Ovarian 17α-hydroxyprogesterone responses to GnRH analog testing in oligomenorrheic insulin-dependent diabetic adolescents

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Abstract

Objective: To investigate the pituitary-ovarian function in adolescent girls with insulin-dependent diabetes mellitus (IDDM).

Design: Clinical case-control study.

Methods: The GnRH analog leuprolide acetate was administered subcutaneously to 16 adolescents with IDDM (seven eumenorrheic and nine oligomenorrheic) and 13 controls between 0800 and 0900 h. Blood samples were collected at baseline and 0·5, 3, 6 and 24 h after leuprolide to measure levels of gonadotropins. 17α-hydroxyprogesterone (17-OHP), androgens and estradiol.

Results: Mean baseline serum LH levels were significantly higher in eumenorrheic compared with oligomenorrheic IDDM patients, while peak LH responses to GnRH analog testing were similar in all subjects. Oligomenorrheic IDDM girls showed, as a group, a distinct 17-OHP response to GnRH analog stimulation, which in five out of nine girls was in the range of functional ovarian hyperandrogenism (≥ 8·6 nmol/l). Androgen and estradiol levels were not significantly altered in any group. No correlation was found between steroid levels and HbA1c, although the latter were significantly higher in oligomenorrheic than in eumenorrheic patients.

Conclusion: About 50% of the oligomenorrheic IDDM adolescents had an increased ovarian 17-OHP response to GnRH analog stimulation in the range of functional ovarian hyperandrogenism. Factors other than metabolic control, such as stress, may play an etiologic role in IDDM ovarian dysfunction.

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Introduction

Women with insulin-dependent diabetes mellitus (IDDM) have an increased incidence of menstrual abnormalities, particularly oligo- and amenorrhea and, less frequently, polymenorrhea (1–3); moreover women with IDDM have a shorter fertile life because of slightly delayed menarche and earlier menopause (1, 2). The delayed menarche appears to be more frequent when IDDM starts before 10 years of chronological age, while a later development of the disease does not seem to affect menarcheal age (2).

Studies on pituitary-gonadal function in adult women with IDDM and amenorrhea are controversial. Some authors have found low basal luteinizing hormone (LH) levels and decreased LH responses to gonadotropin-releasing hormone (GnRH) despite low estrogen levels (4, 5), while others have reported normal LH responses to GnRH challenge but fewer LH pulses/secretory episodes, suggesting a compromise of the GnRH pulse generator (6). Furthermore, ovarian steroid secretion seems to be altered in IDDM patients: while normally menstruating women with IDDM have normal estrogen and normal or, more often, elevated androgen levels, amenorrheic IDDM patients show normal or decreased estrogen and androgen concentrations (3, 7, 8). Despite the increased androgen levels, free hormone fractions remain in the normal range in eumenorrheic patients, thus explaining the maintenance of a normal menstrual pattern and the absence of hyperandrogenic manifestations (3).

GnRH analogs induce gonadal androgen secretion by producing an acute and sustained release of both pituitary LH and follicle-stimulating hormone (FSH) (9, 10). In this setting, Rosenfield and colleagues have determined that a subset of hyperandrogenic women have exaggerated ovarian 17α-hydroxyprogesterone (17-OHP) responses to GnRH agonists, suggestive of disordered ovarian cytochrome P450c17α activity (9–11). This pattern of ovarian steroidogenic response appears to be particularly frequent in hyperandrogenic adolescents after challenge with the GnRH agonist leuprolide acetate (LA) (12, 13).
We have observed an increased frequency of menstrual disturbances in adolescents with IDDM, some of whom also had other mild signs of hyperandrogenism. As all previous studies were carried out in adult women, we decided to undertake the present study to investigate the pituitary-ovarian function in adolescents with IDDM. We elected to use the GnRH analog LA because, according to our previous experience, short-term LA stimulation is a reliable and simple tool for testing pituitary and gonadal functions concomitantly (12, 13).

Materials and methods

Subjects

Of 50 young women with IDDM periodically controlled in our Regional Diabetic Pediatric Clinic (Parma, Italy), 12 (24%) presented with menstrual disturbances: oligomenorrhea (defined as menstrual cycles >45 days in duration) or secondary amenorrhea (3 or more months without a period). Of these nine (age range: 14-0-20-7 years, mean: 18-6 ± 3-3 years) agreed to participate in the study. All clinical and hormonal data were compared with those obtained in seven IDDM eumenorrheic young women (age range: 15-0-18-7 years, mean: 17-8 ± 3-7 years) and in 13 age- and body mass index-matched controls who agreed to take part in the study. Daily insulin requirements were 0-76 ± 0-21 IU/kg per day in oligomenorrheic and 0-78 ± 0-22 IU/kg per day in eumenorrheic patients.

Control subjects (age range: 15-1-22-1 years, mean: 20-0 ± 2-6 years) had normal general medical and menstrual histories and no family history of diabetes mellitus.

All subjects were on average 4 years beyond menarche (Table 1) and none was taking any medication known to affect reproductive function. Late-onset congenital adrenal hyperplasia due to 21-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies was ruled out in all oligomenorrheic IDDM subjects before their inclusion in the study by means of an adrenocorticotropin (ACTH) test, according to published criteria (14, 15). Pelvic ultrasonography was performed in all IDDM patients with menstrual disorders; polycystic ovaries were excluded in all following published criteria (16). Hirsutism, assessed according to the Ferriman & Gallway score (17), was present only in three of the nine oligomenorrheic patients.

The clinical data of patients and controls are shown in Table 1. The purpose of the study was explained to all participants. Control girls were informed that the study would not benefit them directly. Informed parental consent and assent from minors were obtained. The study and consent form were approved by Institutional Review Committees at both the Barcelona and Parma Hospitals.

Study protocol

After an overnight fast, baseline blood samples were obtained in the supine position between 0800 and 0900 h. Fasting blood glucose levels are reported in Table 1. LA (Procrin; Abbot, Madrid, Spain; 500 μg) was then given subcutaneously, and blood samples were drawn 0-5, 3, 6 and 24 h after challenge for measurement of serum LH, FSH, cortisol, 17-OHP, androstenedione, dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEAS) and estradiol (E2). Blood samples were immediately centrifuged, and serum was separated and frozen at −20 °C until assayed. The timing of blood sampling was selected according to previously published data indicating that the peak gonadotropin response occurred 2–6 h after the challenge, while the peak gonadal steroid response occurred after 20–24 h (9, 12, 13). Values of 17-OHP exceeding the mean ± 2 s.d. of those obtained in controls were considered abnormal (>8-6 nmol/l).

The study was scheduled during the follicular phase (days 3–8) of the menstrual cycle or after at least 3 months of amenorrhea.

Hormonal assays

LH, FSH and E2 were measured by an immunoenzymometric method (Serono Diagnostics, Coinsins, Switzerland and IFCI, Bologna, Italy for E2). The mean intra-assay and interassay coefficients of variation (C.V.)

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Age at menarche (years)</th>
<th>Insulin (IU/kg per day)</th>
<th>HbA1c (%)</th>
<th>Blood glucose levels (mg/dl)</th>
<th>Duration of diabetes (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligomenorrheic (n = 9)</td>
<td>18-6 ± 3-3</td>
<td>23-1 ± 3-2</td>
<td>13-6 ± 1-7</td>
<td>0-76 ± 0-2</td>
<td>10-8 ± 2-7</td>
<td>194-5 ± 44-1</td>
<td>8-9 ± 6-6</td>
</tr>
<tr>
<td>Eumenorrheic (n = 7)</td>
<td>17-5 ± 3-3</td>
<td>22-6 ± 1-1</td>
<td>13-3 ± 0-8</td>
<td>0-78 ± 0-2</td>
<td>8-2 ± 0-9</td>
<td>157-4 ± 24-9</td>
<td>7-2 ± 2-9</td>
</tr>
<tr>
<td>Controls (n = 13)</td>
<td>20-0 ± 2-8</td>
<td>21-1 ± 2-2</td>
<td>12-7 ± 1-6</td>
<td>—</td>
<td>—</td>
<td>84-5 ± 4-0</td>
<td>—</td>
</tr>
</tbody>
</table>

BMI: body mass index; HbA1c normal values <6%.

* P < 0.05 oligomenorrheic vs eumenorrheic IDDM patients.
were 4.8% and 6.1% for LH (calculated at 18.2 and 571U/l), 3.9% and 5.7% for FSH (calculated at 10.6 and 24.21U/l) and 4.8% and 9.6% for E2 (calculated at 284.9 and 961.8 pmol/l) respectively. HbA1C was determined by a Bio-Rad microcolumn test (Hercules, CA, USA). Androstenedione, DHEA, DHEAS, testosterone, and 17-OHP were measured by RIA as previously described (18, 19). The intra-assay and interassay C.V. values were 2.4% and 12% for androstenedione (calculated at 2.54 and 11.17 nmol/l), 2.7% and 19% for DHEA (calculated at 3.5 and 29.6 nmol), 2% and 17% for DHEAS (calculated at 433.6 and 1598 μmol/l), 3.8 and 8.7 for testosterone (calculated at 1.52 and 19.1 nmol/l), and 1.8% and 6.4% for 17-OHP (calculated at 3.9 and 5.7 nmol/l) respectively.

### Statistical analysis

Values are reported as the mean ± s.d. Hormone levels among independent groups were compared by one-way ANOVA corrected by Scheffe’s test for multiple comparisons. Correlation between variables were assessed by linear regression analysis; \( P < 0.05 \) was considered significant.

### Results

#### GnRH analog test

HbA1C levels were significantly higher in oligomenorrheic compared with eumenorrheic IDDM patients (Table 1).

Baseline LH levels were significantly higher in eumenorrheic compared with oligomenorrheic IDDM patients (\( P < 0.02 \)), while no statistical difference existed between the values of the oligomenorrheic subjects and the controls, and between the latter and the eumenorrheic diabetic adolescents. Peak LH responses among the three groups were similar. Baseline FSH levels were greater in controls than in patients, reaching statistical significance only in the case of oligomenorrheic IDDM girls (\( P < 0.01 \)).

Baseline and post-LA stimulation levels of E2 and androgen were not significantly different among the three groups. When compared with controls, oligomenorrheic IDDM patients showed a trend to higher E2 responses, while eumenorrheic IDDM patients tended to show higher baseline androstenedione and testosterone levels.

In oligomenorrheic IDDM patients, 17-OHP response to LA stimulation was significantly higher than in controls (\( P < 0.009 \)), while eumenorrheic IDDM girls showed intermediate responses. In five out of nine of the oligomenorrheic patients, the response was in the range of functional ovarian hyperandrogenism (FOH) (17-OHP ≥ 8.6 nmol/l) (Fig. 1).

Four oligomenorrheic patients, two of whom also had 17-OHP hyper-responses to LA stimulation and elevated androstenedione and/or testosterone basal levels, showed exaggerated 17-OHP responses to ACTH testing (>10.6 nmol/l but <36 nmol/l).

Basal and LA-stimulated hormonal values are reported in Table 2.

### Correlations among all parameters tested

In all IDDM girls, a negative correlation between age at menarche and onset of diabetes was found (\( r = -0.62, P < 0.02 \)). Post-LA 17-OHP levels correlated positively with baseline and stimulated testosterone values in all subjects (\( r = 0.64, P < 0.001 \) and \( r = 0.73, P < 0.00003 \) respectively) (Fig. 2).

No correlations were found between 17-OHP responses to GnRH analog stimulation and 17-OHP responses to ACTH testing, or between HbA1C and basal or post-LA steroid levels.

### Discussion

Menstrual irregularities are common in insulin-treated diabetic women, mainly when the onset of the disease occurs in prepubertal years (1, 2). In 24% of our IDDM postpubertal patients menstrual disturbances were already present at adolescence with a predominance of oligomenorrhea versus amenorrhea, thus stressing the early onset of these problems. The delayed age at menarche with respect to the local population (13.6 ± 1.68 years versus 12.4 ± 1.0 years) (20) further reflects an early impact of the disease on the hypothalamic-pituitary-gonadal axis.

Oligomenorrheic girls showed baseline LH levels significantly lower than eumenorrheic patients, although the LH response after GnRH analog challenge in both groups of patients was similar. This is in agreement with
previous published data (6), and suggests that the menstrual dysfunction present in poorly controlled IDDM patients may reflect disturbances of the GnRH pulse generator rather than abnormalities in pituitary or ovarian function. An enhanced dopaminergic tone, present especially in poorly controlled diabetic women, has been proposed as a contributing factor in the GnRH-altered control (4, 21, 22).

Baseline androgen and E2 levels were not significantly different in the three groups studied. However, in accordance with previous published papers (3, 7), we observed a trend of higher levels of androstenedione in eumenorrheic IDDM patients and lower E2 levels in both groups of patients compared with controls.

Oligomenorrheic IDDM adolescents showed, as a group, a distinct 17-OHP response to LA challenge, which in five out of nine patients was in the range of FOH (9, 13, 23). Four of our oligomenorrheic patients, two of whom also had 17-OHP hyper-responses to LA stimulation, showed exaggerated 17-OHP responses to adrenal testing, but not in the range to be consistently and homozgyous late-onset congenital adrenal hyperplasia (14, 15).

Women with hirsutism and polycystic ovary syndrome, in whom this pattern of adrenal 17-OHP hyper-response is associated with hyper-responsiveness of the 3α-hydroxysteroids DHEA and 17-hydroxyprogrenolone, have been described as having functional adrenal hyperandrogenism (FAH) (24, 25). Dysregulation of the adrenal cytochrome P450c17α seems to explain most cases of idiopathic FAH better. It has been suggested that a similar dysregulation of ovarian steroidogenesis causes the exaggerated 17-OHP response to GnRH analog testing characteristic of FOH (11, 12, 13). In our oligomenorrheic patients, basal androgen levels were usually within the normal range, except in the two subjects who also presented with elevated 17-OHP responses to LA and ACTH stimulation and in another patient who had only an ovarian 17-OHP hyper-response.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Oligomenorrheic (n = 9)</th>
<th>Eumenorrheic (n = 7)</th>
<th>Controls (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (IU/l)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Basal</td>
<td>2.5 ± 1.0*</td>
<td>5.5 ± 3.1</td>
<td>3.8 ± 1.6</td>
</tr>
<tr>
<td>3h</td>
<td>42.5 ± 25.0</td>
<td>56.5 ± 42.9</td>
<td>55.9 ± 38.5</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>3.9 ± 1.4†</td>
<td>4.5 ± 0.6</td>
<td>6.2 ± 2.1</td>
</tr>
<tr>
<td>3h</td>
<td>15.9 ± 5.7</td>
<td>18.1 ± 7.5</td>
<td>35.4 ± 28.6</td>
</tr>
<tr>
<td>E2 (pmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>75.5 ± 44.1</td>
<td>56.8 ± 29.6</td>
<td>119.4 ± 67.7</td>
</tr>
<tr>
<td>24 h</td>
<td>571.2 ± 428.4</td>
<td>131.2 ± 59.4</td>
<td>414.0 ± 230.0</td>
</tr>
<tr>
<td>17-OHP (nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>2.9 ± 1.5</td>
<td>3.7 ± 1.3</td>
<td>3.0 ± 1.7</td>
</tr>
<tr>
<td>24 h</td>
<td>9.9 ± 5.4†</td>
<td>5.9 ± 1.7</td>
<td>5.2 ± 1.7</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>9.3 ± 3.1</td>
<td>13.7 ± 6.2</td>
<td>8.8 ± 2.6</td>
</tr>
<tr>
<td>24 h</td>
<td>10.5 ± 3.6</td>
<td>12.3 ± 7.6</td>
<td>9.8 ± 2.4</td>
</tr>
<tr>
<td>DHEA (nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>26.4 ± 14.9</td>
<td>25.2 ± 10.9</td>
<td>30.8 ± 13.4</td>
</tr>
<tr>
<td>24 h</td>
<td>23.9 ± 7.0</td>
<td>22.0 ± 4.9</td>
<td>27.9 ± 9.9</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>2.4 ± 1.6</td>
<td>2.6 ± 2.1</td>
<td>1.5 ± 0.7</td>
</tr>
<tr>
<td>24 h</td>
<td>2.4 ± 1.4</td>
<td>2.0 ± 1.1</td>
<td>1.5 ± 0.6</td>
</tr>
</tbody>
</table>

* P < 0.02 vs eumenorrheic IDDM girls; † P < 0.01 vs controls; ‡ P < 0.009 vs controls.

Figure 2 Correlation between 17-OHP and testosterone levels after LA stimulation in all subjects; oligomenorrheic IDDM patients (OM), eumenorrheic IDDM patients (EU) and controls (C).
These results of ovarian 17-OHP hyper-response are in contrast with the partial hypogonadotropic hypogonadism observed in amenorrheic IDDM women (3–5).

It is noteworthy that hyperandrogenism is uncommon in IDDM and in a previous series of amenorrheic IDDM patients none had polycystic ovary syndrome or adrenal hyperfunction as triggering factors compared with 18% of amenorrheic non-IDDM women (3).

Oligomenorrheic patients had significantly higher HbAIC levels than eumenorrheic IDDM girls, indicating a lower degree of metabolic control. The lack of correlation between 17-OHP responses to LA stimulation and HbAIC levels suggests that factors other than metabolic control may play an etiologic role in IDDM hypothalamic-pituitary-ovarian dysfunction.

In conclusion, approximately half of our IDDM adolescent girls (five out of nine) with menstrual irregularities showed a 17-OHP response to GnRH agonist stimulation in the range of FOH, associated with a slight, but not significant elevation of androgen levels (basal and/or ACTH and/or LA-stimulated) and/or with mild clinical signs of hyperandrogenism such as hirsutism. As studies performed in amenorrheic IDDM women do not demonstrate an increased frequency of hyperandrogenism, we could hypothesize that the ovarian hyper-responsiveness present in many oligomenorrheic IDDM adolescents might eventually evolve into partial hypogonadotropic insufficiency. A progressive regulation of the GnRH pulse generator could be responsible for this (6).

The differences in ovarian steroid secretion patterns but not in gonadotropin secretion in adolescent and adult IDDM oligomenorrheic females suggest different physiopathologic mechanisms for the menstrual disorders in the two age groups. These could be elucidated by a long-term follow up of these patients.

Acknowledgements

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