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Procollagen Type I Amino-Terminal Propeptide: Pediatric Reference Data and Relationship with Procollagen Type I Carboxyl-Terminal Propeptide, *Patricia M. Crofton,[1,2*] Nancy Evans,[2] Mervyn R.H. Taylor,[3,4] and Celia V. Holland[5]* ([1] Department of Paediatric Biochemistry, Royal Hospital for Sick Children, Edinburgh, UK; [2] Section of Child Life and Health, Department of Reproductive and Developmental Sciences, University of Edinburgh, Edinburgh, UK; [3]The National Children's Hospital, Tallaght, Ireland; [4]Department of Paediatrics, Trinity College, Dublin, Ireland; [5] Department of Zoology, Trinity College, Dublin, Ireland; * address correspondence to this author at: Department of Paediatric Biochemistry, Royal Hospital for Sick Children, Sciennes Road, Edinburgh EH9 1LF, United Kingdom; fax 44-131-536-0410, e-mail patricia.crofton@luht.scot.nhs.uk)

Type I collagen is the predominant collagen in bone and soft tissue. The rate of synthesis of type I collagen can be assessed by measuring plasma concentrations of the C-terminal (PICP) and N-terminal (PINP) propeptides released during extracellular processing of its procollagen precursor *(1)*. However, the propeptides have different clearance routes, PICP being cleared by mannose receptors *(2)* and PINP by scavenger receptors *(3)* in liver endothelial cells. Clearance of PICP may be modulated by the hormonal milieu, whereas scavenger receptors apparently are not influenced by hormones *(4, 5)*. Within-individual biological variability is similar for PICP and PINP *(6)*, but PINP displays greater dynamic changes than PICP in response to disease and interventions *(7, 8)*. PINP has been shown to be a useful marker of bone formation in adults *(7–12)*.

During childhood growth, markers of bone turnover circulate at higher concentrations than in adults and correlate with height velocity *(13, 14)*. These markers have been used to investigate bone dynamics in childhood disorders of bone and growth *(13–15)*, but a lack of appropriate reference data has hampered use of PINP in pediatrics. Here, we report age- and sex-related reference data for plasma PINP in children from birth to 19 years of age. We also investigated the relationship between PINP and PICP to determine whether their relative clearance rates differ through childhood and adolescence.

Surplus plasma remaining after routine biochemical tests had been completed was retrieved for 43 neonates, infants, and children (23 males) younger than 5 years, who presented with various minor conditions that were considered not to have either a short- or long-term effect on growth; children with systemic disease or concurrent infections were excluded. Samples were deidentified and stored at −70 °C until analysis.

We also analyzed stored plasma from 284 children (140 males), ages 4–19 years, who had participated in an earlier population-based epidemiologic study on the seroprevalence of toxocariasis in Irish schoolchildren *(16)*. Ten samples from each gender and age group were analyzed, except for girls 4 years of age and boys 16 and 18 years of age, for whom only 4, 6, and 4 samples,

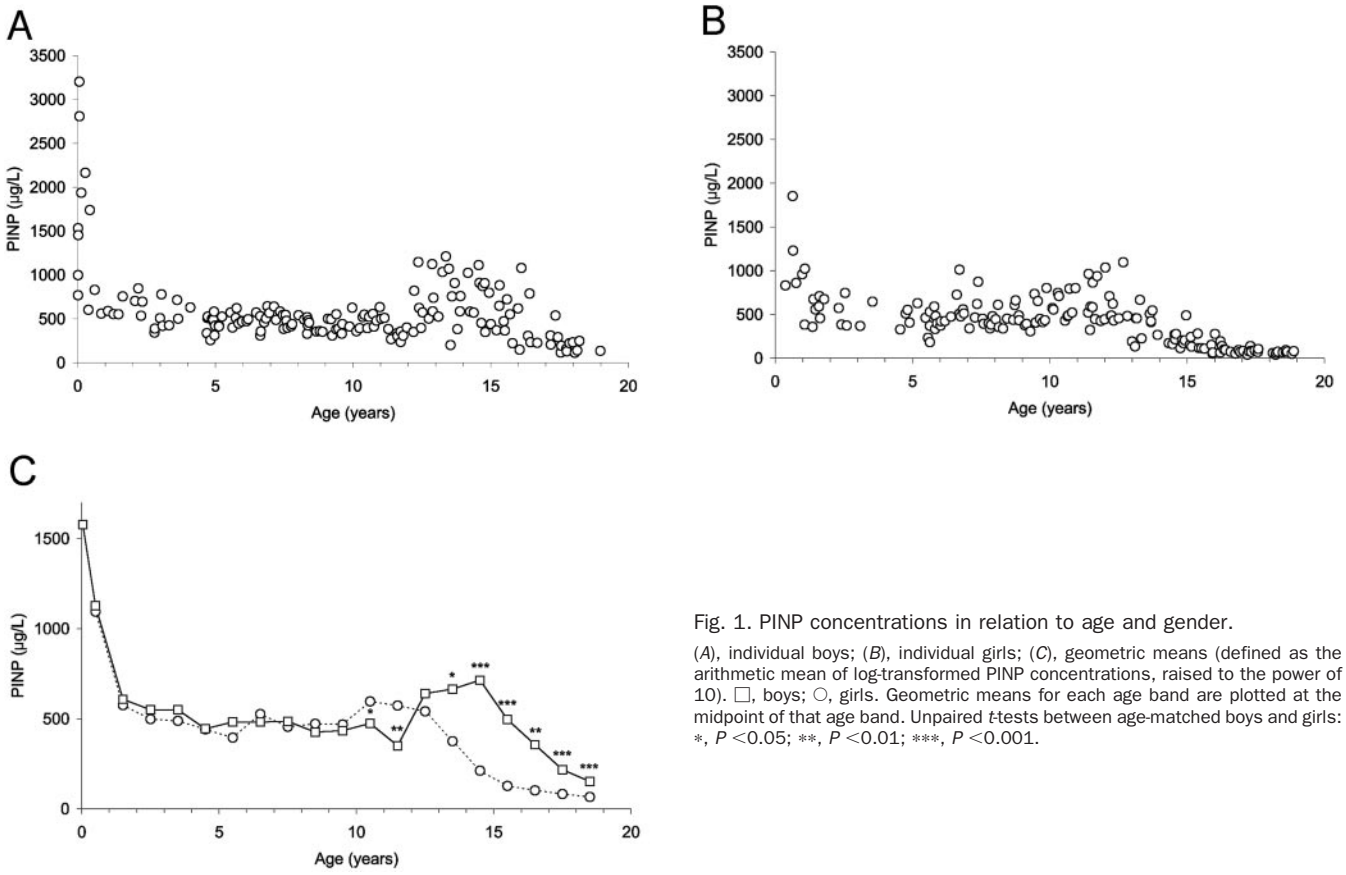

Fig. 1. PINP concentrations in relation to age and gender.

(*A*), individual boys; (*B*), individual girls; (*C*), geometric means (defined as the arithmetic mean of log-transformed PINP concentrations, raised to the power of 10). □, boys; ○, girls. Geometric means for each age band are plotted at the midpoint of that age band. Unpaired *t*-tests between age-matched boys and girls: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

respectively, were available. Samples were collected between 0900 and 1500. All children were well enough to attend school that day. No pubertal staging was undertaken because it would have been ethically inappropriate in this context. Blood samples were collected for the original study with the informed consent of children and parents and after approval by the local ethics committee. The excess plasma remaining after completion of that study was made anonymous and stored at −70 °C until analysis.

We measured PINP with a RIA (INTACT PINP; Orion Diagnostica) *(1)*. Before analysis, we diluted samples with zero calibrator to achieve concentrations within the calibration curve; typical dilutions were 1 in 50 for neonates younger than 1 month, 1 in 20 for infants 1–3 months of age, 1 in 10 for children 3 months to 17 years of age, and 1 in 5 for older adolescents. Between-assay CVs were 4.9% at 30 μg/L, 9.0% at 184 μg/L, and 3.9% at 564 μg/L. PICP was measured by RIA (Orion Diagnostica) in a subset of 123 samples from children 1–19 years of age *(17)*. Between-assay CVs were 7.8% at 94 μg/L and 5.2% at 320 μg/L.

The data were analyzed separately for neonates (postnatal age less than 1 month), for infants (1 month to 1 year), for each gender and year of age thereafter, and also for various combinations of ages. Statistical tests were performed after log transformation to render the distribution gaussian. PINP in males and females in each age group were compared by use of unpaired *t*-tests. Within each gender, changes with age were assessed by one-way ANOVA, followed by Fisher protected least-significant difference as a post hoc test. On the basis of the ages at which statistically significant changes occurred, results for adjacent age groups were then combined to derive appropriate age- and gender-related ranges and 95% confidence intervals (defined as the arithmetic mean of the log-transformed data ± 2 SD, raised to the power of 10). Means (SD) of the log-transformed data are also presented to facilitate calculation of SD scores by age and gender. The relationship between PINP and PICP was explored by use of Spearman rank correlation with correction for ties. Unpaired *t*-tests were used to compare the PICP/PINP ratio between males and females.

The PINP concentrations, plotted by age and gender in individual children, are shown in Fig. 1, A and B. The geometric means by age are displayed in Fig. 1C. Highest concentrations occurred in neonates, with slightly lower concentrations in infants and a further marked decrease after 1 year of age in both genderes. PINP showed significant variation with age in both boys and girls older than 1 year (ANOVA, $P < 0.0001$). Post hoc testing indicated that no significant change occurred in either gender between ages 1 and 10 years. In girls, PINP then increased slightly to a peak between 10 and 13 years before decreasing progressively to low concentrations (Fig. 1, B and C). In boys, PINP increased later to a slightly higher peak

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

Age range	n	Median, μg/L	Range, μg/L	Log-transformed mean (SD)[a]	95% confidence interval,[b] μg/L
Males and females					
<1 month	7	1454	770–3203	3.181 (0.226)	
1 month to 1 year	11	959	560–2165	3.046 (0.209)	424–2916
1–10 years	147	478	184–1024	2.679 (0.119)[c]	277–824
Males					
10–12 years	20	398	237–635	2.611 (0.115)	240–693
12–15 years	30	748	202–1212	2.828 (0.189)[c]	282–1604
15–16 years	10	513	223–883	2.695 (0.174)[d]	223–1103
16–17 years	6	270	148–1080	2.552 (0.338)	
17–19 years	14	200	113–538	2.294 (0.192)[c]	81–476
Females					
10–13 years	30	539	192–1097	2.756 (0.161)[d]	272–1194
13–14 years	10	440	136–668	2.575 (0.208)[c]	144–980
14–15 years	10	206	116–490	2.328 (0.169)[c]	98–462
15–17 years	20	112	54–282	2.059 (0.220)[c]	42–316
17–19 years	19	76	41–140	1.866 (0.128)[c]	41–133

[a] Mean (SD) of log-transformed PINP concentrations (in μg/L).
[b] Arithmetic mean of log-transformed data (in μg/L) ± 2 SD, raised to the power of 10. Not calculated for groups with n <10.
[c,d] Compared with the previous age/gender group (ANOVA followed by post hoc testing): [c] $P < 0.001$; [d] $P < 0.05$.

between 12 and 15 years before gradually decreasing (Fig. 1, A and C).

Individuals 1 month to 10 years of age showed no significant differences in PINP between males and females ($P > 0.15$). However, girls 10–12 years of age had higher PINP concentrations than did age-matched boys, whereas girls 13–19 years had lower concentrations than did age-matched boys (Fig. 1C).

The medians, ranges, logarithmic means (SD), and derived 95% confidence intervals for PINP based on the age groups at which statistically significant changes occurred are shown in Table 1. Combined reference data are given for boys and girls younger than 10 years because there were no statistically significant gender differences in these age groups, but separate reference data are presented for the two genders in older children.

PICP and PINP were correlated in samples from 100 children 1–15 years of age ($r_s = 0.70$; $P < 0.0001$). In this age group, the median PINP/PICP ratio was 1.35 (range, 0.82–2.44), did not differ between the genders ($P = 0.17$), and did not change with age. By contrast, 11 older girls 15–19 years of age had much lower PINP/PICP ratios (median, 0.83; range, 0.37–1.15; $P < 0.0001$), and the correlation between the two markers was lost ($r_s = 0.10$; $P = 0.77$). Twelve boys 15–19 years of age had PINP/PICP ratios (median, 1.11; range, 0.66–2.04) that were slightly lower than in younger children ($P = 0.01$), and the two markers remained highly correlated ($r_s = 0.90$; $P < 0.0001$). Among these older adolescents, girls had lower PINP/PICP ratios than did boys ($P = 0.02$), and there was a direct relationship between the PINP/PICP ratio and PINP concentration [$r_s = 0.65$ ($P = 0.03$) for girls; $r_s = 0.81$ ($P = 0.001$) for boys].

This is the first study to report reference data for PINP across the pediatric age range. The variations in PINP with age and gender were similar to patterns observed previously for most other markers of collagen formation and breakdown in children *(13, 14, 17, 18)* and reflect the pediatric growth curve. Unlike PICP *(17, 19)*, PINP showed the expected pubertal increases in both girls and boys, suggesting that it may be a better marker of type I collagen synthesis than PICP in adolescence. The timing of peak concentrations of PINP in relation to chronologic age coincided with the timing of peak height velocity in each gender on a population basis. Our data are consistent with earlier studies of PINP conducted on smaller numbers of apparently healthy children over a more limited age range compared with our study *(5, 20, 21)*.

It has previously been reported that children have higher plasma concentrations of PINP relative to PICP compared with adults *(5)*. We have established that the higher PINP/PICP ratio in children remained constant in both genders up to 15 years of age. During this period, the two propeptides were correlated, as expected because they are released in equimolar amounts during type I collagen synthesis. After age 15 years, the PINP/PICP ratio decreased rapidly in girls as PINP decreased, and the correlation between the propeptides disappeared. In boys 15–19 years of age, the PINP/PICP ratio decreased less markedly than in girls, and the correlation between the propeptides remained strong, but (as in the girls) lower PINP concentrations were associated with lower PINP/PICP ratios. The most likely cause of these changing PINP/PICP ratios with age is enhanced clearance of PICP by the mannose receptor in children, although reduced clearance of PINP by the scavenger receptor cannot be excluded. Regardless of the mechanism, our study confirms that PINP is a more sensitive marker of type I collagen synthesis than PICP in children.

In summary, we report age- and gender-related refer-

ence data for PINP from birth to 19 years of age. Furthermore, we have confirmed that PINP is a more sensitive marker of type I collagen synthesis than PICP in the pediatric age group.

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High-Throughput Liquid Chromatography–Tandem Mass Spectrometry Assay for Plasma Theophylline and Its Metabolites, *Junghan Song,[1,2] Kyoung Un Park,[1,2] Hyung Doo Park,[1] Yeomin Yoon,[3] and Jin Q. Kim[1*]* ([1] Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Korea; [2] Department of Laboratory Medicine, Seoul National University Bundang Hospital, Gyeonggi-do, Korea; [3] Department of Laboratory Medicine, Cheju National University College of Medicine, Jeju, Korea; * address correspondence to this author at: Department of Laboratory Medicine, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul 110-799, Korea; fax 82-2-745-6653, e-mail jqkim@plaza.snu.ac.kr)

Theophylline is metabolized to 1,3-dimethyluric acid (1,3-DMU), 3-methylxanthine (3MX), and 1-methylxanthine (1MX) mainly by CYP1A2, and 1MX is rapidly converted to 1-methyluric acid (1MU) by xanthine oxidase *(1–3)*. Individuals differ in terms of their rates of theophylline metabolism and the resulting serum concentrations *(1)*; moreover, some theophylline metabolites, such as 3MX, are known to have bronchodilator activity. Thus, we need to determine the plasma concentrations of theophylline and its metabolites simultaneously to ensure the safe use of theophylline, especially in patients with renal insufficiency, in whom serious side effects can occur if metabolites are allowed to accumulate.

Many HPLC methods have been used to measure theophylline and its metabolites. However, most of these techniques require the avoidance of caffeine or require a longer separation time because of the interfering effect of caffeine metabolites such as 1,7-dimethylxanthine (1,7-DMX), 3,7-dimethylxanthine, and theophylline (1,3-dimethylxanthine) *(4)*. Recently, liquid chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods to determine theophylline and caffeine metabolites have been developed *(5, 6)*. However, these methods require two runs per sample, with negative and positive ionization, and the total HPLC run time is ~60 min. These methods therefore are not suitable for the high-throughput determinations of theophylline and its metabolites required for routine therapeutic drug monitoring. In the present study, we developed a simple and rapid LC-MS/MS method for the simultaneous determination of plasma theophylline and