Studies on changes in the concentration of serum adrenal androgens in pubertal twins

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Abstract. A cross-sectional study was undertaken to investigate the correlation of the level of serum adrenal androgens with clinical signs of puberty and to assess the participation of genetic factors in the onset and progression of puberty. Serum concentrations of 11-deoxy-17-ketosteroids (11-deoxy-17-KS), dehydroepiandrosterone (DHEA) and androstenedione (4-A-dione) were measured in 74 monozygotic and 24 dizygotic twin pairs during the years of puberty. The mean serum concentrations of the adrenal androgens increased significantly with the advancement of bone age. A comparison of the adrenal androgen concentrations at a bone age of 15 years revealed the trend of earlier progression of puberty in girls than in boys. Serum 11-deoxy-17-KS and DHEA levels correlated fairly well with the stages of pubic and axillary hair growth in both sexes. No correlation of adrenal androgen levels with pituitary gonadotrophins or prolactin was observed in either sex during puberty. These results strongly suggest the importance of the specific action of adrenal androgens on the onset and progression of puberty, and further suggest that the maturation of the hypothalamic-pituitary-gonadal axis is not involved primarily in the maturation of the adrenal cortex during puberty.

From the viewpoint of twin zygosity, intra-pair differences of serum adrenal androgen levels were compared between monozygotic and dizygotic pairs of twins. Serum 11-deoxy-17-KS and 4-A-dione levels in monozygotic pairs of twins showed a significantly higher intrapair similarity than in dizygotic pairs of twins. These findings suggest that during puberty the maturation of the adrenal cortex is regulated by genetic factors.

The development of secondary sex characteristics starts with puberty, and is followed by the attainment of the reproductive capacity. The main processes in endocrine systems during adolescence are the changes in the hypothalamic-pituitary-gonadal function. On the other hand, there has been shown a dramatic increase in urinary excretion of 17-ketosteroids (KS) during the course of pubertal development mainly due to increased production of adrenal androgens. Recent studies have also shown a progressive rise in the serum concentration of dehydroepiandrosterone (DHEA), DHEA sulphate and androstenedione (4-A-dione) during puberty (Sizonenko & Paunier 1975; Sizonenko et al. 1976; Lee & Migeon 1975; Lee et al. 1976; De Peretti & Forest 1978; Parker et al. 1978; Ducharme et al. 1976; Hopper & Yen 1975; Reiter et al. 1977). These observations suggested the possibility that adrenal androgens may play an important role in the onset and development of puberty, although the physiological effects of these steroids is still uncertain.

The present study was undertaken in an attempt to clarify the significance of adrenal androgens in the development of puberty by evaluating the changes in serum adrenal androgen levels in relation to bone age and secondary sex characteristics, and furthermore to analyze the suspected role of pituitary gonadotrophins in the pubertal production of adrenal androgens by assessing the interrelationships between the serum levels of these hormones. In addition, the implication of genetic factors in the pubertal development of the individual was investigated by comparing the intra-pair similarity in the serum concentration of adrenal androgens between monozygotic and dizygotic pairs of pubertal twins.
Material and Methods

The study comprised 98 pairs of healthy twins, aged 12 to 15 years. Zygosity determination was made by Inoue and co-workers on the basis of polysymptomatic diagnosis, and in consequence 74 pairs of twins were considered monozygotic and 24 pairs dizygotic. The dizygotic pairs consisted of 19 pairs of the same sex and 5 pairs of different sex. Testicular enlargement, pubic and axillary hair growth and development of breasts were evaluated according to our criteria. A classification of testicular size was designed: Class C = smaller than end of index finger, Class B = approximately thumb-end size, and Class A = larger than Class B size. The development of breasts in girls was graded according to three classes: Class C = budding or minimally developed, Class A = well developed, and Class B = intermediate development between the other two classes. As to pubic hair (PH) and axillary hair (AH) growth, PH1 and AH1 indicated no hair, PH2 and AH2 minimal, and PH3 and AH3 moderate to full development of hair. The bone age of each subject was on the criteria of Greulich & Pyle (1955) utilizing a X-ray of the hand and wrist. For comparison, the serum adrenal androgen concentration in normal healthy adults (aged 20 to 39 years) was determined.

Blood samples were obtained between the hours of 08.00 and 11.00 a.m. following overnight fast. Serum was separated immediately and stored at −20°C until assayed. Serum 11-deoxy-17-KS was measured by radioimmunoassay using an anti-11-deoxy-17-KS antibody. Serum 11-deoxy-17-KS measured by this radioimmunoassay accounts for nearly 100% of the DHEA sulphate, 45% of the androsterone sulphate and 35% of the aetiocholanolone sulphate in the serum (Sekihara & Ohsawa 1974a). The value obtained for 11-deoxy-17-KS may be taken to represent the serum DHEA sulphate concentration. Serum DHEA was also measured by the non-chromatographic radioimmunoassay procedure using a mixture of two different antisera (i.e. anti-3-hydroxy-AΔ antiserum and anti-11-deoxy-17-KS antiserum) (Sekihara & Ohsawa 1974b). These two antisera were kindly supplied by Dr. Sekihara. Serum 4-A-dione was measured by a specific radioimmunoassay as reported previously (Akamine et al. 1974). The lower limits of sensitivity were 10 μg/100 ml for 11-deoxy-17-KS, 0.03 μg/100 ml for DHEA and 8 ng/100 ml for 4-A-dione. Intraclass variation expressed as a coefficient of variation was 13.2% for 11-deoxy-17-KS, 13.0% for DHEA and 11.3% for 4-A-dione. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (Prl) levels were measured by specific radioimmunoassay as described elsewhere (Wasada et al. 1978). The coefficient of correlation and regression line between the serum levels of adrenal androgens and gonadotrophins as well as Prl were determined by the method of least squares. For statistical analysis on the comparison of intra-pair similarity of serum adrenal androgen levels between monozygotic and dizygotic twins, the following index of intra-pair difference was employed, i.e. the intra-pair difference in serum levels was divided by the mean value of the two

\[ \frac{|A-B|}{A+B} \]

where A and B represent the serum hormone level of each twin. The Student's t-test was applied to the statistical assessment.

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Results

Bone ages vs. serum adrenal androgen levels (Fig. 1)

Bone ages of 187 children (12 to 15 years in chronological age) ranged from 10 to 17 years. The mean serum concentrations of 11-deoxy-17-KS, DHEA and 4-A-dione at different stages of bone age are shown in Fig. 1. In boys, serum 11-deoxy-17-KS increased at 14 years and serum 4-A-dione at 13 years of bone age with more rapid increases thereafter, while serum DHEA showed a tendency to rise steadily between the bone age of 12 to 16–17 years. The mean serum levels of 11-deoxy-17-KS, DHEA and 4-A-dione in boys at a bone age of 15 years were 51.6, 20.7 and 53.0% of the mean value in young adult males, respectively. In girls, serum 11-deoxy-17-KS and DHEA concentrations rose at the bone age of 12 years, followed by an abrupt increase of 11-deoxy-17-KS levels and a progressive increase of DHEA levels. Serum 4-A-dione levels showed a gradual increase from 14 to 16 years of bone age. Compared with the adult levels, the mean serum concentrations of 11-deoxy-17-KS, DHEA and 4-A-dione in girls at a bone age of 11 years were 78.8, 75.4 and 86.0%, respectively. When compared between the two sexes, serum 11-deoxy-17-KS levels were significantly higher in girls than in boys at a bone age of 14 and 15 years, but no significant difference was observed in serum DHEA and 4-A-dione levels between boys and girls in any bone age group.

Pubic hair stages vs. serum adrenal androgen levels (Fig. 2)

In boys, serum DHEA and 4-A-dione concentrations increased progressively with pubic hair development, but no significant change in the mean serum level of 11-deoxy-17-KS was found at different stages of pubic hair. In girls, serum 11-deoxy-17-KS and DHEA levels increased significantly
Development of breasts vs. serum adrenal androgen levels (Fig. 5)
The development of breasts in girls was classified into three groups as described above. Serum 11-deoxy-17-KS and DHEA levels increased steadily with the development of breasts, but serum 4-A-dione levels remained unchanged.

Correlation between serum levels of adrenal androgens and gonadotrophins (Figs. 6 and 7)
Statistical analysis showed that there was a significant positive correlation between the serum levels of 4-A-dione and LH as well as FSH in both sexes. In boys, a significant positive correlation was also found between levels of 4-A-dione and PRL, and serum levels of DHEA and LH.

with the extent of pubic hair development, while serum 4-A-dione levels showed no significant change in the three groups.

Axillary hair stages vs. serum adrenal androgen levels (Fig. 3)
Significant elevation of serum 11-deoxy-17-KS and 4-A-dione levels occurred between the stage of AH1 and AH2 in boys, but did not in girls. Serum DHEA levels increased significantly at the stage of AH3 in both sexes.

Testicular size vs. serum adrenal androgen levels (Fig. 4)
There was no difference in the mean serum concentration of 11-deoxy-17-KS and DHEA between the three stages graded according to testicular size, but in 4-A-dione, a significant difference was observed only between C and A groups.

Development of Breasts

Serum 11-deoxy-17-KS, DHEA and 4-A-dione levels in relation to breast development. See legends in Fig. 1.
Correlation between adrenal androgens and gonadotrophins (also prolactin) in boys. \( r \) = the coefficient of correlation, and \( P \) = the statistical significance of the correlation assessed.

**Table 1.**
The mean values, variance and standard deviation (sd) for indices of intra-pair difference of serum adrenal androgen levels in monozygotic and dizygotic pairs of twins

<table>
<thead>
<tr>
<th></th>
<th>Monozygotic</th>
<th>Dizygotic</th>
<th>( P^{**} )</th>
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<tbody>
<tr>
<td></td>
<td>([A-B]/\frac{A+B}{2})</td>
<td>([A-B]/\frac{A+B}{2})</td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Mean</td>
<td>Variance</td>
<td>sd</td>
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<tr>
<td>11-deoxy-17-KS</td>
<td>74</td>
<td>0.28</td>
<td>0.06</td>
</tr>
<tr>
<td>DHEA</td>
<td>70</td>
<td>0.45</td>
<td>0.09</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>67</td>
<td>0.15</td>
<td>0.02</td>
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* Index of intra-pair difference, A and B represent the serum hormone level of each twin.
** Analyzed by Student’s t-test. *** Not significant.
Intra-pair similarity of serum adrenal androgen levels in monozygotic and dizygotic pairs of twins

The statistical data of serum adrenal androgen concentrations in monozygotic and dizygotic pairs of twins are given in Table 1. The mean value of index of intra-pair difference in serum levels of 11-deoxy-17-KS and 4-A-dione for monozygotic pairs is significantly less than that for dizygotic pairs of twin. There was no significance in serum DHEA concentration between the two zygosities.

Discussion

Serum concentrations of DHEA and 4-A-dione in adults obtained in this study were generally similar to those reported by a number of other investigators (De Peretti & Forest 1978; Sekihara et al. 1974; Gandy & Peterson 1968; Moshang & Rudd 1970). Serum concentrations of 11-deoxy-17-KS in our subjects were comparable to the value for DHEA sulphate reported in subjects at the corresponding ages (Lee & Migeon 1975; Lee et al. 1976; Yamaji & Ibayashi 1969).

The results presented in this report clearly show that during puberty the mean concentrations of serum 11-deoxy-17-KS, DHEA and 4-A-dione increase with the advancement of bone age, which is a more reliable index of somatic maturation than chronological age. The pattern of the serum 11-deoxy-17-KS level in boys as well as in girls is characterized by a rapid rise at puberty. Serum DHEA, on the other hand, showed a gradual increase in boys and girls during puberty. Similar patterns of the rise in serum DHEA sulphate and DHEA levels during puberty have been demonstrated by Lee & Migeon (1975) and Sizonenko et al. (1976). Hopper & Yen (1975) reported, however, that during puberty there was a progressive and parallel increase in serum DHEA and DHEA sulphate concentrations in boys, whereas the rise of serum DHEA and DHEA sulphate concentrations was not parallel in girls, in whom serum DHEA sulphate rose progressively but serum DHEA showed an abrupt increase. The reasons for this
discrepancy are unclear. It might be due to the small numbers of subjects in some of these studies, and is probably due to the fluctuation of serum DHEA concentration by its episodic secretion. As to serum 4-A-dione levels during puberty, relatively limited data are available (Lee & Migeon 1975; Lee et al. 1976; Parker et al. 1978). In the present study serum 4-A-dione showed a rapid rise in boys and a progressive increase in girls.

The presence of a sex difference in serum adrenal androgen levels at puberty has been reported by Hopper & Yen (1975), but not by others (Korth-Schutz et al. 1976; Reiter et al. 1977; De Peretti & Forest 1978). When one compares the serum levels at a bone age of 15 years with those in adulthood, the mean serum concentrations of adrenal androgens in boys were below about 50% of the adult levels, whereas those in girls had already reached about 80% of the female adult levels. These findings confirm the earlier development of puberty in girls than boys.

Appreciable sexual maturation in both sexes is associated with the period when the serum concentrations of testicular androgens in boys and ovarian oestrogens in girls have begun to rise rapidly towards adult levels. However, the development of pubic and axillary hair in girls is believed to be mainly due to increasing concentrations of serum adrenal androgens (Albright et al. 1942). The present study provides lines of evidence to support the existence of an association of adrenal androgens with pubic and axillary hair growth in girls and presumably in boys. On the other hand, no apparent correlation was observed between the serum level of 11-deoxy-17-KS or of DHEA and the stages of testicular size. A significant rise in serum 4-A-dione concentration was noted only at stage A according to the testicular size criteria. This rise might be partly due to the peripheral conversion of testosterone to 4-A-dione.

Although the mechanism responsible for the dramatic increase of adrenal androgens during puberty remains a matter of controversy, the pre-pubertal and pubertal increase of adrenal androgens may be due to the maturation of the hypothalamic-pituitary-adrenocortical axis. The hypothalamic factor inducing this maturation remains unknown. ACTH has been suggested as stimulating the secretion of adrenal androgens, but there has been no proof that ACTH, by itself, triggers the secretion of adrenal androgens, as there is found to be no significant change in the cortisol production rate when calculated on the basis of the body surface and body weight at puberty (Kenny et al. 1966). LH, PRL and gonadal sex steroids have been postulated to be the hormones which may stimulate the secretion of adrenal androgens directly or modulate the adrenal responsiveness to ACTH. In our previous study, serum LH, FSH and PRL showed a progressive increase with the advancement of bone age in pubertal twins (Wasada et al. 1978). Accordingly, in this study the interrelationship between pituitary gonadotrophins and adrenal androgens was analyzed. There was no correlation between serum LH (and FSH) and serum DHEA (and 11-deoxy-17-KS), while serum 4-A-dione showed a significant correlation with serum LH (and FSH). Almost all DHEA sulphate and DHEA in peripheral blood are secreted by the adrenal cortex (Yamaji & Ibayashi 1969; Nieschlag et al. 1973). On the other hand, 4-A-dione is derived from both adrenal and gonads, i.e. at least 50% of serum 4-A-dione is secreted from the ovary in female (Abraham et al. 1969), and about 36% of serum 4-A-dione is the product of a peripheral conversion from serum testosterone in the male (Horton & Tait 1966). Therefore, it seems probable that the increased secretion of pituitary gonadotrophins and adrenal androgens during puberty occur independently. This is also supported by the observations that during puberty there is no relationship between gonadotrophin secretions and adrenal androgens (Lee et al. 1975), and further that administering hCG and LRH to the agonal patients and normal subjects does not cause a significant rise in the serum levels of adrenal androgens (Lee et al. 1975; Reiter et al. 1977). Therefore the maturation of the hypothalamic-pituitary-gonadal axis is thought not to be a primary factor in the maturation of the adrenal cortex. On the contrary, several investigators have demonstrated that the adrenal androgen secretion increases earlier than gonadal sex hormone in pre-pubertal children, and suggested that the adrenal androgen may play a role in the maturation of hypothalamic-pituitary-gonadal axis (Ducharme et al. 1976; De Peretti & Forest 1978; Sizonenko & Paunier 1975; Sizonenko et al. 1976).

The onset and development of puberty seems to be regulated by individually inherited genetic factors, since a familial occurrence of sexual precocity or delayed adolescence is often experienced. The twin study method is thought to be the best for evaluating the participation of genetic factors, be-
cause monozygotic pairs of twins are more homologous in genetic backgrounds than dizygotic pairs. Fischbein (1977) demonstrated a higher intra-pair similarity in somatic maturity (i.e. age at menarche, development of secondary sex characteristics, peak height velocity and peak weight velocity) in monozygotic twins than in dizygotic twins at puberty. In the present study serum 11-deoxy-17-KS and 4-A-dione levels in monozygotic pairs of twins showed significantly higher intra-pair similarity than in dizygotic twin pairs. These findings, together with our previous observation on serum LH and FSH levels in pubertal twins, suggest that genetic factors probably contribute to the pair's parallel progress in puberty, and support the hypothesis that the onset and development of puberty is an intrinsic process which is genetically determined.

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