TNF-α and Hyperandrogenism: A Clinical, Biochemical, and Molecular Genetic Study

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To evaluate the role of TNF-α in the pathogenesis of hyperandrogenism, we have evaluated the serum TNF-α levels, as well as several polymorphisms in the promoter region of the TNF-α gene, in a group of 60 hyperandrogenic patients and 27 healthy controls matched for body mass index.

Hyperandrogenic patients presented with mildly increased serum TNF-α levels as compared with controls (mean[median] ± SD: 7.2[7.0] ± 3.3 pg/ml vs. 5.6[4.4] ± 4.0 pg/ml, P < 0.02). Although no differences in body mass index and insulin resistance indexes were observed between patients and controls, when subjects were classified by body weight, serum TNF-α was increased only in lean patients as compared with lean controls, but this difference was not statistically significant when comparing obese patients with obese controls.

The TNF-α gene polymorphisms studied here (−1196C/T, −1125G/C, −1031T/C, −863C/A, −857C/T, −316G/A, −238G/A, and −163G/A) were equally distributed in hyperandrogenic patients and controls. However, carriers of the −308A variant presented with increased basal and leuprolide-stimulated serum androgens and 17-hydroxyprogesterone levels when considering patients and controls as a group. No differences were observed in serum TNF-α levels, body mass index, and insulin resistance indexes, depending on the presence or absence of these variants.

In conclusion, our present results suggest that the TNF-α system might contribute to the pathogenesis of hyperandrogenism, independent of obesity and insulin resistance. However, elucidation of the precise mechanisms underlying the relationship between the TNF-α system and androgen excess is needed before considering TNF-α as a significant contributing factor to the development of hyperandrogenism. (J Clin Endocrinol Metab 86: 3761–3767, 2001)

HYPERANDROGENISM, OR ANDROGEN excess, is possibly the most frequent endocrine disorder in women of reproductive age. Hirsutism is a clinical manifestation of androgen excess and is present in 7.1% of Spanish women in this age range (1). The most common cause of hirsutism is polycystic ovary syndrome (PCOS), as defined by endocrine criteria (2), showing a 6.5% prevalence in Spanish women (1).

The increasing evidence that hyperandrogenism and PCOS have a genetic basis (3, 4) has stimulated research into the genes involved in the pathogenesis of these disorders. Despite significant efforts, the precise genetic mechanisms leading to hyperandrogenism remain unknown, suggesting that this disorder is a complex trait in terms of inheritance.

TNF-α influences the reproductive axis, inducing changes that closely resemble those found in patients with PCOS and hyperandrogenism. TNF-α stimulates proliferation and steroidogenesis in rat theca cells in vitro (5, 6), facilitating the effects of insulin and IGF-I in a dose-dependent and additive fashion (6). Moreover, TNFα may be involved in apoptosis and anovulation in the rat ovary (7). In humans, increased serum TNFα levels have been found in lean PCOS patients (8), but no differences in the follicular TNFα content have been found between normal and polycystic ovaries (9).

TNFα has several metabolic activities. Hyperexpression of TNF-α in adipose and muscle tissue has been proposed to play a key role in the development of insulin resistance in humans (10, 11) by decreasing the tyrosine kinase activity of the insulin receptor (12). This effect is mediated by the insulin-receptor substrate-1 (12), which is associated with an increased expression of TNFα receptors in adipose tissue (13, 14), and can be antagonized by troglitazone in experimental animals (15). Although TNFα acts through autocrine-paracrine mechanisms in adipose and muscle tissue, increased serum levels of TNFα have been found in several conditions associated with insulin resistance, such as type 2 diabetes mellitus (16) and obesity (17).

TNFα is also a significant source of genetic variability. There are several single nucleotide polymorphisms in the regulatory region of the TNFα gene, and some of them have been proposed to play a role in the pathogenesis of insulin resistance, type 2 diabetes mellitus, and obesity (18–20), among other disorders.

Hyperandrogenism, including PCOS and hirsutism with normal ovulation, is present in as many as 50% of women diagnosed with type 2 diabetes mellitus (21); conversely, impaired glucose tolerance is a frequent finding in PCOS and non-PCOS hyperandrogenic patients (22, 23). The association of obesity with hyperandrogenism is also well known (24). Therefore, the genes related to type 2 diabetes mellitus and obesity are considered candidate genes for the inheritance of hyperandrogenism (25).

The present study was undertaken to further delimitate the influence of TNFα on the pathogenesis of female hyperandrogenism, using a clinical, biochemical, and molecular genetic approach.
Materials and Methods

Subjects

Sixty consecutive hyperandrogenic patients, aged 24.3 ± (SD) 6.6 yr with a body mass index (BMI) 29.0 ± (SD) 8.4 kg/m², were studied. Hirsutism, as defined by the presence of excessive body hair distributed in an androgen-dependent pattern, with a modified Ferriman-Gallwey score (26) of 8 or more, was present in 57 of the patients with a score of 14.6 ± (SD) 5.3.

Menstrual cycle intervals were evaluated on recall for every patient. Oligomenorrhea was defined as the presence of 6 or more cycles of more than 36 days in the previous year, and amenorrhea by lack of vaginal bleeding for 3 consecutive months (27). Oligomenorrhea and amenorrhea were considered as indicative of ovulatory dysfunction, but no further effort was made to demonstrate oligo-ovulation. Oligomenorrhea or amenorrhea were present in 21 of the 60 patients, including 18 of the 57 hirsute patients and 3 women who did not have hirsutism (their hirsutism scores were 6, 7, and 7) but presented with oligomenorrhea and increased serum total T levels.

The reference values for the analytical procedures were obtained from a control group of 27 normal menstruating women, aged 30.2 ± (SD) 8.7 yr, matched for BMI (28.9 ± (SD) 17.7 kg/m²), who did not have signs and symptoms of hyperandrogenism or family history of endocrine diseases.

None of the patients had hypertension, features of Cushing’s disease, or drug-induced hirsutism. Hyperprolactinemia and congenital adrenal hyperplasia were ruled out because all the patients presented basal PRL levels <24 µg/liter, together with ACTH-stimulated 17-hydroxyprogesterone (17-OHP) levels <30 nmol/liter and ACTH-stimulated 11-deoxycorticisol levels (S) <26 nmol/liter (which is the mean + 2 sd of the values obtained from the control group).

Data from some patients and controls, regarding different aspects of the pathophysiology of hirsutism, have been previously published (28). The patients and controls had not taken hormonal medications, including contraceptive pills, for the last 6 months. All the patients and controls were Caucasian. The ethics committee of the Hospital Ramón y Cajal approved the study, and informed consent was obtained from each patient and control.

Study protocol, hormone profiles, and diagnostic categories of hyperandrogenism

Studies were performed between days 5 and 10 of the menstrual cycle or during amenorrhea, after excluding pregnancy by proper testing. The patients reported to the Endocrine-Metabolic testing room between 0800 and 0900 h after a 12-h overnight fast. An indwelling iv line was placed in a forearm vein, and after 15–30 min, basal blood samples were obtained for the measurement of T(0), total T, Δ4-androstenedione (Δ4-A), 17-OHP (17-hydroxyandrostenedione-suited (DHA)), LH, FSH, E2, SHBG, glucose, and insulin. Immediately after taking basal samples, a 250-µg iv bolus of 1–24 ACTH (Synacthen, Ciba-Geigy, Basel, Switzerland) was injected and blood samples were obtained at 0 and 60 min for the measurement of S, 17-OHP, and Δ4-A.

Finally, in all the patients and 19 controls, a 10-µg/kg body weight sc dose of leuprolide was injected (29), and samples were obtained at 0900 h on the next day for measurement of LH, FSH, E2, F, T, LH, 17-OHP, and Δ4-A. Serum LH, FSH, and E2 served to confirm the stimulatory effect of leuprolide on gonadotropin and ovarian steroid secretion, and F levels were measured in all the subjects to rule out an interference of the stimulatory effect of the ACTH test performed the day before, on serum 17-OHP and Δ4-A levels. Samples were immediately centrifuged and serum was separated and frozen at −20°C until assayed.

Serum TFF levels were measured within a single assay by solid-phase, two-site chemiluminescent enzyme immunometric technique (Immulett, Diagnostic Products Corp., Los Angeles, CA) with a sensitivity of 1.7 pg/ml and a mean intra-assay coefficient of variation of 3.2%. The technical characteristics of the assays employed for hormone measurements have been reported previously (30, 31). The free testosterone (FT) concentration was determined after separation of SHBG-concentrations, assuming a serum albumin concentration of 43 g/liter, and taking a value of 1 × 10^{-8} liter/mol for the association constant of SHBG for T and a value of 3.6 × 10^{-8} liter/mol for that of albumin for T (32).

Insulin resistance was estimated from fasting glucose and insulin levels using the homeostatic model assessment (HOMAIR) (33) and using the quantitative insulin sensitivity check index (QUICKI) (34).

Patients were classified clinically as follows: Women presenting with hirsutism or hyperandrogenemia and menstrual dysfunction (n = 21) were diagnosed with PCOS according to the criteria derived from the National Institute for Child Health and Human Development 1990s conference (2). Hirsute patients with increased circulating concentrations of T, FT, DHEAS, and/or Δ4-A and regular menstrual cycles were classified to have hyperandrogenemic hirsutism (n = 28). Hirsute patients with normal serum T, FT, DHEAS, and Δ4-A levels and regular menstrual cycles were diagnosed with idiopathic hirsutism (n = 11).

Independently, the presence or absence of functional ovarian hyperandrogenism (FOH), as defined by increased 17-OHP levels after leuprolide administration, was also considered.

The 95th percentile upper limits of normality for basal serum androgens, derived from the control group of 27 healthy women, were 2.15 nmol/liter, 35 pmol/liter, 9.5 gmol/liter, and 15.7 nmol/liter for T, FT, DHEAS, and Δ4-A, respectively. The 95th percentile upper limit of normality for the leuprolide test, derived from 19 women in the control group, was a serum 17-OHP level of 7.6 nmol/liter.

DNA extraction and genotype analysis

Genomic DNA was extracted from leukocytes obtained from whole blood samples, using commercial DNA purification kits (Wizard Genomic DNA purification kit, Promega Corp., Madison, WI, and Nu- cleon BAC C3, Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom). PCR primers were designed to amplify 2 fragments of the promoter of TNFα gene. The first fragment, spanning from −1232 to −732 relative to the TNFα gene transcription start site, comprises a DNA region that contains the −1196C/T, −1125G/C, −1031T/C, −863C/A, and −857C/T polymorphisms described previously (35). Primer sequences were 5’-TCT GCT TGT GTG TGT TCT GCT G-3’ (sense) and 5’-ATG AAG CTC TCA CTT ACA CTC GAG G-3’ (antisense). A second fragment, spanning from −444 to −88 and containing the −316G/A, −388G/A, −238G/A, and −163C/A polymorphisms (36), was also amplified. Primer sequences were 5’-CAA CCG ACT CAG CCT TAT GAA G-3’ (sense) and 5’-TGG AGA AGA CCC TGA GCT CAT C-3’ (antisense).

DNA sequences were amplified in an automated thermocycler (GeneAmp PCR System 2400, PE Applied Biosystems, Foster City, CA), using a 25-µl reaction mix containing 1 U of AmpliTaq polymerase (PE Applied Biosystems). PCR conditions for both fragments included an initial denaturing step at 94°C for 1 min, 30 cycles at 94°C for 1 min, at 60°C for 1 min, and a final extension at 72°C for 10 min.

After PCR amplification, alleles for each polymorphism were identified by direct sequence as follows. PCR products were purified using the Wizard DNA purification kit (QIAGEN GmbH, Hilden, Germany). Sequencing analysis was carried out using the dRhodamine Terminator Cycle DNA Sequencing Ready Reaction Kit (PE Biosystems, Warrington, UK) using the antisense primer for the first fragment of the TNFα gene promoter and the sense primer for the second fragment. The cycle-sequencing products were precipitated and analyzed by an ABI 310 automated sequencer (PE Applied Biosystems) according to the manufacturer’s instructions.

Statistical analysis

Results are expressed as mean ± SD, unless otherwise stated. The Kolmogorov-Smirnov statistic, with a Lil- liefors significance level for testing normality, was applied to continuous variables. Because most of the variables did not follow a normal distribution, nonparametric tests were applied. The Mann-Whitney U - Wilcoxon rank sum W test or the Kruskall-Wallis test were used to compare variables among the groups of subjects, as appropriate. For variables showing significant differences between the groups by the Kruskall-Wallis test, repeated Mann-Whitney U-Wilcoxon rank sum W tests were used to identify the differences between each pair of groups, applying an a priori downward correction to the level of significance to compensate for multiple comparisons (i.e. P < 0.0125 is needed to reach statistically significant differences when comparisons involve four groups) (37). A χ² test was used for discontinuous variables, and Spear-
man’s nonparametric correlation analysis was also used. P < 0.05 was considered statistically significant, with the exception stated above. Power analysis was performed using the G*Power software (38). Because power analysis requires parametric tests instead of the nonparametric tests used here, sample sizes for 0.80 statistical power were calculated for the equivalent parametric tests (i.e., t test or one-way ANOVA), and the results were corrected for the asymptotic relative efficiency of the nonparametric tests relative to their parametric equivalents (39).

**Results**

**Serum TNFα levels and hormone profiles**

When considered as a whole, the group of 60 hyperandrogenic patients presented with mildly increased serum TNFα levels as compared with the healthy controls, although there was a considerable overlap among these groups (Fig. 1).

Patients presented increased serum T, FT, basal and ACTH-stimulated Δ4-A and 17-OHP, and DHEAS concentrations, and decreased SHBG levels, as compared with controls (Table 1). No differences were observed in basal LH, FSH, E2, and insulin resistance as measured by HOMAIR and QUICKI indexes (Table 1). Serum TNFα levels showed weak but statistically significant correlations, with basal and ACTH-stimulated serum Δ4-A concentrations (r = 0.286, P < 0.01; and r = 0.222, P < 0.05, respectively), when considering patients and controls as a whole. No other statistically significant correlations were observed (data not shown).

When considering the clinical diagnostic categories of hyperandrogenism, the differences in serum TNFα levels among controls and patients with PCOS (n = 21), hyperandrogenemic hirsutism (n = 28), and idiopathic hirsutism (n = 11) did not reach statistical significance (controls, 5.6[4.4] ± 4.0 pg/ml; PCOS, 7.3[6.7] ± 3.7 pg/ml; hyperandrogenemic hirsutism 6.8[7.0] ± 2.7 pg/ml; and idiopathic hirsutism, 8.1[7.6] ± 4.1 pg/ml; P = 0.099). However, the statistical power was not enough to rule out such a difference (a total sample size of 209 subjects is needed for 0.80 power).

Fourteen patients (8 from the PCOS group and 6 from the group with hyperandrogenic hirsutism) were diagnosed with FOH (40) according to increased 17-OHP levels after the leuprolide challenge (χ² = 3.936, P < 0.05 by Fisher’s exact test for the association between FOH and PCOS in patients). Serum TNFα levels were increased, compared with controls only in the group of non-FOH patients, and FOH patients showed intermediate values that were not different with respect to those of controls and non-FOH patients (controls, 5.6[4.4] ± 4.0 pg/ml; FOH, 6.1[4.9] ± 3.2 pg/ml, non-FOH, 7.6[7.2] ± 3.3 pg/ml, P = 0.05). No differences were observed in HOMAIR or QUICKI indexes of insulin resistance or in the BMI when considering the diagnostic categories of hyperandrogenism or the presence or absence of FOH (data not shown).

**Single nucleotide polymorphisms in the TNFα gene**

None of the polymorphisms in the promoter of the TNFα gene studied here was associated with patient or control status (Table 2), with the diagnostic category of hyperandrogenism (data not shown) or with the presence or absence of FOH (data not shown).

The frequencies of these polymorphisms in hyperandrogenic patients and healthy controls are summarized in Table 2. However, because of the limited sample size, our study does not have enough statistical power to detect true but small differences in the proportions of affected and non-affected individuals among patients and controls (Table 2). Nevertheless, none of the subjects presented the −163A and the −1196T variants, and only one patient presented the −1125C variant.

We then studied the influence of these polymorphisms on the serum TNFα levels and on the hormonal profiles. When considering hyperandrogenic patients and controls as a whole, carriers of the −308A variant presented with increased basal FT, 17-OHP, Δ4-A, and DHEAS levels and increased serum T, FT, 17-OHP, and Δ4-A levels after leuprolide administration, compared with subjects with wild-type (−308G) alleles (Table 3). No differences were observed in serum TNFα, basal T, ACTH-stimulated 17-OHP, and Δ4-A and basal and leuprolide-stimulated LH, FSH, and E2 levels or in insulin resistance as measured by HOMAIR and QUICKI indexes (Table 3). All these parameters showed no statistically significant differences when studying patients and controls separately.

Also, no differences were observed in serum determinations among individuals with wild-type alleles, compared with subjects hetero- or homozygous for the −238A, −316A, −857T, −863A, and −1031C variants (data not shown).

**Influence of obesity in serum TNFα levels and association with TNFα gene polymorphisms**

As stated above, the BMI was used to match patients and controls, explaining why the study groups showed no statistically significant differences for this variable. For comparisons between lean and obese subjects (obesity was defined by a BMI ≥ 25 kg/m²), patients and controls were studied as a whole. Obese subjects presented higher serum TNFα concentrations (7.4[7.1] ± 4.0 vs. 5.6[5.0] ± 2.6 pg/ml, P < 0.05), higher basal and leuprolide-stimulated FT levels
Obese controls (7.8 ± 7.9) were not different between the 37 obese patients and the 17 hyperandrogenic patients and healthy controls. The leuprolide test was performed in 19 of the 27 controls. No statistically significant associations were observed when considering hetero- and homozygous subjects separately.

These results persisted when studying patients alone, but differences in HOMAIR and QUICKI remained statistically significant (data not shown). The possible involvement of TNFα in the pathogenesis of hyperandrogenism is based on several recent findings. TNFα might be related to increased ovarian steroid secretion, anovulation, and ovarian apoptosis in animals (5–7), features that resemble those of hyperandrogenism in humans.

Also, as reviewed by Hotamisligil (41), virtually all animal and human models of obesity and insulin resistance are associated with TNFα messenger RNA and protein hyper-expression. As stated above, obesity and insulin resistance are frequent findings in hyperandrogenic women (42).
increased only in lean patients, compared with lean controls, with projected increases in serum TNF-α levels were higher in obese patients, compared with controls. Therefore, baseline serum TNF-α levels were increased, although with significant overlap, in patients and controls. Our present results suggest that TNF-α acts mostly if not completely by autocrine or paracrine mechanisms (43), it is also possible that the fluctuations in serum TNF-α levels may have no pathogenic significance, representing only secondary events not actually related to the development of hyperandrogenism.

Nevertheless, in our series the TNF-α system also influenced hyperandrogenism from a genetic perspective. Although none of the polymorphisms studied here was more frequent in hyperandrogenic patients, the −308A variant in the promoter of the TNF-α gene clearly influenced the phenotype, resulting in increased basal and leuprolide-stimulated androgen concentrations in the group of carriers of this variant.

The −308G/A polymorphism in the promoter of the TNF-α gene has been studied extensively. The −308A variant, which is associated with human leukocyte antigens A1, B8, and DR3 alleles (44), is a much more powerful transcription activator, compared with the −308G allele (45, 46), explaining the increased TNF-α production found in these individuals (47).

Based on these previous studies, we hypothesize that carriers of the −308A allele might have increased TNF-α production in several tissues, including the ovary. In such a case, the increased TNF-α levels may stimulate ovarian Δ4-A secretion in theca cells as occurs in experimental animals (5). However, definite proof would require in vitro studies that are far beyond the methodology of our present study.

Because the increased androgen secretion in −308A carriers was observed when considering patients and controls as a whole, the −308A variant in the promoter of the TNF-α

<table>
<thead>
<tr>
<th>Table 3: Serum TNFα levels and basal, ACTH-stimulated, and leuprolide-stimulated serum hormone concentrations in carriers of the −308A variant, compared with subjects with wild-type alleles (−308G)</th>
<th>Variable</th>
<th>−308A (n = 22)</th>
<th>−308G (n = 59)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα (pg/ml)</td>
<td>6.9(6.5) ± 4.2</td>
<td>6.8(6.5) ± 3.5</td>
<td>0.992</td>
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<tr>
<td>Basal T (nmol/liter)</td>
<td>2.3(2.0) ± 0.8</td>
<td>1.9(1.7) ± 0.8</td>
<td>0.051</td>
<td></td>
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<tr>
<td>Leuprolide-stimulated T (nmol/liter)</td>
<td>2.5(2.5) ± 1.1</td>
<td>2.0(1.9) ± 0.8</td>
<td>&lt;0.05b</td>
<td></td>
</tr>
<tr>
<td>Basal FT (pmol/liter)</td>
<td>42(37) ± 21</td>
<td>32(28) ± 19</td>
<td>&lt;0.05b</td>
<td></td>
</tr>
<tr>
<td>Leuprolide-stimulated FT (pmol/liter)</td>
<td>48(46) ± 21</td>
<td>36(33) ± 19</td>
<td>&lt;0.05b</td>
<td></td>
</tr>
<tr>
<td>SHBG (nmol/liter)</td>
<td>37(30) ± 19</td>
<td>47(40) ± 28</td>
<td>0.153</td>
<td></td>
</tr>
<tr>
<td>Basal 17-OHP (nmol/liter)</td>
<td>4.0(3.6) ± 2.6</td>
<td>2.8(2.5) ± 1.8</td>
<td>&lt;0.05b</td>
<td></td>
</tr>
<tr>
<td>ACTH-stimulated 17-OHP (nmol/liter)</td>
<td>9.5(8.4) ± 4.6</td>
<td>8.8(7.4) ± 5.3</td>
<td>0.179</td>
<td></td>
</tr>
<tr>
<td>Leuprolide-stimulated 17-OHP (nmol/liter)</td>
<td>6.8(5.8) ± 3.2</td>
<td>5.4(4.1) ± 4.8</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Basal Δ4-A (nmol/liter)</td>
<td>14.1(13.3) ± 4.5</td>
<td>11.8(11.8) ± 4.6</td>
<td>&lt;0.05b</td>
<td></td>
</tr>
<tr>
<td>ACTH-stimulated Δ4-A (nmol/liter)</td>
<td>15.9(13.8) ± 5.1</td>
<td>15.1(15.2) ± 5.1</td>
<td>0.698</td>
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<tr>
<td>Leuprolide-stimulated Δ4-A (nmol/liter)</td>
<td>15.6(14.4) ± 5.3</td>
<td>12.7(11.9) ± 5.1</td>
<td>&lt;0.05b</td>
<td></td>
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<tr>
<td>DHEAS level (μmol/liter)</td>
<td>8.6(7.7) ± 5.1</td>
<td>6.4(5.9) ± 3.4</td>
<td>&lt;0.05b</td>
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</tr>
<tr>
<td>Basal LH (IU/liter)</td>
<td>5.8(4.6) ± 4.1</td>
<td>5.1(4.2) ± 3.8</td>
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<tr>
<td>Leuprolide-stimulated LH (IU/liter) &amp; n</td>
<td>35.8(30.9) ± 20.6</td>
<td>27.9(26.7) ± 15.3</td>
<td>0.164</td>
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<tr>
<td>Basal FSH (IU/liter)</td>
<td>5.2(5.1) ± 1.5</td>
<td>5.7(5.0) ± 2.6</td>
<td>0.803</td>
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<td>Leuprolide-stimulated FSH (IU/liter) &amp; n</td>
<td>13.5(12.8) ± 5.2</td>
<td>12.6(11.3) ± 5.5</td>
<td>0.325</td>
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<tr>
<td>Basal E2 (pmol/liter)</td>
<td>224(192) ± 139</td>
<td>222(142) ± 174</td>
<td>0.342</td>
<td></td>
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<tr>
<td>Leuprolide-stimulated E2 (pmol/liter) &amp; n</td>
<td>628(525) ± 258</td>
<td>617(532) ± 301</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>HOMAIR</td>
<td>3.1(1.3) ± 1.4</td>
<td>2.9(2.5) ± 1.7</td>
<td>0.284</td>
<td></td>
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<tr>
<td>QUICKI</td>
<td>0.33(0.32) ± 0.02</td>
<td>0.34(0.33) ± 0.05</td>
<td>0.284</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.1(29.8) ± 6.5</td>
<td>28.9(26.3) ± 8.9</td>
<td>0.222</td>
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</tbody>
</table>

* Because the leuprolide test was performed in only 19 controls, these comparisons include 20 carriers of the −308A variant and 53 subjects with −308G alleles.

b Although no statistically significant differences in BMI, HOMAIR, and QUICKI were observed, analysis of covariance was performed to rule out a possible influence of these variables on the differences found in serum hormone levels. Normality was ensured by logarithmic transformation before analysis of covariance. After controlling for BMI, HOMAIR, and QUICKI, leuprolide-stimulated T, basal and leuprolide-stimulated Δ4-A serum concentrations remained significantly increased in carriers of the −308A variant as compared with subjects homozygous for the −308G allele.

Obesity alone modulates serum TNFα levels, which, as has been described by others (16, 17), were higher in obese subjects, compared with lean individuals. Obese subjects also presented a higher degree of insulin resistance, lower SHBG, and increased FT, compared with lean subjects. When subjects were classified by body weight, serum TNFα levels were increased only in lean patients, compared with lean controls, but this difference was not maintained when comparing obese patients with obese controls. Gonzalez et al. (8) recently reported similar results in PCOS patients.

Therefore, our present results confirm that serum TNFα levels increase mainly because of obesity both in controls and hyperandrogenic women but also point to a different mechanism in relation to hyperandrogenism. Interestingly, the higher serum TNFα levels were found in the less severe hyperandrogenic women according to the leuprolide test, and serum TNFα levels tended to be higher in women with idiopathic hirsutism.

Whether these tendencies reflect a pathogenic mechanism cannot be solved by our present results but, because TNFα
gene should be considered only as a contributing factor to the development of hyperandrogenism instead of the main etiologic factor for this condition. Interestingly, serum TNFα levels were similar in −308A carriers and in subjects presenting wild-type alleles, pointing to a local effect of TNFα on androgen secreting tissue rather than to an endocrine effect mediated by circulating TNFα.

On the contrary, neither the −308G/A polymorphism nor any of the other TNFα gene polymorphisms studied here were associated with patient or control status. Milner et al. (48) recently reported similar results, failing to demonstrate an association of the −308G/A polymorphism with PCOS in their series. However, Milner et al. (48) did not observe differences in serum androgens depending on the −308G/A polymorphism, but they measured only T and Δ4-A.

The association of variants in the promoter of the TNFα gene with obesity and insulin resistance is controversial, and there have been negative (36, 49–52) and positive (18–20) reports for these associations over the last years. In our series, none of the polymorphisms in the promoter of the TNFα gene were associated with obesity, and these polymorphisms did not influence serum TNFα levels, BMI, HOMAIR, or QUICKI, which were not different among the carriers of these variants and subjects presenting wild-type alleles. Albeit we have assessed insulin resistance by relatively insensitive methods, our present results cannot demonstrate any association between obesity and insulin resistance with the polymorphisms in the promoter of the TNFα gene studied here.

Sample size and statistical power merits an explanation. Statistical power, as the complement of type II error, reflects the ability of a study to detect a true difference (37). Therefore, low power indicates an elevated probability of concluding erroneously that there are no differences or associations in a study. As a rule, a 0.80 power is considered adequate (37). Our present study lacks enough power to accurately rule out that the proportions of affected and non-affected individuals for the polymorphisms in the TNFα gene were actually different among hyperandrogenic patients and controls.

Yet even in the case that these differences actually exist, they are too small (the higher was a 10-point difference in the percentage of affected individuals among patients and controls) to represent a major mechanism in the pathogenesis of hyperandrogenism. Moreover, as stated above, the study by Milner et al. (48) ruled out an association of the −308A variant with PCOS, and the sample size in this study provided adequate statistical power.

In conclusion, our present results suggest that the TNFα system might play a role in the pathogenesis of hyperandrogenism, independent of obesity and insulin resistance. Not only serum TNFα levels were increased in patients, compared with controls, but also the −308G/A polymorphism in the promoter of the TNFα gene modulated ovarian function, resulting in increased serum androgen levels in carriers of the −308A variant. However, elucidation of the precise mechanisms underlying the relationship between the TNFα system and androgen excess is needed before considering TNFα as a significant contributing factor to the development of hyperandrogenism.

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