PLASMA CONCENTRATION OF OESTRADIOL-17β
IN PREMATURE THELARCHE AND
IN DIFFERENT TYPES OF SEXUAL PRECOCITY

By

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ABSTRACT

Plasma \(\text{OE}_2\) concentration was measured by radioimmunoassay in patients with premature thelarche, with precocious puberty and in 29 normal controls. The mean plasma \(\text{OE}_2\) was 1.5 pg/ml (0–7.2) in normal pre-pubertal girls, 23.8 ± 17.8 (SD) in pubertal girls, 50.2 (± 19.4) in the follicular phase, and 94.2 (± 19.5) in the luteal phase of normal adult females. Ten girls with premature thelarche had a mean of 7.7 ± 6.6 pg/ml. Three of them showed higher values than the other 7, suggesting that in these cases, elevated levels of plasma \(\text{OE}_2\) might have played a role in the development of breast tissue. Ten untreated girls with idiopathic precocious sexual development had a mean of 51.6 ± 42.9 pg/ml while 6 patients treated with 150 mg per week of medroxyprogesterone acetate had a mean of 11.4 ± 2.5 pg/ml. Two patients with Down’s syndrome, hypothyroidism and sexual precocity had plasma \(\text{OE}_2\) of 144 and 31.5 which fell to 24.7 and 8 pg/ml, respectively, after thyroid replacement. One girl with a granulosa cell tumour had a basal value of 304 pg/ml and a concentration of 27 pg/ml after surgery.

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Since the introduction of sensitive and specific radioimmunoassays, information on plasma oestrogens in children has become available. It has thus been possible to determine normal values before puberty as well as changes occurring in girls during sexual development (Jenner et al. 1972; Morera et al. 1972; Bidlingmaier et al. 1973; Winter & Faiman 1973). Plasma oestrogens should be of help in the diagnosis of some abnormalities of sexual development in girls, such as premature thelarche and sexual precocity.

Premature thelarche has been defined as the premature presence of isolated hypertrophy of the breast not accompanied by any other sign of sexual maturation. Since other oestrogen target tissues are not stimulated, it has been postulated that there is a hypersensitivity of the breast tissue to low levels of oestrogens secreted during childhood (Wilkins 1965). Precocious sexual development can originate from several causes. In the most common of them, idiopathic sexual precocity, the normal process of sexual maturation takes place at an abnormally early age. Since the normal adult feed-back regulatory mechanism is operating, plasma levels of LH, FSH and oestradiol-17β (Oe2) should be the same as in the developing or adult female and should respond to stimulatory and suppressive influences. This does not apply to other causes of sexual precocity.

The present report presents our studies on plasma Oe2 in girls with premature thelarche and in girls with precocious puberty of different types as well as on the influence of therapy on these levels. Furthermore, ovarian activity in normal girls, as reflected by the concentration of Oe2 in plasma, was correlated with secondary sexual characteristics.

**CLINICAL MATERIAL**

Twenty-nine patients and 29 normal control subjects were studied.

*Normal controls.* – Normal controls were classified into three groups, 7 prepubertal girls aged 2 to 7 years, 12 pubertal girls aged 8–9½ to 14 years corresponding to P3, P3 and P4 stages of development (Jenner et al. 1972), and 10 adult females, aged 24 to 35 years, with regular menstrual cycles who were not receiving any hormonal medication.

*Patients with premature thelarche.* – Ten girls, aged 1 to 7–10½ years, were studied. They were classified on the basis of the presence of breast development before the age of 8 years in the absence of any other sign of sexual development.

*Patients with precocious puberty.* – Nineteen girls were included. No aetiological factor or clinical association could be detected in 13 girls and they were classified as idiopathic precocious puberty. Two additional girls had a history of lesion of the central nervous system. In two other girls precocious puberty was associated with hypothyroidism and Down’s syndrome. One girl had Albright’s syndrome and another had a feminizing ovarian tumour.
All subjects were clinically evaluated and their sexual development was classified using a modification of the criteria Marshall and Tanner (Jenner et al. 1972). Bone ages were determined from X-ray films of the hands and wrists in accordance with the standards of Greulich & Pyle (1955).

Plasma concentration of Oe2 was measured by radioimmunoassay.

Urocytograms were carried out simultaneously and the oestrogenic effect estimated and graded from 0 to ++++. This procedure presents practical advantages over vaginal smears in children. The cells of the urethra and trigone are oestrogen responders that undergo parallel changes to vaginal cells (Lencioni 1972).

**Plasma oestradiol-17β radioimmunoassay**

The technique used was a modification of Abraham's method (Abraham 1971). A brief description of this assay follows:

\[2.4.6,7-^3\text{H}]\text{oestradiol-17β} \text{ (S. A. 90 Ci/mmol) was obtained from New England Nuclear Corporation; the steroid was purified by paper chromatography before use. Antibody to oestradiol-17β-succinyl-BSA was kindly supplied by Dr. Guy E. Abraham.}

Heparinized venous blood was obtained, centrifuged and the plasma was stored at \(-20^\circ\text{C}\) until analysis. Plasma aliquots from 0.5 to 5 ml were used. Samples were extracted three times with three volumes of ethyl ether; the combined ether extract was evaporated to dryness.

Chromatographic separation of Oe2 was accomplished using Celite microcolumns. Dextran-coated charcoal was used for separation of free from antibody-bound hormone.

Water blanks were obtained by extracting and completely processing 5 ml of deionized water. Blanks were subtracted before calculations and values corrected for procedural losses.

The sensitivity of the assay was 2.5 pg/ml, calculated as any value greater than two standard deviations above the mean blank value.

**RESULTS**

**Normal controls**

Plasma Oe2 levels were undetectable in most prepubertal girls, and were less than 2.5 pg/ml. The mean for the whole group was 1.5 pg/ml. Only 2 out of 7 girls had measurable Oe2, the maximal value being 7.2 pg/ml.

In pubertal girls, plasma Oe2 ranged from 5.4 to 54.3 pg/ml with a mean of 23.8. In adult females levels varied between 32.6 and 248 pg/ml. When they were grouped according to the phase of the menstrual cycle, the Oe2 concentration was 50.2 ± 19.4 (σd) in the follicular phase, and 94.2 ± 19.5 (σd) in the luteal phase. In four subjects, blood was withdrawn from days 12 to 15, in order to detect the pre-ovulatory rise of Oe2. They had a mean of 221 pg/ml ± 30.9 (σd).

**Premature thelarche**

Table 1 shows clinical data and plasma concentrations of Oe2 in 10 girls with premature thelarche. Patients have been grouped according to chronological age. Some patients had low or undetectable levels but others showed
Table 1.
Clinical data and plasma oestradiol-17β in premature thelarche.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Chronological age</th>
<th>Bone age</th>
<th>Breast development</th>
<th>Urocytogram</th>
<th>Plasma Oe2 pg/ml</th>
<th>Clinical evolution</th>
<th>Plasma Oe2 pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. E.</td>
<td>1-9/12</td>
<td>1-9/12</td>
<td>2</td>
<td>0</td>
<td>9.4</td>
<td>↓</td>
<td>&lt; 2.5</td>
</tr>
<tr>
<td>J. C.</td>
<td>1-9/12</td>
<td>2-9/12</td>
<td>3</td>
<td>+</td>
<td>9</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>C. S.</td>
<td>1-10/12</td>
<td>2-9/12</td>
<td>2</td>
<td>0</td>
<td>3.9</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>N. R.</td>
<td>2-9/12</td>
<td>2-9/12</td>
<td>3</td>
<td>0</td>
<td>8.8</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>K. A.</td>
<td>2-5/12</td>
<td>2-9/12</td>
<td>2</td>
<td>0</td>
<td>&lt; 2.5</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>S. B.</td>
<td>3-9/12</td>
<td>3-9/12</td>
<td>2</td>
<td>0</td>
<td>&lt; 2.5</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>C. P.</td>
<td>5-9/12</td>
<td>6-9/12</td>
<td>2</td>
<td>0</td>
<td>&lt; 2.5</td>
<td>↓</td>
<td>&lt; 2.5</td>
</tr>
<tr>
<td>G. E.</td>
<td>7-3/12</td>
<td>7-10/12</td>
<td>2</td>
<td>+</td>
<td>15</td>
<td>→</td>
<td>16.8</td>
</tr>
<tr>
<td>L. S.</td>
<td>7-4/12</td>
<td>8-9/12</td>
<td>2</td>
<td>+</td>
<td>18.9</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>A. P.</td>
<td>7-10/12</td>
<td>8-9/12</td>
<td>2</td>
<td>+</td>
<td>12.5</td>
<td>↓</td>
<td></td>
</tr>
</tbody>
</table>

1) One to 5, from Marshall and Tanner classification, modified by Jenner et al. (1972).
2) Oestrogenic effect on urocytogram: 0 to +++.
   U: Unknown.
4) Two years after the first determination.

Concentrations that were elevated as compared to prepubertal girls. The 3 oldest girls had the highest values and they also showed some oestrogenic effect in their urocytograms. This oestrogenic activity was seen in only 1 of the other girls.

Fig. 1 compares Oe2 levels of this and the other groups of patients.
Clinical evolution, evaluated several months after the first examination, showed regression of breast development in most cases (Table 1). It was possible to determine Oe2 concentration again in only 3 patients.

Patient G. E. who failed to regress had a relatively high Oe2, while the other two, who showed clinical regression, had undetectable levels.

Precocious puberty

Clinical data and plasma Oe2 values in girls with various types of precocious puberty are summarized in Table 2.

Basal plasma Oe2 levels in 10 girls with idiopathic precocious puberty ranged between 19 and 154 pg/ml (mean ± sd, 51.6 ± 42.9). They were significantly greater (P < 0.02) than in the group of normal pubertal girls (mean ± sd,
Oestradiol plasma values in normal females, premature thelarche and idiopathic precocious puberty. Mean values are indicated by horizontal lines and standard deviations by vertical lines.

23.8 ± 17.8) and not statistically different from normal adult females during the follicular phase of the menstrual cycle (Fig. 1).

Plasma Oestradiol (Oestradiol) concentration was also greater ($P < 0.0025$) in precocious puberty than in girls with premature thelarche. (Mean ± sd, 7.7 ± 6.6).

In 3 girls (A. I., M. F. B. and L. F.) receiving medroxyprogesterone acetate (MPA) in a dose of 150 mg im weekly for at least 3 months, plasma Oestradiol concentration fell from 26.3 to 10.5, 19 to 9.2 and 42.3 to 14.4 pg/ml. Patients S. F., A. F. and C. P., on MPA therapy for 4 years showed plasma Oestradiol levels of 11.6, 8.5 and 14.2 pg/ml. The mean for the group of patients with idiopathic precocious puberty treated with MPA was 11.4 pg/ml (sd ± 2.5). In all these patients, low plasma Oestradiol levels agreed with good clinical response to treatment. In 2 patients with precocious puberty secondary to a central nervous system lesion, basal Oestradiol concentrations were 26.6 and 69 pg/ml, falling in the last case to 27 pg/ml with MPA treatment. A girl with an Allbright's syndrome, had an Oestradiol concentration of 44.8 pg/ml before treatment with MPA, and 20 pg/ml while receiving treatment.

S. P. and E. D. B. were 2 girls who had Down's syndrome associated with hypothyroidism and advanced sexual development, including breast enlargement, vaginal bleeding, marked oestrogenic effect on their urocytograms, and absent pubic hair without advanced bone age. All these signs regressed with
Table 2.
Clinical data and oestradiol-17β in female precocious puberty.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Chronological age</th>
<th>Bone age</th>
<th>Stage of breasts</th>
<th>Pubertal development(^1)</th>
<th>Menses</th>
<th>Urethral(^2) cytology</th>
<th>Aetiology</th>
<th>Plasma O(_{2}) pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pubic hair</td>
<td>Axillary hair</td>
<td></td>
<td></td>
<td>Untreated patients</td>
</tr>
<tr>
<td>B. F.</td>
<td>5–8/12</td>
<td>10–5/12</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>+</td>
<td>83</td>
</tr>
<tr>
<td>P. Z.</td>
<td>7–5/12</td>
<td>11</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>++</td>
<td>154</td>
</tr>
<tr>
<td>F. F.</td>
<td>6–8/12</td>
<td>8–9/12</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>–</td>
<td>++</td>
<td>80.8</td>
</tr>
<tr>
<td>G. P.</td>
<td>6–9/12</td>
<td>7–9/12</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>++</td>
<td>33</td>
</tr>
<tr>
<td>M. T. D.</td>
<td>4–9/12</td>
<td>8–9/12</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>++</td>
<td>19.4</td>
</tr>
<tr>
<td>M. L. S.</td>
<td>4–8/12</td>
<td>10–9/12</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>++</td>
<td>37.4</td>
</tr>
<tr>
<td>V. R.</td>
<td>6–9/12</td>
<td>9–9/12</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>–</td>
<td>+</td>
<td>21.1</td>
</tr>
<tr>
<td>A. I.</td>
<td>5–11/12</td>
<td>8–9/12</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>+</td>
<td>26.3</td>
</tr>
<tr>
<td>M. B. F.</td>
<td>2–5/12</td>
<td>4–9/12</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>++</td>
<td>19</td>
</tr>
<tr>
<td>Case</td>
<td>Age</td>
<td>Pubertal Stage</td>
<td>Testicular Size</td>
<td>Oestrogenic Effect</td>
<td>Diagnosis</td>
<td>Testicular Volume</td>
<td>Thyroid Volume</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>-------</td>
<td>----------------</td>
<td>-----------------</td>
<td>--------------------</td>
<td>---------------------------</td>
<td>-------------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td>L. F</td>
<td>2-8/12</td>
<td>3-8/12</td>
<td>3</td>
<td>1</td>
<td>Idiopathic</td>
<td>42.3</td>
<td>14.4^3</td>
<td></td>
</tr>
<tr>
<td>S. F</td>
<td>8-8/12</td>
<td>10-8/12</td>
<td>1</td>
<td>4</td>
<td>Idiopathic</td>
<td>-</td>
<td>11.6^3</td>
<td></td>
</tr>
<tr>
<td>A. F</td>
<td>10-5/12</td>
<td>13-8/12</td>
<td>2</td>
<td>4</td>
<td>Idiopathic</td>
<td>-</td>
<td>8.5^3</td>
<td></td>
</tr>
<tr>
<td>C. P</td>
<td>7-8/12</td>
<td>9-8/12</td>
<td>2</td>
<td>2</td>
<td>Idiopathic</td>
<td>-</td>
<td>14.2^3</td>
<td></td>
</tr>
<tr>
<td>D. C</td>
<td>5-8/12</td>
<td>9-8/12</td>
<td>3</td>
<td>2</td>
<td>Cerebral lesion</td>
<td>26.6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>G. C</td>
<td>6-18/12</td>
<td>11-9/12</td>
<td>4</td>
<td>2</td>
<td>Cerebral lesion</td>
<td>69</td>
<td>27^3</td>
<td></td>
</tr>
<tr>
<td>P. S</td>
<td>7 months</td>
<td>3-9/12</td>
<td>3</td>
<td>3</td>
<td>Albright's syndrome</td>
<td>44.8</td>
<td>20^3</td>
<td></td>
</tr>
<tr>
<td>S. P</td>
<td>7-8/12</td>
<td>6-9/12</td>
<td>3</td>
<td>1</td>
<td>Down's syndrome and hypothyroidism</td>
<td>144</td>
<td>24.7^4</td>
<td></td>
</tr>
<tr>
<td>E. D. B</td>
<td>10-5/12</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>Down's syndrome and hypothyroidism</td>
<td>31.5</td>
<td>8^4</td>
<td></td>
</tr>
<tr>
<td>P. G</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>Granulosa cell tumour</td>
<td>304</td>
<td>27^5</td>
<td></td>
</tr>
</tbody>
</table>

1) Stages of pubertal development: 1 to 5 from Marshall and Tanner classification, modified by Jenner et al. (1972).
2) Oestrogenic effect on urocytogram: 0 to +++.
3) On medroxyprogesterone acetate.
4) On thyroid therapy.
5) One week after surgery.
Effect of treatment on plasma Oe₂ in patients with different types of sexual precocity.

thyroid therapy. Basal plasma Oe₂ concentrations were 144 and 31.5 pg/ml decreasing to 24.7 and 8, respectively, after 2 months of thyroid extract therapy.

P. G., a 6-year-old girl, with a rapid premature sexual development, had an ovarian granulosa cell tumour. Plasma Oe₂ before surgery was 304 pg/ml, and 1 week later decreased to 27 pg/ml. A new clinical examination 3 months later showed no signs of sexual development. Fig. 2 shows the plasma Oe₂ response to treatment in precocious puberty.

**DISCUSSION**

Our Oe₂ plasma concentrations showed good correlations with clinical signs of oestrogenic effects in prepubertal and adolescent normal girls.

This is similar to what has been previously reported by other investigators (Jenner et al. 1972; Bidlingmaier et al. 1973; Winter & Faiman 1973). Seven of our 10 girls with premature thelarche had prepubertal levels of Oe₂. They were 5 years old or younger, and in all of them there was spontaneous regression of the breasts. Two of them had undetectable plasma Oe₂ at this time. The other 3 girls, who were 7 years old, had higher values and they also showed urocytograms with moderate oestrogenic activity. It has been assumed that premature thelarche results from a specific higher sensitivity of breast

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tissue to prepubertal oestrogen levels since other oestrogen target tissues do not show any stimulatory effect. Our findings in the 7 younger girls confirmed this hypothesis and were in agreement with those of Jenner et al. (1972).

However, as indicated by the findings in the other 3 patients, elevation of plasma oestrogens could be responsible for the breast development in some of these girls. Jenner et al. (1972) also found Oe₂ concentrations higher than normal in 2 of their 6 patients. Collett-Solberg & Grumbach (1965) found a "mild oestrogenic effect" in the urocytogram of 8 out of 9 girls with premature thelarche. It is known that during childhood different degrees of follicular maturation can be seen in the ovaries from primordial to Graafian follicles. The theca interna also shows vascularization and partial luteinization (Spivack 1934; Kraus & Neubecker 1962; Merrill 1963). However, these changes have been described before to be more prominent around the second year of life (Merrill 1963).

The fact that the 3 older girls showed evidences of ovarian activity might suggest that they were developing the first manifestations of a complete sexual development. However, in 2 of the 3 girls, who were followed, there was a regression of breast tissue in a few months in one, and lack of progression of sexual development in the other. This girl had a similar plasma Oe₂ concentration and physical signs 2 years later suggesting she is not yet starting complete sexual maturation; on the other hand, the bone age was not significantly advanced in the three cases. In summary, transient ovarian activity in some cases or target tissue receptor hypersensitivity in others could be responsible for premature thelarche.

All patients with idiopathic precocious puberty had elevated Oe₂ plasma concentrations for their ages but showed wide variations. No correlation between grades of sexual development and levels of Oe₂ was observed. Clinically, they could be compared to non-menstruating adolescent girls since none of them showed signs of menarche. The finding of pubertal or normal adult levels of plasma Oe₂ is consistent with the pathogenesis of the disease. This is comparable to the normal adult levels of plasma testosterone found in boys with idiopathic precocious sexual development (Rivarola et al. 1968). Precocious puberty can be associated with a variety of cerebral injuries. It is not clear why such different lesions as tumours or encephalitis of several types can stimulate gonadotrophin secretion. It is reasonable to assume that an injury could be responsible for blocking inhibitory stimuli as has been demonstrated in experimental animals (Van der Werff ten Bosch 1969). Our two patients with cerebral lesions showed plasma Oe₂ values similar to those of the patients with idiopathic precocious puberty. Even if we accept the theory of the blockade of inhibitory stimuli, it seems that other inhibitory influences are still operating because of the absence of an uncontrolled secretion of gonadotrophins and oestrogens.
Treatment with 150 mg of MPA per week decreased plasma Oe2 in patients with idiopathic sexual precocity. Evaluation of the effect of MPA on oestrogen secretion by urinary oestrogens had been very inconsistent (Kaplan et al. 1968; Severi et al. 1970; Hahn et al. 1964) but correlated well with clinical response when plasma Oe2 was determined (Jenner et al. 1972; Jeppson et al. 1973). Mishell et al. (1972), and Briggs & Briggs (1972) found some inhibition of plasma Oe2 levels in women receiving MPA as a contraceptive, but values were still 10 times higher than in post-menopausal women. In these studies, however, the dose was much lower than given in our cases. MPA can act by two mechanisms in sexual precocity, i.e. inhibition of gonadotrophin secretion and peripheral anti-oestrogenic effect of progestational agents. From our results, it is concluded that MPA, if given in high doses, decreases plasma Oe2 in patients with idiopathic sexual precocity. This is also similar to what has been reported for the effect of MPA on testosterone levels in boys with idiopathic precocious sexual development (Rivarola et al. 1968).

Measurements of plasma Oe2 gives useful information in other types of isosexual precocity. Our patient with a granulosa cell tumour had a value much higher than any others, suggesting that this determination could have a diagnostic value. This finding is in agreement with that of Jenner et al. (1972).

Two girls with Down's syndrome, hypothyroidism and sexual precocity had elevated Oe2 plasma concentrations that decreased on thyroid therapy. Signs of puberty also regressed. Association of sexual precocity with hypothyroidism and Down's syndrome has been reported (Hubble 1963; Aarskog 1969; Costin et al. 1972; Pabst et al. 1967) but the mechanism of this is not clear. It has been suggested that prolonged hypersecretion of thyrotrophin releasing hormone could stimulate pituitary secretion of gonadotropins. It is interesting that, in our subjects, Oe2 was diminished by thyroid treatment.

REFERENCES


Received on February 10th, 1975.