Collagen Markers Deoxypyridinoline and Hydroxylysine Glycosides: Pediatric Reference Data and Use for Growth Prediction in Growth Hormone-deficient Children

Frank Rauch, Mareile Georg, Angelika Stabrey, Christina Neu, Werner F. Blum, Thomas Remer, Friedrich Manz, and Eckhard Schoenau

Background: In children and adolescents, markers of bone and collagen metabolism reflect the dynamics of skeletal growth and development. The aim of this study was to assess the relationship of the urinary collagen markers deoxypyridinoline (DPD) and hydroxylysine (Hyl) and its glycosides [galactosyl-Hyl (Gal-Hyl) and glucosyl-Gal-Hyl] with growth.

Methods: Urine samples from 240 apparently healthy children and adolescents (6–19 years; 124 girls) and from 51 prepubertal children with growth hormone (GH) deficiency (3–14 years; 14 girls) were analyzed. Urinary Hyl and its glycosides were quantified by HPLC, and DPD was assessed by chemiluminescence assay. Urinary concentrations of all markers were related to urinary creatinine.

Results: Multiple regression analysis revealed that only age and height velocity were independently associated with these markers in healthy children. In GH-deficient patients, the urinary excretion of both analytes after 4 weeks of GH therapy correlated significantly with the height increase during the first treatment year (r = 0.79 for Gal-Hyl; r = 0.70 for DPD; P < 0.001 each). In a multivariate linear regression model using Gal-Hyl concentrations at 4 weeks, baseline concentrations of insulin-like growth factor 1 and height velocity after 3 months accounted for 80% of the variability in height gain during the first treatment year. A model using DPD concentrations at 4 weeks, in place of Gal-Hyl concentrations, as well as baseline concentrations of insulin-like growth factor 1 and height velocity after 3 months accounted for 83% of the variability.

Conclusions: These urinary bone and collagen markers give some early indication of growth response, but the prediction of an individual marker is too imprecise to serve as a basis for clinical decisions. Markers of bone and collagen metabolism might be more useful as components of multivariate growth prediction models.

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Skeletal growth is a complex process that includes at least three different tissue mechanisms: (a) growth in length, (b) growth in width, and (c) bone maintenance. Growth in length occurs mostly by endochondral bone formation at the level of growth plates and metaphyses. Bone growth in width is accomplished by what Frost has termed “bone modeling”. Bone modeling refers to the sculpting of a bone’s inner and outer shape and involves osteoclast and osteoblast action on opposite sides of a given piece of bone. Bone maintenance is achieved by remodeling, which is performed by the same effector cells as modeling. However, in remodeling, osteoclasts and osteoblasts sequentially resorb and produce bone on the same bone surface with little effect on the amount or shape of the bone.

All three mechanisms characterizing skeletal growth involve the formation and degradation of collagen matrix. It is therefore not surprising that various biochemical markers derived from collagen reflect longitudinal growth for children. We have shown previously that this is also true for the urinary excretion of two collagen breakdown products, galactosyl hydroxylysine...
(Gal-Hyl)\(^4\) and deoxypyridinoline (DPD). Both of these markers are derived from Hyl residues of collagen chains. DPD arises extracellularly from the covalent linkage of three Hyl residues (9), whereas Gal-Hyl is a product of the posttranslational glycosylation of collagen at certain Hyl sites (10). Large proportions of the amounts of Gal-Hyl and DPD found in urine are derived from bone collagen and, therefore, are considered markers of bone turnover (10). A proportion of Gal-Hyl residues are further glycosylated to glucosyl(Glc)-Gal-Hyl. Concentrations of Glc-Gal-Hyl are higher in skin than in bone collagen (11). For this reason, Glc-Gal-Hyl is thought to reflect skin collagen metabolism rather than bone metabolism. Unglycosylated Hyl, Gal-Hyl, Glc-Gal-Hyl, and DPD are released during collagen degradation and are not reused for new collagen formation (10, 11).

Our earlier results (6–8) with Gal-Hyl and DPD prompted us to prospectively test the growth prediction potential of Gal-Hyl and DPD. Furthermore, we established a new reference database for these markers by studying a large group of healthy children. By slightly modifying our initial chromatographic method to measure Gal-Hyl (7), we were also able to investigate the urinary excretion of Glc-Gal-Hyl and Hyl.

**Patients and Methods**

**HEALTHY CHILDREN**

The healthy study group comprised 240 children and adolescents (124 girls, 116 boys; 6–19 years). The children were participants in the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study (12), an ongoing observational study investigating the interrelations of nutrition, growth, and metabolism in healthy children. This study is performed at the Research Institute for Child Nutrition in Dortmund, Germany. The cohort was initially recruited for an anthropometric study in a representative sample of school children of Dortmund and later through personal recommendations of parents whose children were already participating. Overall, the study population mostly comprises children of middle class families, and all participants are of Caucasian origin. On an annual basis, all participants undergo a full medical history and examination, which begin at infancy.

Height was determined to the next succeeding 1 mm with a wall-mounted Harpenden stadiometer. The mean of three measurements was noted. We measured weight to the nearest 0.1 kg, using digital electronic scales with the children clothed in underwear. The stage of sexual development was determined with the grading system defined by Tanner (13). Assessment of pubertal stage was refused by five boys and seven girls. Urine samples (24-h) were obtained once from all study participants. No efforts were made to control the diet of the participants before urine collection. All individuals showed normal physical development and were free of any signs of serious health impairment.

Informed consent was obtained from the children’s parents or from the volunteers who were ≥18 years of age. In addition, written consent was also obtained from participants who were 14–17 years of age.

**PATIENTS WITH GROWTH HORMONE DEFICIENCY**

Fifty-one prepubertal children with growth hormone (GH) deficiency (14 girls, 37 boys; 3–14 years; mean ± SD, 8.0 ± 2.3 years) from 27 German centers for pediatric endocrinology participated in this prospective longitudinal trial, which has been described in detail elsewhere (14). The diagnosis of GH deficiency was based on a height velocity (HV) below the 25th percentile for age and a maximal GH response of <10 μg/L in at least two provocative tests. To avoid interference with growth attributable to pubertal development, only patients whose bone age was <10 years for boys and <9 years for girls and who had no clinical signs of puberty (pubic hair, breast development) were included in the study. Patients with a disorder of GH secretion secondary to chronic illness and malignancy were also excluded from the study.

GH replacement therapy was carried out with recombinant human GH at a standard dose of 0.07 IU·kg\(^{-1}\)·day\(^{-1}\) (0.023 mg·kg\(^{-1}\)·day\(^{-1}\); Humatrope®, Novo Nordisk, Princeton, NJ). Therapy was started at puberty, and the dose was adjusted to maintain a juvenile GH level (14). Therapy was continued for at least 2 years with a maximum of 8 years.

**Table 1. Anthropometric characteristics of the healthy study population.**

<table>
<thead>
<tr>
<th>Age, years</th>
<th>n</th>
<th>Weight, kg</th>
<th>Height, cm</th>
<th>HV, cm/year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6–7</td>
<td>20</td>
<td>23.6 ± 3.4</td>
<td>122.1 ± 5.0</td>
<td>6.2 ± 0.9</td>
</tr>
<tr>
<td>8–9</td>
<td>21</td>
<td>30.2 ± 6.6</td>
<td>133.5 ± 6.7</td>
<td>6.0 ± 1.6</td>
</tr>
<tr>
<td>10–11</td>
<td>28</td>
<td>43.5 ± 10.5</td>
<td>151.4 ± 8.1</td>
<td>6.5 ± 1.8</td>
</tr>
<tr>
<td>12–13</td>
<td>23</td>
<td>51.5 ± 13.5</td>
<td>158.2 ± 8.1</td>
<td>5.4 ± 2.3</td>
</tr>
<tr>
<td>14–15</td>
<td>15</td>
<td>61.0 ± 17.5</td>
<td>170.9 ± 7.3</td>
<td>2.0 ± 2.6</td>
</tr>
<tr>
<td>16–19</td>
<td>24</td>
<td>61.8 ± 11.1</td>
<td>168.9 ± 8.1</td>
<td>0.4 ± 0.6</td>
</tr>
<tr>
<td>Boys</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6–7</td>
<td>20</td>
<td>24.1 ± 4.8</td>
<td>122.4 ± 5.6</td>
<td>6.5 ± 1.0</td>
</tr>
<tr>
<td>8–9</td>
<td>15</td>
<td>29.8 ± 4.6</td>
<td>134.7 ± 6.9</td>
<td>6.4 ± 0.8</td>
</tr>
<tr>
<td>10–11</td>
<td>33</td>
<td>40.9 ± 8.7</td>
<td>149.2 ± 9.2</td>
<td>5.6 ± 1.5</td>
</tr>
<tr>
<td>12–13</td>
<td>19</td>
<td>51.1 ± 9.8</td>
<td>159.8 ± 8.8</td>
<td>7.0 ± 2.7</td>
</tr>
<tr>
<td>14–15</td>
<td>14</td>
<td>60.3 ± 7.0</td>
<td>174.0 ± 6.2</td>
<td>7.2 ± 3.0</td>
</tr>
<tr>
<td>16–19</td>
<td>15</td>
<td>69.0 ± 9.7</td>
<td>178.6 ± 8.9</td>
<td>1.5 ± 2.0</td>
</tr>
</tbody>
</table>

*Nonstandard abbreviations: Gal-Hyl, galactosyl-hydroxylysine; DPD, deoxypyridinoline; Glc, glucosyl; GH, growth hormone; HV, height velocity; HV±3, height after 3 months of therapy; HV±12, height after 12 months of therapy; Cr, creatinine; and IGF, insulin-like growth factor.

*Values are mean ± SD.
Lilly Deutschland GmbH). No other hormones were administered during the study period. Urine samples (24 h) were obtained before therapy and after 2, 4, 12, and 26 weeks of treatment. Urine was collected at home just before the visit, and samples were handed to the attending physician. Height was measured at baseline and after 3 and 12 months of therapy. HV after 3 months (HV+3) and after 12 months (HV+12) of therapy were calculated from these measurements. The study on GH-deficient patients was approved by all local ethical review boards of the participating centers, and written informed consent was obtained from the parents of the patients.

Table 3. Urinary excretion of Hyl compounds and DPD. 

<table>
<thead>
<tr>
<th>Pubertal stage</th>
<th>n</th>
<th>Glc-Gal-Hyl/Cr, μmol/mmol</th>
<th>Gal-Hyl/Cr, μmol/mmol</th>
<th>Unglyc. Hyl/Cr, μmol/mmol</th>
<th>Total Hyl/Cr, μmol/mmol</th>
<th>DPD/Cr, mmol/mmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls</td>
<td>1</td>
<td>47</td>
<td>4.6 ± 0.9</td>
<td>5.5 ± 0.9</td>
<td>2.1 ± 0.7</td>
<td>12.1 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12</td>
<td>4.8 ± 1.6</td>
<td>5.9 ± 1.8</td>
<td>2.1 ± 0.8</td>
<td>12.8 ± 4.3</td>
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<tr>
<td></td>
<td>3</td>
<td>11</td>
<td>4.4 ± 1.1</td>
<td>5.6 ± 1.4</td>
<td>2.0 ± 0.9</td>
<td>11.9 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9</td>
<td>4.1 ± 1.1</td>
<td>4.9 ± 1.7</td>
<td>2.2 ± 1.0</td>
<td>11.3 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>38</td>
<td>2.8 ± 0.7</td>
<td>2.9 ± 0.9</td>
<td>1.3 ± 0.7</td>
<td>7.0 ± 2.0</td>
</tr>
<tr>
<td>Boys</td>
<td>1</td>
<td>58</td>
<td>4.4 ± 1.2</td>
<td>5.2 ± 1.4</td>
<td>2.4 ± 1.0</td>
<td>12.0 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11</td>
<td>4.3 ± 1.4</td>
<td>5.3 ± 1.9</td>
<td>1.9 ± 0.8</td>
<td>11.5 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>4.3 ± 0.2</td>
<td>5.2 ± 0.9</td>
<td>1.9 ± 0.5</td>
<td>11.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>12</td>
<td>3.9 ± 0.7</td>
<td>5.0 ± 0.9</td>
<td>1.9 ± 0.9</td>
<td>10.8 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>22</td>
<td>3.1 ± 1.0</td>
<td>3.4 ± 1.5</td>
<td>1.3 ± 0.4</td>
<td>7.8 ± 2.7</td>
</tr>
</tbody>
</table>

P

<table>
<thead>
<tr>
<th>Sex</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pubertal stage</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Interaction</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.03</td>
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</tbody>
</table>

* Variation with pubertal stage in healthy children and adolescents. Values are mean ± SD.
* Unglyc., unglycosylated; NS, not significant.
* Significance of the effect of sex.
* Significance of the effect of pubertal stage.
* Significance of the two-way interaction term between sex and pubertal stage (by two-way ANOVA).
Urine samples were stored at −20°C until analysis. We measured Glc-Gal-Hyl, Gal-Hyl, and unglycosylated Hyl, using HPLC basically as we described previously (7). Samples were derivatized with dansyl and separated on a C18 column with a solvent gradient system between two buffers containing 125 mL/L and 500 mL/L acetonitrile. Our only modification was to use a slightly different buffer gradient in this study from the buffer gradient in our previous report (7), allowing the additional separation of the peaks corresponding to Glc-Gal-Hyl and unglycosylated Hyl. Control urine samples (n = 8) with known concentrations of unglycosylated Hyl, Gal-Hyl, and Glc-Gal-Hyl were added to each analytical run of 40 samples for quality control. Mean intraassay variability for unglycosylated Hyl (at a concentration of 42.2 μmol/L), Gal-Hyl (at 65.0 μmol/L), and Glc-Gal-Hyl (at 53.7 μmol/L) was 2.6%, 2.4%, and 3.3%, respectively. Interassay variability was 5.5%, 2.5%, and 6.5%, respectively. Total Hyl was calculated as the sum of Glc-Gal-Hyl, Gal-Hyl, and unglycosylated Hyl.

Urinary DPD was determined with a commercially available chemiluminescence assay, according to the manufacturer’s instructions (IMMULITE® Pyrilinks®-D; Diagnostic Products Corporation). Urinary creatinine (Cr) was quantified by the Jaffe method. Insulin-like growth factor 1 (IGF-1) was measured with an IGF-binding, protein-blocked RIA in the presence of a large excess of IGF-2 (Mediagnost), as described previously (15).

ANALYTICAL METHODS

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STATISTICAL ANALYSES

The significance of differences between sexes, age groups, and pubertal stages were tested by ANOVA. For post hoc testing for significance among individual groups, Bonferroni’s adjustment was used. Associations were given as Pearson’s correlation coefficients. Stepwise multiple regression analyses were performed in the forward mode. We transformed results in children with GH deficiency into SD scores using the formula: SD score = [(result in patient) − (age- and sex-specific mean value in the healthy population)]/(age- and sex-specific SD in the healthy population).

All tests were two-tailed, and P < 0.05 was considered significant. These calculations were performed with SPSS software (Ver. 6.0 for Windows; SPSS Inc.).
Results

RESULTS IN HEALTHY CHILDREN AND ADOLESCENTS

Anthropometric characteristics of the healthy study population are shown in Table 1. The variation with age in the urinary excretion of Hyl glycosides, unglycosylated Hyl, and DPD is shown in Table 2. The pattern of these changes with age was similar for all indices, with the highest values in the youngest children and lowest results in the oldest age group. Two-way ANOVA showed that the effect of age was highly significant for each index, whereas no significant effect of sex was found. However, the interaction term between age and sex reached significance for the ratios of Gal-Hyl/Cr and DPD/Cr, showing that the variation with age was different between girls and boys. This was attributable to higher values for Gal-Hyl/Cr in 6- to 7-year-old girls and lower values for Gal-Hyl/Cr and DPD/Cr in girls at 14–15 years of age.

The variation of these indices with pubertal stage is shown in Table 3. Two-way ANOVA showed no effect of sex, whereas pubertal stage had a highly significant influence on all indices. The interaction term between sex and pubertal stage was of borderline significance only for DPD/Cr, attributable to higher results in prepubertal girls (Table 3).

For consistency with the literature on this topic (5), we are presenting urinary markers of collagen metabolism relative to urinary Cr concentrations. Because timed urine samples were obtained, it was also possible to separate the developmental changes in collagen markers and Cr from one another. The variation with age and pubertal stage in the daily urinary excretion of Gal-Hyl, DPD, and Cr is shown in Fig. 1. Cr excretion increased steadily with age and pubertal stage. In contrast, Gal-Hyl and DPD increased to a peak at 12–13 years of age in girls and 12–15 years in boys and decreased thereafter.

Multiple regression analyses were performed to test which of the anthropometric characteristics (age, pubertal stage, weight, height, and HV) were independently associated with collagen markers. As shown in Table 4, only indices with a significant contribution are included in the equations. All correlations are highly significant (P <0.0001 each).

Table 4. Multiple regression analysis in healthy children and adolescents.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Marker</th>
<th>Regression term\textsuperscript{b}</th>
<th>( \beta )</th>
<th>( r^2 )</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glc-Gal-Hyl/Cr, ( \mu )mol/mmol</td>
<td>5.02</td>
<td>-0.14 (age)</td>
<td>-0.41</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.13 (HV)</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Gal-Hyl/Cr, ( \mu )mol/mmol</td>
<td>5.05</td>
<td>-0.15 (age)</td>
<td>-0.32</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.27 (HV)</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Unglyc.\textsuperscript{c} Hyl/Cr, ( \mu )mol/mmol</td>
<td>+2.25</td>
<td>-0.08 (age)</td>
<td>-0.34</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.06 (HV)</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Total Hyl/Cr, ( \mu )mol/mmol</td>
<td>+12.76</td>
<td>-0.39 (age)</td>
<td>-0.40</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.46 (HV)</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>DPD/Cr, nmol/mmol</td>
<td>+19.9</td>
<td>-0.74 (age)</td>
<td>-0.39</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.59 (HV)</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Prediction of Hyl compounds and DPD from sex, age, pubertal stage, weight, height, and HV. Only indices with a significant contribution are included in the equations. All correlations are highly significant (P <0.0001 each).

\textsuperscript{b} Age, years; HV, cm/year.

\textsuperscript{c} Unglyc., unglycosylated.

Gal-Hyl/Cr in 6- to 7-year-old girls and lower values for Gal-Hyl/Cr and DPD/Cr in girls at 14–15 years of age.

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\textsuperscript{b} Age, years; HV, cm/year.

\textsuperscript{c} Unglyc., unglycosylated.

Studies in children with GH deficiency

The course of the collagen markers in children with GH deficiency before and during GH treatment is shown in Fig. 2. Compared with baseline results, concentrations of

![Fig. 2. Changes in urinary Hyl glycosides, unglycosylated Hyl, and DPD during recombinant human GH therapy in children with GH deficiency. All values are expressed as SD scores (SDS). Black filled circles, total Hyl/Cr; gray filled circles, DPD/Cr; crosses, Gal-Hyl/Cr; triangles, Glc-Gal-Hyl/Cr; squares, Hyl/Cr.](image-url)
DPD/Cr, Gal-Hyl/Cr, and unglycosylated Hyl/Cr already had increased after 2 weeks of treatment \( (P < 0.01) \), whereas the increase in Glc-Gal-Hyl/Cr was significant only after 4 weeks \( (P < 0.001) \). Thereafter, all markers remained significantly above baseline values throughout the first 6 months of therapy \( (P < 0.01) \).

Next, we evaluated the relationship between the short-term response of these collagen markers and growth in the first year of treatment. The correlation coefficients for the relationship between these indices and HV+12 were highest after 4 weeks of therapy, with correlation coefficients ranging from 0.42 to 0.79. This relationship for Gal-Hyl/Cr and DPD/Cr is shown in Fig. 3.

A multivariate linear regression model using Gal-Hyl/Cr at 4 weeks, HV+3, and baseline concentrations of IGF-1 as predictive variables accounted for 80% of the variability in HV+12 \( (r = 0.91; P < 0.0001) \); regression equation: HV+12 (in cm/year) = 4.08 + (0.00505 × mmol Gal-Hyl/mmol Cr) + [0.313 × HV+3 (in cm/year)] − [0.010 × μg/L IGF-1]. When DPD/Cr was used instead of Gal-Hyl/Cr, the model explained 83% of the variability in HV+12 \( (r = 0.92; P < 0.0001) \); regression equation: HV+12 (in cm/year) = 4.63 + (0.00518 × nmol DPD/

**Discussion**

In this study, we examined the relationship between growth and the urinary excretion of DPD, Hyl, and Hyl glycosides. Several authors have studied the urinary DPD/Cr ratio in healthy children and have reported broadly similar changes with age as in the present study \( (6,8,16−21) \). We examined previously urinary Gal-Hyl/Cr ratios in a smaller group of healthy individuals from 4 to 18 years of age with results very similar to those in the present study \( (7) \). The urinary excretion of Glc-Gal-Hyl and unglycosylated Hyl has been investigated in only small groups of children and adolescents \( (11,22,23) \). In accordance with Segrest et al. \( (11) \) and Krane et al. \( (22) \), we found higher urinary Gal-Hyl than Glc-Gal-Hyl during growth, whereas the amounts of both glycosides were about equal in the oldest study group with slow or no growth. This is consistent with the finding that Gal-Hyl is the predominant Hyl glycoside in bone collagen, whereas Glc-Gal-Hyl prevails in collagen from other sources, such as skin \( (11) \).

It may be surprising that, in accordance with earlier studies \( (6,7,18) \), no clear pubertal peak was discernible for any of the markers of the present study. On the other hand, there is little doubt that bone turnover does increase during puberty, because all three mechanisms contributing to bone turnover in growing individuals (longitudinal growth, modeling and remodeling) accelerate at this time of life \( (24−26) \). As shown in this study, there is some evidence of a pubertal peak in the daily excretion of collagen breakdown products, but this pubertal increase is obfuscated by the concomitant increase in Cr excretion. Nevertheless, the Cr-related values correlate well with individual HV. It would therefore be advantageous to analyze these results as a function of the temporal relationship with the age at peak HV rather than as a function of age or Tanner stage, as was done in the present study. However, for the younger participants of this study, age at peak HV will be known several years from now, and therefore this analysis cannot be done at present.

The relationship between HV and collagen markers has consequences for the application of these indices in clinical practice. When Gal-Hyl/Cr and DPD/Cr are used as diagnostic markers in metabolic bone disease, it is important to know whether the disease process affects bone metabolism apart from its effect on HV. This question can be addressed with the results presented in Table 4. These multiple regression data allow for calculation of age- and HV-dependent SD scores with the general equation: SD score = (measured result − predicted result)/SD of the prediction. For example, assume that a boy (13.4 years) has a Gal-Hyl/Cr result of 3.5 μmol/mmol. If his
HV is 12 cm/year, the regression predicts a result of \( 5.05 - (0.15 \times 13.4) + (0.27 \times 12) = 6.3 \mu \text{mol/mmol} \). The SD of the prediction is 1.19 \( \mu \text{mol/mmol} \) (Table 4), and therefore the SD score is \( (3.5 - 6.3)/1.19 = -2.3 \). This would be interpreted as a low value. However, if the HV of this boy is only 3 cm/year, analogous calculations yield a SD score of \(-0.29\), which would be regarded as a normal result. Thus, if the HV of a patient is known, individualized reference intervals can be calculated from the multiple regression data.

Since Jasin et al. (27) first reported on the relationship between collagen metabolism and HV four decades ago, dozens of reports have concluded that bone and collagen markers are “potentially useful” indicators of growth. Until now, however, very few attempts have been made to actually put this potential to use. Predicting the response to GH treatment could be one of the clinically most relevant situations for the use of growth indicators. The individual therapeutic effect on growth varies greatly among individuals, and indeed it would be helpful to have a way to evaluate responsiveness after a short period of therapy. At present, therapeutic success is typically assessed only after 1 year of therapy, and the dosage is increased when growth is not satisfactory. A sufficiently precise prognosis of whether the growth response will match the expectations would allow a faster dose adjustment.

Several authors (17, 28–32) have examined the relationship between the short-term response of bone and collagen markers and growth in the first year of therapy. The observed correlations between these biochemical markers and HV+12 were lower than or, at best, similar to those we found for Gal-Hyl, Glc-Gal-Hyl, and DPD. Although these associations are highly statistically significant, it is clear that the accuracy of a growth prediction on the basis of these markers is insufficient for practical purposes. We therefore have developed a multivariate regression model comprising variables that reflect different aspects of growth regulation (14). In the original model, DPD/Cr was used as a marker of bone metabolism, but as shown in the present report, similar results can be obtained when Gal-Hyl/Cr is used instead. The accuracy of the prediction that can be achieved in this manner might be adequate for clinical use. However, this model obviously still requires validation in a larger group of individuals before any firm conclusions can be reached.

In conclusion, in this study we present reference data for urinary markers of bone and collagen metabolism and examine their relation to growth. These markers give some early indication of therapeutic success of GH therapy in children with GH deficiency, but the prediction of an individual marker is too imprecise to serve as a basis for clinical decisions. Markers of bone and collagen metabolism might be more useful as components of multivariate growth prediction models.


