaphyseal inwaisting, and at the metaphysis/growth plate junction during removal of primary spongiosa (33, 44–46). Bone resorption markers decrease rapidly following bisphosphonate therapy and are commonly used for monitoring. Bone formation markers may decrease as long as they derive from the suppressed remodeling process. As raw levels of bone formation and resorption markers decline during infancy and late adolescence, it is impossible to differentiate treatment-induced changes from physiological, age-related changes. Therefore, the use of bone marker SDS calculated from our reference curves may improve the monitoring of bone metabolism in infants and children with osteogenesis imperfecta or other conditions undergoing short- or long-term antiresorptive therapy.

Weak positive correlations among markers of bone formation and bone resorption were found in our study. Not surprisingly, SDS for bone formation markers (BALP and

OC) were significantly correlated as were SDS for collagen degradation markers (ICTP and CTX). Interestingly, the greatest correlation coefficient was found between SDS for the two phosphatases, likely reflecting their activity in the continuous remodeling process. However, in general only weak correlations were detected, as markers reflect different biological processes at many different regions and bone surfaces during skeletal growth, which itself is nonlinear (47). Markers are also released during different stages of the bone formation, resorption or growth processes, may have different elimination pathways and serum half-lives, affecting their relation at distinct time points during growth. Similarly, some of the positive correlations between anthropometric SDS and bone markers SDS could be by chance. However, the positive correlations between SDS for lnBALP, height, and weight suggest greater bone formation in children tall or heavy for age. Taller children usually have greater growth velocity and greater weight bearing, inducing larger bone formation in response to superior mechanical strains. The fact that the resorption markers TRAP5b and lnICTP SDS also correlated with weight SDS would not be contradicting as, *e.g.*, because increased periosteal modeling would also lead to removal of bone from the endocortical surface (48, 49).

In line with previous reports, we found generally greater serum concentrations of bone markers in infancy and in mid-puberty (11, 50–55). These findings indicate that both bone formation and resorption are accelerated during periods of growth spurt. Not surprisingly, later peaks were observed for lnBALP, lnICTP, and TRAP5b in boys, reflecting their later pubertal development and thus bone mass accrual. In both boys and girls, concentrations of most bone markers declined during late puberty with lowest values in the transition to adulthood. Because growth and puberty usually are completed by late adolescence, markers of bone formation and resorption converge into adult values. One has to extrapolate our data for ages older than 17 yr because subject numbers were low in this age group.

Reference data for serum bone markers in children have been previously published (11, 13, 50–60), but mostly for single bone markers and in relatively small numbers. This is the first study implementing curve fitting procedures for five recognized markers of bone formation and resorption in the same large healthy pediatric population. Standardized blood sampling and analytical procedures were used to avoid any bias due to diurnal variation. Reference values for TRAP5b have been published for Chinese (50) but not for Caucasian children. One limitation of the study is the lack of data for young adults, as concentrations of most markers had not plateaued at age 18. Therefore, an extension of reference values to young adulthood would be required. Further, the gross majority of children in this study were not fasting, which may have introduced additional variability. However, overnight fasting, as recommended for adults (2, 61), is often impracticable for infants, younger children or the chronically ill. In addition, the clinical impact of feeding *vs.* fasting in adults was reportedly small, apart from serum CTX (2, 61, 62), and detailed information is missing for most bone marker assays, in particular for children. However, as monitoring is the main purpose of using bone markers, the individual one-off measurement is much less important than the course over time. Using our reference curves, clinicians can choose which regimen (fasting or not) is best for the individual patient but then should stick to the chosen regimen for all subsequent measurements. This approach will facilitate monitoring for patients and doctors. Finally, mean CTX concentrations in our study were greater compared with Scottish reference data in a substantially smaller population (51) despite using a similar study design and analytical methods and observing a similar age-related slope of CTX curves in both sexes. As both study populations were nonfasting, other factors like altitude, climate, lifestyle, or average vitamin D status between populations may serve as an explanation for this discrepancy.

Conclusions

The presented sex- and age-specific reference curves and the possibility of calculating SDS will facilitate monitoring of antiresorptive therapy or disease progression in children with metabolic bone disease. Potentially, our curves may also help in assessing the response to other treatments of a variety of diseases causing secondary osteoporosis, the prediction of growth response to GH therapy and the progression of cancer-induced bone disease in children. The use of markers for one-off diagnostic purposes is precluded, because severe diseases may affect both epiphyseal growth and bone metabolism. We recommend using a set of formation and resorption markers rather than single markers in the longitudinal assessment of bone metabolism, because sensitivities and predictive values of single markers are usually poor.

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