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Androgen Dependence of Hirsutism, Acne, and Alopecia in Women

Retrospective Analysis of 228 Patients Investigated for Hyperandrogenism

THESE

préparée sous la direction du Docteur Fulgencio Gomez, Privat-Docent et Maître d'Enseignement et de Recherche
avec la co-direction du Professeur Rolf C. Gaillard
et présentée à la Faculté de biologie et de médecine de l'Université de Lausanne pour l'obtention du grade de

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par

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Androgen dependence of hirsutism, acne, and alopecia in woman
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RAPPORT DE SYNTHESE

Androgen Dependence of Hirsutism, Acne, and Alopecia in Women. Retrospective Analysis of 228 Patients Investigated for Hyperandrogenism.

Dépendance aux androgènes de l'hirsutisme, l'acné et l'alopécie chez la femme. Analyse rétrospective de 228 patientes investiguées pour hyperandrogénie.

L'hirsutisme, l'acné et l'alopécie chez la femme sont souvent associés à des troubles menstruels et à une production excessive d'androgènes, raison pour laquelle ces symptômes cutanés font l'objet d'évaluation endocrinienne. L'hyperandrogénie affecte 5 à 10 % des femmes en âge de reproduction et constitue un motif fréquent de consultation. Récemment, les sociétés d'endocrinologie ont émis des recommandations sur l'investigation et le traitement de l'hyperandrogénie.

Longtemps confrontés à la demande de patientes souffrant d'hirsutisme, d'acné ou d'alopécie, nous avons décidé d'effectuer une approche diagnostique et thérapeutique comportant des dosages hormonaux et un traitement antiandrogénique. Un grand nombre de patientes a été ainsi étudié au fil des années. Les paramètres mesurés incluaient la testostérone plasmatique totale, l'androstènedione, le sulfate de déhydroépiandrostérone (DHEAS), la sex hormone-binding globulin (SHBG) et la testostérone salivaire. Cette dernière est considérée comme un bon reflet de la testostérone libre plasmatique, indépendamment des protéines de liaison. L'analyse rétrospective des dossiers nous a permis de comparer nos données avec celles de la littérature.

Des 318 dossiers de patientes ayant consulté notre Service pour hirsutisme, acné ou alopécie pendant 6 ans, 228 ont pu être retenus pour une évaluation adéquate. Chez les patientes présentant ces symptômes de façon isolée, les taux d'androgènes et la prévalence de l'oligo-aménorhée étaient plus élevés en cas d'hirsutisme qu'en cas d'alopécie, avec des valeurs intermédiaires en cas d'acné. Aucun des androgènes mesurés ne permettait, à lui seul, d'identifier tous les cas d'hyperandrogénie, mais la testostérone salivaire a montré la meilleure corrélation positive avec l'hirsutisme, alors que la testostérone plasmatique totale montrait la moins bonne corrélation, et l'androstènedione, le DHEAS et la SHBG des corrélations intermédiaires (corrélation négative pour la SHBG). De plus, au cours du traitement antiandrogénique, la testostérone salivaire a montré l'abaissement proportionnel le plus marqué de tous les androgènes mesurés. Comparées aux patientes originaires d'Europe centrale, les patientes originaires d'Europe du sud consultaient avec des degrés d'hirsutisme supérieurs, mais aucune différence n'a été observée dans les corrélations entre l'hirsutisme et les taux hormonaux de ces deux groupes. En l'absence d'un nombre suffisant d'échographies ovariennes, la prévalence du syndrome des ovaires polykystiques a été probablement sous-estimée (63 patientes, 27.6 % des cas), au bénéfice du diagnostic d'hyperandrogénie avec euménorrhée (101, 44.3 %) ; les autres diagnostics étaient : androgènes normaux (51, 22.4%), SHBG basse isolée (7, 3.1%), hyperplasie surrénalienne congénitale non-classique (4, 1.8%), et tumeur ovarienne (2, 0.9%).

Nous avons comparé les divers traitements médicaux de l'hirsutisme publiés au cours des 25 dernières années, quant à leur efficacité et leur coût. La sensibilisation à l'insuline avec metformin est moins efficace, mais aussi moins chère. L'anti-androgène flutamide et l'inhibiteur de la 5-α reductase finastéride figurent parmi les traitements les plus performants, mais ils sont aussi les plus chers. Le traitement anti-androgénique et de suppression hormonale avec acétate de cyprotérone et éthinyl-oestradiol, utilisé dans cette étude, est également parmi les plus efficaces, tout en étant nettement moins cher.

Cette étude est la première comparant directement les taux d'androgènes et la prévalence de l'oligo-aménorrhée dans les 3 symptômes cutanés d'hyperandrogénie, hirsutisme, acné et alopécie, et elle démontre leur différente dépendance aux androgènes. La salive apparaît comme un milieu de choix pour identifier ces patientes et la recommandation actuelle de doser la testostérone plasmatique totale en premier, pour distinguer l'hyperandrogénie de l'hirsutisme idiopathique, nous paraît inadéquate. Nous proposons, au contraire, d'abandonner ce dosage au profit de celui de la testostérone salivaire. Par ailleurs, notre étude infirme l'hypothèse d'une sensibilité cutanée accrue aux androgènes chez les femmes originaires du sud de l'Europe. Finalement, elle est la seule à comparer les effets cliniques, les changements biologiques et le coût annuel des traitements publiés de l'hirsutisme.

Androgen Dependence of Hirsutism, Acne, and Alopecia in Women

Retrospective Analysis of 228 Patients Investigated for Hyperandrogenism

Sandra Karrer-Voegeli, MD, François Rey, PhD, Marianne J. Reymond, PhD, Jean-Yves Meuwly, MD, Rolf C. Gaillard, MD, and Fulgencio Gomez, MD

Abstract: Hirsutism, acne, alopecia, and oligo-amenorrhea are clinical expressions of hyperandrogenism, one of the most frequent endocrine disorders in women of reproductive age. Women referred to our endocrine clinics for skin symptoms of hyperandrogenism underwent a laboratory workup to evaluate hormone measurements and received antiandrogen therapy. We retrospectively analyzed the outcome of 228 consecutive patients investigated over 6 years.

Patients with hirsutism had higher levels of androstenedione, dehydroepiandrosterone sulfate (DHEAS), and salivary testosterone; lower levels of sex hormone-binding globulin (SHBG); and a higher prevalence of oligo-amenorrhea than patients with alopecia, while patients with acne showed intermediate values. Hirsutism score correlated positively with androstenedione, DHEAS, and salivary testosterone, and correlated negatively with SHBG; salivary testosterone showed the highest correlation coefficient. Total testosterone was not significantly different among patients with hirsutism, alopecia, or acne, and did not significantly correlate with hirsutism score. Hirsutism and oligo-amenorrhea were the most sensitive symptoms of hyperandrogenism, and no androgenic parameter alone allowed us to identify all cases of hyperandrogenism.

Patients of central European origin sought consultation with milder hirsutism scores than patients of southern European origin. There was, however, no difference in the clinical-biological correlation between these groups, arguing against differences in skin sensitivity to androgens.

Polycystic ovary syndrome, defined as hyperandrogenism (hirsutism or elevated androgens) and oligo-amenorrhea, was diagnosed in 63 patients (27.6%), an underestimate compared with other reports that include systematic ovarian ultrasound studies. Neither pelvic ultrasound, used in a limited number of cases, nor the luteinizing hormone/follicle-stimulating hormone ratio helped to distinguish patients with polycystic ovary syndrome from the other diagnostic groups. These included hyperandrogenism (hirsutism or elevated androgens) and eumenorrhea (101 patients; 44.3%); normal androgens (acne or alopecia and eumenorrhea) (51 patients; 22.4%); isolated low SHBG (7 patients; 3.1%); nonclassical congenital adrenal hyperplasia (4 patients; 1.8% of total, 4.9% of patients undergoing cosyntropin stimulation tests); and ovarian tumor (2 patients; 0.9%).

Ethinylestradiol and high-dose cyproterone acetate treatment lowered the hirsutism score to 53.5% of baseline at 1 year, and was also effective in treating acne and alopecia. The clinical benefit is ascribed to the peripheral antiandrogenic effect of cyproterone acetate as well as the hormone-suppressive effect of this combination. Salivary testosterone showed the most marked proportional decrease of all the androgens under treatment. Cost-effectiveness and tolerance of ethinylestradiol and high-dose cyproterone acetate compared well with other antiandrogenic drug therapies for hirsutism. The less potent therapy with spironolactone only, a peripheral antiandrogen without hormone-suppressive effect, was effective in treating isolated alopecia in patients with normal androgens.

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Abbreviations: 17-OHP = 17-hydroxyprogesterone, DHEA = dehydroepiandrosterone, DHEAS = dehydroepiandrosterone sulphate, FSH = follicle-stimulating hormone, LH = luteinizing hormone, NCAH = nonclassical congenital adrenal hyperplasia, SHBG = sex hormone-binding globulin.

INTRODUCTION

Hirsutism, acne, and scalp hair loss in women are a frequent cause of referral to endocrine clinics, since they are often associated with menstrual abnormalities and are usually ascribed to hyperandrogenism.[50] Hyperandrogenism is one of the most frequent endocrine disorders, considered to affect 5%–10% of reproductive-aged women in different regions of the world.[6]

Androgens are necessary for the physiologic development of the pilosebaceous unit. Under the action of androgens, villous hair becomes terminal hair in skin areas where women have considerably less hair than men.[14,38] Studies have demonstrated increased androgen production by the ovaries and the adrenals of women with hirsutism.[46] Androgens are also involved in the development of acne, a disorder of the pilosebaceous unit that occurs physiologically during the rise of androgen levels at the onset of puberty in both sexes.[32,33] Acne can develop in adult women as a manifestation of hyperandrogenism, even in the absence of hirsutism.[47] Androgen excess in women can also cause hair loss of the scalp region leading to alopecia; women with hyperandrogenism may present with alopecia, associated with acne and hirsutism or as an isolated skin symptom.[19] The elevated scalp sensitivity to androgens may be explained by the high concentration in this tissue of type 1 5α-reductase, the enzyme that converts testosterone into the more potent androgen dihydrotestosterone.[61]

Although it is assumed that women with skin symptoms of hyperandrogenism have an increased rate of ovarian and adrenal androgen production,[64] there is no good correlation between symptoms and circulating testosterone levels. One of the reasons for this poor correlation is that most total testosterone circulates bound to albumin and to sex hormone-binding globulin (SHBG), whereas free testosterone, which is supposed to be the major determinant of the hormonal action in peripheral tissues, constitutes only about 2% of total testosterone. Moreover, increased androgen production, as well as increased body

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weight, results in decreased SHBG levels, and therefore moderate increases in testosterone production may not be sufficient to raise serum total testosterone, particularly in overweight women.[13,27] Under these conditions, free testosterone may well be elevated and may show good clinical correlation,[11] but measuring it has limitations in terms of sensitivity, accuracy, and practicability.[48,51] For that reason salivary testosterone has been proposed as a surrogate measurement for circulating free testosterone,[48] and has demonstrated a better correlation to the degree of hirsutism than total testosterone or SHBG.[53] Another reason for poor correlation is that conversion rates of androgen precursors dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and androstenedione, into testosterone and dihydrotestosterone, are not evaluated on clinical grounds although they may present important individual variations.[29]

Different pathogenetic mechanisms may play a role in acne and hirsutism. It has been shown that the dihydrotestosterone metabolite 3α androstanediol and its glucuronide are produced in significantly smaller amounts in women presenting with acne than in women presenting with hirsutism, despite identical total testosterone and dihydrotestosterone levels.[62] This suggests that a different tissue response to androgens determines the different clinical expressions of hyperandrogenism. Furthermore, in the case of acne, beyond androgen stimulation of sebaceous gland growth and sebum production, additional factors contribute to the formation of microcomedones, such as sebum clogging in hair follicles, colonization with *Propionibacterium acnes*, and tissue inflammatory response. This may explain the poor correlation between symptom severity and androgen levels.[2] The causes of hair loss in women can be multiple, and while hyperandrogenism is rare in dermatologic clinics,[56] it is seen frequently in endocrine clinics,[19] reflecting an obvious selection bias.

Individual susceptibility and ethnic factors play a role in the clinical manifestations of hyperandrogenism. Studies in twins indicate that genetic factors markedly influence testosterone secretion and processing to dihydrotestosterone and to 3α-androstanediol,[37] and variants in the gene encoding for type 1 5α-reductase, the predominant isoenzyme responsible for dihydrotestosterone formation in hair follicles, are associated with the degree of hirsutism in affected women.[21] On the other hand, normal and hyperandrogenic women of different ethnic origins show differences in circulating androgens and urinary metabolites, suggesting ethnic differences in androgen secretion and action,[31,65] although some of these observations may have been affected by environmental confounding factors.[55]

Notwithstanding the anticipated difficulty of obtaining a representative picture of the androgenic status of patients and formal proof of the relevance of androgen levels to hirsutism, acne, and alopecia in women seeking medical advice for those symptoms, we considered it important to measure androgens in order to confirm the hormonal imbalance, to establish a diagnosis, and to make the appropriate therapeutic decisions. This has been our practice over the years. In the current study, we retrospectively reviewed the results of the endocrine workup and antiandrogen therapy carried out in women referred to our endocrine clinics for hirsutism, acne, and hair loss and/or alopecia.

PATIENTS AND METHODS

We reviewed the records of 318 consecutive nonmenopausal adult women, who consulted our endocrine clinics for hirsutism, acne, and diffuse nonscarring hair loss and/or alopecia (hereafter termed alopecia), suspected to be due to hyperandrogenism. Most patients were referred by general practitioners. We limited the study to the referral period between January 1991 and April 1997, since in the following years hospital records lacked sufficient family data on new patients for an appropriate assessment of their regional origin. To facilitate the retrospective analysis, we created a database (Microsoft Access 2.0) and entered the clinical and laboratory data.

Clinical Features

Clinical features included the regional distribution of body hair to establish a total hirsutism score according to Ferriman and Gallwey.[18] Only terminal hair was considered, and none of the patients was taking drugs known to induce generalized hypertrichosis, such as glucocorticoids or antiepileptic drugs. The total hirsutism score is the sum of regional hair scores in 11 areas of the skin, including the face, the trunk, and the extremities, graded from 0 to 4. A hormonal hirsutism score in 9 areas, excluding forearms and legs, was also defined on the assumption that hair growth in those locations was not androgen dependent.[23] However in our experience, hair on forearms and legs is also increased in hirsute patients and decreases under antiandrogenic treatment, proving androgen dependence, and therefore we based our analysis on the total score. Patients were considered to be hirsute when they presented a total hirsutism score ≥9 according to Ferriman and Gallwey[18] (this corresponded to a hormonal hirsutism score ≥5 in all the patients).

The presence or absence of acne and alopecia was recorded for all patients, but no attempt was made to quantify acne, and the female or male pattern of alopecia was not systematically recorded.[30] Potential causes of telogen effluvium, such as recent parturition, serious illness, or obvious nutritional deficiency, were ruled out in all patients, but hidden iron deficiency was not systematically sought by measuring serum ferritin and C-reactive protein.

Regarding menstrual status, patients were considered to be eumenorrheic when they reported regular menses at normal intervals, and to be oligo-amenorrheic when they reported amenorrhea (absent menstrual periods for ≥3 months), or oligomenorrhea (irregular menses with fewer than 9 menstrual periods per year).

At the initial evaluation and during follow-up, all patients were systematically examined by 2 observers, one a physician with a background in internal medicine undergoing endocrine training (approximately 50% were women over the period studied), and one of the authors (FG) as supervisor. Consensus on the hirsutism score, on the presence of acne or alopecia, and on the response to therapy was recorded.

Hormone Laboratory Workup

The hormone laboratory workup on the initial evaluation was performed in mid-follicular phase for patients with normal menstrual periods, and during amenorrhea or in the absence of ovulation based on progesterone levels for patients with oligo-amenorrhea. Hyperprolactinemia and ovarian failure were excluded as causes of amenorrhea. Two patients with hirsutism were excluded because of markedly elevated prolactin levels, ascribed to idiopathic hyperprolactinemia on eventual investigations, and none of the patients referred for hirsutism, acne, or alopecia had premature ovarian failure. None of the patients had clinical Cushing syndrome on first examination or on follow-up, but no systematic endocrine testing was performed to unveil occult Cushing syndrome. All patients with classical congenital adrenal hyperplasia who were seen in our adult endocrine clinics over the period studied had been on corticosteroid replacement

TABLE 1. Patient Estimation and Physician Assessment of Hirsutism, Acne, and Alopecia in 279 Patients*

Condition	No. of Patients With Condition	
	By Patient Estimation	By Physician Assessment
Hirsutism	111	80
Acne	11	18
Alopecia	77	67
Hirsutism and alopecia	19	14
Hirsutism and acne	45	61
Alopecia and acne	6	14
Hirsutism, alopecia and acne	10	17
Total no. of patients	279	271

*As explained in text, 51 of 279 patients were eventually excluded from study.

therapy since childhood and were not considered for inclusion in the study; none of the patients included had a history or clinical examination consistent with classical congenital adrenal hyperplasia.

The following hormonal data were evaluated for the purpose of this study: total testosterone, salivary testosterone, androstenedione, DHEAS, SHBG, luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio, and 17-hydroxyprogesterone (17-OHP), before and 60 minutes after a 250 μg intravenous cosyntropin-stimulating test. The following assay methods were used: radioimmunoassay for serum androstenedione (Diagnostic Systems Laboratories, USA), serum DHEAS (Diagnostic Systems Laboratories, USA), serum 17-OHP (CISbio International, France), and plasma total testosterone (Diagnostic Products Corporation, USA); immunoradiometric assay for serum SHBG (Orion Diagnostica, Finland); and microparticle enzyme immunoassay IMX and AXSIM (Abbott, USA) for serum LH and FSH. For salivary testosterone, saliva was collected by free salivation into polystyrene disposable tubes. After sample extraction with n-hexane-diethyl-ether, testosterone was measured using a specific and sensitive radioimmunoassay developed in our laboratory and previously described,[35] with a mean inter-assay coefficient of variation of 8.8% on a salivary pool of salivary testosterone 120 pmol/L. The reference intervals for all the above methods were established in this laboratory. Because of the retrospective nature of the study, not all the data were available for each patient.

Ovarian Ultrasound Study

In a limited number of cases, ovarian ultrasound study was performed via the transabdominal route under the supervision of one of the authors (JYM). Ovarian volume in mL was calculated with a simplified formula for an ellipsoid (1/2 × length [cm] × width [cm] × thickness [cm]); ovarian stroma was quantitatively estimated; and the number and disposition of follicles or cysts were recorded.

Skin Symptoms

In 39 of the initial 318 patients, skin symptoms could not be assessed accurately from the data on record, so those patients were excluded from the study. In the remaining 279 patients, the patient's estimation of the presence of hirsutism, acne, and alopecia was compared with the physician's assessment of these symptoms. Patients tended to overestimate, or physicians to underestimate, the presence of hirsutism, whereas the reverse occurred for acne (Table 1). For subsequent analysis, patients were distributed for skin symptoms according to the physician's assessment.

In 8 patients, no hirsutism, acne, or alopecia was diagnosed, so they were excluded from the study. Forty-three patients were on oral contraception when they consulted for the first time and were also excluded from the study. The remaining 228 patients are described below. None of them had received any sex hormone treatment for at least 3 months before the study, and none was pregnant. In all cases, skin symptoms were considered "patient-important," since previous cosmetic measures had been judged insufficient by patients and referring physicians, and further investigation was sought.

Antiandrogenic Treatment

Patient follow-up during antiandrogenic treatment was recorded. Patients with hirsutism and/or acne were offered

TABLE 2. Clinical and Biological Characteristics of Patients With Isolated Hirsutism, Acne, or Alopecia

	Hirsutism (n = 64)	Acne (n = 16)	Alopecia (n = 53)	Reference Interval
Age (yr)	30.3 ± 12.6† (64)	29.8 ± 7.6* (16)	36.2 ± 11.3 (53)	—
BMI (kg/m^2)	24.7 ± 5.1 (57)	24.0 ± 5.1 (16)	24.8 ± 6.7 (49)	—
T (nmol/L)	1.6 ± 0.7 (60)	1.6 ± 0.7 (16)	1.4 ± 0.7 (40)	0.7–2.8
Ts (pmol/L)	78.1 ± 31.4† (53)	72.8 ± 21.7 (16)	61.4 ± 31.7 (47)	15–100
A (nmol/L)	8.8 ± 4.2† (57)	7.9 ± 3.3 (14)	6.4 ± 3.6 (48)	3.5–10.0
DHEAS (μmol/L)	8.0 ± 3.3† (56)	7.4 ± 3.3 (14)	5.7 ± 2.7 (49)	2.2–9.6
SHBG (nmol/L)	33.4 ± 17.4† (56)	41.9 ± 17.5 (14)	44.0 ± 17.8 (42)	30–80
No. of patients with any abnormal values	48/64‡	9/16	17/53	—
No. of patients with oligo-amenorrhea	26/62§	6/16‡	4/43	—

Mean ± SD (no. of patients). Patients with mixed skin symptoms (n = 95) not shown.

*$P < 0.05$.

†$P < 0.01$ vs. Alopecia, Student t test.

‡$P < 0.05$.

§$P < 0.01$ vs. Alopecia, $\chi 2$.

Abbreviations: BMI, body mass index; T, total testosterone; Ts, salivary testosterone; A, androstenedione; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin.

TABLE 3. Clinical and Biological Characteristics of Patients According to the Presence or Absence of Hirsutism

	Hirsutism (Isolated or With Acne and/or Alopecia) (n = 148)	No Hirsutism (Acne and/or Alopecia) (n = 80)	Reference Interval
Age (yr)	28.4 ± 11.3* (148)	33.6 ± 11.9 (80)	—
BMI (kg/m^2)	24.3 ± 5.1 (148)	24.4 ± 6.4 (79)	—
T (nmol/L)	1.7 ± 0.8 (144)	1.5 ± 0.7 (67)	0.7–2.8
Ts (pmol/L)	81.4 ± 33.6* (132)	63.7 ± 27.4 (73)	15–100
A (nmol/L)	9.0 ± 3.9* (141)	6.7 ± 3.3 (72)	3.5–10.0
DHEAS (μmol/L)	7.9 ± 3.5* (139)	6.2 ± 2.9 (72)	2.2–9.6
SHBG (nmol/L)	34.8 ± 18.3* (131)	44.4 ± 19.1 (66)	30–80
No. of patients with any abnormal values	109/148‡	26/80	—
No. of patients with oligo-amenorrhea	57/146†	13/72	—

Mean ± SD (no. of patients).
*$P < 0.01$, Student t test.
†$P < 0.05$.
‡$P < 0.01$, $\chi 2$, vs. No hirsutism.
Abbreviations: see previous table.

symptomatic antiandrogenic treatment with ethinylestradiol and high-dose cyproterone acetate in a "reverse cycle" sequence,[22] consisting of ethinylestradiol 35 μg/day for 21 consecutive days, cyproterone acetate 100 mg/day, during the first 11 days of ethinylestradiol administration, and a drug-free interval of 7 days, for 28-day cycles. The general practice was to halve the daily dose of cyproterone acetate after 6 cycles, with no attempt to treat acne with retinoic acid derivatives until then. To monitor treatment, patients were asked to report at 3-month intervals, 1 month apart from the previous depilation to verify the effect on hirsutism, acne, and alopecia.

Patients with isolated alopecia and no biological hyperandrogenism were offered treatment with spironolactone, at doses gradually increasing up to 100 mg/day, and the effect was evaluated at 1- to 3-month intervals. On control visits, patients were interviewed for drug tolerance.

All patients who were prescribed antiandrogenic therapy were initially followed for tolerance in our clinics, but approximately 40% of them eventually pursued the treatment under the surveillance of referring physicians, and monitoring for efficacy was not in our records. Patients with ovarian tumor (see below) were offered surgical treatment.

RESULTS

Clinical Hyperandrogenism

To ascertain the androgen dependence of hirsutism, acne, and alopecia, we compared androgen and SHBG levels in patients presenting with 1 of these symptoms alone (Table 2). No significant differences were observed in total testosterone values. Higher levels of salivary testosterone, androstenedione, and DHEAS, and lower levels of SHBG were observed in patients with isolated hirsutism compared to patients with isolated alopecia, whereas patients with isolated acne showed intermediate values not significantly different from the other 2 groups. Differences in SHBG were not explained by differences in body mass index. The proportion of patients with 1 or more abnormal values was higher in the hirsutism group than in the alopecia group, and an intermediate, not significantly different proportion was observed in the acne group. Although moderately older, patients with alopecia had oligo-amenorrhea less often than patients with hirsutism or acne.

From these data we concluded that hirsutism was more closely associated with biological hyperandrogenism than alopecia and acne. Furthermore, we compared the clinical and biological characteristics of patients with hirsutism, isolated or in combination with acne and/or alopecia, with those of patients presenting with acne and/or alopecia but no hirsutism (Table 3). Patients with hirsutism had significantly higher levels of salivary testosterone, androstenedione, and DHEAS; had lower levels of SHBG; and had a higher prevalence of abnormal values and of oligo-amenorrhea than patients without hirsutism.

Biological Hyperandrogenism

We evaluated the different androgens and SHBG as biological markers of clinical hyperandrogenism by comparing them with the quantitative hirsutism score. Salivary testosterone, androstenedione, and DHEAS showed a significant positive correlation, and SHBG showed a significant negative correlation with the hirsutism score, whereas total testosterone did not show a significant correlation (Table 4). Salivary testosterone had the highest correlation coefficient with hirsutism score. Hirsute and nonhirsute patients displaying elevated androgens or low SHBG levels are shown in Table 5. Significantly more patients with hirsutism had elevated androstenedione and DHEAS, lowered SHBG, or any of the above, than patients without hirsutism. A similar trend was observed for salivary testosterone although it did not reach statistical significance, whereas total testosterone was elevated in a small, similar proportion of patients with and without hirsutism.

Although differences in SHBG were apparently unrelated to differences in body mass index (see Table 3), to avoid a potential confounding factor low SHBG values were not considered as defining biological hyperandrogenism, and the presence of elevated androgens was required. Under this definition, "idiopathic hirsutism," which implies normal androgens and eumenorrhea, occurred in 50 of 146 (34%) patients with hirsutism, including 22 of 62 (35.5%) patients with isolated hirsutism. Similarly, "idiopathic acne and/or alopecia" occurred in 49 of 72 (68%) patients, including 6 of 16 (37.5%) with isolated acne and 31 of 43 (72%) with isolated alopecia.

TABLE 4. Linear Correlation With Total Hirsutism Score*

	Hormonal Hirsutism Score†	T (nmol/L)	Ts (pmol/L)	A (nmol/L)	DHEAS (μmol/L)	SHBG (nmol/L)
Correlation coefficient	0.993	0.137	0.309	0.249	0.280	-0.289
No. of pairs	228	211	205	213	211	197
P Value	<0.001	NS	<0.001	<0.001	<0.001	<0.001

*Total hirsutism score is the sum of terminal hair scores in 11 areas of the skin, graded from 0 to 4, according to Ferriman and Gallwey.[18]
†Hormonal hirsutism score is the total hirsutism score minus the scores in forearms and legs.[23] Hirsutism in women was defined as a total hirsutism score ≥9.
Abbreviations: see previous table.

TABLE 5. Patients Presenting With Elevated Androgens or Decreased SHBG

	Hirsutism (n = 148) No. (%)	No Hirsutism (n = 80) No. (%)
Increased T	10/144 (6.9)	4/67 (6.0)
Increased Ts	32/132 (24.2)	8/73 (11.0)
Increased A	47/141 (33.3)*	9/72 (12.5)
Increased DHEAS	38/139 (27.3)*	9/72 (12.5)
Decreased SHBG	66/131 (50.4)*	18/66 (27.3)
Any abnormality	109/148 (73.6)†	26/80 (32.5)

Patients in the "No hirsutism group" presented with acne and/or alopecia.
*$P < 0.05$.
†$P < 0.01$, $\chi 2$, vs. No hirsutism.
Abbreviations: See previous tables.

Regional Origin of Patients

In most cases, the patient's regional origin could be established taking into account the origin of both parents. The 2 main groups were patients originating principally from Switzerland and France (hereafter termed central Europe, n = 142), or from Italy, Spain, and Portugal (hereafter termed southern Europe, n = 67). We compared the clinical and biological characteristics of these 2 main groups. There were no significant differences in the proportion of patients of central European origin compared with southern European origin regarding hirsutism (59.2% vs. 67.2%, respectively) or acne and/or alopecia (40.8% vs. 32.8%, respectively). Although women from central Europe tended to consult with milder hirsutism scores than women from southern Europe, for a similar degree of hirsutism their hormone levels were similar (Table 6).

Diagnosis

For diagnostic purposes, "hyperandrogenism" in premenopausal women was defined as the presence of hirsutism (considered here as synonymous with "clinical hyperandrogenism") and/or elevated androgen levels ("biological hyperandrogenism," defined as "hyperandrogenemia or elevated salivary testosterone"), independent of SHBG levels and of the menstrual status.

Patients were distributed in the following 6 diagnostic groups (Table 7):

1) Hyperandrogenism and eumenorrhea (n = 101).
2) Hyperandrogenism and oligo-amenorrhea (n = 63).
3) Normal androgens (n = 51). This group consisted of patients with acne and/or alopecia but no hirsutism or biological hyperandrogenism (9 oligo-amenorrheic and 42 eumenorrheic patients). To avoid the SHBG confounding factor, patients with low SHBG were not included in this group.
4) Isolated low SHBG (n = 7). This group consisted of patients with acne and/or alopecia and low SHBG but no hirsutism or biological hyperandrogenism (2 oligo-amenorrheic and 5 eumenorrheic patients).
5) Nonclassical (late-onset) congenital adrenal hyperplasia[5] (NCAH) (n = 4). This was diagnosed when 17-OHP was ≥30 nmol/L 60 min after 250 μg intravenous cosyntropin (2 patients originated from southern Europe and 2 from the Middle East; 3 patients presented with hirsutism and acne and 1 with hirsutism; 3 had normal menses and 1 had chronic anovulation).

TABLE 6. Androgens and SHBG Levels in Patients With Hirsutism, According to Regional Origin in Europe

	Degree of Hirsutism			
	Central European Origin		Southern European Origin	
	Moderate (n = 41)	Severe (n = 43)	Moderate (n = 11)	Severe (n = 34)
Total score	11.2 ± 2.2 (41)	19.8 ± 5.3† (43)	10.2 ± 1.6 (11)	21.8 ± 5.2† (34)
Hormonal score	7.6 ± 1.4 (41)	15.2 ± 4.4† (43)	7.2 ± 1.7 (11)	16.8 ± 4.7† (34)
T (nmol/L)	1.5 ± 0.8 (39)	1.7 ± 0.8 (42)	1.6 ± 1.0 (9)	1.8 ± 0.9 (34)
Ts (pmol/L)	66.7 ± 30.0 (39)	87.3 ± 39.3* (35)	62.2 ± 22.0 (10)	89.4 ± 32.5* (33)
A (nmol/L)	7.6 ± 3.6 (40)	9.0 ± 4.6 (42)	7.2 ± 2.5 (8)	9.3 ± 3.6 (34)
DHEAS (μmol/L)	7.1 ± 3.1 (38)	8.4 ± 3.8 (41)	8.0 ± 3.1 (8)	7.8 ± 3.5 (34)
SHBG (nmol/L)	39.2 ± 18.7 (35)	32.6 ± 17.4 (34)	37.9 ± 22.8 (9)	34.5 ± 19.3 (32)

For abbreviations and reference intervals, see previous tables.
Mean ± SD (no. of patients). Moderate degree of hirsutism = total score 9–12; Severe degree of hirsutism = total score 16–32.
*$P < 0.05$.
†$P = 0.01$ vs. Moderate within the same regional origin group, Student t test.

TABLE 7. Hormone Levels According to Diagnosis in Patients Presenting With Hirsutism and/or Acne and/or Alopecia

	Hyperandrogenism and Eumenorrhea (n = 101)	Hyperandrogenism and Oligo-Amenorrhea/ PCOS (n = 63)	Normal Androgens (n = 51)	Isolated Low SHBG (n = 7)	NCAH (n = 4)	Ovarian Tumor (n = 2)
BMI (kg/m^2)	23.9 ± 4.3 (101)	24.9 ± 5.7 (63)	23.6 ± 5.6 (44)	30.0 ± 12.6‡¶ (7)	22.6 ± 2.0 (4)	25.2, 23.5 (2)
T (nmol/L)	1.6 ± 0.8§ (99)	1.8 ± 0.8§ (62)	1.2 ± 0.5 (42)	1.1 ± 0.2 (7)	1.5 ± 0.3 (4)	14.6, 18.2 (2)
Ts (pmol/L)	78.0 ± 32.7*§ (92)	91.1 ± 32.5§ (56)	55.5 ± 18.7 (51)	63.8 ± 12.7 (5)	120.3 ± 12.7 (4)	294, 403 (2)
A (nmol/L)	8.4 ± 3.8†§ (96)	10.2 ± 3.9§ (61)	5.6 ± 1.7 (45)	5.2 ± 1.1 (7)	10.8 ± 1.5 (4)	5.2, 4.5 (2)
DHEAS (μmol/L)	7.9 ± 3.1§ (95)	8.1 ± 3.9§ (59)	5.1 ± 2.4 (46)	5.5 ± 2.5 (6)	9.6 ± 4.8 (4)	2.0, 0.6 (2)
SHBG (nmol/L)	35.6 ± 19.7§ (92)	34.2 ± 17.0§ (53)	59.1 ± 20.2 (47)	20.5 ± 5.4 (7)	47.0, 49.0 (2)	24.0, 19.0 (2)
LH/FSH	1.6 ± 1.0 (71)	1.6 ± 1.0 (48)	1.3 ± 1.0 (27)	1.0 ± 0.3 (3)	-	-
Basal 17-OHP (nmol/L)	3.0 ± 1.9 (43)	3.5 ± 2.2 (30)	1.2 ± 0.2 (3)	3.9 ± 3.6 (3)	6.6 ± 2.9 (4)	9.5, 1.0 (2)
60-min 17-OHP (nmol/L)	7.8 ± 3.2 (43)	7.1 ± 3.1 (30)	5.4 ± 2.9 (3)	7.7 ± 3.2 (3)	82.9 ± 64.2 (4)	12.5, 6.7 (2)

Mean ± SD (no. of patients). Diagnosis groups defined in text.

*$P < 0.05$.

†$P < 0.005$ vs. Hyperandrogenism and oligo-amenorrhea.

‡$P < 0.05$.

§$P < 0.001$ vs. Normal androgens.

¶$P < 0.05$ vs. Hyperandrogenism and eumenorrhea, Student t test.

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; NCAH, nonclassical congenital adrenal hyperplasia; PCOS, polycystic ovary syndrome; 17-OHP, 17-hydroxyprogesterone. Reference interval for basal mid-follicular phase 17-OHP: 1.0–4.0 nmol/L. For other abbreviations and reference intervals, see previous tables.

6) Ovarian tumor (n = 2) (1 patient presented with hirsutism, the other with alopecia, and both with amenorrhea).

Considering all these diagnoses together, patients with hyperandrogenism had a higher prevalence of oligo-amenorrhea (75 of 170; 44.1%) than patients with normal androgens (11 of 58; 19.0%; $p < 0.02$, χ^2).

The 63 patients with hyperandrogenism and oligo-amenorrhea met the criteria for the diagnosis of polycystic ovary syndrome according to the Rotterdam consensus definition,[52] which requires at least 2 of the following 3 characteristics: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovarian morphology on ultrasound study (and exclusion of other etiologies such as congenital adrenal hyperplasia, androgen-secreting tumors, and Cushing syndrome). However, only 46 of the 228 patients in the study had pelvic ultrasound, including the 40 patients shown in Table 8 and, owing to insufficient data, it was not possible to determine whether in the other diagnostic groups there were patients with polycystic ovarian morphology who would also meet the criteria of polycystic ovary syndrome. Patients in the hyperandrogenism and oligo-amenorrhea group had higher salivary testosterone and androstenedione levels than patients in the hyperandrogenism and eumenorrhea group (see Table 7), and patients in these 2 groups showed significantly lower SHBG levels than patients in the normal androgens group (and by definition, higher total testosterone, salivary testosterone, androstenedione, and DHEAS levels). However, there were no significant differences among these 3 groups regarding mean LH/FSH ratio, number of patients with LH/FSH >2 or >1 (not shown), mean basal 17-OHP levels, number of patients with elevated basal 17-OHP (not shown), and ultrasound appearance of the ovaries (see Table 8).

The cosyntropin test was performed in 85 patients. Cosyntropin-stimulated 17-OHP levels >30 nmol/L were observed in the 4 patients with NCAH by definition, whereas in the remaining diagnostic categories the level was <16 nmol/L. However, basal 17-OHP levels above normal were observed in only 3 of the 4 patients in whom NCAH was diagnosed on the basis of the test, whereas similarly elevated basal levels in 17 of the remaining 81 patients were not confirmed as NCAH by the cosyntropin test (these patients are distributed among 4 other diagnoses). The 2 patients with ovarian tumor displayed strikingly high total testosterone values, 5 and 6 times the upper normal limit, indicating tumor hyperandrogenemia,[26] and similarly high salivary testosterone values, 3 and 4 times the upper normal limit.

Antiandrogenic Treatment

Treatment efficacy was assessed based on physicians' evaluation of hirsutism scores, and on patients' estimation of acne and alopecia.

Ethinylestradiol and high-dose cyproterone acetate treatment was administered to 124 of the 148 patients with hirsutism, isolated or with acne and/or alopecia, and to 20 of the 27 patients with acne, isolated or with alopecia. The effect of ethinylestradiol and cyproterone acetate on the hirsutism score after 3 or

TABLE 8. Ovarian Characteristics From Transabdominal Ultrasound Study in Patients With Hyperandrogenism

Characteristic	Hyperandrogenism and Eumenorrhea (n = 15)	Hyperandrogenism and Oligo-Amenorrhea (n = 25)
Volume (mL)	9.4 ± 4.8	10.1 ± 4.1
No. of follicles	3.5 ± 3.5	4.6 ± 4.3
Stroma (%)	78 ± 19	73 ± 22

Mean ± SD.

TABLE 9. Effect of Ethinylestradiol and High-Dose Cyproterone Acetate Treatment on Hirsutism Score

	Months on Treatment				
	Baseline	3	6	9	12
Hirsutism score	18.3 ± 6.3 (74)	13.2 ± 6.6* (74)	11.6 ± 6.4* (54)	10.6 ± 5.5*† (38)	9.8 ± 4.4*‡ (30)

Patients from the diagnostic groups Hyperandrogenism and eumenorrhea, and Hyperandrogenism and oligo-amenorrhea. Baseline hirsutism score range: 10–35.

Mean ± SD (no. of patients).

*$P < 0.01$ vs. baseline.

†$P < 0.05$ and ‡$P < 0.01$ vs. 3 mo, Student t test.

more months was recorded in 74 patients, 42 eumenorrheic and 32 oligo-amenorrheic (Table 9). The hirsutism score decreased progressively in all patients followed in our clinics, with a significant decrease apparent at 3 months, and a value of 53.5% of baseline at 1 year. On-treatment hormone values are compared to pre-treatment values in Table 10. All androgens decreased significantly, the most marked proportional decrease being that of salivary testosterone and the least being that of DHEAS; at the same time, SHBG showed a fourfold increase. No significant correlation was found between baseline androgens or SHBG and the decrease of the hirsutism score during treatment. Acne improved in 40 of the 47 patients, with or without hirsutism, in whom the response was evaluated after 3 or more months, based on patients' estimation (26 hyperandrogenic eumenorrhea, 15 hyperandrogenic oligo-amenorrhea, 5 normal androgens, and 1 NCAH). Furthermore, 16 of 24 patients who were not complaining of acne, and were not considered as having acne on the initial evaluation, reported "improvement of acne" during ethinylestradiol and high-dose cyproterone acetate treatment. Alopecia under this treatment was reported to be improved by 16 of 26 patients, unchanged by 7, and worsened by 3 patients.

Spironolactone was administered to 40 of the 53 patients presenting with isolated alopecia, and follow-up for treatment efficacy was recorded in 22 of them. Twelve patients reported improvement, 8 no change, and 2 reported worsening. Six of the patients who showed no change reported improvement on ethinylestradiol and high-dose cyproterone acetate added to spironolactone.

One of the 2 patients with the diagnosis of ovarian tumor underwent successful surgical ablation of a small Sertoli-Leydig cell tumor. The other patient refused surgical exploration and was lost to follow-up.

Ninety-five of the 124 patients treated with ethinylestradiol and cyproterone acetate showed good tolerance to treatment, while 11 complained of breast tenderness, 10 of headache, and 8 of heavy legs. These symptoms usually occurred at the beginning of treatment and subsided as treatment went on, and no treatment discontinuation was recorded in this retrospective evaluation. Absent or irregular menstrual response to drug withdrawal between cycles was not systematically recorded so no precise figures can be given here, but, in the authors' experience, this occurs in a limited number of patients, relatively overweight, and subsides after cyproterone acetate dose halving. Inter-menstrual bleeding was reported by 1 patient on spironolactone only.

DISCUSSION

Patients referred to our endocrine clinics for cutaneous symptoms of hyperandrogenism were evaluated and treated with antiandrogenic drugs on the basis that these symptoms were of subjective, esthetic, and social importance to them. On the initial evaluation and on the follow-up, a relatively large number of hormones were measured, and this rendered meaningful a study on the apparent androgen dependence of hirsutism, acne, or alopecia, on the yield of individual hormone measurements to disclose hyperandrogenism, and on response to therapy. Although some of the observers changed over the years, consistency of clinical data throughout the study was guaranteed by the supervision of all cases by one of the authors (FG). The main limitation of the study, inherent to its retrospective nature, is that data are missing, particularly on ovarian ultrasound.

Importance of Skin Symptoms to Patients

The discordant opinions between patients and physicians regarding the presence of skin symptoms illustrates the "patient-importance" of these symptoms, and suggests that women living in our community tolerate excess body hair and alopecia less well than acne. The elevated prevalence of oligo-amenorrhea in women with hirsutism (42% in the case of isolated hirsutism) may be an additional reason to seek medical advice, but this does not apply to those with isolated alopecia, since only 9% of those patients presented with menstrual disturbances. Social and cultural factors may also be involved, as suggested by the trend that women of central European origin consulted with milder hirsutism scores than women of southern European origin. In this context, it is interesting to note the absence of discrepancies in the clinical-biological correlation between these 2 groups, arguing against differences in skin sensitivity to androgens. Although these observations on hirsute patients do not necessarily apply to the population at large, they speak against the hypothesis that southern European women are more skin-sensitive to androgens than central European women.

TABLE 10. Hormone Changes During Treatment With Ethinylestradiol and High-Dose Cyproterone Acetate

	Baseline	Treatment	Percentage Change
T (nmol/L) (28)	2.1 ± 0.9	1.4 ± 0.7*	−33.3
Ts pmol/L) (32)	109.8 ± 50.4	50.4 ± 26.5*	−54.1
A (nmol/L) (35)	10.8 ± 3.5	6.1 ± 2.1*	−41.5
DHEAS (μmol/L) (30)	10.0 ± 4.7	7.1 ± 3.1*	−29.0
SHBG (nmol/L) (35)	28.8 ± 14.8	122.9 ± 52.0*	326.7

Mean ± SD (no. of patients).

*$P < 0.01$ vs. Baseline, Student t test.

Abbreviations and reference intervals: see previous tables.

TABLE 11. Prevalence of Hyperandrogenism and Oligo-Amenorrhea in Women With Hirsutism, Acne, and Alopecia, Present and Previous Reports

First Author, Year	Ref.	No. of Patients	T (nmol/L)	FT (pmol/L)	Ts (pmol/L)		
						(% Reported as Abnormal)	
Hirsutism, isolated or associated with acne or alopecia							
Pugeat, 1981	46	21	1.5 ± 0.5	37.5 ± 12.1			
Reingold, 1987	47	13	—	44.0 ± 19.0 (58.8)		—	
Ruutiainen, 1987	53	48	2.3 ± 0.8 (39.6)	29.1 ± 11.8 (89.6)		237.6 ± 66.7 (67.9)	
Futterweit, 1988	19	42	1.8 ± 0.7	—		—	
	—	—				(Elevated androgens, 90.2)	
Osredkar, 1989	45	50	2.6 ± 1.5 (26.0)	—		52.0 ± 18.0 (68.0)	
Toscano, 1993	62	52	1.6 ± 0.3	—		—	
	—	—				(Elevated androgens, 57.7)	
Orio, 2003	44	100	2.6 ± 0.3 (33.0)	—		—	
Azziz, 2004	6	858*	(38.1)	(55.6)		—	
Carmina, 2006	9	950†	(70.2)	(67.4)		—	
Present report	—	148	1.7 ± 0.8 (6.9)	—		81.4 ± 33.6 (24.2)	
Acne without hirsutism							
Reingold, 1987	47	21	—	—	37.1 ± 14.7 (13.8)	—	
Toscano, 1993	62	90	1.6 ± 0.5	—		—	
	—	—				(Elevated androgens, 53.3)	
Present report, isolated acne	—	16	1.6 ± 0.7 (6.3)	—		72.8 ± 21.7 (12.5)	
Isolated alopecia							
Futterweit, 1988	19	67	1.21 ± 0.5	—		—	
	—	—				(Elevated androgens, 31.5)	
Present report	—	53	1.4 ± 0.7 (5.0)	—		61.4 ± 31.7 (12.8)	

First Author, Year	Ref.	DHEAS (µmol/L)	SHBG (mol/L)	Oligo-Amenorrhea (%)
Hirsutism, isolated or associated with acne or alopecia				
Pugeat, 1981	46	6.2 ± 1.6	20 ± 10	(33.3)
Reingold, 1987	47	—	—	—
Ruutiainen, 1987	53	(45.8)	36.3 ± 18.6 (64.6)	—
Futterweit, 1988	19	6.6 ± 3.1	26.0 ± 14.4	—
	—	—	—	(29.3)
Osredkar, 1989	45	5.5 ± 1.7 (28.0)	43.0 ± 16.0 (60.0)	—
Toscano, 1993	62	9.2 ± 2.4	25.0 ± 15.2	(28.8)
	—	—	—	—
Orio, 2003	44	6.7 ± 2.8 (27.0)	27.6 ± 5.1	(100.0)‡
Azziz, 2004	6	(40.6)	—	(88.2)
Carmina, 2006	9	(44.3)	—	(54.8)
Present report	—	7.9 ± 3.5 (27.3)	34.8 ± 18.3 (50.4)	(39.9)
Acne without hirsutism				
Reingold, 1987	47	—	—	—
Toscano, 1993	62	10.5 ± 4.5	29.9 ± 14.8	—
	—	—	—	(17.8)
Present report, isolated acne	—	7.4 ± 3.3 (18.8)	41.9 ± 17.5 (25.0)	(37.5)
Isolated alopecia				
Futterweit, 1988	19	4.33 ± 2.1	30.8 ± 11.2	(23.9)
	—	—	—	—
Present report	—	5.7 ± 2.7 (10.2)	44.0 ± 17.8 (26.2)	(9.3)

Mean ± SD (% increased androgens or decreased SHBG).
*Patients with clinical hyperandrogenism (hirsutism in 75.5%).
†Patients with clinical hyperandrogenism (hirsutism in 95%).
‡Diagnosis of PCOS required for inclusion.
Abbreviations: FT, free testosterone. For other abbreviations, see previous tables.

Clinical Hyperandrogenism

The higher androgen levels and increased incidence of oligo-amenorrhea in patients with hirsutism indicate that this symptom is more androgen dependent than acne and alopecia. Nevertheless, 26% of our patients with isolated alopecia had elevated androgen levels, a figure that is lower than the percentage of women with isolated hirsutism (76.5%), but higher than the figures usually reported in dermatologic series. Indeed, due to the low yield of androgens measured in patients with isolated nonscarring alopecia, dermatologists recommend performing those analyses only if additional symptoms such as menstrual disturbances are present.[43] Had we followed that recommendation, hyperandrogenism would have remained undiagnosed in 6 of the 10 patients presenting with eumenorrhea and isolated alopecia who did have elevated androgens. Unidentified selection bias in patients referred to endocrine clinics might explain the higher incidence of hyperandrogenism in our patients with isolated alopecia. Moreover, if iron deficiency had been systematically sought and such patients excluded from the analysis, the proportion of women with isolated alopecia and hyperandrogenism might have been higher. Due to increasing awareness of the role of decreased iron stores in diffuse alopecia, screening these patients for low ferritin with normal C-reactive protein has become common practice in recent years. In the current study, however, the number of patients screened was too small to yield meaningful information. Patients with isolated acne had intermediate androgen and SHBG levels, not significantly different from patients with isolated hirsutism or alopecia, indicating intermediate androgen dependence of acne.

To our knowledge, this is the first study directly comparing the prevalence of elevated androgens and oligo-amenorrhea in all 3 skin symptoms of hyperandrogenism: hirsutism, acne, and alopecia. In Table 11 we compare our results with the experience of others. Some large studies on polycystic ovary syndrome include data on hirsutism that cannot be analyzed separately.[7,11] When direct comparison for skin symptoms was possible in published work, women with hirsutism disclosed more often hyperandrogenism and oligo-amenorrhea than women with acne[47,62] or alopecia,[19] similar to the findings in the current study. The high prevalence of oligo-amenorrhea in some studies reflects selection bias, since menstrual or ovulatory abnormalities were criteria sufficient for inclusion.[6,44]

Biological Hyperandrogenism

Several studies report androgen levels similar to those observed in the current study (see Table 11), and report that the prevalence of elevated free testosterone or elevated salivary testosterone is also higher than that of elevated total testosterone.

Although salivary testosterone, androstenedione, DHEAS, and SHBG all showed significant correlation with the hirsutism score in the current study, no single parameter was sufficient to identify all cases of hyperandrogenism. Measuring all parameters yielded the highest proportion of patients with biological hyperandrogenism. Low SHBG values may result not only from increased androgen action, but also from adiposity and insulin resistance, and therefore caution should be exerted to interpret such values.[13] However, since our patients with and without hirsutism had identical body mass index, we interpreted the differences in SHBG as differences of androgenicity. The prevalence of idiopathic hirsutism, defined as normal androgens and eumenorrhea, was 34% in the current study, although it rarely exceeds 20% if hidden anovulation is taken into account as a marker of increased androgen action (40% of hirsute eumenorrheic women present hidden anovulation[4]). On the other hand, community-recruited asymptomatic women with the metabolic syndrome and a relatively high calculated free testosterone have oligomenorrhea more often than controls but not more skin symptoms, indicating that abnormal menses may be a particularly sensitive index of androgenicity.[28]

Contrary to the other parameters evaluated, circulating total testosterone did not significantly correlate with the hirsutism score, and showed poor sensitivity to identify hyperandrogenism. The Endocrine Society guideline's recommendation[36] that total testosterone should be measured as the first step to distinguish hyperandrogenism from idiopathic hirsutism does

TABLE 12. Prevalence of Specific Diagnosis in Patients Presenting With Hirsutism, Present and Previous Reports

First Author, Year	Ref.	No. of Patients	Referral (% With Hirsutism)	PCOS (%)	NCAH (%)	ASN (%)	Comments
Carmina, 2006	9	950	Hirsutism (95)	72.1*	4.3	0.1 ov 0.1 adr	—
Moghetti, 2000	40	40	Hirsutism (100)	52.5†	—	—	NCAH deliberately excluded from study§
Ruutiainen, 1987	53	48	Hirsutism (100)	31.3†	—	4.2 adr	—
Spritzer, 2000	57	46	Hirsutism (100)	43.2†	—	—	NCAH deliberately excluded from study§
Present report	—	148	Hirsutism (100)	39.0†	2.7¶	0.7 ov	—
Azziz, 2004	6	873	Hyperandrogenism‡ (75.5)	82.0†	2.1	0.2 ov	0.7% CAH 3.8% HAIRAN
Escobar-Morreale, 2008	16	270	Hyperandrogenism‡ (Unspecified)	63.3†	2.2	—	—

*Ovarian ultrasound study used for diagnosis.
†Ovarian ultrasound study not used for diagnosis.
‡Includes patients with oligo-amenorrhea or anovulation, independent of skin symptomns.
§Unspecified number of patients with NCAH excluded from study.
¶4.7 of all patients in the study undergoing the cosyntropin test.
Abbreviations: ASN, androgen-secreting neoplasm (ov = ovarian, adr = adrenal); CAH, classical congenital adrenal hyperplasia; HAIRAN, hyperandrogenic insulin-resistant acanthosis nigricans; NCAH, nonclassical congenital adrenal hyperplasia; PCOS, polycystic ovary syndrome.

not seem appropriate since, at least in this laboratory, hyperandrogenism would have remained unidentified in the majority of hirsute women. In our opinion there is no rationale to continue systematic screening with total testosterone. The possible concern of missing androgen-secreting tumors, a diagnosis that is traditionally based on the demonstration of strongly elevated total testosterone levels, is not justified since similarly elevated salivary testosterone levels, with similar diagnostic relevance,

TABLE 13. Antiandrogenic Treatment in Patients With Hirsutism, Present and Previous Reports

First Author, Year	Ref.	No. of Patients	Mean Values on Treatment (% of Baseline)						Side Effects* (% of Patients)		Treatment (mo)
			Hirsutism Score	T	FT	Ts	A	DHEAS	Mild	Severe	
Antiandrogens and hormone suppression											
EE and high-dose CA											
Baxendale, 1983	8	15					46				3
Holdaway, 1985	24	34	60.0	60.0	45.0	—	74	100	64.7	11.8	9
Spritzer, 2000	57	9 PCOS	54.5	49.0	—	—	61	—	22.2	0.0	12
		14 IH	56.5	70.0			100		14.3	0.0	12
Present study	—	30	53.5	66.6	—	45.9	56.5	71.0	23.0	0.0	12
EE and low-dose CA											
Luque-Ramírez, 2007	34	15	67.0†		36.0†		57.0†	72.0†			6
Şahin, 2001	54	16	62.8	75.0	48.2			75.5	0.0	0.0	12
Tartagni, 2000	60	10 PCOS	60.0†	—	34.2	—	76.3	75.6	10.0	0.0	6
		15 IH	62.8†		48.6		77.8	48.4	10.0	0.0	6
Finasteride, EE, and low-dose CA											
Şahin, 2001	54	18	51.1	106.6	64.5			80.7	0.0	0.0	12
Tartagni, 2000	60	10 PCOS	54.5†	—	26.7	—	85.7	71.8	20.0	0.0	6
		15 IH	50.0†		41.6		71.4	44.0	20.0	0.0	6
Spironolactone and OC											
Azziz, 2004	6	123	40.9	—	—	—	—	—	36.0	0.0	12
Flutamide and OC											
Castelo-Branco, 2008	10	58	54.5	—	—	—	—	—	41.0	10.0‡	—
Antiandrogens only											
Spironolactone											
Ashraf Ganie, 2004	1	34	67.4	54.3	—	—	—	89.9	26.5	2.9	6
Moghetti, 2000	40	10	59.2†	—	98.8	—	—	107.3	50.0	0.0	6
Spritzer, 2000	57	9 PCOS	76.2	100.0	—	—	100.0	—	40.0	0.0	12
		11 IH	66.7	100.0			100.0		45.5	0.0	12
Flutamide											
Castelo-Branco, 2008	10	25	53.4	—	—	—	—	—	25.0	10.0‡	—
Gambineri, 2004	20	9	72.7†	72.5	73.5	—	107.9	126.9	10.0	0.0	6
Ibáñez, 2002	25	10	55.3	71.3	—	—	85.3	98.3	0.1	0.0	9
Moghetti, 2000	40	10	63.4†	—	82.7	—	—	68.8	—	—	6
Finasteride											
Moghetti, 2000	40	10	70.6†	—	121.1	—	—	87.3	—	—	6
Insulin sensitizers											
Metformin											
Ashraf Ganie, 2004	1	35	80.0	59.5	—	—	—	87.4	22.9	11.4	6
Gambineri, 2004	20	10	92.6†	72.5	73.5	—	107.9	126.9	10.0	0.0	6
Ibáñez, 2002	25	8	68.9	49.3	—	—	86.3	83.3	12.5	0.0	9
Luque-Ramírez, 2007	34	12	88.2†	—	70.8†	—	85.7†	119.0†	—	10.5	6
Metformin and flutamide											
Gambineri, 2004	20	10	66.7†	53.6	38.6	—	67.5	70.3	20.0	0.0	6
Ibáñez, 2002	25	13	55.5	51.0	—	—	56.2	57.5	15.4	0.0	9

*Severe side effects required discontinuation of treatment.

†Figures estimated from graphics.

‡Flutamide only or with OC.

Abbreviations: EE, ethinylestradiol; CA, cyproterone acetate; OC, oral contraceptive; PCOS, polycystic ovary syndrome; IH, idiopathic hirsutism. For other abbreviations, see previous tables.

would probably be found, as illustrated by the 2 patients with ovarian tumor in the current study.

Measuring free testosterone or bioavailable testosterone has been proposed as a sensitive adjunct to measuring total testosterone when necessary for the evaluation of hyperandrogenism,[36] but the existing methods have several drawbacks. Testosterone circulates in plasma unbound (free, approximately 2%), bound to the specific plasma protein SHBG, and weakly bound to nonspecific proteins such as albumin. The SHBG-bound fraction is biologically inactive because of the high binding affinity of SHBG for testosterone. Free testosterone measures the free fraction, bioavailable testosterone includes free plus weakly bound to albumin. The gold standard for true plasma free testosterone measurement is equilibrium dialysis,[51] but this expensive and cumbersome technique has no place in a clinical setting. Therefore, plasma free testosterone is either measured with direct radioimmunoassay or calculated from total testosterone and SHBG values, whereas calculated bioavailable testosterone also includes albumin. In a previous study we showed that direct plasma free testosterone by radioimmunoassay was not representative of true free testosterone under changing conditions of binding proteins.[48] On the other hand, calculated free testosterone is influenced by factors that modify SHBG levels, including body weight, estrogens, and androgens. Therefore, to avoid methodologic pitfalls and confounding factors, we did not use direct radioimmunoassay or calculated free testosterone, but used instead salivary testosterone as an independent sensitive assay of androgenicity. In the current study, salivary testosterone correlated better to the degree of hirsutism than did total testosterone, androstenedione, DHEAS, or SHBG.

We have previously shown[49] that salivary testosterone exceeds calculated free testosterone by a factor of 2 in normal women, and by a factor of 3 in hirsute women with identical total testosterone levels, suggesting that salivary testosterone reflects more than a mere passive flux between blood and saliva but reflects also tissue androgen impregnation and action. In addition, increased testosterone generation from precursor androstenedione in the salivary glands of hirsute women was inferred from studies on the androstenedione-testosterone ratios in plasma and saliva.[59] Our suggestion that saliva was a medium of choice to identify patients with hyperandrogenism is sustained by the present data on the clinical-biological relationship, and we propose that salivary testosterone be considered as a simple and the most efficient parameter to screen for androgenization in women.

Diagnosis

Hyperandrogenic oligo-amenorrheic patients showed significantly higher salivary testosterone and androstenedione than hyperandrogenic eumenorrheic patients, while the other hormonal parameters evaluated were not significantly different, confirming the highest sensitivity of salivary testosterone and androstenedione to characterize androgenicity in women. Patients with hyperandrogenism and oligo-amenorrhea meeting the criteria of polycystic ovary syndrome, according to the Rotterdam[52] and the Androgen Excess Society[3] definitions, represent 27.6% of all patients in the study, and 39.0% of those with hirsutism (see Table 3). This is an underestimate according to the Rotterdam criteria, because ovarian morphology, largely unavailable, was not taken into account for the diagnosis of polycystic ovary syndrome in this study. In Table 12 we compare the prevalence of polycystic ovary syndrome and other specific diagnoses in different series of hyperandrogenism. Studies that do not take into account ovarian morphology for diagnosis,[40,53,57] including the present study, report a lower prevalence of polycystic ovary syndrome than studies that systematically

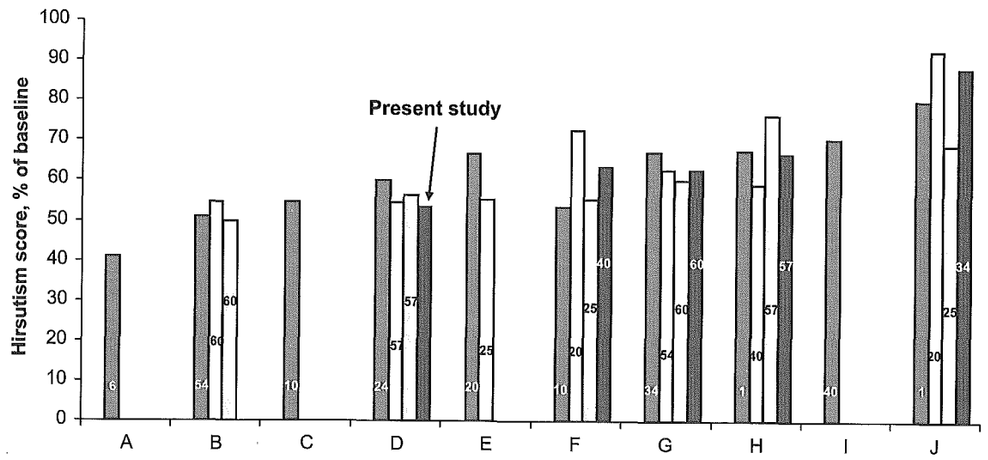

FIGURE 1. Comparison of antiandrogenic treatments for hirsutism in present and previous studies, from Table 13. Each bar represents the mean hirsutism score on treatment in a study, as a percentage of baseline score as specified in Table 13. The reference number is indicated in the bar (references 57 and 60 give separate data on patients with polycystic ovary syndrome and patients with idiopathic hirsutism, so they are included twice per relevant treatment). Treatments are displayed in order of their effectiveness in decreasing the hirsutism score. To estimate the cost-effectiveness of the different therapies, we calculated the approximate yearly cost of each treatment in Switzerland (1 Swiss franc [CHF] ≅ 1 US dollar). Treatments: A = spironolactone and oral contraceptive (544 CHF); B = finasteride plus ethinylestradiol and low-dose cyproterone acetate (343 or 546 CHF, depending on the dose of finasteride); C = flutamide plus oral contraceptive (696 CHF); D = ethinylestradiol and high-dose cyproterone acetate, including the present study (440 CHF); E = flutamide plus metformin (606 or 1132 CHF, depending on the dose of flutamide); F = flutamide only (526 or 1052 CHF depending on the dose); G = ethinylestradiol and low-dose cyproterone acetate (170 CHF); H = spironolactone only (374 CHF); I = finasteride only (376 CHF); and J = metformin only (80 CHF).

perform pelvic ultrasound.[9] Studies on clinical hyperandrogenism, including patients with menstrual or ovulatory abnormalities independent of skin symptoms, also show a high prevalence of polycystic ovary syndrome.[6,16] Although leaving polycystic ovary syndrome undiagnosed implies neglecting an increased risk of metabolic syndrome in these patients, it must be pointed out that the inclusion of the ultrasound criteria in the definition of polycystic ovary syndrome remains controversial, that the presence of polycystic ovarian morphology alone does not predict the development of polycystic ovary syndrome,[41] and that women with oligo-amenorrhea and polycystic ovarian morphology but normal androgens have milder metabolic features of the polycystic ovary syndrome.[15] The relatively low number of ovarian follicles described in the ultrasound study of our patients indicates the poor performance of the transabdominal route, which should not be recommended to evaluate polycystic ovaries whenever transvaginal ultrasonography can be performed. Our data also sustain the lack of usefulness of an elevated LH/FSH ratio as a means to identify women with polycystic ovary syndrome using the present-day gonadotropin assays.[39]

Screening for NCAH can be performed by measuring basal 17-OHP and reserving the cosyntropin test to confirm elevated basal levels in a second step. This 2-step procedure would have missed NCAH diagnosis in 1 of 4 cases in the current series, and would not have confirmed the diagnosis in 17 of 81 other cases. Thus, if the clinical setting allows, systematic testing with cosyntropin should be preferred. The prevalence of NCAH among hyperandrogenic women from central Europe and southern Europe was recently found to be 2.7% and 2.2%, respectively,[16,17] lower than that observed in the current study (4 cases out of 85 patients with hyperandrogenism undergoing cosyntropin test, or 4.7%). These differences may be more apparent than real since, owing to the small numbers, a single positive case would considerably change the proportions. However, selection bias may have played a role: on the one hand, to qualify for hirsutism in the current study, a higher Ferriman and Gallwey score was required (score of 9) than in the 2 above-mentioned studies (scores of 8 and 7, respectively), and therefore the patients we tested had a higher degree of androgenicity. On the other hand, 2 of our 4 patients originated from the Middle East, an area with a higher prevalence of NCAH than Europe. We note that the prevalence of NCAH in clinical hyperandrogenism was 4.3% in a large study in Sicily.[9]

Although systematic screening was not performed to unveil occult Cushing syndrome, none of the patients initially studied and followed presented clinically apparent Cushing syndrome as a cause of skin or reproductive symptoms.

Antiandrogenic Treatment

In Table 13 and Figure 1, we compare different studies on antiandrogenic therapy for hirsutism. The antiandrogenic efficacy of ethinylestradiol and high-dose cyproterone acetate in the present study results from both hormone suppression (gonadotropins and, subsequently, ovarian androgens) and androgen receptor blockade by cyproterone acetate. The role of peripheral receptor blockade is underlined by the decrease in the hirsutism score with flutamide[10,20,25,40] and spironolactone[1,40,57] only, which are receptor antagonists without gonadotropin suppressive effect, and by the lesser effect of ethinylestradiol and low-dose cyproterone acetate (42 mg cyproterone acetate/cycle)[34,54,60] compared with ethinylestradiol and high-dose cyproterone acetate (550-1100 mg cyproterone acetate/cycle) in the present and previous studies,[24,57] despite similar gonadotropin suppression. The evidence that high-dose cyproterone acetate results in no additional benefit on hirsutism compared with low-dose cyproterone acetate is based on only 1 study[63] with relatively small numbers, and it was suggested that the effect of larger doses may become apparent in expanded studies.

The role of dihydrotestosterone on hirsutism is confirmed by the efficacy of finasteride, a 5α-reductase inhibitor, which appeared similar to flutamide or spironolactone as a single drug.[40] The role of hormone suppression is demonstrated by the more intense effect on hirsutism when ethinylestradiol and low-dose cyproterone acetate or other OC combination is added to antiandrogens or finasteride.[6,10,54,60] Other treatments for the skin symptoms of hyperandrogenism have emerged in recent years, based on the observation that most women with polycystic ovary syndrome are insulin resistant, independent of obesity, and based on the assumption that the resulting hyperinsulinemia contributes to hyperandrogenemia by stimulating androgen synthesis in the ovarian theca cells. Therefore, an antiandrogenic effect was expected from lowering insulin levels with insulin sensitizers. In this context, metformin is being widely used to treat women with the polycystic ovary syndrome desiring fertility. However, as shown in Table 13, inconsistent and modest decreases in androgenemia are observed with metformin. These may be sufficient to contribute to the observed improvement in ovulation and fertility,[42] but not to induce any relevant effect on hirsutism, where antiandrogens are clearly more efficient.[12,58] Consequently, the effect of metformin on hirsutism was also modest and inconsistent.[1,20,25,34]

The available antiandrogenic therapies for hirsutism are listed by efficacy in Figure 1, with mention of their respective costs. Metformin, finasteride, and spironolactone only, and ethinylestradiol and low-dose cyproterone acetate, are the least expensive and the least effective. Flutamide only was more effective on hirsutism than the above drugs, but is considerably more expensive. The most prominent effect on hirsutism was reported in a study[6] with spironolactone and oral contraceptive, a treatment with an intermediate cost. With the exception of the above study, the ethinylestradiol and high-dose cyproterone acetate used in the current study resulted in a score reduction similar to the most efficient and more expensive treatments listed. On the other hand, in the current study, ethinylestradiol and cyproterone acetate treatment was less beneficial on alopecia than on hirsutism or acne, corroborating the lesser androgen dependence of alopecia. This lesser dependence was the rationale for treating patients with isolated alopecia and normal androgens with spironolactone only, without hormone suppression. This less potent therapy was almost as effective on isolated alopecia as ethinylestradiol and high-dose cyproterone acetate on alopecia with acne or hirsutism.

Side effects of antiandrogens and hormone suppression were typically estrogenic (headache, depression, loss of libido, nausea, breast tenderness, heavy legs, and hypertension), and were particularly frequent in the older study listed in Table 13 (Holdaway et al[24] from 1985), which used high ethinylestradiol doses (50μg/day). Spironolactone was related to inter-menstrual bleeding; ethinylestradiol and high-dose cyproterone acetate, delayed menstruation; and metformin, gastritis and diarrhea.

Hyperandrogenemia has been reported to be an independent risk factor for the development of the metabolic syndrome in women,[28] and there could be concern that hormone therapy aggravates the metabolic trend of these patients. However, recent studies[34] show that the association of ethinylestradiol and cyproterone acetate (at the low dose of a contraceptive pill) does not result in any worsening of the metabolic profile of women with the polycystic ovary syndrome, while being more efficient than metformin in reducing the hirsutism score. Further studies

are necessary to determine whether higher cyproterone acetate doses have any effect on the metabolic profile of these patients, and to determine whether hirsutism itself constitutes a metabolic risk factor, independent of androgen levels, SHBG levels, and the body mass index.

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