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PRELIMINARY REPORT

Effect of Adrenal Androgen and Estrogen on Bone Maturation and Bone Mineral Density

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To clarify the independent physiological roles of adrenal androgen and estrogen on bone growth, we compared the lumbar spine bone mineral density (BMD) in prepubertal girls with virilizing congenital adrenal hyperplasia (CAH) (n = 17) and girls with central precocious puberty (CPP) (n = 18). When BMD was analyzed according to chronologic age, no significant differences were found between CPP and CAH patients. However, when adjusted to bone age, BMD was statistically higher in CAH than in CPP subjects. This finding suggests that adrenal androgen, as well as estrogen, plays an important role in increasing BMD. Adrenal androgen may act on bone not only as androgen, but as estrogen after having been metabolized into an aromatized bone-active compound in peripheral tissues, such as bone and fat. Therefore, adrenal androgen may have a more important role in increasing BMD than previously realized.

THE SEX STEROIDS androgen and estrogen play an important role in increasing and maintaining bone mineral density (BMD).[1-6] Two recent case studies have demonstrated that estrogen plays a more critical role than androgen in bone maturation and bone mineralization.[7,8] However, more clinical evidence of the effects of these sex steroids on actual bone growth is needed to clarify their possible physiologic roles in the regulation of bone maturation and BMD. We therefore compared BMD among patients with two endocrine diseases: congenital adrenal hyperplasia (CAH) and central precocious puberty (CPP). Since patients with CAH and CPP have high levels of adrenal androgen and ovarian estrogen, respectively, these 2 diseases are thought to be good models for investigating the physiologic roles of the sex steroids on bone growth.

MATERIALS AND METHODS

The subjects of this study were 17 prepubertal girls (age 5 to 8 years) with CAH (21-hydroxylase deficiency) and 18 girls (age 5 to 8 years) with untreated CPP. The CAH patients had been receiving treatment since the neonatal period. The concentrations of their plasma sex steroids during treatment were as follows: testosterone, 0.35 to 2.0 nmol/L; androstenedione, 1.0 to 2.0 nmol/L (normal for prepubertal girls, <1 nmol/L); and estradiol, less than 35 pmol/L (normal for prepubertal girls, <35 pmol/L). The CPP patients had historys of breast development before the age of 8 years. At the time of initial examination, their plasma estradiol concentrations were 110 to 240 pmol/L, and their testosterone concentrations were less than 0.2 nmol/L.

Bone Age Assessment

Bone age of patients with CPP and CAH was assessed using the Tanner Whitehouse-2 (RUS) method for Japanese children.[9] This method is based on data from more than 500 Japanese children, aged 3 to 18 years.

BMD Assessment

Dual-energy x-ray absorptiometry was performed using a Hologic QDR 2000W densitometer (Hologic Inc, Waltham, MA). Bone mineral content was measured in grams per centimeter, and BMD (measured in grams per square centimeter) was calculated for the second, third, and fourth lumbar vertebrae. Precision errors in children are 1% to 2%. The BMD values were compared with normal Japanese control data,[10-12] and the standard deviation scores for chronologic age and bone age were calculated accordingly.

Bone age is of great importance in evaluating growth disorders in children because the chronologic age does not necessarily correspond

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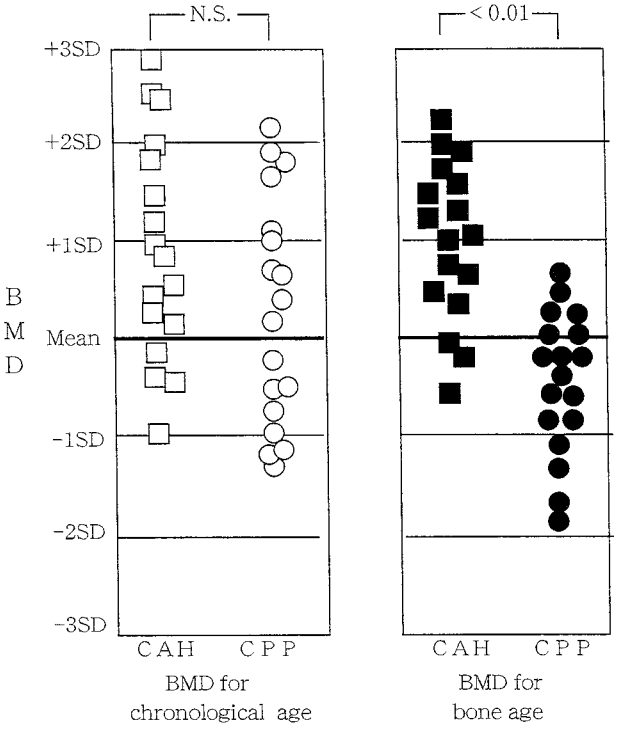

Fig 1. Comparison of BMD SD scores for chronologic age and for bone age in the lumbar spine of girls with CAH or CPP. N.S., not significant.

with the bone age of these children. Discrepancies between bone age advancement and growth are particularly likely to occur in children with elevated levels of estrogen and androgen. These children usually show accelerated bone age.[13] Therefore, BMD values adjusted to bone age, not to chronologic age, seem to be appropriate for evaluating altered bone mineral status in patients with CPP and CAH. Practically, we compared bone age in the patient with the same chronologic age in the reference population. The statistical analysis was performed using a *t* test and the Mann-Whitney test. A *P* value less than .05 was considered statistically significant.

Effect of Adrenal Androgen on Bone

To examine the effect of adrenal androgen on bone growth and bone metabolism in CAH patients, correlations between serum levels of testosterone (which is thought to reflect the serum level of adrenal androgen) and osteocalcin (a biochemical marker of bone formation) and the linear growth velocity were retrospectively assessed in 5 of the 17 CAH patients. Retrospective data were collected for a 3-year period, with data points obtained at 6-month intervals. Growth velocity was expressed as the growth velocity SD score (SDS) for the chronologic age. Venous sampling was performed at between 10 AM and noon (before lunch). The assay procedures for these biochemical parameters have been described elsewhere.[1] Correlations between biochemical variables and growth velocity values were assessed by linear regression analysis using StatView software, version 4.5 (StatView, Berkeley, CA). A *P* value less than .05 was considered statistically significant.

RESULTS

Although the bone age/chronologic age (BA/CA) ratio did not show a statistically significant difference between patients with CAH and those with CPP (1.16 ± 0.16 *v* 1.27 ± 0.15, $P = .01$), bone maturation (bone age) in CPP patients tended to advance more rapidly than in CAH patients.

BMD adjusted to chronologic age showed no statistically significant difference between CPP and CAH patients ($P = .092$), whereas BMD adjusted to bone age was significantly higher in CAH patients than CPP patients ($P = .009$ by *t* test, and $P < .01$ by Mann-Whitney test) (Fig 1).

The correlations between growth velocity and testosterone levels ($r = .45$, $P = .02$), as well as growth velocity and osteocalcin levels ($r = .55$, $P = .001$), in CAH patients are shown in Fig 2.

DISCUSSION

Although the sex steroids androgen and estrogen are considered to have an important role in increasing and maintaining BMD,[1-6] a comparison of spinal BMD adjusted to bone age in patients with CAH and CPP indicated that the BMD of CAH patients was higher than that of CPP patients. This finding can be explained by the observation that the BA/CA ratio was higher in CPP patients than in CAH patients. In other words, adrenal androgen appears to have a smaller role in bone maturation than estrogen despite the fact that both estrogen and adrenal androgen are involved in increasing BMD. Such a situation would account for the difference in BMD gains in relation to bone age in CPP and CAH patients seen in the present study. Thus, adrenal androgen is thought to play an

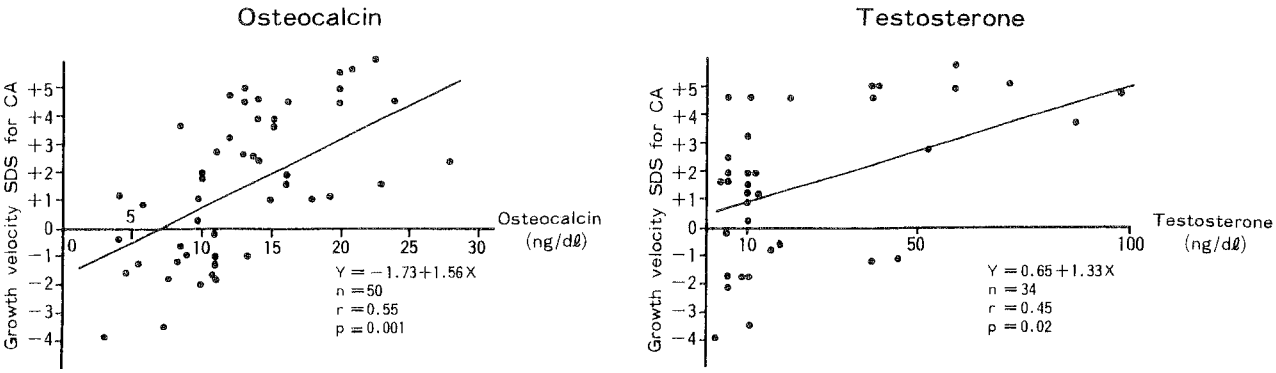

Fig 2. Correlations between serum levels of testosterone and osteocalcin and growth velocity in patients with CAH.

important role in increasing BMD, even though its effect is smaller than that of estrogen.

Although the recommended daily hydrocortisone dosage for the management of patients with CAH ($\simeq$25 mg/m^2/d) only slightly exceeds the level of cortisol normally produced by the adrenal gland (12 to 13 mg/m^2/d), such dosages given during childhood could impair bone mineral acquisition.[14-16] However, the spinal BMD of CAH patients was not lower than that of CPP patients, despite long-term corticosteroid treatment. This finding suggests that adrenal androgen may have significant effect on BMD, compensating for the adverse effect of the glucocorticoids. The important role of adrenal androgen in increasing BMD is supported by another report describing reduced BMD values in patients with Addison's disease.[17]

To examine the relation between adrenal androgen and bone metabolism, adrenal androgen (androstenedione or dehydroepiandrosterone sulfate) levels should be measured over time. Unfortunately, this information was not available in the present retrospective analysis. Nevertheless, serum testosterone levels, which are thought to indirectly reflect serum adrenal androgen levels, showed a positive correlation with growth velocity in CAH patients. In addition, the positive correlation between serum osteocalcin levels and growth velocity seems to indicate that bone growth promoted by adrenal androgen (testosterone) is also associated with an increase in osteocalcin production. These findings support the theory that adrenal androgen affects bone metabolism.

Adrenal androgens, such as dehydroepiandrosterone sulfate and androstenedione, may act directly on bone cells by binding to androgen receptors. Alternatively, adrenal androgens may act on bone cells after being converted to testosterone and dihydrotestosterone.[18] A third possibility is that adrenal androgens are metabolized by 3-β-hydroxysteroid dehydrogenase into an aromatized bone-active compound, such as estradiol or estrone,[19] in peripheral tissues, such as bone or fat. Our findings suggest that adrenal androgen may have a important role in increasing BMD than previously realized. This may be concerned the built of bone during mid-childhood before puberty. Thus, the effect of adrenal androgen on bone metabolism during childhood should be further investigated.

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