REPLACEMENT ORAL ETHINYL Estradiol THERAPY FOR GONADAL DYSGENESIS: GROWTH AND ADRENAL ANDROGEN STUDIES

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ABSTRACT

We have studied growth and adrenal dehydroepiandrosterone (DHA) responses to iv synthetic adrenocorticotropic hormone (ACTH, Cortrosyn) in 6 girls with gonadal dysgenesis before and during treatment with low-dose ethinyl estradiol (EOe2). In all patients there was a satisfactory induction of secondary sexual characteristics including increase in breasts and pubic hair and onset of withdrawal bleeding within 6 months of

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therapy. Height velocity increased from 2.8 ± 0.9 cm/year pre-treatment to 5.3 ± 1.5 cm/year ($P < 0.02$) in the first year. There was deceleration to 1.9 ± 1.1 cm/year in the second year. There was no disproportionate advancement in bone age and thus, presumably, no loss of ultimate height. We could demonstrate no change in basal or ACTH-stimulated levels of DHA, a specific adrenal androgen, to account for the increased pubic hair and growth in these patients.

Treatment of patients with gonadal dysgenesis at adolescence is directed toward maximizing both ultimate height potential and appropriate sexual maturation. Oestrogen in high doses will advance bone age and retard bone growth, probably via suppression of somatomedin action (Saenger et al. 1976; Wetenhall et al. 1975; Zachmann et al. 1975). In low doses, however, oestrogen itself may have a growth promoting effect, either directly or through stimulation of enhanced adrenal androgen production. The clinical observation that girls with gonadal dysgenesis show accelerated pubic hair growth concomitant with initiation of oestrogen therapy has suggested enhancement of adrenal androgen production. In vitro evidence suggests that oestradiol will inhibit the enzyme $\Delta^5$-3$\beta$-hydroxysteroid dehydrogenase ($\Delta^5\beta$-ol), thus promoting production of more $\Delta^5$-steroids (such as DHA) relative to $\Delta^4$-steroids (such as cortisol) (Goldman 1967, 1968, 1969). One study comparing urinary excretion products of $\Delta^5$- to $\Delta^4$-steroids after 6 weeks of oestrogen treatment (Sobrinho et al. 1971), and another examining serum levels of androgen in treated post-menopausal women (Abraham & Maroulis 1975) support this hypothesis. On the other hand, there are now at least 4 studies which have failed to show change in single values of DHA or DHAS during oestrogen treatment in patients with gonadal dysgenesis or after menopause (Anderson & Yen 1976; Lee & Gareis 1975; Rose et al. 1977; Rosenfield & Fang 1974) or after ACTH stimulation (Anderson & Yen 1976).

In an attempt to resolve these conflicting data, both on growth and on adrenal androgen patterns, we studied the effect of low dose oestrogen on the growth response and on the plasma DHA and cortisol levels following ACTH stimulation in 6 subjects with gonadal dysgenesis.

MATERIALS AND METHODS

Clinical studies

Six teenage girls with gonadal dysgenesis volunteered for the study. They all had elevated serum gonadotrophin levels and were judged to be psychologically ready for oestrogen therapy (Table 1). Informed written consent was obtained from the patients and their parents. Heights were measured on the same stadiometer and the mean of 10 individual measurements was recorded at approximately 3 month intervals. Bone
Table 1.
Clinical and growth data on 6 subjects with gonadal dysgenesis during therapy with ethinyloestradiol (EOe₂).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Karyotype</th>
<th>Age at onset of EOe₂ years</th>
<th>Height before EOe₂ cm</th>
<th>Growth velocity</th>
<th>Before EOe₂ years</th>
<th>Bone age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before EOe₂ cm/year</td>
<td>in 1st year cm/year</td>
<td>in 2nd year cm/year</td>
</tr>
<tr>
<td>I</td>
<td>45 XO</td>
<td>13-4/12</td>
<td>135.2</td>
<td>–</td>
<td>5.5</td>
<td>–</td>
</tr>
<tr>
<td>II†</td>
<td>45 XO</td>
<td>13-2/12</td>
<td>135.3</td>
<td>4.3</td>
<td>4.4</td>
<td>2.1</td>
</tr>
<tr>
<td>III</td>
<td>45 XO</td>
<td>13-7/12</td>
<td>132.0</td>
<td>3.1</td>
<td>6.6</td>
<td>0.4</td>
</tr>
<tr>
<td>IV</td>
<td>45 XO/46 XX frag</td>
<td>13-10/12</td>
<td>127.0</td>
<td>2.5</td>
<td>7.2</td>
<td>2.1</td>
</tr>
<tr>
<td>V</td>
<td>45 XO/46 XiXq</td>
<td>14-2/12</td>
<td>140.6</td>
<td>2.1</td>
<td>4.3</td>
<td>3.0</td>
</tr>
<tr>
<td>VI</td>
<td>45 XO</td>
<td>15/5/12</td>
<td>134.5</td>
<td>2.2</td>
<td>3.8</td>
<td>–</td>
</tr>
<tr>
<td>Mean* ± 1 sd</td>
<td></td>
<td></td>
<td></td>
<td>2.8</td>
<td>5.3*</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* Subjects II–VI
† Previously received 6 months of oxandrolone
( ) # months of EOe₂
ages were read one of us (A.W.L.) at the end of the study using the Greulich-Pyle atlas (Greulich & Pyle 1959). The mean maturity of all carpal and phalangeal epiphyseal centres in the left hand and wrist was recorded. Five girls were given ethinyl-oestradiol (EOe2), 10 µg po daily for 3 months followed by 20 µg daily. Patients II and V were given 50 µg after the next 3 months. Patient VI was started on a dose of 20 µg and then increased to 50 µg daily after 3 months. In all patients, as soon as breakthrough bleeding occurred, cyclic withdrawal using Provera was begun: 5 mg of Provera was given once daily by mouth from day 21 to day 25 of each month in addition to oestrogen. No medication was taken until the first day of the next month when oestrogen alone was resumed. Some girls chose to have 60-day cycles, and in this case the Provera was added every other month. At the end of two years, medication was changed to a combination low-dose oestrogen-progestin contraceptive pill. The 6 girls initially consented to undergo 8 h iv ACTH stimulation tests, using 400 µg of tetracosactine (Cortrosyn), in 500 ml N.S. with blood sampling at 0, 4 and 8 h at 3 monthly intervals during the first year of oestrogen therapy. Patient I dropped out of the study after 9 months because of excessive breakthrough bleeding (due to non-compliance with medication) and hypertension. This girls was necessarily treated with a short course of high-dose oestrogen-progestin and then chose to discontinue oestrogen for several months.

**Hormone assays**

Serum cortisol was measured by radioimmunoassay as previously described (Ruder et al. 1972). Dehydroepiandrosterone (DHA) was measured by radioimmunoassay using a rabbit antiserum to DHA-3-hemisuccinate-BSA. The cross-reactivity of the antiserum to other compounds (compared to DHA) was: 71% to DHA sulphate, 1.6% to Δ4-androstenedione, 0.5% to 3β-androstenediol, and less than 0.1% to progesterone, 17-hydroxyprogesterone, pregnenolone, 17-hydroxyprogrenolone, testosterone, cortisol, oestradiol, and cholesterol. Ether extracts of 0.5 ml serum were dried under air, re-dissolved in cyclohexane-methanol (80:15:5), and chromatographed on Sephadex LH-20 columns (Pharmacia). Aliquots of the dried eluates dissolved in 1 ml buffer (0.5 mg/ml bovine gamma globulin Fraction II (Pentex) in Dulbecco’s phosphate buffered saline). Each sample was monitored for recovery and assayed in duplicate in a total volume of 1 ml containing antiserum at a final dilution of 1:10000 and [1,2-3H]DHA (New England Nuclear, 40-60 Ci/mmol as tracer. After overnight incubation at 4°C, bound and free tracer were separated by centrifugation after a 20 min incubation with Dextran-coated charcoal (0.5% Dextran T70 (Pharmacia), 0.05% charcoal (Darco G70, MC & B)). The assay blank was 0.01 nm/l using plasma from a post-menopausal Addisonian patient on dexamethasone replacement. The sensitivity was 0.02 nm/l, the intra-assay variation 5% and the inter-assay variation 10% at 50% bound. The measurement of known quantities of DHA added to blank plasma at multiple doses gave results within ±6% of the true value.

**Calculation of results**

Results of the radioimmunoassays were calculated using the computer programme of Rodbard (Faden & Rodbard 1975). Comparisons of height velocities, serum DHA and cortisol were made using paired t-tests.
RESULTS

Growth and development

All patients experienced increased development of breasts, pubic and axillary hair. The pattern of development was identical to that seen in normal puberty. All girls had onset of withdrawal bleeding within 6 months of initiation of treatment.

Height velocities and bone ages are summarized in Table 1. The mean pretreatment growth velocity of the 5 patients on whom it was available was $2.8 \pm 0.9$ cm/year. This significantly increased ($P < 0.02$) in the first treatment year to $5.3 \pm 1.5$ cm/year. In 4 patients followed 6–9 months into the second year of treatment, height velocity decreased to $1.9 \pm 1.1$ cm/year. There was no significant change in predicted height during the course of the treatment as estimated from the Bayley-Pinneau tables (Greulich & Pyle 1959) (Table 2).

Adrenal steroids

Basal and ACTH-stimulated serum values of DHA and cortisol are summarized in Fig. 1 and Table 3. There was no significant change in basal levels of DHA during oestrogen therapy. Although the mean difference between basal and peak levels of DHA during ACTH stimulation rose after initiation of

![Fig. 1](https://example.com/f1.png)

Scrub dehydroepiandrosterone (DHA) levels at 0, 4 and 8 h (3 adjacent bars) during an infusion of 400 μg of synthetic ACTH in girls with gonadal dysgenesis before and during treatment with ethinyloestradiol (EOc2). Each bar represents the mean ± 1 sd for the sum of 2–6 individual ACTH tests. The daily dose of ethinyloestradiol and numbers of months of therapy are indicated in the figure.
Table 2.
Predicted heights estimated from the Bayley-Pinneau† tables during therapy with ethinyloestradiol (EOe₂).

<table>
<thead>
<tr>
<th>Subject*</th>
<th>Predicted height at onset of EOe₂ (cm) (mean ± 1 sd)</th>
<th>Predicted height after EOe₂ (cm) (mean ± 1 sd)</th>
<th># months of EOe₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>137.9 (134.9-141.5)</td>
<td>143.2 (141.5-145.9)</td>
<td>22</td>
</tr>
<tr>
<td>IV</td>
<td>147.3 (143.8-151.1)</td>
<td>141.5 (136.9-144.5)</td>
<td>16</td>
</tr>
<tr>
<td>V</td>
<td>151.4 (146.6-156.7)</td>
<td>149.9 (148.1-151.9)</td>
<td>22</td>
</tr>
<tr>
<td>VI</td>
<td>145.3 (140.5-150.1)</td>
<td>145.0 (140.7-145.8)</td>
<td>13</td>
</tr>
</tbody>
</table>

* Subjects I and II were not included because of insufficient data.
† Greulich & Pyle (1959).

Table 3.
Basal levels and maximally stimulated increments (Δmax) of DHA and cortisol in response to 400 μg og ACTH iv during therapy with ethinyloestradiol (EOe₂).

<table>
<thead>
<tr>
<th>Time on† EOe₂</th>
<th>DHA (nm/l) basal</th>
<th>DHA Δmax</th>
<th>Cortisol (μM/l) basal</th>
<th>Cortisol Δmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Rx (6)</td>
<td>171 ± 0.80</td>
<td>1.48 ± .73</td>
<td>52.0 ± 13.5</td>
<td>123.3 ± 23.4</td>
</tr>
<tr>
<td>3 months (6)</td>
<td>1.64 ± 0.88</td>
<td>1.57 ± 1.28</td>
<td>67.0 ± 8.0</td>
<td>172.7 ± 37.5</td>
</tr>
<tr>
<td>6 months (4)</td>
<td>0.96 ± 0.17</td>
<td>1.83 ± 1.19</td>
<td>83.0 ± 19.8</td>
<td>173.2 ± 37.8</td>
</tr>
<tr>
<td>10 months (5)</td>
<td>0.93 ± 0.50</td>
<td>1.92 ± 0.86</td>
<td>123.0 ± 37.2</td>
<td>154.2 ± 39.4</td>
</tr>
<tr>
<td>16 months (4)</td>
<td>1.05 ± 0.36</td>
<td>3.22 ± 1.52</td>
<td>73.0 ± 22.0</td>
<td>201.1 ± 57.6</td>
</tr>
<tr>
<td>22 months (2)</td>
<td>1.06 ± 0.71</td>
<td>1.90 ± 0.87</td>
<td>77.0 ± 73.8</td>
<td>153.6 ± 70.0</td>
</tr>
</tbody>
</table>

( ) = Number of subjects.
* = ± 1 sd.
† See Fig. 2 for doses of ethinyloestradiol.

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Table 4.  
A comparison of reported growth velocities during hormonal therapy for gonadal dysgenesis.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Treatment</th>
<th>Pre-therapy</th>
<th>1st year cm/year</th>
<th>2nd year cm/year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johanson et al. (1969)</td>
<td>Fluoxymestrone</td>
<td>3.3 ± 0.8</td>
<td>6.5 ± 1.3</td>
<td>4.6</td>
</tr>
<tr>
<td>Danowski et al. (1967)</td>
<td>Oxandrolone</td>
<td>1.8 (0.5–3.8)</td>
<td>5.3 (2.1–8.7)</td>
<td>– – – – – –</td>
</tr>
<tr>
<td>Moore et al. (1977)†</td>
<td>Oxandrolone</td>
<td>3.0 ± 0.5</td>
<td>5.3 ± 0.8</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>Rosenfield &amp; Fang (1974)</td>
<td>DepoOe¿ (im)</td>
<td>1.9 ± 0.5</td>
<td>3.3 ± 0.2</td>
<td>– – – – – –</td>
</tr>
<tr>
<td>Current study</td>
<td>Ethinyloestradiol (po)</td>
<td>2.8 ± 0.9</td>
<td>5.3 ± 1.5</td>
<td>1.9 ± 1.1</td>
</tr>
</tbody>
</table>

† group IIa.

Oestrogen therapy, there was no systematic increase in any of the individual patients. Throughout oestrogen treatment, there was an increase levels of plasma cortisol (Table 3).

DISCUSSION

Development

We have found that replacement doses of ethinyloestradiol are satisfactory to induce feminization in agonadal girls. Starting with a dose as low as 10 µg EOe¿, a natural, gradual appearance of secondary sexual characteristics is achieved similar to that observed in spontaneous puberty. We had no problems with some of the commoner side effects of oestrogen therapy such as nausea, glucose intolerance or oedema. One girl developed hypertension and breakthrough bleeding; the former was borderline prior to onset of therapy, and the latter was secondary to non-compliance concerning cyclic Provera. Because of the recent studies suggesting an increase in endometrial cancer in women on long-term unopposed oestrogen (Smith et al. 1975; Ziel & Finkle 1975), we advise changing to a low oestrogen-progestin combination oral contraceptive after completion of development of secondary sex characteristics. However, we have no data that sequential oestrogen and progestin in such patients is carcinogenic.

Growth

Treatment of girls with Turner's syndrome in such a way as to maximize their height potential has been a controversial issue for many years. The use
of androgens prior to institution of oestrogen therapy has been favoured by several investigators (Danowski et al. 1967; Johanson et al. 1969; Moore et al. 1977; Rosenbloom & Frias 1973; Rosenbloom 1974). These groups feel that therapy with androgens before feminization with oestrogens may accelerate growth velocity and perhaps enhance ultimate height (Moore et al. 1977). Comparisons of height velocity in androgen treated vs. oestrogen treated patients have often been retrospective and without regard to the dose of oestrogen used. In some cases comparisons are based on series collected from different clinics (Brook et al. 1974). When factors such as oestrogen dose (Rosenfield et al. 1973; Rosenfield & Fang 1974), karyotype (Snider & Solomon 1974) and mid-parental height (Brook et al. 1974) are carefully considered, it becomes less clear that small doses of oestrogen are detrimental to growth in Turner's syndrome, although large doses of oestrogen certainly do retard growth and accelerate bone age (Saenger et al. 1976; Wetterhall et al. 1975; Zachmann et al. 1975). In fact, oestrogen has been demonstrated to increase growth hormone secretion independent of its inhibiting effect on somatomedin (Wiedemann et al. 1976). We have compared our data on growth velocity to 4 studies in the literature (Table 4); no real difference in growth velocity among the various treatment regimens is apparent. Until the patients involved in all such studies have finally completed their growth, the ultimate beneficial or detrimental effects of either androgen or oestrogen therapy cannot be completely assessed. We can only state that oral ethinyloestradiol in replacement doses appears to have a similar effect on growth velocity without significant acceleration of bone age compared to parenteral oestrogen or androgen therapy and is without the risk of masculinizing side effects of the latter.

Adrenal androgens

It has been postulated that the growth promoting effects of low doses of oestrogen are a result of direct stimulation of oestrogen on adrenal androgen production, primarily the $\Delta^5$-steroids such as dehydroepiandrosterone (DHA) (Zachmann et al. 1975). The proposed mechanism of this increased secretion is based on in vitro evidence that oestradiol will inhibit the enzyme $3\beta$-hydroxysteroid dehydrogenase and thus promote more $\Delta^5$-steroids (such as DHA) relative to $\Delta^4$-steroids (such as cortisol) (Goldman 1967, 1968, 1969). We have been unable in these few patients to document a rise in DHA during oestrogen therapy despite the clinical evidence of enhancement of growth velocity and rapid appearance of pubic and axillary hair during oestrogen treatment. The basal levels of DHA prior to therapy in our patients were comparable to levels reported for mid to late pubertal girls in the literature (De Peretti & Forest 1976; Ducharme et al. 1976; Korth-Schutz et al. 1976; Rosenfield et al. 1973). There was no significant difference between the basal
DHA levels or the maximal stimulated ($\Delta_{\text{max}}$) DHA levels at 4 vs. 8 h throughout the treatment course. When each patient's individual responses of DHA to ACTH were analyzed, there were no consistent increase in $\Delta_{\text{max}}$. Thus, we cannot confirm the hypothesis of Sobrinho et al. (1971) who reported that short-term oestrogen therapy increased $\Delta^\delta$-urinary steroid metabolites, or of Abraham & Maroulis (1975) who found a specific elevation in DHA, DHA sulphate and pregnenolone in post-menopausal women treated with oestrogen. Our data confirm what Rosenfield & Fang (1974) and Lee & Gareis (1975) have suggested from studies of single serum samples; that is, there is no effect of oestrogen on serum DHA levels. In addition, using stimulation with ACTH, we did not find any significant increase in adrenal androgen reserve during induction of puberty with oestrogen.

It is interesting to speculate why we may not have found increased adrenal androgens during oestrogen therapy despite increased growth and pubic hair. It is possible that the 4-hour time period for initial DHA sampling may have been too late to pick up a subtle difference in adrenal responsiveness. Heterozygotes for 21-hydroxylase deficiency (congenital adrenal hyperplasia) show a significantly elevated response of serum 17-hydroxyprogesterone (17P) after ACTH stimulation at 2 h, but the blood levels are not different from controls at 3 h (Lee & Gareis 1975). Also, DHA may not be the particular steroid involved in somatic or hair growth stimulation and there may be other adrenal androgens (such as androstenedione if 11\beta-hydroxylase were blocked) which are more specifically stimulated by oestrogen. It is possible that oestrogen acts at the end organ (bone or hair) to enhance metabolic effects of androgen or that oestrogen itself promotes linear and hair growth. Finally, it may be that oestrogen is important for initiation of adrenarche but is not necessary for the continued increases of adrenal androgens seen throughout puberty.

During oestrogen therapy we found that serum cortisol rose dramatically. This was presumably due to a secondary effect of oestrogen on corticosteroid binding globulin (Dixon et al. 1967).

In summary, we have found that replacement doses of oestrogen are useful in feminizing agonadal girls without compromising ultimate height. Our data cannot support the hypothesis that oestrogens enhance adrenal DHA production to account for the growth of pubic hair or acceleration of growth velocity.

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REFERENCES


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