mean values of urea or creatinine measurements on either analyzer (Table 1).

To determine whether the heat treatment degraded glucose in vitreous humor, glucose was added to four different specimens that had been heated once to reduce their viscosity and allow reproducible pipetting. Glucose was added because it is usually detectable only in vitreous humor from patients with marked hyperglycemia. The glucose concentrations of these specimens before and after repeating the heat treatment and centrifugation were 12.5 and 13.1 mmol/L, 11.8 and 12.0 mmol/L, 11.7 and 11.6 mmol/L, and 11.9 and 12.1 mmol/L, respectively. These results indicated that glucose, like urea and creatinine, was not degraded in vitreous humor subjected to these conditions. It is even possible that the heat treatment could slow the rate of change of vitreous humor glucose concentrations by denaturing key glycolytic enzymes.

Heating vitreous humor to 100°C for 5 min was a simple and safe method for improving the precision of electrolyte measurements on the Hitachi 911 and 737 analyzers without any adverse effects on the measurement of urea, glucose, or creatinine. We found that centrifuging vitreous humor specimens alone, as suggested by others (2), did not have the same effect. We postulate that the beneficial effects of heating derived from the reduced viscosity of the treated specimens. Like previous workers (8), we found large differences in the reliability of vitreous humor measurements on different analyzers, presumably caused by differences in the mechanics of pipetting, pumping, and dilution. Heat treatment obviously would not be suitable before the measurement of volatile or labile substances. We are examining whether treatment with enzymes that break down mucopolysaccharides might be a suitable alternative in these situations (10).

It is interesting to speculate that some of the unusual results of biochemical testing of vitreous humor reported in the past may be explained by variable specimen viscosity and poor analytical precision. Coe and Apple (8) showed significant variations in sodium, urea, and glucose results for some specimens between different analyzers and commented on the diagnostic confusion that this could produce. They reported good precision (CV <4.3%) for all of their tests on a single pooled vitreous control. Their instruments may not have been as susceptible to the precision problems that we encountered, although it is also possible that their single pooled control may not have reflected the wide variation in viscosity that can occur between individual specimens. Balasooriya et al. (6) and Madaea et al. (7) showed large differences in potassium concentrations between the left and right eyes of some patients and used this to argue that potassium measurements are not useful for calculating the postmortem interval. Neither of these groups performed repeat measurements on individual specimens; therefore, it is possible that some of the apparent eye-to-eye variation was the result of analytical variation.

References

Bone Alkaline Phosphatase Isoenzyme and Carboxy-Terminal Propeptide of Type-I Procollagen in Healthy Chinese Girls and Boys, Keh-Sung Tsai,1,2 Men-Hwaing Jang,3 Sandy Huey-Jen Hsu,4 Wern-Cherng Cheng,1 and Mei-Hwei Chang2 (Departments of 1 Laboratory Medicine and 2 Pediatrics, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China; 3 Department of Laboratory Medicine, Taipei City Psychiatric Center, Taipei, Taiwan, Republic of China; and 4 address correspondence to this author at: Department of Laboratory Medicine, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei, Taiwan, Republic of China; fax 886-2-23224263, e-mail kstsaimd@pcmail.com.tw)

The bones of children grow at a faster rate during the first few years of childhood and puberty. Recently, advances in assays for biochemical markers of bone formation have provided noninvasive means to study bone growth and metabolism in children (1–14). Because of the variations in the rate of bone growth in different age groups and possible ethnic differences, age-specific reference ranges for bone formation markers must be established in a particular pediatric population.

Conventional bone formation markers such as osteocalcin (1) have been shown to correlate with serum concentrations of insulin-like growth factor-1 and testosterone in children and adolescents. In pathological conditions, osteocalcin (2–4) is lower in growth hormone-deficient children and increases with replacement therapy. Osteocalcin, however, is labile and possesses problems in sample processing and storage (15). Recently, two other bone formation markers in serum, bone alkaline phosphatase isoenzyme (BAP) (16) and the carboxy-terminal
healthy Taiwanese girls and boys

changes in serum concentrations of BAP and PICP in the aim of this study was to investigate the age-related decreases in these two markers. Both markers are chemically much more stable than osteocalcin; therefore, their measured concentrations should be less sensitive to processing and storage conditions. The limits of these assays.

The horizontal line in each panel represents the mean + 2 SD values of healthy Chinese adults, ages 20–50 years, of the same gender [from Tsai et al. (18)].

Table 1. Mean values (± SD) of PICP and BAP in different age groups of both genders.

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Girls</th>
<th>Boys</th>
<th>Girls</th>
<th>Boys</th>
<th>Girls</th>
<th>Boys</th>
<th>Girls</th>
<th>Boys</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td>20</td>
<td>20</td>
<td>43</td>
<td>50</td>
<td>37</td>
<td>41</td>
<td>10</td>
<td>9</td>
<td>118</td>
<td>68</td>
</tr>
<tr>
<td>P = 0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAP, μg/L</td>
<td>122±19</td>
<td>104±21</td>
<td>106±16</td>
<td>94±17</td>
<td>112.4±16</td>
<td>114±21</td>
<td>39±23</td>
<td>59±19</td>
<td>17±6</td>
<td>20±8</td>
</tr>
</tbody>
</table>
| *Values for young adults were from Tsai et al. (18).*

propeptide of type-I procollagen (PICP) (17), were shown to be sensitive and specific markers of bone formation. BAP has been shown to be lower in children with growth hormone deficiency (5). When these children were treated with growth hormone, BAP showed a substantial increase (5). Both PICP and BAP were also used to monitor the response to growth-promoting agents in short apparently healthy children (6). On the other hand, in children with precocious puberty treated with gonadotropin-releasing hormone agonists, PICP decreased, suggesting a favorable retarding effect on skeletal growth (7). PICP was reported to be lower in children treated with glucocorticoid for asthma (8) or inflammatory bowel disease (9). The serum concentration correlated with the growth velocities in children and adolescents having inflammatory bowel disease with or without corticosteroid therapy (9). These two markers are chemically much more stable than osteocalcin; therefore, their measured concentrations should be less sensitive to processing and storage conditions. The aim of this study was to investigate the age-related changes in serum concentrations of BAP and PICP in healthy Taiwanese girls and boys <18 years of age.

We collected fasting morning serum samples from 110 girls and 120 boys from the urban Taipei area in 1997. All of them gave blood for the purpose of hepatitis screening and were found to have normal liver, kidney, and thyroid function. None of them was receiving any medication or had diseases that could affect bone metabolism. After venipuncture, serum samples were aliquoted and stored at −70 °C until analysis. The procedures were in accordance with the revised Helsinki declaration in 1983.

Serum BAP was measured with immunocatalytic kits (Metra Biosystem). The intraassay imprecision (CV) was 8% and the interassay CV was 11% at 25 U/L in our laboratory. Serum PICP was measured with radioimmunoassay kits (Orion Diagnostic). The intraassay CV was 7% and the interassay CV was 9% at 285 μg/L. All of the samples showed concentrations well above the detection limits of these assays.

The values of BAP and PICP of the different age groups are shown in Table 1. Both BAP and PICP showed sigmoid regression curves with increasing age (Fig. 1). Both markers showed mean values approximately fourfold higher than the upper reference limit for adults in the first 3 years of life in both genders. The PICP then was substantially lower after 3 years of age in both girls and boys until puberty. Prepubertal PICP was one- to twofold higher than the upper reference limit for adults. During puberty, PICP increased again to a mean of 250 μg/L, approximately twofold higher than the upper limit of adults in each gender. After puberty, both girls (ages, 13–18 years) and boys (ages, 15–18 years) showed mean PICP concentrations at approximately the upper reference limit for adults. In contrast to the substantial decrease in PICP, BAP showed sustained high values in prepubertal girls and boys, a phenomenon similar to that of osteocalcin (13, 14). However, unlike the lack of higher osteocalcin in the first few years of life (13, 14), BAP was higher during the first 3 years of life than the prepubertal values during the next 5 years. After puberty, BAP showed a gradual decrease in girls and boys. In general, girls showed decreased postpubertal values of both markers 2 years earlier than boys, reflecting the earlier completion of puberty in girls. The correlation between PICP and BAP was significant in boys (n = 120; r = 0.261; P = 0.004), in...
to be good tools for clinical and physiologic research. Although various bone markers have been used extensively in physiologic and clinical research in adults, much less information is available in children. Metabolic bone disorders in childhood are caused mainly by defects in osteoblastic functions, which emphasizes the important role of bone formation markers in pediatrics. The two markers examined in this study are stable, suitable for long-term storage, and showed little overlap between the adult values and the childhood/adolescent values. Unlike osteocalcin, they clearly showed higher concentrations in infancy, when the rate of growth was fastest. Both appear to be good tools for clinical and physiologic research.

References


