Bone Alkaline Phosphatase and Height Velocity in Short Normal Children Undergoing Growth-Promoting Treatments: Longitudinal Study

Patricia M. Crofton,1 Heather F. Stirling,2,3 and Christopher J. H. Kelnar2

We studied the temporal and quantitative relation between bone alkaline phosphatase (ALP) and height velocity in 62 short normal children as part of a prospective randomized study to compare placebo, growth hormone, oxandrolone, and testosterone, singly and in combination, in promoting short-term growth acceleration and increased final height. The pretreatment cross-sectional correlation between bone ALP and height velocity was poor (P ≥0.25), but was much higher (P = 0.0001) 3 months after treatment started. In each treatment group, there was a parallel relation between bone ALP and height velocity through time. Individual children showed a variety of growth responses over 12–42 months, but in almost all cases bone ALP paralleled height velocity. Within individual children, bone ALP was strongly correlated with 6-month height velocity (r >0.9 in 30% of the children, r >0.7 in 70%). We conclude that bone ALP is a useful short-term marker of growth in short normal children treated with growth hormone.

Indexing Terms: growth hormone/oxandrolone/testosterone/pe- diatric chemistry/isoenzymes/somatotropin/enzyme activity/variation, source of

Until relatively recently, treatment with growth hormone (GH) was reserved exclusively for children with demonstrable GH deficiency, all of whom could be predicted to grow rapidly in response to therapy. With the advent of biosynthetic GH, available in virtually unlimited quantities, there has been an upsurge of interest in treating other clinical groups, in whom growth response to exogenous GH may be less predictable (1). Although an increase over predicted adult height is sometimes regarded as the ultimate goal of treatment, an interim acceleration in height velocity may be of equal or greater psychological importance to the child, and is therefore an equally important clinical goal. The pattern of short-term growth response to treatment through time may help to elucidate the relative contributions of endogenous and exogenous factors, and to develop rational therapies. However, the measurement of height velocity is insensitive and im-精准 over short time periods, retrospective in nature, and operator- and instrument-dependent (2–4). A biochemical marker that could be measured accurately and precisely and that could reflect relatively short-term fluctuations in growth would clearly provide a valuable objective tool in the assessment of an early response to growth-promoting treatments.

Linear growth, particularly that under the control of GH, occurs predominantly at the epiphyseal growth plate of the long bones (5). Growth is also associated with extensive remodeling of bone, involving both osteelastic and osteoblastic phases. The bone isoform of alkaline phosphatase (ALP; EC 3.1.3.1) is present in mature osteoblasts (6), in the hypertrophic chondrocytes of the epiphyseal growth plate, and in the matrix vesicles associated with bone mineralization (5); it is the predominant isoform in the plasma of growing children. Cross-sectional studies have demonstrated that the activities of both total (7, 8) and bone (9–13) ALP in plasma parallel the childhood height velocity curve, with highest activities during infancy, smaller increases during puberty (the peak occurring earlier and lower in girls than in boys), and a postpubertal decrease to much lower adult values. The coincidence in timing of the height velocity and total ALP peaks during puberty in both boys and girls has been confirmed in a longitudinal study (14). However, to our knowledge, only one semi-longitudinal study has investigated the quantitative relation between total ALP and height velocity (15). A significant correlation was found between the two for measurements taken at yearly intervals during normal puberty; the bone isoform was not measured. Although in healthy children, changes in total ALP largely reflect changes in bone ALP, the same is not necessarily true of other clinical groups in whom the underlying disease state or drug treatment often has a significant effect on liver ALP. It is therefore necessary to establish the relation of bone ALP, rather than total ALP, to growth.

A long-term prospective intervention study, partially placebo-controlled on a double-blind basis, is currently being carried out on well-defined groups of healthy children with short stature but no demonstrable abnormality of GH secretion; its overall aim is to compare the efficacy of various growth-promoting treatments in causing short-term acceleration of growth and ultimate increase (if any) in final height. As part of that longitudinal study, we report here the detailed temporal and quantitative relation between bone ALP and height velocity in a cohort of these short normal children for whom a complete data set was available, both
during growth response to treatment and during their own pubertal growth spurts.

Patients and Methods

Patients and Treatments

Sixty-two children, ages 4.7–16.5 years, who were attending a tertiary referral center for growth disorders, participated in the study. Children were excluded if they had evidence of systemic disease, were receiving long-term medication that might compromise growth, had malnutrition or diabetes, or showed significant psychosocial pathology. Full informed consent was obtained from both children and parents, and the study was approved by the local ethical committee. Participants in the study were divided into three groups, allocated to a number of different treatment schedules as detailed below and in Table 1.

Group A was 24 children (14 boys and 10 girls) with familial short stature without significant growth delay. Their height was at or below the third centile for chronological age, and their height velocity standard deviation score was ≤0 at enrollment. They were all clinically and biochemically prepubertal at the start of the study, with a bone age <8 years and a bone age delay <2 years. Only children with peak GH responses of >20 mIU/L in response to insulin-induced hypoglycemia or clonidine were included in the study. These children were initially randomized into three subgroups. The first received no treatment for 2 years, but thereafter were treated with biosynthetic GH (Norditropin®, supplied by NovoNordisk, Gentofte, Denmark) in a dose of 15 IU/m² per week, divided into daily subcutaneous injections. The second subgroup received daily GH (24 IU/m² per week) and the third received daily placebo injections, both on a double-blind basis and both commencing at the time of enrollment in the study. After the code was broken at the end of a year of treatment, the placebo group was started on daily GH at the lower dose of 15 IU/m² per week, whereas the group that had received GH continued treatment at the higher dose.

Group B was 26 boys with familial short stature in late puberty at the time of enrollment. They had a chronological age <14 years, a bone age >8 years, a bone age delay <2 years, and their height was at or below the third centile for chronological age. Before inclusion in the study each boy underwent a GH stimulation test (insulin hypoglycemia or clonidine). A few had peak GH responses <20 mIU/L but, because they were not primed beforehand with sex steroids, this was not considered to disqualify them from the study [peak GH response did not predict response to treatment in this group (unpublished observations)]. None had classical GH deficiency. The boys were randomized to four subgroups. The first received daily GH alone (24 IU/m² per week), the second oxandrolone alone (2.5 mg/day orally), the third a combination of GH and oxandrolone at the same dosages, and the fourth no treatment for the first year, followed by daily GH at 15 IU/m² per week in subsequent years. Oxandrolone was discontinued when the testes reached 6 mL in volume.

Group C was 12 boys with constitutional delay in growth and puberty. Their chronological age was >14 years but they were still either prepubertal or only in early puberty (testicular volume <6 mL) at enrollment. Like the boys in group B, they did not necessarily all achieve peak GH responses of >20 mIU/L during unprimed stimulation tests, but none had evidence of classical GH deficiency. They were randomized to three subgroups. The first received daily GH alone (24 IU/m² per week), the second testosterone alone (testosterone undecanoate, 40 mg orally on alternate days), and the third a combination of GH and testosterone at the same dosages.

Anthropometry

Height measurements were made by the same examiner throughout and were recorded for a minimum of 6 months, but more usually 12 months, before a child was enrolled in the study. During the study, height was recorded every 3 months. Height velocities were calculated on each occasion and expressed as either 3-month or 6-month height velocities (the difference between the current height and that measured either 3 or 6 months earlier, respectively, both corrected for the exact time interval between the two measurements and normalized to centimeters per year).

Blood Samples

For those children receiving active treatment (including the placebo group), heparinized blood samples were collected at baseline (pretreatment), after 3 and 6 months of treatment, and then at 6-month intervals throughout treatment. These intervals were chosen partly for ethical reasons, to minimize blood sampling in healthy children, and to restrict sampling to the intervals appropriate for other biochemical tests of possible deleterious side-effects of treatment. The chosen sample times also coincided with measurements of height, which would have been inappropriate at shorter intervals. Children who were randomized to the no-treatment subgroups had blood samples collected at yearly intervals until they started GH treat-

---

Table 1. Numbers of children in each clinical group allocated to different treatments.  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo followed by GH*</td>
<td>6</td>
<td>—</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>GH</td>
<td>18 (8)*</td>
<td>14 (8)*</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>Oxandrolone</td>
<td>—</td>
<td>4</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>GH + oxandrolone</td>
<td>—</td>
<td>8</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>Testosterone</td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>GH + testosterone</td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>26</td>
<td>12</td>
<td>62</td>
</tr>
</tbody>
</table>

* One year of placebo, followed by GH treatment (15 IU/m² per week).

b Numbers in parentheses indicate those children in whom GH treatment was administered at the lower dose of 15 IU/m² per week after 2 years (group A) or 1 year (group B) of no treatment. The remaining children received 24 IU/m² per week from the date of their admission to the study.
ment, at which point the same schedule of sampling was followed as for the other treatment groups. After centrifugation, plasma ALP was measured within 24 h. A separate aliquot of plasma was stored frozen at −20°C until ALP isoenzyme measurement could take place. ALP isoenzymes were stable for at least a year under these conditions.

**Analytical Methods**

Total plasma ALP activity was measured at 37°C, with p-nitrophenyl phosphate as substrate in diethanolamine buffer (16). The reference range for children in our laboratory is 250–800 U/L; the corresponding reference range for adults is 50–250 U/L. ALP isoenzymes were measured by wheat germ lectin affinity electrophoresis in agarose gel (12). The reference range of the bone isofrom in children by this method is 180–700 U/L (12). The interassay CV for bone ALP was 5.4%.

**Results**

In a cross-sectional analysis of the pretreatment data among all 62 children, there was no significant correlation of the absolute values of bone ALP activity and height velocity, whether the latter was measured over the previous 12 months (r = 0.03, P = 0.80), 6 months (r = 0.06, P = 0.64) or 3 months (r = 0.15, P = 0.25). However, among the 59 children for whom both baseline and 3-month data were available, there was a highly significant correlation between the bone ALP measured at 3 months and the height velocity over those first 3 months of treatment (r = 0.46, P = 0.0002). There was a slightly stronger correlation when both were expressed as their increments over baseline values (r = 0.50, P = 0.0001).

Among the children treated with GH only (Table 1), there was no significant difference between groups A, B, and C in terms of their increments in bone ALP and height velocity at 3, 6, and 12 months (three-factor analysis of variance, single repeated measure model: P > 0.2); these children were therefore combined into a single GH-treated group.

Figure 1 shows the changes in bone ALP and height velocity over the first year of treatment in each treatment group for those 59 children for whom a full data set was available. Analysis of variance (one-factor, single repeated measure model) indicated that there was an overall significant response to treatment by both bone ALP and height velocity in each of the treatment groups except the placebo group (Table 2). During GH treatment (whether alone or in combination with oxandrolone or testosterone), the major response by both bone ALP and height velocity occurred in the first 3 months, with a parallel relation between bone ALP and height velocity during the first year.

There was, however, considerable variability in the response to treatment of individual children. This may have been at least partly attributable to the observation that 21 of the 59 children entered puberty during the first year (defined as a testicular volume >6 mL for boys or Tanner breast stage >1 for girls), although all but one of the children were prepubertal at the time of commencing active treatment. Fig. 2 shows some examples of that variability in individual boys treated with GH (either alone or combined with oxandrolone or testosterone) for 18 to 30 months. Whatever the pattern of response, in almost all cases (both in those illustrated and those not shown), the changes in bone ALP closely paralleled the changes in height velocity.

Figure 3 shows the within-child correlations in all 62 children between the bone ALP at each time point and the corresponding height velocity over the previous 3 months and over the previous 6 months. Bone ALP was more highly correlated with the integrated height velocity measured over the previous 6 months than with that over the most recent 3 months, with 71% of the correlation coefficients within individual children exceeding 0.7 and 31% exceeding 0.9.

To define the quantitative relation between increments (compared with the pretreatment baseline) in bone ALP (y) and in 6-month height velocity (x), regression lines were calculated for the 11 children who fulfilled the following criteria: (a) >4 consecutive data points (median 6, range 5–8), (b) r ≥0.90, and (c) P < 0.02 (Fig. 4). The mean intercept was 8 U/L (SD 31 U/L, range −37 to 73 U/L) and was not significantly different from zero in 10 of the 11 children (95% confidence limits). When the data were expressed in
Table 2. One-factor analysis of variance (repeated-measures model) of serial changes in height velocity (HV) and bone ALP after 0, 3, 6, and 12 months of treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>n</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Placebo</td>
<td>6</td>
<td>0.3</td>
<td>0.826</td>
</tr>
<tr>
<td>ABC</td>
<td>GH</td>
<td>34</td>
<td>26.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>B</td>
<td>Oxandrolone</td>
<td>4</td>
<td>5.8</td>
<td>0.018</td>
</tr>
<tr>
<td>B</td>
<td>GH + oxandrolone</td>
<td>7</td>
<td>20.7</td>
<td>0.001</td>
</tr>
<tr>
<td>C</td>
<td>Testosterone</td>
<td>4</td>
<td>49.8</td>
<td>0.009</td>
</tr>
<tr>
<td>C</td>
<td>GH + testosterone</td>
<td>4</td>
<td>8.3</td>
<td>0.058</td>
</tr>
</tbody>
</table>

* See Table 1.

absolute terms rather than as increments, the mean intercept was 265 U/L (SD 100 U/L, range 187–525 U/L). Although the slopes of the regression lines varied by a factor of 2 (mean 40.2, SD 10.6, range 25.4–58.5), these differences did not reach significance (95% confidence limits).

Discussion

In this study, height was recorded in a tertiary referral center by the same experienced observer throughout, who used a carefully set up stadiometer.
Even under such ideal conditions, however, there is an inherent imprecision (SD ±0.4 cm) for each height measurement, largely attributable to the flexibility and posture of the child (2–4). Since height velocity is based on two consecutive measurements, its imprecision is even larger and becomes proportionately greater the closer together the two measurements are made. This limits its sensitivity and reliability in detecting true fluctuations in growth, particularly over short periods (4). If more than one observer is used (as would be the case in routine practice, even in specialist centers), there may be systematic biases of 0.3 cm or greater for each height measurement, even in experienced hands (3). The combination of systematic bias and imprecision may give rise to an error of as much as 1 cm in the increment between two consecutive height measurements, on which calculations of height velocity are based, i.e., error of ±4 cm/year in height velocity calculated at 3-month intervals (3). It is in this context that the concept of a sensitive biochemical marker that reflects growth, that can be measured simply and precisely, and that is free from observer bias, becomes attractive, particularly in clinical situations in which rapid changes in growth are anticipated.

Bone ALP was, on theoretical grounds, a good candidate growth marker since it is present in the cells actively involved in bone growth: mature osteoblasts, hypertrophic chondrocytes, and matrix vesicles (5, 6). However, in our study, the correlation between pretreatment bone ALP and height velocity was poor. This may be at least partly attributable to the relatively narrow range in pretreatment height velocity in the children and in agreement with previous studies investigating other biochemical markers of growth: procollagen type I C-terminal propeptide (PICP), osteocalcin, and procollagen type III N-terminal propeptide (PIIINP) (17–22). Although some of these studies showed correlations when a very wide range of growth rates was included (from patients with GH deficiency to tall pubertal subjects), inspection of the data shows much poorer correlations within homogeneous diagnostic categories—for example, in short, normal, prepubertal children. In our study, the correlation between bone ALP and height velocity was much stronger 3 months after treatment was started and was strongest (P = 0.0001) when both were expressed as increments over pretreatment values.

All of the treatments except placebo resulted in increased growth and bone ALP. Combining GH with either oxandrolone or testosterone appeared to have a greater effect on both height velocity and bone ALP responses than GH alone during the first year of treatment, but this conclusion must remain tentative because numbers were small in the former two treatment categories. In the testosterone-treated group, the bone ALP increment initially lagged behind the height velocity increase in three of four boys, although it subsequently correlated well with height velocity. In the same three boys, other growth markers (PICP and PIIINP) also showed very little change at 3 months compared with baseline concentrations, in contrast to significant increases in all other treatment groups except placebo (data not shown). A further cohort of 6 testosterone-treated boys in whom only total ALP was measured had a mean increment of 104 U/L (range 18–263 U/L) compared with a mean height velocity increment of 3.35 cm/year (range 1.77–5.01 cm/year) in the first 3 months of treatment, suggesting that the three boys in our study with delayed bone ALP responses were not entirely typical of testosterone-treated boys as a whole. A delay in osteoblast proliferation and maturation normally associated with growth might explain a delayed bone ALP and PICP response, but not a delayed PIIINP response. GH stimulates linear growth of the long bones, whereas testosterone acts both directly to promote spinal growth and indirectly via stimulation of GH release (23). However, examination of sitting height velocity and subischial height velocity in the testosterone-treated boys did not suggest that differential growth at different skeletal sites could explain the differences in early biochemical response in bone ALP and PICP. Perhaps the three boys with delayed biochemical response had enhanced clearance of bone ALP, PICP, and PIIINP, all of which are removed from the circulation by liver endothelial cells, although different receptors are involved.

In all groups treated with GH, whether alone or in combination with oxandrolone or testosterone, there was a marked response in bone ALP and height velocity within the first 3 months of treatment, with an overall parallel relation between the two indicators. Variable patterns of response were seen in individual children, presumably partly because, unlike GH-deficient children, they were also secreting their own
endogenous GH and their growth was the outcome of both endogenous and exogenous factors. Other contributing factors were compliance, whether or not they entered their own endogenous pubertal growth spurt, and whether puberty was induced by exogenous testosterone in addition to GH. In almost all cases, bone ALP and height velocity changed in parallel, within the limits of error for height velocity measurements.

We examined the within-child correlations between serial measurements of bone ALP and height velocity, either over the most recent 3 months at each time point or integrated over the previous 6 months. Correlations were greatest with height velocity measured at 6-month intervals, with 70% of the correlation coefficients being >0.7 and 30% being >0.9. This may reflect the greater error associated with height velocities calculated from height measurements only 3 months apart. However, a possible alternative or additional explanation relates to the localization and timing of expression of ALP in bone and the sites of action of GH. GH appears to act simultaneously on the germinai, proliferating, and hypertrophic layers of epiphyseal cartilage, either directly via GH receptors or indirectly via insulin-like growth factor I (5). Bone ALP is found only in the hypertrophic layer, in which it is located on the plasma membrane of chondrocytes and in extracellular matrix vesicles (5). It is also expressed by osteoblasts, not during the early proliferative cycle (which expresses PICP), but in the later maturation phase (6).

As growth is stimulated by GH, there is an expansion in the population of growth plate chondrocytes at all stages of differentiation, resulting in elongation of the growth plate and an early increase in bone ALP. As the chondrocytes from the germinai and proliferative layers (which do not express ALP) further differentiate to form the hypertrophic layer, which is then invaded by osteoblasts from the metaphysis to form new bone, there is a further increase in bone ALP. The time course of these events has not yet been established in the growing child, but it would explain why there was some lag in attaining maximal expression of bone ALP. There is some support for this in the literature: GH treatment of GH-deficient children resulted in an earlier increase in PICP than in bone ALP, although height velocity was not measured (24, 25).

The cause of the few poor correlations between bone ALP and height velocity in individual children appeared to be multifactorial, including single erroneous height measurements (distorting both the preceding and subsequent calculations of height velocity) and lack of response to treatment (resulting in minimal changes in both height velocity and bone ALP).

In an attempt to discover whether the variability between children was due to different slopes in their individual regression lines between bone ALP and height velocity, we selected only those children who had well-defined regression lines, with high and significant correlations. We found that the slopes varied by a factor of 2 but, owing to the limited number of data points, the differences did not reach significance. Nevertheless, it seems likely that different children have different set points between their growth and bone ALP activity, resulting in very high intraindividual correlations, but lower interindividual correlations. Once the set points of an individual have been ascertained (intercept and slope), growth can be predicted from bone ALP measurements.

In conclusion, bone ALP activity closely parallels height velocity changes through time in short normal children undergoing growth-promoting treatments. Although pretreatment correlations were poor in the unstimulated baseline state, as has been observed for other markers, correlations between bone ALP and height velocity were much higher once growth had been stimulated, especially when both were expressed as increments compared with baseline values. Within individual children, the correlations were greatest between bone ALP and height velocity measured over the previous 6 months, allowing prediction of growth in an individual child by precise and objective measurements of bone ALP once their relation in that particular child had been established.

We thank NovoNordisk, Gentofte, Denmark, for supplying Norditropin® for use in this study. We are greatly indebted to Janet Darling for her help in handling the blood samples from the children in the study and to Eckhard Schönaud for measurements of collagen propeptides.

References