

Serum concentrations of carboxyl-terminal propeptide of type I procollagen, amino-terminal propeptide of type III procollagen, cross-linked carboxyl-terminal telopeptide of type I collagen, and their interrelationships in schoolchildren

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We report pediatric age- and sex-specific 95% reference intervals for procollagen type I C-terminal propeptide (PICP), the cross-linked C-terminal telopeptide of type I collagen (ICTP), and procollagen type III N-terminal propeptide (P3NP), measured in plasma from 302 schoolchildren (156 boys, 146 girls) ages 4–19 years. All three markers displayed a significant variation with age (ANOVA $P \leq 0.0015$). PICP showed no detectable increase during adolescence for either sex, but decreased towards adult concentrations after the age of puberty, with an earlier decrease for girls than for boys ($P < 0.01$). ICTP and P3NP both increased in pubertal-aged children ($P < 0.05$), with an earlier increase in girls than in boys ($P < 0.05$), before decreasing towards adult concentrations ($P < 0.01$). All three collagen markers were highly correlated with one another ($P < 0.001$). The patterns observed mirrored the childhood growth curve and reflected the high turnover of bone and soft tissue during childhood growth.

Childhood growth involves an orderly process of soft tissue synthesis, linear bone growth, and extensive bone modeling. Type III collagen is found in soft connective tissues throughout the body, but is negligible in bone. During the final stages of its biosynthesis, procollagen type III N-terminal propeptide (P3NP) is cleaved from the parent molecule and released into the circulation in stoi-

chiometric amounts [1]⁴. Its concentration in serum, therefore, reflects soft tissue growth and proliferation. Type I collagen is ubiquitously distributed and is the predominant collagen both in soft tissue and in bone, where it forms the matrix for subsequent mineralization. During the modeling of bone that accompanies growth, type I collagen is synthesized by osteoblasts during their early proliferative phase, releasing procollagen type I C-terminal propeptide (PICP) into the circulation [2, 3]. PICP therefore reflects a combination of bone and soft tissue growth. Bone modeling associated with growth also involves degradation of existing bone, releasing fragments of cross-linked type I collagen into the circulation. The cross-linked C-terminal telopeptide of type I collagen (ICTP) is one of these fragments and is, at present, the only marker of bone collagen degradation that can be measured in serum [3].

We, and others [4–8], have demonstrated that plasma concentrations of bone alkaline phosphatase, P3NP, PICP, and ICTP in children with growth disorders reflect height velocity and may be used to give an early indication of height velocity response to growth-promoting treatments. These markers may be of considerable potential value in assessing the effects of the underlying disorder and therapeutic interventions on bone turnover and growth in children in other clinical conditions. In such studies, use of a panel of biochemical markers that reflect different aspects of bone and soft tissue turnover is essential. However, good age- and sex-related reference data are needed for their interpretation. We have previously reported children's reference data for bone alkaline phos-

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⁴ Nonstandard abbreviations: P3NP, procollagen type III N-terminal propeptide; PICP, procollagen type I C-terminal propeptide; ICTP, cross-linked telopeptide of type I collagen.

phatase [9]. We present here reference data for P3NP, PICP, and ICTP, obtained from a group of school-attending children, and also the interrelationships between these markers.

Materials and Methods

SUBJECTS

Blood was collected from 2129 schoolchildren from Counties Dublin, Kildare, and Wicklow, Ireland, as part of a population-based epidemiological study on the seroprevalence of toxocariasis [10]. All children were well enough to attend school that day. No formal pubertal staging was undertaken as it would have been ethically inappropriate in this context. Blood samples were taken with the informed consent of both children and parents, and the study was approved by the local ethics committee. All samples were collected between 0900 and 1500. The excess sera remaining after completion of that study were stored at -20°C until analysis. Such conditions of storage have no effect on the measurement of collagen markers.

Collagen markers were measured on stored samples from 302 of these children (156 boys, 146 girls) ages 4–19 years. Between 10 and 14 samples from each sex in each year-group were analyzed, except for girls age 4 years and boys age 18 years from whom only four samples each were available.

METHODS

Assays. We measured PICP, ICTP, and P3NP by radioimmunoassay (Orion Diagnostica), with methods described previously [11–13]. Before analysis, we diluted samples appropriately in 154 mmol/L sodium chloride to achieve concentrations within the calibration curve; in prepubertal and pubertal children typical dilutions were 1 in 4 for PICP and 1 in 2 for ICTP and P3NP. In brief, we mixed diluted plasma (100 μL for PICP and ICTP, 200 μL for P3NP) with 200 μL of polyclonal antiserum (rabbit) and 200 μL of ^{125}I -labeled tracer. After a 2-h incubation at 37°C , we added 500 μL of second antibody covalently bound to solid particles and allowed the tubes to stand for 30 min at room temperature. After centrifugation, we removed the supernatant and counted the precipitated antibody–antigen complex in a gamma counter. Calibration curves were constructed with five calibrators by spline function curve-fitting. All samples were analyzed in duplicate. Between-run CVs were 7.8% at 94 $\mu\text{g}/\text{L}$ and 5.2% at 320 $\mu\text{g}/\text{L}$ for PICP, 6.3% at 8.7 $\mu\text{g}/\text{L}$ and 9.2% at 33.8 $\mu\text{g}/\text{L}$ for ICTP, and 5.6% at 4.6 $\mu\text{g}/\text{L}$ and 6.4% at 10.4 $\mu\text{g}/\text{L}$ for P3NP.

Statistical methods. The data were analyzed separately for each sex and each year of age (e.g., the 4-year-old age-band comprised children ≥ 4.0 to < 5.0 years) and also for all ages combined, to assess whether untransformed or log-transformed data gave a better fit to a gaussian distribution: Means, medians, SD values, and indices of

skewness and kurtosis were compared with transformed and untransformed data. After log-transformation, data from males and females in each yearly age-band were compared with unpaired *t*-tests. Within each sex, changes with age were assessed by one-way ANOVA of the log-transformed data, followed by Fisher-protected least significant difference as a post hoc test. On the basis of the ages at which statistically significant changes in each collagen marker occurred, results in adjacent age-bands were then combined to derive appropriate age- and sex-related reference intervals. The 95% reference interval was defined as the arithmetic mean of the log-transformed data ± 2 SD, raised to the power of 10. Means \pm SD of the log-transformed data have also been presented to allow calculation of SD scores by age and sex. All statistical tests were two-tailed, and $P < 0.05$ was regarded as significant.

Results

The concentrations of the collagen markers, plotted by age and sex, are shown in Figs. 1–3, a and b. We found that for PICP and P3NP the untransformed data were positively skewed; log transformation gave a much-improved fit to a gaussian distribution and tended to equalize variances in the different age-groups. For ICTP, there was little difference in these respects between transformed and untransformed data; both gave acceptable fits to a gaussian distribution. Log transformation was therefore used for all three collagen markers for simplicity.

The geometric means of each marker by sex and age are displayed in Figs. 1–3, c. PICP, ICTP, and P3NP all showed a significant variation with age for both sexes (ANOVA, $P \leq 0.0015$). For PICP in boys, however, post hoc testing demonstrated that no statistically significant change occurred before the age of 17 years. PICP concentrations in boys ages 17 and 18 years were lower than at all other ages ($P < 0.01$). In girls, there was no significant change in PICP between the ages of 4 and 12 years. PICP concentrations in 13- and 14-year-old girls were lower than in younger girls ($P < 0.01$) and lower still in girls ages 15–18 years ($P < 0.01$). PICP concentrations did not differ between the sexes until age 13, thereafter reaching maximum difference at ages 15–16 years ($P < 0.001$).

For both ICTP and P3NP in boys, post hoc testing indicated no significant change between ages 4 and 11 years. Boys ages 12–16 years had higher concentrations than younger boys ($P < 0.05$) and older boys ages 17–18 years ($P < 0.05$). In girls, the two markers showed slightly different patterns. For ICTP, there were no consistent significant change between the ages of 4 and 8 years. Girls ages 9–13 years had higher concentrations than girls ages 6–8 years ($P < 0.05$) and all older girls ($P < 0.01$). There was a further progressive decrease in ICTP in girls from ages 14–17 years ($P < 0.05$). For P3NP in girls, there was no significant change between ages 4 and 10 years. Girls ages 11–12 years had higher concentrations than younger girls ($P < 0.05$) and girls ages 14–18 years ($P < 0.01$).

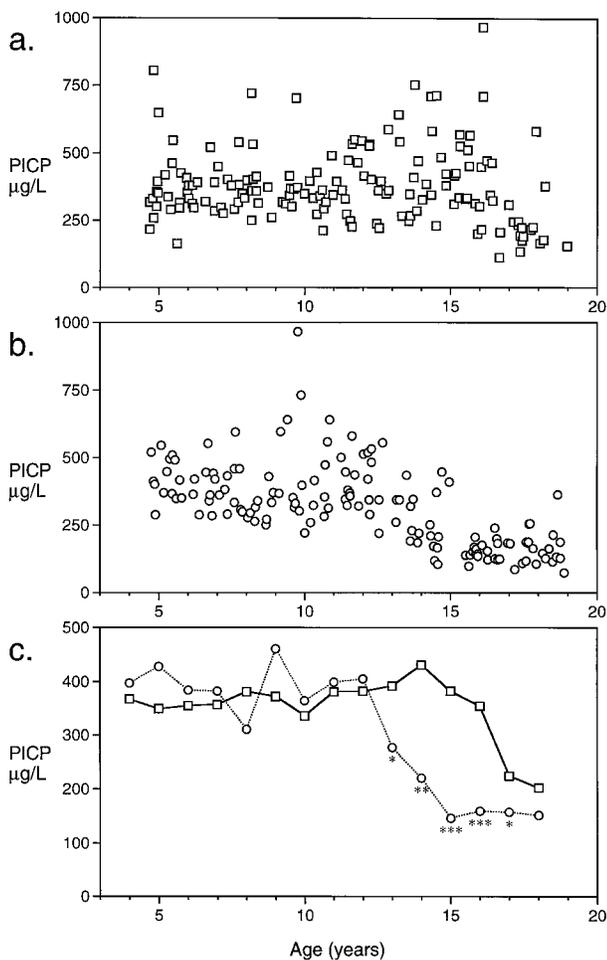


Fig. 1. PICP concentrations in relation to age and sex. *a*, individual boys; *b*, individual girls; *c*, geometric means (defined as the arithmetic mean of log-transformed PICP concentrations, raised to the power of 10). Squares represent boys, and circles girls. Unpaired *t* tests between age-matched boys and girls: *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

Concentrations declined progressively then, with 13- to 14-year-old girls having higher P3NP concentrations than 15- to 18-year-old girls (*P* < 0.01). At ages 9 and 11 years, girls had significantly higher concentrations of both ICTP and P3NP than did boys (*P* < 0.05). Conversely, between ages 14 and 17 years, boys had higher concentrations of these collagen markers than did girls (*P* < 0.01).

Table 1 shows age- and sex-specific logarithmic means ± SD and derived 95% reference intervals for the collagen markers, on the basis of the above age-groupings. Cross-correlations between PICP, ICTP, and P3NP measurements on the same samples of serum, both for the study group as a whole and subdivided into prepubertal-aged children, are given in Table 2.

Discussion

Although PICP [6, 7, 14, 15] and P3NP [13, 14] reference data in children have been published previously, this is the first study to report these markers and ICTP in the same group of children. ICTP, validated by bone histo-

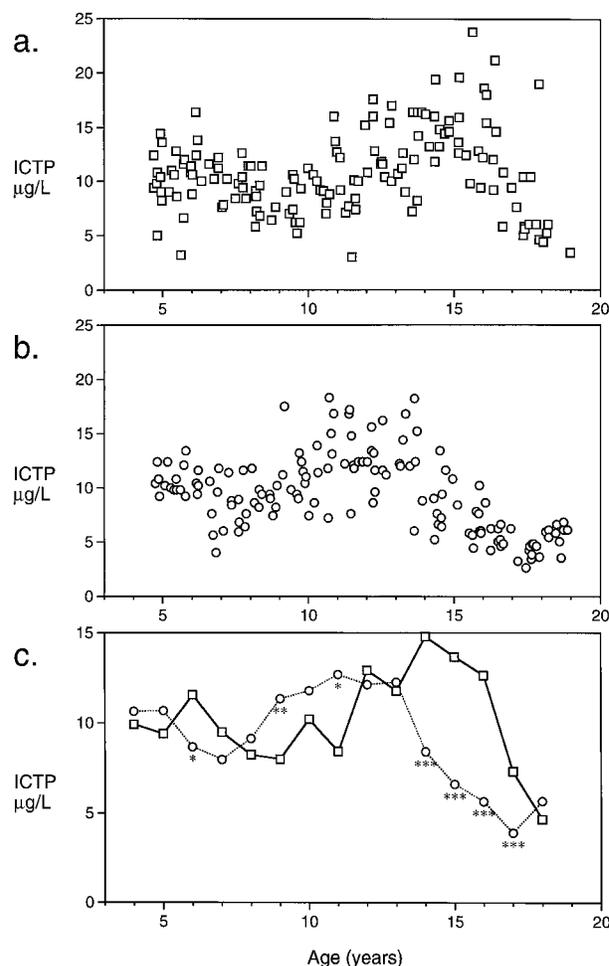


Fig. 2. ICTP concentrations in relation to age and sex. *a*, individual boys; *b*, individual girls; *c*, geometric means (defined as in Fig. 1). Symbols as for Fig. 1.

morphometry [16] and calcium kinetic studies [17], is the only marker of bone collagen degradation that can be measured in serum and can therefore be compared directly with the markers of collagen synthesis measured in the same sample. Other markers of bone collagen degradation—pyridinoline, deoxypyridinoline, various collagen peptides, galactosyl-hydroxylysine, and hydroxyproline—can be measured in urine only. Wide intraindividual day-to-day biological variation in pyridinium cross-link excretion has been reported in both adults [18–21] and children [22], making the test insensitive and difficult to interpret. An additional practical point is the difficulty in collecting timed urine specimens in young children; if random samples are collected, concentrations must be related to creatinine, the excretion of which may itself be variable and subject to such factors as muscle mass and catabolic state.

We found that log-transformation was required to achieve a gaussian distribution for PICP and P3NP. Some [14, 15] but not all [6, 7, 13] previous authors have applied a similar transformation. For PICP, our reference data

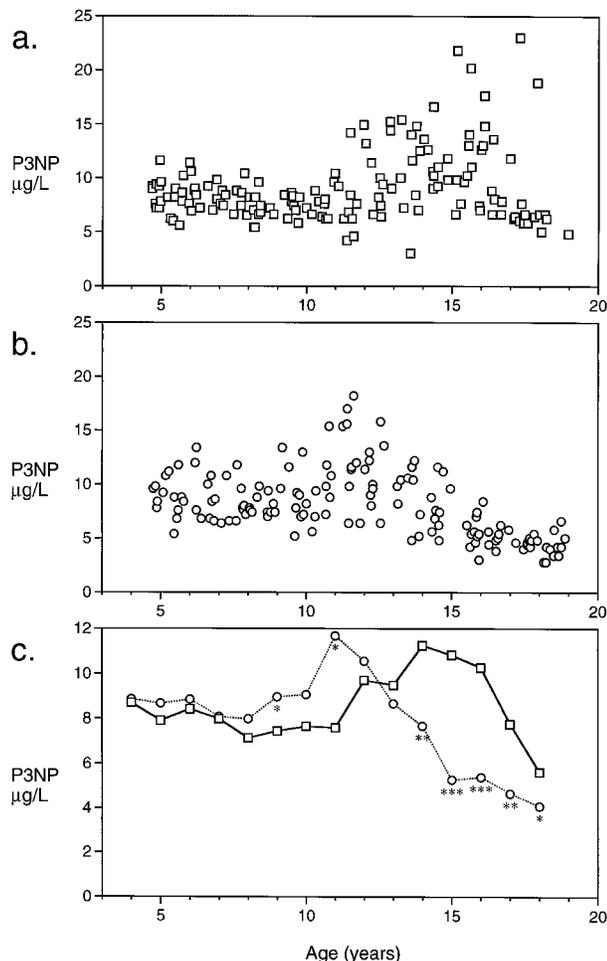


Fig. 3. P3NP concentrations in relation to age and sex. a, individual boys; b, individual girls; c, geometric means (defined as in Fig. 1). Symbols as for Fig. 1.

were very similar to those obtained by other authors when the same assay method was used [6, 14, 15], with a similar lack of increase during the pubertal growth spurt. Increased secretion of growth hormone and sex steroids during this period may affect the clearance of PICP via the mannose receptor of liver endothelial cells [23]. By contrast, Saggese et al. [7] did find increases in PICP during puberty in both boys and girls although their prepubertal reference data were similar to our own; this discrepancy remains unexplained.

Our P3NP reference data were also very similar to those obtained by others by the same assay method [13, 14], with an earlier peak in girls than in boys, reflecting the sex differences in timing of peak growth velocity during puberty. The increased ICTP that we observed during puberty is qualitatively similar to pubertal increases in pyridinoline and deoxypyridinoline excretion previously reported in children [24]. However, unlike that earlier study, we observed sex differences in the timing of the pubertal increase, suggesting that ICTP may reflect

Table 1. Age- and sex-specific reference data.

Analyte	Sex	Age range, years	n	Log-transformed mean (SD) ^a	95% reference interval, ^b µg/L
PICP	M	4–16	141	2.5699 (0.1424)	193–716
		17–18	15	2.3377 (0.1587)	105–452
	F	4–12	84	2.5910 (0.1194)	225–676
		13–14	20	2.3921 (0.1806)	108–567
ICTP	M	4–11	83	0.9693 (0.1310)	5.1–17.0
		12–16	52	1.1169 (0.1200)	7.5–22.8
	F	17–18	14	0.8066 (0.1896)	2.7–15.3
		4–8	44	0.9631 (0.1055)	5.7–14.9
P3NP	M	9–13	50	1.0803 (0.1102)	7.2–20.0
		14–15	20	0.8706 (0.1272)	4.1–13.3
	F	16–18	30	0.6977 (0.1149)	2.9–8.5
		4–11	83	0.8927 (0.0930)	5.1–12.0
P3NP	M	12–16	54	1.0128 (0.1524)	5.1–20.8
		17–18	16	0.8540 (0.1878)	3.0–17.0
	F	4–10	65	0.9338 (0.0995)	5.4–13.6
		11–12	20	1.0455 (0.1397)	5.8–21.1
P3NP	F	13–14	20	0.9107 (0.1340)	4.4–15.1
		15–18	41	0.6799 (0.1052)	3.0–7.8

^a The mean (and SD) of log-transformed collagen concentrations (in µg/L).

^b Arithmetic mean of log-transformed data ± SD, raised to the power of 10.

the timing of the pubertal growth spurt more closely than pyridinium cross-link excretion.

All three collagen markers were highly correlated with one another when all the data were pooled, reflecting their close association with growth. The lower (but still statistically significant) correlations in prepubertal than in pubertal children can be explained by the smaller variation in growth rates among the former. Notably, in each group of children we observed higher correlations between P3NP and ICTP than between PICP and ICTP. A similar phenomenon was found in our earlier study on short, healthy children undergoing growth-promoting treatments [8]. The explanation may be twofold. Firstly, a small proportion of P3NP is incorporated into newly synthesized soft tissue collagen fibrils, so that its concentration in serum may partially reflect the soft tissue collagen degradation that occurs during tissue modeling. In individual children, this may coincide with the degradation of type I collagen occurring at the same time (releasing ICTP into the circulation), either as part of the normal saltatory growth pattern [25] or during subclinical infections that may affect short-term growth [26]. We have found no evidence of analytical cross-reaction between the two assays and in other longitudinal clinical studies have found dissociation between P3NP and ICTP responses to disease and therapeutic interventions (unpublished observations). Secondly, normal growth-associated bone modeling in children does not involve tight coupling of osteoblast and osteoclast activity. Therefore no reason exists to suppose that PICP and ICTP would be highly correlated at a single point in time, except insofar as both reflect a different aspect of growth.

Table 2. Correlation coefficients (and n) between markers.

Group	PICP vs ICTP	PICP vs P3NP	ICTP vs P3NP
All children	0.61 ^a (271)	0.66 ^a (278)	0.76 ^a (264)
Children <9 years	0.24 ^b (91)	0.24 ^b (90)	0.35 ^c (87)
Girls 9–13 years	0.35 ^b (48)	0.61 ^a (50)	0.62 ^a (48)
Boys 12–16 years	0.57 ^a (45)	0.60 ^a (47)	0.69 ^a (44)

^a $P < 0.0001$.^b $P < 0.05$.^c $P = 0.001$.

In this study we have produced age- and sex-related 95% reference intervals for PICP, P3NP, and ICTP, together with log-transformed mean \pm SD values that allow calculation of SD scores. These are now being applied to other pediatric clinical studies currently underway on the effect of disease burden and therapeutic interventions on bone and somatic growth.

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References

- Danne T, Grüters A, Schuppan D, Quantas N, Enders I, Weber B. Relationship of procollagen type III propeptide-related antigens in serum to somatic growth in healthy children and patients with growth disorders. *J Pediatr* 1989;114:256–60.
- Stein GS, Lian JB, Owen TA. Relationship of cell growth to the regulation of tissue-specific gene expression during osteoblast differentiation. *FASEB J* 1990;4:111–23.
- Risteli L, Risteli J. Biochemical markers of bone metabolism. *Ann Med* 1993;25:385–93.
- Tapanainen P, Risteli L, Knip M, Käär M-L, Risteli J. Serum aminoterminal propeptide of type III procollagen: a potential predictor of the response to growth hormone therapy. *J Clin Endocrinol Metab* 1988;67:1244–9.
- Trivedi P, Hindmarsh P, Risteli J, Risteli L, Mowat AP, Brook CGD. Growth velocity, growth hormone therapy, and serum concentrations of the amino-terminal propeptide of type III procollagen. *J Pediatr* 1989;114:225–30.
- Trivedi P, Risteli J, Risteli L, Hindmarsh PC, Brook CG, Mowat AP. Serum concentrations of the type I and III procollagen propeptides as biochemical markers of growth velocity in healthy infants and children with growth disorders. *Pediatr Res* 1991;30:276–80.
- Saggese G, Bertelloni S, Baroncelli GI, Di Nero G. Serum levels of carboxyterminal propeptide of type I procollagen in healthy children from 1st year of life to adulthood and in metabolic bone diseases. *Eur J Pediatr* 1992;151:764–8.
- Crofton PM, Stirling HF, Schönau E, Kelnar CJH. Bone alkaline phosphatase and collagen markers as early predictors of height velocity response to growth-promoting treatments in short normal children. *Clin Endocrinol* 1996;44:385–94.
- Crofton PM. Wheat-germ affinity electrophoresis for alkaline phosphatase isoforms in children: age-dependent reference ranges and changes in liver and bone disease. *Clin Chem* 1992;38:663–70.
- Holland CV, O'Lorain P, Taylor MRH, Kelly A. Sero-epidemiology of toxocariasis in school children. *Parasitology* 1995;110:535–45.
- Melkko J, Niemi S, Risteli L, Risteli J. Radioimmunoassay of the carboxyterminal propeptide of human type I procollagen. *Clin Chem* 1990;36:1328–32.
- Risteli J, Elomaa I, Niemi S, Novamo A, Risteli L. Radioimmunoassay for the pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen: a new serum marker of bone collagen degradation. *Clin Chem* 1993;39:635–40.
- Risteli J, Niemi S, Trivedi P, Mäentausta O, Mowat AP, Risteli L. Rapid equilibrium radioimmunoassay for the amino-terminal propeptide of human type III procollagen. *Clin Chem* 1988;34:715–8.
- Hertel NT, Stoltenberg M, Juul A, Main KM, Müller J, Nielsen CT, et al. Serum concentrations of type I and III procollagen propeptides in healthy children and girls with central precocious puberty during treatment with gonadotrophin-releasing hormone analog and cyproterone acetate. *J Clin Endocrinol Metab* 1993;76:924–7.
- Bouillon R, Bex M, Van Herck E, Laureys J, Dooms L, Lesaffre E, Ravussin E. Influence of age, sex, and insulin on osteoblast function: osteoblast dysfunction in diabetes mellitus. *J Clin Endocrinol Metab* 1995;80:1194–202.
- Eriksen EF, Charles P, Melsen F, Mosekilde L, Risteli L, Risteli J. Serum markers of type I collagen formation and degradation in metabolic bone disease: correlation with bone histomorphometry. *J Bone Miner Res* 1993;8:127–32.
- Charles P, Mosekilde L, Risteli L, Risteli J, Eriksen EF. Assessment of bone remodeling using biochemical indicators of type I collagen synthesis and degradation: relation to calcium kinetics. *Bone Miner* 1994;24:81–94.
- McLaren AM, Isdale AH, Whiting PH, Bird HA, Robins SP. Physiological variations in the urinary excretion of pyridinium crosslinks of collagen. *Br J Rheumatol* 1993;32:307–12.
- Colwell A, Russell RGG, Eastell R. Factors affecting the assay of urinary 3-hydroxypyridinium crosslinks of collagen as markers of bone resorption. *Eur J Clin Invest* 1993;23:341–9.
- Popp-Snijders C, Lips P, Netelenbos JC. Intraindividual variation in bone resorption markers in urine. *Ann Clin Biochem* 1996;33:347–8.
- Panteghini M, Pagani F. Biological variation in urinary excretion of pyridinium crosslinks: recommendations for the optimum specimen. *Ann Clin Biochem* 1996;33:36–42.
- Shaw NJ, Dutton J, Fraser WD, Smith CS. Urinary pyridinoline and deoxypyridinoline excretion in children. *Clin Endocrinol* 1995;42:607–12.
- Smedsrød B, Melkko J, Risteli L, Risteli J. Circulating C-terminal propeptide of type I procollagen is cleared mainly via the mannose receptor in liver endothelial cells. *Biochem J* 1990;271:345–50.
- Rauch F, Schönau E, Woitge H, Remer T, Seibel M. Urinary excretion of hydroxypyridinium cross-links of collagen reflects skeletal growth velocity in normal children. *Exp Clin Endocrinol* 1994;102:94–7.
- Thalange NKS, Foster PJ, Gill MS, Price DA, Clayton PE. Model of normal prepubertal growth. *Arch Dis Child* 1996;75:427–31.
- Wales JKH, Milner RDG. Knemometry in assessment of linear growth. *Arch Dis Child* 1987;62:166–71.