Functional hyperandrogenism detected by corticotropin and GnRH-analogue stimulation tests in women affected by apparently idiopathic hirsutism

R. Rossi, L. Tauchmanovà, A. Luciano, R. Valentino, S. Savastano, C. Battista, M. Di Martino, and G. Lombardi

Department of Molecular and Clinical Endocrinology and Oncology, University "Federico II", Naples, Italy

ABSTRACT. The etiologic diagnosis of hirsutism is often difficult. Previous studies have reported normal basal androgen and SHBG concentrations in 33-50% of hirsute women, suggesting the presence of an "idiopathic" form of hirsutism as the most frequent cause of this problem. The recent use of GnRH-analogues together with the corticotropin stimulation test allows better understanding of whether the cause of hirsutism is androgen excess and, if so, whether the origin of the latter is ovarian, adrenal or both. The present study evaluated adrenal and ovarian function in 48 young hirsute women as well as in 78 normal women matched for body mass index and age, who acted as control group. To determine ovarian function, a single 100-µg dose of GnRH analogue triptorelin was injected sc; thereafter, gonadotropins, 17-hydroxyprogesterone (17-OHP), △4-androstenedione (△4), total testosterone (T) and estradiol were determined. To better understand the adrenal function, 250 µg of 1,24 ACTH were administrated as iv infusion for 5 h, and plasma cortisol (F), 17-OHP, △4, DHEAS, T, 11-desossicortisol were measured. The combined use of these two stimulation tests was able to detect mild to moderate abnormalities in the steroidogenesis of ovaries alone (23%), adrenals alone (16.6%), or both (35.4%) in most hirsute women (75%) with otherwise normal baseline androgen concentrations. In particular, patients showed significantly increased responses of 17-OHP, △4, total T, 11-desossicortisol, and F to 1,24-ACTH administration. Moreover, they also had significantly higher 17-OHP and T responses to triptorelin. In conclusion, milder forms of functional ovarian and/or adrenal hyperandrogenism, similar to those found in clearly hyperandrogenic women, were observed and could be an underlying mechanism of idiopathic hirsutism.

(J. Endocrinol. Invest. *24:* 491-498, 2001)

INTRODUCTION

Hirsutism, oligomenorrhea and acne are reported to be the most common endocrine disorders in adolescent and reproductive-age women in Southern Italy (1). Usually these symptoms persist for years, can result in emotional problems and depression, and can deteriorate the quality of life (2). The etiologic diagnosis of hirsutism is often difficult. Normal basal androgen and SHBG concentrations have been reported in 33 to 50% of hirsute women, suggesting an "idiopathic" form of hirsutism as the most frequent cause of this problem (3). This disorder has been defined as the development of androgen-dependent terminal body hair in women in regions where terminal hair is normally not found (4), without signs of virilization, menstrual irregularities or hyperandrogenemia; the possible mechanisms underlying hair excess not related to androgen secretion rates have been postulated by various authors (5-10).

There is now increasing evidence that subtle alterations in androgen production and metabolism may underlie the apparently idiopathic form of hirsutism (11). Although it may not be easy to determine the cause of hyperandrogenism, the use of the corti-

Key-words: Idiopathic hirsutism, functional hyperandrogenism, ovarian hyperandrogenism, adrenal hyperandrogenism, steroidogenesis dysregulation, GnRH-analogue test, corticotropin test, triptorelin, dexamethasone low-dose test.

Correspondence: Dr. Riccardo Rossi, Piazza degli Artisti 17, 80129 Napoli, Italy.

E-mail: tauchman@unina.it

Accepted November 30, 2000.

cotropin stimulation test (1, 11, 12) together with the recent diagnostic use of GnRH-analogues (13-16) allow clinicians to understand whether androgen excess is also present in patients with apparently idiopathic hirsutism, and whether the origin of this disorder is ovarian, adrenal, or both. To this aim, several authors have evaluated the ovarian production of androgens after both acute and prolonged GnRH-analogue administration, the first to stimulate (13-16), and the second to suppress ovarian hormonal secretion (11, 17). Abnormal response to either acute or chronic GnRH-analogue administration was found predictive of ovarian origin of androgen excess (11, 13-16). The acute stimulation test has the advantage of being a short, direct, and specific test of pituitary-ovarian-function, capable to disclose the nature of ovarian steroidogenesis (13, 14, 16).
In order to better understand the etiology of idiopathic hirsutism, the present study evaluated ovarian and adrenal function using the GnRH-analogue triptorelin acute stimulation test (GnRHa) and the corticotropin long stimulation test, in 48 women with apparently idiopathic hirsutism. The combination of these tests was able to reveal mild to moderate abnormalities in the steroidogenesis of ovaries, adrenals, or both, in most hirsute women with normal baseline androgen concentrations.

SUBJECTS AND METHODS

The study population was composed of 48 women with peripuberal or adult-onset hirsutism with a Ferriman-Galwey score ≥12 (4), normal values of the main androgens – *i.e.* T, DHEAS and △4-androstenedione (△4A) – normal levels of 17-hydroxyprogesterone (17-OHP) and SHBG concentrations, and regular menstrual cycles (every 25-35 days). Ovulation was assumed to be regular in all women on the basis of regular cycles, reported symptoms of ovulation, such as mid-cycle pain, pre-menstrual discomfort and breast tenderness. Moreover, serum progesterone was determined in a random subset of 12 women in days 20-22 of their menstrual cycle and resulted always >6 ng/ml (range, 7.4-16.7), thus confirming previous ovulation (18). The women included in the study were 16 to 34 years old and were a part of a group of 123 hirsute women referred to our department for the evaluation of their androgen pattern. Seventy-five women were excluded after the initial evaluation because of abnormal baseline concentrations of one or more androgens or 17-OHP, considering the mean of 3 different determinations. None of the 48 women included in this study had drug-induced hirsutism, thyroid dysfunction, hyperprolactinemia, Cushing's syndrome or severe insulin resistance, which were excluded by anamnestic data and standard endocrine tests.
Seventy-eight normal women without signs of hyperandrogenism or a family history of endocrine disease, not taking any medication, whose ages were between 20 and 31 years, entered the study as controls for 1,24-ACTH and dexamethasone (DXM) tests, while 36 of them also underwent GnRHa testing. Both patients and controls were evaluated during the early follicular phase of their menstrual cycle (days 3 to 6) and at least 6 months after discontinuing estrogen and progestin therapy.
Informed consent was obtained from all subjects and the study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki.

Design of the study

BMI, expressed as weight divided by height square, fasting glucose and C-peptide concentrations were determined the first day of hospitalization. Transparietal pelvic ultrasonography was performed in all patients, using a 3.5-MHz transducer. The diagnosis of polycystic ovary was considered on the basis of criteria previously published (19).
ACTH test: Two-hundred and fifty micrograms of 1,24-ACTH (Synacthen, Ciba-Geigy, Balsel, Switzerland) were administrated as a continuous 250 ml saline iv infusion for 5 h, beginning at 08:00 h of the first day, and plasma F, 17-OHP, △4, DHEAS, total T, 11-deoxycortisol (S) were measured at time 0, 300 and 360 min.
DXM test: A low-dose 2-mg suppression test was carried out by an oral administration of 0.5 mg DXM from the second day, four times a day for 2 days; serum steroids were measured at 08:00 h the following morning (fourth day).
GnRHa test: A dose of 0.5 mg DXM was continued four times a day for 2 more days, which means that DXM was administered 2 days before and during the whole sampling period following the administration of GnRHa. On the fourth day, from 07:00 h, 2 blood samples were collected every 30 min for baseline measurements of serum gonadotropins and steroid hormones (17-OHP, △4, 17β-estradiol and total T). Then, at 08:00 h a single 100-μg dose of GnRHa triptorelin (D-Trp6-GnRH, Decapaptyl, Ipsen, Italy) was injected sc, and blood samples were collected after 0.5, 1, 2, 3, 4, 20 and 24 h. Gonadotropin levels were measured in all samples and, after 20 and 24 h, steroid hormones were also determined.
The response to GnRHa was considered abnormal if the plasma 17-OHP peak was greater than 6.2 nmol/l, the △4 peak greater than 5.7 nmol/l and

the total T peak above 1.4 nmol/l. These values represent 2 SD above the mean responses of controls. The response to 1,24-ACTH was considered abnormal if the maximum peak exceeded by at least 2 SD the mean of the control series. The cut-off value was set at 17.5 nmol/l, 13 nmol/l and 1257 nmol/l for 17-OHP, S and F respectively; and at 22.3 nmol/l, 15.5 µmol/l and 2.9 nmol/l for △4, DHEAS, and total T respectively.

The response to the DXM test was considered abnormal when steroids did not decrease below 50% of their baseline value; whereas F should fall to below 140 nmol/l (5 µg/dl) (20).

Hormonal analysis in serum: All measurements were performed in duplicate, within the same assay, using commercially available kits: F, T, estradiol and DHEAS, using Immulite, solid phase chemioluminescent enzyme immunoassay (DPC, Los Angeles, CA 90045-5597, USA); △4, 17-OHP with RIA Diagnostic Systems Laboratories (Webster, TX, USA); LH and FSH with RIA Biodata S.p.A. (Rimini, Italy). Reference intervals were as follows: F: 5-20 ng/dl, S: 10-250 ng/dl, 17-OHP: 10-200 ng/dl, △4: 1.0-4 pg/ml, DHEAS: 35-400 mcg/dl, T: 30-100 ng/dl. The conversion factors to SI units (nmol/l) were F: 27.6; S: 0.029; 17-OHP: 0.0303; △4: 0.035; DHEAS: 0.027 and T: 0.0346. Reference values for FSH and LH were 5-20 and 5-25 IU/l, respectively (conversion factor to SI units=1, both).

Statistical analysis: Results were expressed as mean±SD in the text and tables. Inter-group differences both at baseline and after stimulation tests were compared using the one-way ANOVA test and the Student's *t*-tests. The non-parametric method (Mann-Whitney U-test) was used when the Wilk-Shapiro test was not consistent with a Gaussian distribution of the data, *i.e.* for evaluation of 17-OHP and S concentrations. A *p* value less than 0.05 was considered significant.

RESULTS

Patients characteristics and baseline steroid pattern

The group of hirsute women had a Ferriman-Gallwey score of 16±3.6. Ultrasonographic features of polycystic ovaries were found in 7 patients (14%). A LH/FSH ratio >2 was found in 12 women (25%), only 5 of whom with ultrasound evidence of polycystic ovaries. Nevertheless, baseline androgen concentrations fell within the normal range in all participants, but the group of patients was characterized by significantly higher basal values of the

Table 1 - *Hormone values at baseline and in response to 250 mcg of 1,24-ACTH iv.*

Variable	Patients (no.=48)	Controls (no.=78)	*p* value
17-OHP (nmol/l)			
baseline	4.24±2.4	1.5±0.7	<0.001
peak	20.3±9.2	9.1±4.2	<0.001
△ after ACTH	14±10.3	6.6±5.6	<0.001
S (nmol/l)			
baseline	5.9±2.7	2.31±0.87	<0.001
peak	21±8.1	7.2±2.8	<0.001
△ after ACTH	13±9.4	7.82±5.37	<0.001
△4 (nmol/l)			
baseline	9.7±3.8	7.2±2.1	<0.001
peak	17.1±6.9	13.3±4.5	<0.001
△ after ACTH	7.3±6.8	5.2±3.1	<0.05
DHEAS (µmol/l)			
baseline	5.6±2.25	4.4±2.4	<0.005
peak	8.45±3.9	8.3±3.6	NS
△ after ACTH	2.58±2.9	2.53±1.8	NS
Testosterone (nmol/l)			
baseline	2.42±0.68	1.42±0.4	<0.001
peak	3.1±1.38	1.9±0.5	<0.001
△ after ACTH	0.7±1.38	0.7±0.7	NS
Cortisol (nmol/l)			
baseline	416.1±210	316.5±123	<0.005
peak	1490±678	1045±103.7	<0.001
△ after ACTH	1084±546	706±265	<0.001

All values are mean±SD; The response to corticotropin administration was expressed as the peak and △ (net increment of steroid). 17-OHP: 17-hydroxyprogesterone; NS: not significant; S: 11-deoxycortisol.

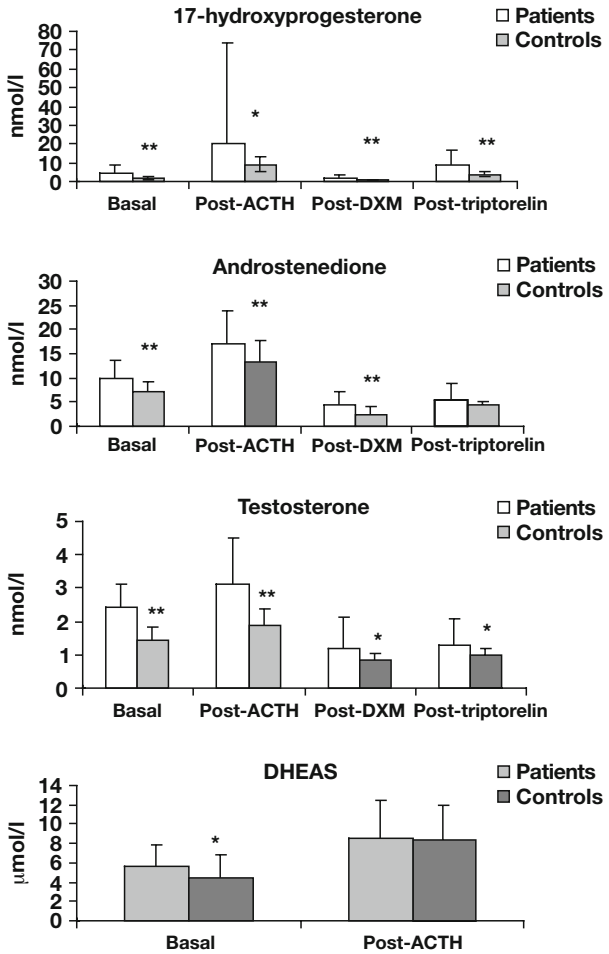

Fig. 1 - *Circulating concentrations of 17-hydroxyprogesterone (17-OHP), △4-androstenedione and DHEAS at baseline, after dexamethasone (DXM) and maximal responses to 1,24-ACTH and triptorelin administration. *at least p<0.05; **at least p<0.001 vs controls.*

main androgen – △4, T ($p<0.001$, both) and DHEAS ($p<0.05$) – and by significantly higher values of other steroids – *i.e.*, 17-OHP, S ($p<0.001$, both) and F ($p<0.005$). No difference was found between patients and controls in terms of BMI (24.6±3.9 *vs* 24.3±4.1), fasting glucose or C-peptide concentrations (5.3±0.8 *vs* 5.1±0.7 mmol/l and 0.68±0.5 *vs* 0.65±0.34 nmol/l respectively).

Steroid pattern after stimulation tests

ACTH testing: The results are shown in Table 1 and Figure 1. Compared to controls, the group of patients had a significantly increased response of 17-OHP, S, △4, and F to 1,24-ACTH administration considering both the peak and the net increment of steroids, which represented the change in circulating levels from 0 to 360 min after acute corticotropin administration (△ of the response). Total T showed an increased peak in patients compared to controls, but the △ response was similar in the two groups. This is possible since baseline T values were significantly different in two groups. When considering the single subjects, an abnormal response of at least one steroid to 1,24-ACTH administration was found in 25/48 (52%) patients, and an abnormal response of at least two steroids was found in 19 (39.6%); in particular, elevated 17-OHP response in 20 patients, increased S response in 14 patients, elevated △4 response in 5 patients, enhanced F response in 5 of them; DHEAS and T hyperresponsiveness in 2 and 3 women, respectively. However, no women had ACTH-stimulated S and 17-OHP values exceeding 30 nmol/l (1000 ng/dl), which is suggestive of 11β-hydroxylase or 21-hydroxylase deficiency (1, 15, 21-23), while post-ACTH DHEAS levels did not exceed by 4 SD the normal control values, thus excluding 3β-hydroxy-steroid dehydrogenase deficiency. Moreover, in 2 women with increased DHEAS response, DHEA/△4 ratio was also determined and was found not to exceed by 4 SD the value of normal women (4.1 and 3.9 *vs* 3.3±1.2 of controls) (1, 24). Women with abnormal response of at least one steroid to 1,24-ACTH administration were considered as having adrenal functional hyperandrogenism (15).

DMX testing: F, 17-OHP, △4, DHEA-S and T decreased after DXM administration in all the women considered; nevertheless mean post-DXM 17-OHP, △4 and T values were significantly higher in patients than in controls ($p<0.001$, $p<0.001$ and $p<0.05$, respectively). Individual response to DXM was abnormal in 15 patients for at least one steroid, and in 8 of them for 2 steroids (17-OHP and △4 in all except one woman, in whom 17-OHP and T did not decrease sufficiently). All these women had abnormal response to the GnRHa administration, suggesting ovarian functional hyperandrogenism. Moreover, F did not fall below 140 nmol/l in 3 patients who had normal adrenal gland morphology at abdominal computed tomography scan.

GnRHa testing: The mean 17-OHP response to triptorelin was significantly higher among patients than controls ($p<0.001$). Total T post-triptorelin values were also increased albeit with a milder significance ($p<0.05$), while no significant difference in △4 response was found between the two groups (Table 2, Fig. 1). Considering each single patient, the response of at least one steroid to triptorelin was abnormal in 28 women (58%), who were thus considered as having ovarian functional hyperandrogenism (14, 15). Furthermore, both 17-OHP and △4 hyperresponsiveness were shown in 10 patients,

Table 2 - *Hormone values at baseline (during dexamethasone treatment) and in response to GnRH-analogue triptorelin.*

	Patients (no.=48)	Controls (no.=36)
LH/FSH ratio	1.37	1.19
FSH (IU/l)		
basal	6.86±3.6	8±1.4
maximum	29.9±11.2*	44.6±6.1
LH (IU/l)		
basal	10.5±6.8	10.6±3.5
early response	33.3±21§	23.5±3.5
maximum	138±69	117±23
17-hydroxyprogesterone (nmol/l)		
basal	1.94±1.65*	0.9±0.32
maximum	9.2±7.4*	3.6±1.3
△4-androstenedione (nmol/l)		
basal	4.37±2.8*	2.51±1.51
maximum	5.43±3.41	4.5±0.6
Testosterone (nmol/l)		
basal	1.2±0.93§	0.83±0.2
maximum	1.27±0.8§	1.0±0.2
Estradiol (pmol/l)		
basal	152±11	162±18
maximum	629±239	502±48

*$p<0.001$ and §$p<0.05$ *vs* controls. All values are mean±SD, maximal values were defined as a peak for gonadotropins and as the mean value at 16 to 24 h for steroids. Early response of LH means its value at 30 min.

while T hyperresponsiveness only in two. A significant increase in early LH response (at 30 min) was found in patients *vs* controls ($p<0.05$) and in 19 hirsute women (39,6%) when considered individually. On the other hand, the lack of any increase in estradiol levels after triptorelin administration was revealed in two women with otherwise normal steroid and gonadotropin response to stimulus, indicating possible aromatase dysregulation (15).
The abnormal steroid responses to both the adrenal and ovarian stimulation tests were observed in 17 (35.4%) patients (Fig. 2), who also showed a high variability of △4 and DHEAS concentrations stimulated by 1,24-ACTH.

DISCUSSION

All the women included in this study were considered as having idiopathic hirsutism after initial evaluation; this diagnosis was based on the absence of menstrual irregularities, basal hyperandrogenemia or virilization. The percentage of such women was 39% in our series of hirsute women evaluated (48 out of 123). Several different abnormalities in steroid profiles were found in the present study using the combination of corticotropin and GnRH-analogue stimulation tests; this suggests that hirsutism in these women can no longer be considered idiopathic. By this approach, the percentage of hirsute women with normal adrenal and ovarian function was reduced subsequently to 9.8% of the overall series of hirsute women (12 of 123 patients).
According to these test results, hirsutism was considered to be caused by ovarian dysfunction alone in 11 women (23%), by ovarian and adrenal dysfunction in 17 (35.4%) and by adrenal dysfunction alone in 8 (16.6%) (Fig. 2).
We observed that the association of the 1,24-ACTH and triptorelin acute stimulation tests is a simple, non-invasive and useful way to determine whether

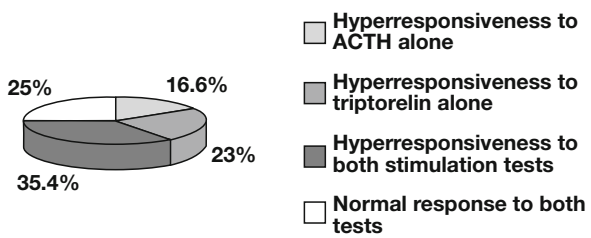

Fig. 2 - *Relative expression of adrenal, ovarian functional hyperandrogenism in 48 women with apparently idiopathic hirsutism; 16.6% of women had hyperresponsiveness of at least one steroid to ACTH test alone, 23% to triptorelin test alone, 35.4% to both tests and 25% had normal responses. In summary, adrenal hyperandrogenism occurred in 52% (16.6+35.4%), and ovarian hyperandrogenism occurred in 58.4% (23+35.4%) of patients.*

androgen excess may be the underlying mechanism of hirsutism in women with the apparently idiopathic form of this disorder and whether it is of adrenal and/or ovarian origin. The ovarian response to triptorelin was evaluated during DXM administration in an attempt to quantify the amount of ovarian steroid response to GnRHa under the suppression of adrenal androgen production. The long 1,24-ACTH test was performed to achieve the most effective stimulus on adrenal steroidogenesis, especially on androgens, in keeping with the data published and with our previous studies comparing the ACTH short test (time 0-60 min) with the ACTH long test (time 0-300-360 min) (1, 21).

Ovarian hyperresponsiveness to triptorelin defined by △4 and T, but predominantly 17-OHP, was observed in 58% women and was similar to that found by other authors in women with polycystic ovaries and functional ovarian hyperandrogenism; this suggests an activation of P450c17 cytochrome that includes △4-17α-hydroxylase and △4-17,20-lyase activities, with predominant abnormalities of the first over the second in such women (14-16). The appearance of ovarian abnormalities in our patients was supported also by the presence of an LH/FSH ratio >2 in 12 patients, by ultrasonographic evidence of polycystic ovaries in 7 and by early LH hyperresponsiveness to triptorelin in 19. All these abnormalities have already been found in association with ovarian functional hyperandrogenism, whereas the latter has been correlated with hypothalamic-pituitary-ovarian axis dysregulation in series of hyperandrogenic women (13, 14, 16). Only 15 of the 28 women with ovarian hyperandrogenism detected by triptorelin had impaired androgen suppression after DXM. Although a high degree of concordance has been previously found between acute GnRHa and the DXM suppression tests in relation to the diagnosis of ovarian hyperandrogenism in clearly hyperandrogenic women (14), our data suggest that acute GnRHa test may have some advantages over the DXM test in uncovering functional hyperandrogenism, at least in women with normal basal androgen levels. However, no relationship was observed between the endocrine pattern and ultrasonographic findings. This is in agreement with previous data showing polycystic ovary evidence even in normally ovulating women without any sign of hirsutism (25).

Fifty-two percent of women – all hyperresponsive to 1,24-ACTH – were considered as having adrenal steroid biosynthesis dysregulation, the expression of which appeared widespread and variable. The predominant hyperresponsiveness of 17-OHP and △4 to 1,24-ACTH in some women, and of DHEAS in others suggests that abnormal P450 c17 △4- and △5-pathways could be a relevant underlying mechanism of hirsutism in some cases and the cause of adrenal functional hyperandrogenism. Increase in S and F responses to 1,24-ACTH indicates that other adrenal enzymes may also be hyperactive. Moreover, some women with increased F baseline and stimulated values, not suppressed after DXM administration, may be affected by a mild form of glucocorticoid resistance (26); receptor abnormalities have already been described in women in whom hirsutism was the sole clinical manifestation and who displayed a great biochemical variability of steroid production (26).

Although the predominant dysregulation in the △5-pathways has been proposed by previous studies as the most frequent cause of adrenal functional hyperandrogenism, other studies have reported dominant abnormalities in △4-pathway (15, 27, 28). Nevertheless, our data are in agreement with the hypothesis of a more generalized adrenal hyperactivity – rather than enhanced P45c17 activity alone – as the cause of the adrenal component of hirsutism (22).

Although some authors have proposed various mechanisms of idiopathic hirsutism not related to androgen secretion, such as increased bioavailability of T to target tissues, enhanced skin conversion of T to dihydrotestosterone or increased circulating 5α-androstanediol glucuronide levels (5-8), these hypotheses have not been widely accepted (11). More recently, abnormalities of the androgen receptor function at a molecular level have been investigated and a polymorphism with trinucleotide (CAG) repeats and skewing of X-chromosome inactivation have been proposed as possible mechanisms underlying the apparently idiopathic form of hirsutism (9, 10); however, these data remain to be confirmed. On the contrary, our results are in line with previous proposals suggesting that most women with idiopathic hirsutism may have underlying abnormal ovarian and/or adrenal androgen production (11, 22). The hyperresponsiveness to ovarian acute stimulation together with a wide range of adrenal abnormalities in response to 1,24-ACTH administration suggest that milder, but probably multiple forms of functional ovarian and/or adrenal hyperandrogenism, similar to those found in clearly hyperandrogenic women, frequently lead to "idiopathic hirsutism". Being these forms of androgen excess of milder entity, they do not cause basal hyperandrogenaemia, and can be detected only by dynamic testing of ovarian and adrenal functions.

Finally, the determination of the etiology of hirsutism should be performed in all hirsute women,

regardless of their basal androgen concentrations, to help choose the most appropriate treatment that may consequently result in diminished emotional problems and better psychological outcome, especially given the young age of most of these patients.

ACKNOWLEDGMENTS

We are grateful to Mrs. Rosanna Scala for her assistance in editing the manuscript.

REFERENCES

1. Valentino R., Tommaselli A.P., Savastano S., Dorato M., Rossi R., Lombardi G.
Different dysregulation in adrenal steroid biosynthesis as a prevalent cause of hyperandrogenism in women from Southern Italy.
Fertil. Steril. 1997, *682:* 36-41.
2. Darendeliler F., Bas F., Balaban S., Bundak R., Demirkol D., Saka N., Gunoz H.
Spironolactone therapy in hypertrichosis.
Eur. J. Endocrinol. 1996, *135:* 604-608.
3. Horton R., Lobo R.
Peripheral androgens and the role of androstanediol glucuronide.
Clin. Endocrinol. Metab. 1986, *15:* 293-306.
4. Ferriman D., Gallwey J.D.
Clinical assessment of body hair growth in women.
J. Clin. Endocrinol. Metab. 1961, *21:* 1440.
5. Rittmaster R.S.
Clinical relevance of testosterone and dihydrotestosterone metabolism in women.
Am. J. Med. 1995, *98* (Suppl.): 17S-21S.
6. Paulson R.J., Serafini P.C., Catalino J.A., Lobo R.A.
Measurements of 3α, 17β-androstanediol glucuronide in serum and urine and the correlation with skin 5α-reductase activity.
Fertil. Steril. 1986, *46:* 222-226.
7. Glickman S.P., Rosenfield R.L.
Androgen metabolism by isolated hairs from women with idiopathic hirsutism is usually normal.
J. Invest. Dermatol. 1984, *82:* 62-66.
8. Rittmaster R.S., Zwicker H., Thompson D.L., Konok G., Norman R.W.
Androstanediol glucuronide production in human liver, prostate and skin. Evidence for the importance of the liver 5α-reduced androgen metabolism.
J. Clin. Endocrinol. Metab. 1993, *76:* 977-982.
9. Vottero A., Stratakis C.A., Ghizzoni L., Longui C.A., Karl M., Chrousos G.P.
Androgen receptor-mediated hypersensitivity to androgens in women with nonhyperandrogenic hirsutism: skewing of X-chromosome inactivation.
J. Clin. Endocrinol. Metab. 1999, *84:* 1091-1095.
10. Sawaya M.E., Shalita A.R.
Androgen receptor polymorphisms (CAG repeat lengths) in androgenetic alopecia, hirsutism, and acne.
J. Cutan. Med. Surg. 1998, *3:* 9-15.
11. Escobar-Morreale H.F., Serrano-Gotarredona J., Garcìa-Robles R., Sancho J., Varela C.
Mild adrenal and ovarian steroidogenic abnormalities in hirsute women without hyperandrogenemia: does idiopathic hirsutism exist?
Metabolism 1997, *46:* 902-907.
12. Erel C.T., Senturk L.M., Oral E., Mutlu H., Colgar U., Seysoglu H., Ertungealp E.
Results of the ACTH stimulation test in hirsute women.
J. Reprod. Med. 1999, *44:* 247-252.
13. Barnes R.B., Rosenfield R.L., Burnstein S., Ehrman D.A.
Pituitary-ovarian response to Nafarelin testing in the polycystic ovary syndrome.
N. Engl. J. Med. 1989, *320:* 559-565.
14. Ehrmann D.A., Rosenfield R.L., Barnes R.B., Brigell D.F., Sheikh Z.
Detection of functional ovarian hyperandrogenism in women with androgen excess.
N. Engl. J. Med. 1992, *327:* 157-162.
15. Ehrmann D.A., Barnes R.B., Rosenfield R.L.
Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion.
Endocr. Rev. 1995, *16:* 322-351.
16. Rosenfield R.L., Barnes R.B., Cara J.F., Lucky A.W.
Dysregulation of cytochrome P450c 17α as the cause of polycystic ovary syndrome.
Fertil. Steril. 1990, *53:* 785-791.
17. Rittmaster R.S., Thompson D.L.
Effects of leuprolide and dexamethasone on hair growth and hormone levels in hirsute women: the relative importance of the ovary and the adrenal in the pathogenesis of hirsutism.
J. Clin. Endocrinol. Metab. 1990, *70:* 1096-1102.
18. Billings E.L., Brown J.B., Billings J.J., Burger H.G.
Symptoms and hormonal changes accompanying ovulation.
Lancet 1972, *1:* 282-284.
19. Adams J., Franks S., Polson D.W., Mason H.D., Abdulwahid N., Tucker M., Morris D.V., Price J., Jacobs H.S.
Multifollicular ovaries: Clinical and endocrine features and response to pulsatile gonadotropin releasing hormone.
Lancet 1985, *ii:* 1375-1379.
20. Nieman L., Cutler G.B. Jr.
Cushing's syndrome.
In: DeGroot L.J., Besser M., Burger H.G., Loriaux D.L., Marshall J.C., Odell W.D., Potts J.T. Jr., Rubenstein A.H. (Eds.), Endocrinology.
W.B. Saunders Company, Philadelphia, 1995, p. 1741.
21. New M.I., Lorenzen F., Lener A.J., Kohn B., Oberfield S.E., Pollack M.S., Dupont B., Stoner E., Levy D.J., Pang S., Levine L.S.

Genotyping steroid 21-hydroxylase deficiency. Hormonal reference data.
J. Clin. Endocrinol. Metab. 1983, *57:* 320-325.

22. Eldar Geva T., Hurwitz A., Vecsei P., Palti Z., Mildwidsky A., Rosler A.
Secondary biosynthetic defects in women with late-onset congenital adrenal hyperplasia.
N. Engl. J. Med. 1990, *323:* 855-863.
23. De Simone G., Tommaselli A.P., Rossi R., Valentino R., Lauria R., Scopacasa F., Lombardi G.
Partial deficiency of adrenal 11β-hydroxylase. A possible cause of primary hypertension.
Hypertension 1985, *7:* 204-210.
24. Sakkal-Alkaddour H., Zhang L., Yang X., Chang Y.T., Kappy M., Slover R.S., Jorgensen V., Pang S.
Studies of 3β-hydroxysteroid dehydrogenase genes in infants and children manifesting premature pubarche and increased adrenocorticotropin-stimulated △5-steroid levels.
J. Clin. Endocrinol. Metab. 1996, *81:* 3961-3965.
25. Polson D.W., Adams J., Wadsworth J., Franks S.
Polycystic ovaries – A common finding in normal women.
Lancet 1988, *i:* 870-872.
26. Werner S., Thorèn M., Gustafsson J.A., Brönnegård M.
Glucocorticoid receptor abnormalities in fibroblasts from patients with idiopathic resistance to dexamethasone diagnosed when evaluated for adrenocortical disorders.
J. Clin. Endocrinol. Metab. 1992, *75:* 1005-1009.
27. Azziz R., Bradley E.L., Potter H.D., Boots L.
Adrenal androgen excess in women: Lack of a role for 17-hydroxylase and 17,20-lyase dysregulation.
J. Clin. Endocrinol. Metab. 1995, *80:* 400-405.
28. Barnes R.L., Ehrmann D.A., Brigell D.F., Rosenfield R.L.
Ovarian steroidogenic responses to the gonadotropin-releasing hormone agonist nafarelin in hirsute women thought to have 3β-hydroxysteroid dehydrogenase deficiency.
J. Clin. Endocrinol. Metab. 1993, *76:* 450-455.