

Polycystic ovarian disease unmasked by pulsatile GnRH therapy in a subgroup of women with hypothalamic amenorrhea

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Objective: To present the observation in six out of 120 women treated with pulsatile GnRH for ovulation induction, who developed hyperandrogenemia and polycystic ovaries during treatment.

Design: Clinical observation.

Setting: Department of Gynecologic Endocrinology and Reproductive Medicine, Medical University of Innsbruck, Austria.

Patient(s): A total of 120 women initially diagnosed as suffering from primary or secondary hypothalamic amenorrhea were treated for ovulation induction with pulsatile administration of GnRH for up to 140 days. There was no indication of the presence of polycystic ovaries or hyperandrogenemia before therapy.

Intervention(s): Pulsatile GnRH therapy using the Zyklomat pump.

Main Outcome Measure(s): Ovulatory menstrual cycles.

Result(s): Initially, all patients responded to pulsatile GnRH administration with ovulation and corpus luteum formation. During continuation of treatment, 6 patients developed an increase in LH and LH/FSH ratio as well as a progressive rise in serum T levels resulting in hyperandrogenemia. This was accompanied by the development of polycystic ovaries and cessation of follicular maturation.

Conclusion(s): We conclude from these observations that restoration of normal GnRH stimulation of the pituitary gland can result in the development of hyperandrogenemia and polycystic ovaries, suggesting a pituitary or ovarian defect underlying the pathogenesis of this disorder. (*Fertil Steril*® 2008;89:404–9. ©2008 by American Society for Reproductive Medicine.)

Key Words: Hyperandrogenemia, polycystic ovarian disease, pulsatile GnRH therapy, infertility, amenorrhea

Hyperandrogenemia is the most frequent cause of ovarian failure, affecting up to 10% of women of reproductive age. It is associated with infertility, the development of polycystic ovaries, hirsutism, and metabolic disturbances. In the majority of cases, the clinical signs of hyperandrogenemia develop at the time of puberty or shortly thereafter (1, 2). Because the onset of puberty is caused by the initiation of hypothalamic GnRH release and an increase of pituitary gonadotropin secretion it has been suggested that development of hyperandrogenemia may in some way be dependent upon gonadotropic stimulation of the ovaries (3).

Hypothalamic amenorrhea, the second most frequent cause of ovarian failure in young women, is caused by the

reduction of hypothalamic GnRH release (3–6). Functionally, these patients may be viewed as being prepubertal. Pulsatile GnRH therapy may therefore be viewed as resembling the initiation of puberty in such women (7). We here present observations in patients who initially presented with hypothalamic amenorrhea but then developed hyperandrogenemia during continuous pulsatile GnRH therapy, supporting the view that the cause of hyperandrogenemia may reside primarily within the ovaries.

PATIENTS AND METHODS

Patients

During the observation time, 120 patients were diagnosed as suffering from hypothalamic amenorrhea and treated with pulsatile GnRH for ovulation induction. Of those 120 patients, six, aged 25 to 35 years, suffering from primary (n = 1) and secondary (n = 5) hypothalamic amenorrhea of 3–7 years' duration and infertility, responded abnormally

Received October 5, 2006; revised and accepted February 27, 2007.

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and represent the group presented here. The clinical characteristics of these patients are shown in Table 1.

Diagnostic Procedures

As part of the endocrine workup, basal levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), testosterone (T), dehydroepiandrosterone-sulfate (DHEAS), free testosterone (fT), and sex hormone-binding globuline (SHBG) were measured in all patients. Thyroid disorders were excluded by documentation of normal values of TSH, fT3, and fT4. An ACTH test for detection of heterozygous C₂₁-hydroxylase deficiency was performed according to Lejeune-Lenain et al. (8) in four of six patients. Insulin resistance was evaluated by oral glucose tolerance test (OGTT). The OGTT was performed between 8:00 and 11:00 a.m., after overnight fasting, by administration of a solution containing 75 g glucose dissolved in 300 mL water. Blood samples were drawn before administration of the solution and at 15-minute intervals for 3 hours with determination of glucose and insulin in plasma and calculation of the areas under the curves as described previously (9).

The gestagen test was performed by oral administration of 10 mg medroxyprogesterone-acetate for 10 days (Prodafem; Pfizer Corporation Austria, Vienna, Austria). The test was designated positive when vaginal bleeding occurred within 10 days after the last gestagen administration. When the gestagen test was positive, the clomiphene test was started on cycle day 3–5: 100 mg clomiphene citrate (Clomiphene “Arcana”; Pfizer Austria) was administered daily for 5 days. The clomiphene test was positive when vaginal bleeding started 2–3 weeks after the last intake of clomiphene. When the gestagen test was negative, GnRH test was performed, consisting of the administration of 100 µg GnRH (Relefact LH-RH 0.100 mg ampoules; Aventis Pharma, Vienna, Austria). Blood samples were taken at various time intervals for up to 60 minutes for determination of LH and FSH. According to the results of these tests hypothalamic amenorrhea was diagnosed and graded as reported previously (10). Grading of hirsutism was

performed according to the Ferriman and Gallway criteria (11). Ultrasonography of the ovaries was performed using a Siemens Sonoline Versa Pro ultrasound machine equipped with a 5-MHz vaginal probe. The presence of polycystic ovaries was determined according to the Rotterdam criteria: presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter and/or large ovarian volume (>10 mL) (12).

Pulsatile GnRH Therapy

Pulsatile GnRH therapy was given using a dose of 20 µg GnRH per pulse administered every 90 minutes subcutaneously (sc) over a period of 80–140 days as described previously (6, 10). For pulsatile application, the Disetronic delivery pump (Zyklomat pulse) and Zyklomat sets (Ferring, Kiel, Germany) were used.

Blood samples for the determination of LH, FSH, E₂, progesterone (P), and T levels were collected every 2–4 days during the first 30 days of treatment and once or twice weekly thereafter. Ultrasonography of the ovaries was performed at various time intervals. The presence of polycystic ovaries was determined according to the Rotterdam criteria.

Assays

Levels of LH, FSH, PRL, E₂, P, DHEAS, SHBG, and insulin in serum were determined using commercially available specific enzyme immunoassays obtained from Boehringer Mannheim, Germany, and DPC Biermann, Bad Nauheim, Germany. Testosterone and fT were assayed using specific radioimmunoassays purchased from DPC Biermann. The interassay coefficient of variation (CV) of all assays was below 5%, and the intra-assay CV was less than 2%. The free androgen index was calculated from the concentrations of T and SHBG.

RESULTS

Basal Diagnosis

All patients were diagnosed as suffering from primary or secondary hypothalamic amenorrhea, respectively, based on the

TABLE 1

Clinical characteristics of the patients (#1–#6) before start of pulsatile GnRH application.

	#1	#2	#3	#4	#5	#6
Age	26	32	35	25	31	33
BMI	21	18.7	18.9	18.5	20.7	20.5
Amenorrhea	second	second	second	second	second	P
Duration of amenorrhea	3 yrs	5 yrs	7 yrs	4 yrs	3 yrs	—
Hirsutism	no	no	no	no	no	no
Polycystic ovaries	no	no	no	no	no	no
Gestagen test ^a	neg	pos	neg	neg	neg	pos
Grade	3a	2	3b	3a	3a	2

Note: BMI = body mass index (kg/m²); P = primary.

^a Gestagen tests were performed as described in the text and in reference 10.

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findings of low serum levels of LH and FSH, normal PRL levels, and T concentrations below 0.4 ng/mL. None of the patients examined exhibited insulin resistance as ascertained from the area under the curve of insulin concentrations after oral carbohydrate challenge, nor was there an indication of heterozygous C₂₁-hydroxylase deficiency.

The results of the gestagen, clomiphene, and GnRH tests in patients suffering from secondary amenorrhea revealed three patients suffering from grade 3a, one suffering from grade 2, and one suffering from grade 3b hypothalamic amenorrhea. The patient suffering from primary amenorrhea exhibited grade 2. The body mass index (BMI) of all patients was in the low to normal range; however, none of the patients reported significant weight loss or anorectic episodes during the 2 years before treatment. The pertinent clinical data are shown on Table 2.

At the initial vaginal ultrasonography, none of the patients exhibited signs of polycystic ovaries. The hirsutism score according to the criteria of Ferriman and Gallway was below 4 before treatment in all patients.

Pulsatile GnRH Therapy

Because all patients were initially diagnosed with hypothalamic amenorrhea, treatment with pulsatile administration of GnRH was initiated. The results of pulsatile GnRH therapy are shown in Table 3 and in Figures 1 and 2.

All patients responded initially to pulsatile GnRH administration by an increase of circulating LH and FSH levels, followed by a preovulatory E₂ rise accompanying the increase in the diameter of the leading follicle. All patients ovulated during the first cycle, as indicated by the preovulatory LH surge and the subsequent increase in P that reached levels typical for a normal luteal phase. However, only one of the six patients ovulated during a second cycle of pulsatile GnRH

administration, and the remaining five patients returned to the anovulatory stage. As shown in Figure 2, the P levels during the luteal phase of the second cycle during pulsatile GnRH-therapy in patient #4 were considerably lower than during the first cycle, suggesting the development of corpus luteum insufficiency.

During the period of anovulation after the first or second ovulatory cycle, LH levels in serum rose continuously, resulting in an increase in the LH/FSH ratio from below 1.0 before treatment to up to 9.0 at the end of pulsatile GnRH administration. Estradiol remained low at early to mid-follicular phase concentrations, and there was no rise of P-levels above 1.0 ng/mL, indicating persistent anovulation. However, T levels increased continuously from normal concentrations of less than 0.4 ng/mL up to peak concentrations of 1.5 ng/mL at the end of the pulsatile GnRH treatment. Ultrasonography performed during the anovulatory cycles revealed a progressive change to polycystic ovaries, characterized by an increased number of pearl string–arrayed follicles with a diameter below 10 mm. Three of the six patients reported the appearance of hirsutism during treatment.

At termination of pulsatile GnRH therapy, all patients reverted to the amenorrhic state with low levels of serum gonadotropins and T typical for women suffering from hypothalamic amenorrhea.

DISCUSSION

In spite of intense efforts over the last three decades, the causes of hyperandrogenemia remain enigmatic (13). Several hypotheses have been put forward to understand the etiology of this disorder. Defects in ovarian or adrenal steroidogenesis have been invoked as primary causes of androgen excess (14–16). The demonstration of an increased frequency of heterozygous carriers of C₂₁-hydroxylase deficiency lends some support to this view (17–19). Insulin resistance as part of the

TABLE 2

Basal hormone levels before starting GnRH therapy for each patient (#1–#6).

Hormone: normal value	#1	#2	#3	#4	#5	#6
LH: <10 [mIU/mL]	2.5	4.5	3.2	1.5	<0.5	9.1
FSH: <20 [mIU/mL]	2	<0.5	2.8	2.5	<0.5	6.5
PRL: 1.9–25 [ng/mL]	10	4.8	14.5	12.4	7.8	6
T: 0.0–0.4 [ng/mL]	0.2	0.3	0.2	0.3	0.25	0.3
DHEAS: 500–3000 [ng/mL]	1500	1860	2400	2250	1500	—
SHBG: >4.5 [μgDHT/dl]	2.5	2.7	3.8	3.5	4.5	—
FAI: 0–4.5	0.81	1.1	0.5	0.88	0.56	1.3
fT: 0–2.5 [pg/mL]	2.5	2.6	2.4	<1.5	1.8	—
AUC insulin: <12,000 [μIU × 180 min/mL]	9001	8150	9245	7560	8950	—

Note: AUC = area under the curve of insulin concentration after oral carbohydrate challenge; FAI = free androgen index (T/SHBG); fT = free testosterone.

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TABLE 3**Duration of pulsatile GnRH therapy and day of ovulation for each patient (#1–#6).**

	#1	#2	#3	#4	#5	#6
Duration, dose of therapy	80 days, 20 µg/pulse sc	124 days, 20 µg/pulse sc	90 days, 20 µg/pulse sc	140 days, 20 µg/pulse sc	80 days, 20 µg/pulse sc	90 days, 20 µg/pulse sc
Ovulation ^a	Day 16	Day 16	Day 17	Day 16, Day 44	Day 18	Day 16

^a Day of ovulation was defined as the day of LH surge + 1.

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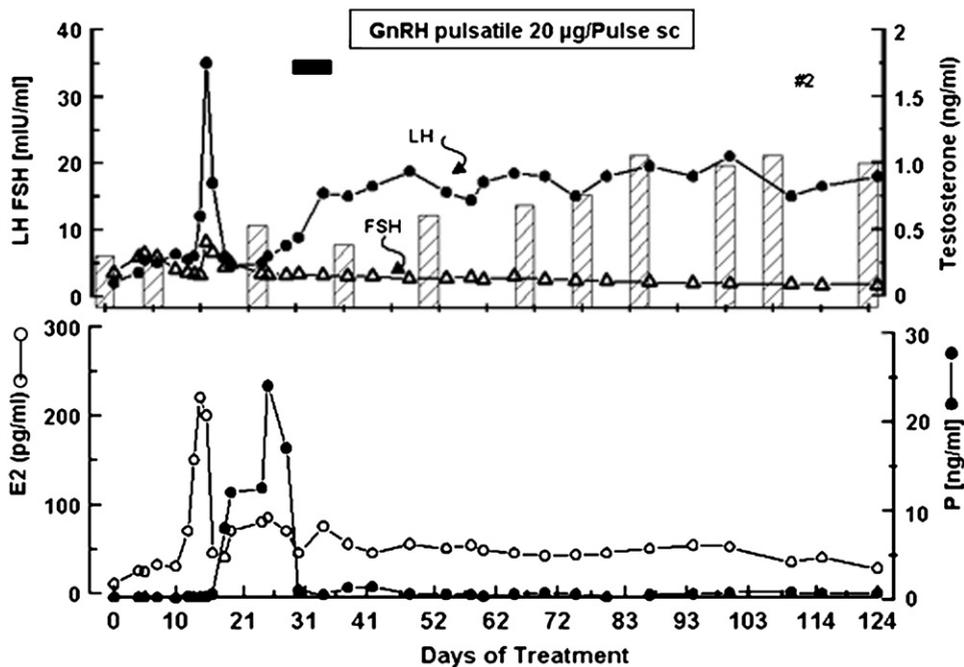
metabolic syndrome is at present discussed widely as being causally involved in the pathogenesis of hyperandrogenemia (20). In addition, disorders of the hypothalamo-pituitary axis, resulting in an increased frequency of LH pulses and an increase in the LH/FSH ratio, have been postulated as etiologic factors (2, 21, 22).

Ovulation induction in patients suffering from hyperandrogenemia is performed mainly by the administration of antiestrogens or gonadotropins, as well as recently by the

additional administration of metformin. The patients reported here were treated with pulsatile GnRH, because they presented initially with the typical signs and symptoms of hypothalamic amenorrhea. Pulsatile GnRH administration is generally not considered to be effective for ovulation induction in hyperandrogenemia. It has been demonstrated by Filicori et al. (23) that pulsatile GnRH therapy given to women with hyperandrogenemia produces abnormal ovarian responses. However, when pulsatile GnRH therapy

FIGURE 1

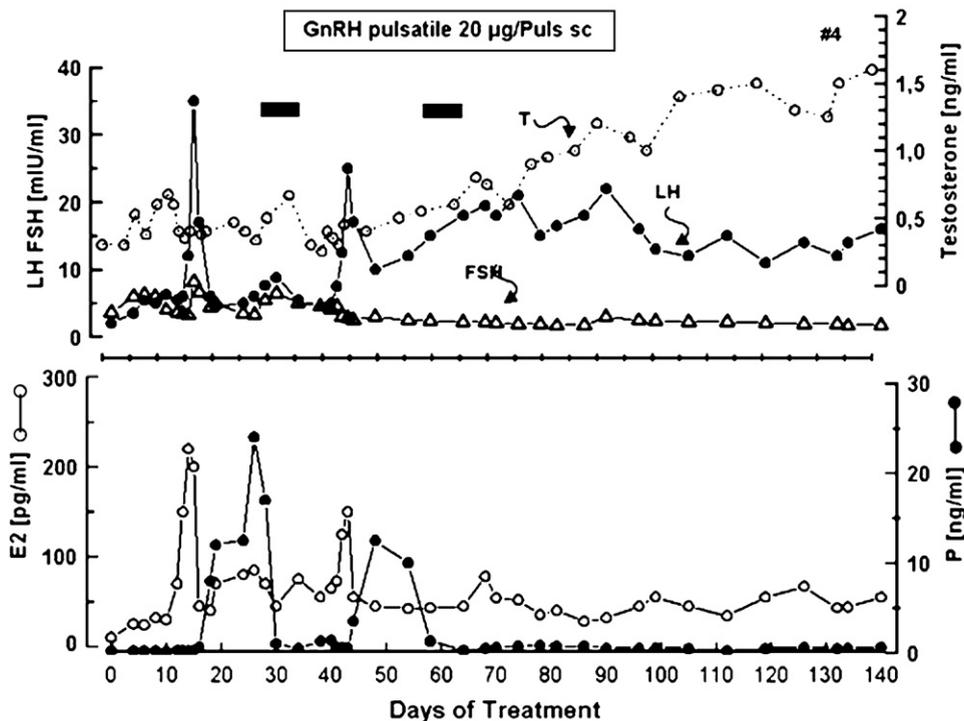
Induction of an ovulatory cycle by pulsatile GnRH treatment in a 32-year-old woman suffering from hypothalamic amenorrhea grade 2. Ovulation occurred on day 16. In the **upper panel**, the time courses of LH (*solid circles*) and FSH (*open triangles*) as well as the concentrations of testosterone in serum (*hatched bars*) are depicted. Serum T levels steadily increased during treatment, reaching peak levels of 2.0 ng/mL at the time when treatment was discontinued. The LH/FSH ratio increased from <1 at the beginning of treatment to 2.0 at the end of therapy. Menstruation is indicated by the *black rectangle*. The time courses of E₂ and P are shown on the **lower panel**. There was one normal preovulatory E₂ rise and a normal luteal phase as determined from the time course of P.



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FIGURE 2

Induction of two ovulatory cycles by pulsatile GnRH treatment in a 25-year-old woman suffering from hypothalamic amenorrhea grade 3a. Time course of LH (solid circles), FSH (open triangles), and T (open circles, dashed line) are shown on the **upper panel**. Serum T increased from <0.4 ng/mL at the beginning of treatment to 1.6 ng/mL at the end of therapy. The LH/FSH ratio rose from below 1 to 9 during treatment. Ovulation occurred on days 16 and 44 as adjudged by the time of the preovulatory LH surge. The time course of E₂ and P (**lower panel**) show lower preovulatory E₂ levels and lower serum P concentrations during the second cycle compared with the first cycle. Menstruation, as indicated by the *black rectangles*, occurred twice at the end of the luteal phases.



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was initiated after down-regulation of the pituitary and ovaries by GnRH analogues, ovulatory cycles could be induced transiently during the time when the pituitary recovered from the effects of the GnRH analogue. These findings are consistent with the present observation of an initially normal response of the pituitary and subsequent disappearance of normal ovarian response to pulsatile GnRH when hyperandrogenemia and polycystic ovaries develop during therapy. It was surprising, however, that development of hyperandrogenemia occurred rather quickly, within one or two cycles.

The data presented here demonstrate that the restoration of gonadotropin secretion by exogenously administered GnRH in certain women is followed by the manifestation of hyperandrogenemia, as indicated by a rise in T and an increase in the LH/FSH ratio, and the development of polycystic ovaries. These observations strongly suggest that in these patients the origins of hyperandrogenemia do not reside within the hypothalamus but rather reside within the ovary, the pituitary gland, or at both of these sites. The present

data do not allow a differentiation between the ovary and the pituitary gland as primary sites of the defects resulting in hyperandrogenemia. Qualitative or quantitative aberrations in pituitary gonadotropin secretion, changes in feedback sensitivity of the pituitary gonadotrophs to ovarian steroids or inhibin or inhibition of ovarian steroidogenic pathways have been discussed in this context (2, 24). However, because there is ample evidence that pituitary gonadotropin secretion can be normalized completely by pulsatile GnRH administration, our observations favor the view of an ovarian rather than pituitary defect as the underlying cause for the development of hyperandrogenemia.

In summary, the present observations demonstrate the existence of a subgroup of women who develop hyperandrogenemia with normalization of hypophysiotropic stimulation with pulsatile GnRH application. It is interesting in this context that of a total of 120 patients treated during the observation period, six developed hyperandrogenemia and polycystic ovaries during treatment. That is, the proportion of women belonging to this subgroup of patients corresponds closely

to the proportion of women suffering from hyperandrogenemia in the general population. Identification of patients suffering from combined hypothalamic hyperandrogenic ovarian failure such as those described here seems to be helpful in further clarifying some aspects of the pathogenetic mechanisms underlying hyperandrogenemia, in particular hyperandrogenemia developing during or immediately after puberty.

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