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, for whom only 4, 6, and 4 samples, respectively, were available. Infants (10) were enrolled as follows: preterm infants (≤37 weeks of gestation), term infants, and children (23 males) younger than 5 years. Neonates (13) were randomized to 2 groups: premature neonates born at ≤37 weeks and those born at ≥37 weeks. Both groups included males and females. Eighteen of the 43 children attended 2 features of bone remodeling is the 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).

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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 genders. 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
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 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 scavenger 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-

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

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

*Mean (SD) of log-transformed PINP concentrations (in µg/L).

Arithmetic mean of log-transformed data (in µg/L) ± 2 SD, raised to the power of 10. Not calculated for groups with n < 10.

Compared with the previous age/gender group (ANOVA followed by post hoc testing): a P < 0.001; b P < 0.05.
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.

We thank Orion Diagnostica for providing the PINP assays used in this study. Collection of the Irish blood samples was supported by the National Children’s Hospital Ladies Guild, the Garfield Weston Foundation, and the Trinity Trust.

References

10. Sciarino JK, Glew RH, Bou-Serhal CE, Clemens JD, Garry PJ, Baumgartner H, et al. Effects of intensive chemotherapy on bone and collagen turnover 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 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.

High-Throughput Liquid Chromatography–Tandem Mass Spectrometry Assay for Plasma Theophylline and Its Metabolites, Junghan Song,1,2 Kyoun Un Park,1,2 Hyung Doo Park,3 Yoomin Yoon,3 and Jin Q. Kim4 (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.

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