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Degree of hyperinsulinemia, independent of androgen levels, is an important determinant of the severity of hirsutism in PCOS

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Abstract

Objective—To determine whether the severity in hyperandrogenemia determines, to a significant degree, the severity of hirsutism in patients with polycystic ovary syndrome (PCOS).

Design—Cross-sectional study.

Setting—Tertiary care academic referral center.

Patient(s)—A total of 749 patients with PCOS.

Intervention(s)—History and physical examination, blood sampling.

Main Outcome Measure(s)—Hirsutism defined by a value of ≥ 6 using the modified Ferriman-Gallwey (mFG) score, age, body mass index (BMI), calculated homeostatic assessment for insulin resistance (HOMA-IR) value, and levels of total (TT) and free (FT) testosterone, dehydroepiandrosterone sulfate (DHEAS) 17-hydroxyprogesterone (17OH-P), and fasting insulin (INS) and glucose (GLC).

Result(s)—Univariate correlations revealed associations between the mFG score and INS, 17OH-P, HOMA-IR, and BMI. Multivariate classification and regression tree analysis indicated that INS had the most significant association with mFG score and that at higher INS levels T played an additional role whereas at lower INS levels 17OH-P had an effect; however, this model accounted for only 8.2% of total variation in mFG score.

Conclusion(s)—Insulin appears to have a direct effect on the severity of hirsutism in PCOS and appears to have a synergistic interaction with TT. Notably, over 90% of the variation in the mFG score was not related to the factors studied and likely reflects intrinsic factors related to pilosebaceous unit function or sensitivity and to other factors not yet assessed.

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Keywords

Polycystic ovary syndrome; testosterone; DHEAS; 17-hydroxyprogesterone; hirsutism; hair

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies, affecting between 6% and 7% of reproductive-age women (1–3). Polycystic ovary syndrome was initially defined by the National Institutes of Child Health and Human Development (NICHD) in 1990 as a syndrome of: 1) hyperandrogenemia and/or clinical evidence of hyperandrogenism; 2) oligo-ovulation; and 3) exclusion of other etiologies (4). The Rotterdam Criteria set forth in 2003 agreed with these diagnostic criteria, adding polycystic-appearing ovaries as an additional defining feature that can be used for diagnosis (5).

Although not always clinically evident, hyperandrogenism is most commonly manifested in several cutaneous symptoms, namely hirsutism, acne, and alopecia (i.e., male pattern baldness). Hirsutism, the development of terminal hair in male pattern distribution, has been documented in up to approximately 70% of patients with PCOS (2). It is well known that androgens have significant effects on hair follicle development and growth, stimulating male pattern hair growth via conversion of vellus to terminal hair types (6). The relationship of androgens to hirsutism is not completely understood, however, and earlier studies have not demonstrated clear correlations between androgen levels and presence of hirsutism.

Although some studies have shown hirsutism to correlate with free testosterone (FT), others have documented that in women with mild hirsutism, only 50% had elevated FT; in women with modestly elevated FT (twofold increase), 33% of women had no hirsutism at all, 27% had moderate, and 40% had mild (7). In another study of over 300 PCOS patients, of those who had evidence of hyperandrogenemia, 63% had clinical hirsutism; of those who were hirsute, 68% were hyperandrogenemic (3).

Because hirsutism is so prevalent among PCOS patients and carries with it an often extensive quality of life impact (8), it is important to attempt to ascertain hormonal correlations and, therefore, possible causations of its severity in order to choose and develop the most efficacious treatment options. As previously described, however, a significant degree of controversy exists regarding the role that androgens play in the development of hirsutism and its severity. Given what we know regarding the pathophysiology of the follicle and hair growth, it is likely that androgens play a role in the development of hirsutism. What is not clear, however, is the extent to which any given androgen contributes to hair growth or, for that matter, if other hormones also play a role in moderating the effect androgens have on the hair follicle. Although several studies have examined this interaction, to date it has not yet been well characterized in large populations. Therefore, we hypothesized that other anabolic factors, including insulin, modulate the effect of androgens on the development of hirsutism. To test this hypothesis, a cross-sectional analysis was performed on data collected in 749 patients with PCOS.

MATERIALS AND METHODS

Subjects

All patients who presented for the evaluation of symptoms potentially related to androgen excess at the University of Alabama at Birmingham between 1987 and 2002 were initially evaluated, and data were collected prospectively in a computerized database (Alpha Four v. 6.0; Alpha Software, Burlington, MA). The study was approved by the Institutional Review Board for Human Use at the University of Alabama at Birmingham. Data from 749 patients diagnosed with PCOS by the 1990 NICHD criteria (4) were analyzed. None of the subjects were premenarchal or postmenopausal, had undergone prior hysterectomy or oophorectomy,

or had been receiving hormonal therapy for at least 3 months before presentation and evaluation.

Clinical evaluation

All patients completed a standardized history form focusing on gynecologic and obstetric history as well as signs and symptoms potentially related to androgen excess and PCOS. The following data were collected during the initial visit: height, weight, body mass index (BMI), age, race, gravidity, parity, degree of ovulatory/menstrual dysfunction, and presence of acne and hirsutism score. Polycystic ovary syndrome was defined by: 1) the presence of hyperandrogenemia or clinical hyperandrogenism; 2) oligo-ovulation; and 3) the exclusion of other disorders. The degree of menstrual dysfunction was determined as previously described (2). Hirsutism was defined by the modified Ferriman-Gallwey (mFG) score, a level of 6 signaling hirsutism, as previously described (9).

Laboratory Analysis

Blood sampling was performed on days 3 through 8 of the menstrual cycle. A 30-mL blood sample was drawn in a plain top tube and stored at -70° until the time of assay. Samples were subsequently analyzed for total testosterone (TT) and FT, sex hormone binding globulin (SHBG) dehydroepiandrosterone sulfate (DHEAS) 17-hydroxyprogesterone (17OH-P), and fasting insulin (INS) and glucose (GLC) levels. Total testosterone was measured by an in-house radioimmunoassay (RIA) based on extraction, column chromatography and double antibody assay using an in-house antibody previously validated (10); SHBG activity was determined by competitive binding and FT was calculated, as previously described (10). The levels of DHEAS and 17OH-P were measured by a direct RIA using commercially available kits (Diagnostic System Laboratories, Webster, TX). In addition, the homeostatic assessment for insulin resistance (HOMA-IR) was calculated from the INS and GLC levels as previously described (11).

Statistical Analysis

Univariate associations between each continuous variable and mFG score were assessed using the nonparametric rank-based Spearman correlations. Simultaneous relation of all predictors and possible confounders was assessed using both multiple linear regression and by classification and regression tree (CART) methods. For all potential predictors except age, BMI, and race, data distribution was better approximated by a gaussian curve on a log scale; therefore, for these variables, both the original value and its base 10 log were considered. Mean mFG score differences across race were assessed by one-way analysis of variance.

RESULTS

The features of the 749 patients included in the study are presented in Table 1. The vast majority of patients were white (82.5%), and none were Hispanic. When analyzed univariately, the difference in mean mFG scores between whites (8.04) and blacks (9.04) was found to be statistically significant ($P=.05$). However, no significant effect of race was detected after controlling for all of the other factors in the multiple regression model.

The univariate correlation coefficients are presented in Table 2. When considered independently, only log INS, log HOMA-IR, log 17OH-P, and BMI were found to be significantly associated with mFG score, using the $P<.05$ criterion. Using univariate analysis, INS was found to have the greatest statistical significance, though the correlation between it and the mFG score was only 0.191.

The multivariate regression equation for mFG score as a function of fasting log insulin, log 17OH-P, and log TT is depicted below; all are significant at $p < 0.03$ or smaller:

$$\begin{aligned} \text{Predicted mFG score} = & 24.024 - [12.37 \log \text{INS} \\ & + 2.075 \log 17\text{OH} - \text{P} \\ & - 9.87 \log \text{TT} \\ & + (7.74 \log \text{INS} \times \log \text{TT})] \end{aligned}$$

Controlling for these three factors, the other seven factors were not statistically significant. This equation implies that although mFG score would be expected to increase as log INS, log 17OH-P, or log TT increases, the rate of increase depends on the interaction among the three. That is, this model also posits a synergistic effect of INS and TT on mFG score such that the log scale effect of INS and TT is more than just the additive effect of each. We note that this model, however, accounts for only 7.5% of the variation in mFG score.

A graph of the multivariate CART analysis is shown in Figure 1. The implication of this analysis is that INS is the most significant variable associated with the severity of the mFG score. When INS level is high, defined in the data as $>53.8 \mu\text{IU/mL}$, CART analysis detects that the next most significant variable is TT, and when it is low ($< 53.8 \mu\text{IU/mL}$) 17OH-P is the next most significant variable. Careful consideration of the tree in this model implies that there is an interaction and synergism between fasting INS, TT, and 17OH-P that affects the severity of hirsutism. However, the CART model accounted for only 8.2% of the variation seen in mFG score in this population.

DISCUSSION

We hypothesized that androgen levels play a significant role in the severity of hirsutism in patients with PCOS. Based on the findings in this study, although TT does have a detectable, albeit modest, effect, it is the level of fasting INS that correlates most with the severity of hirsutism in PCOS, possibly mediated through a mitogenic effect on the pilosebaceous unit (PSU), either directly or via increases in insulin-like growth factor 1 (IGF-1)(12). At higher INS levels, there also exists a synergistic interaction between INS and TT on the development of hirsutism. Alternatively, at lower INS levels, the effect of INS on the development and severity of hirsutism appears to be primarily modulated by 17OH-P, likely reflecting the overall state of ovarian and adrenal steroidogenesis. In general, 17OH-P can be considered to be a nonspecific indicator of steroidogenesis because Δ^4 17,20-lyase activity appears to be minimal in humans, allowing for the exaggerated accumulation of 17OH-P.

The association of fasting INS levels with the severity of hirsutism is not surprising and supports the work of others who have examined this relationship. For example, IGF-1 has been extensively studied regarding its effect on the growth and development of the PSU, and has been observed to be a significant regulator of cell proliferation and differentiation within the follicle (13). Insulin itself may also bind to IGF-1 receptors and has also been found to be a required growth factor for in vitro hair follicular growth (14). Recent studies exploring the association between INS sensitivity and resistance with hirsutism have also found an association between the two. One group of authors examining hirsute and nonhirsute Sicilian women with PCOS noted that those found to be hirsute were more likely to have lower insulin sensitivity, independent of their BMI (15). Another group of investigators, investigating idiopathic hirsutism (IH), observed an association between the presence of IH and insulin resistance (16). In concert with one another, these findings, as well as our own, seem to decidedly indicate a relationship between insulin and body and facial terminal hair

growth and development; whether this is a direct effect of elevated levels of insulin acting at the level of the PSU or a secondary effect mediated through increased androgen production has yet to be elucidated. The present data, though, suggest that the effect of insulin may be more likely direct than mediated through excess androgen production.

Most importantly, however, the present data suggest that greater than 90% of the variation in mFG score is not related to any of the factors studied, and likely reflects intrinsic factors related to PSU function. This highlights the fact that excess hair growth and hirsutism in women with PCOS is multifactorial, complex, and, as yet, incompletely understood. Androgens, which inarguably can be partially implicated in the development of hirsutism in these patients, have not been reliably well correlated with its severity, especially when one considers those patients with IH who, by definition, do not exhibit hyperandrogenemia despite their appearance. Activity levels of 5 α -reductase (which is also affected by androgens, insulin, and IGF-1), the number of genetically determined hair follicles, and the embryonic microenvironment in which the follicles develop have all been implicated as possible contributors to the development of hirsutism. For example, we recently observed both isoforms of 5 α -reductase in the pathogenesis of PCOS, but only the *SRD5A1* haplotypes were associated with degree of hirsutism, suggesting that only this isoform is important in the hair follicle (17). Many other hormonal factors, including growth hormone, glucocorticoids, and thyroid hormone, have also been found to contribute to PSU growth and development (18), although their role in the development and severity of hirsutism has not yet been ascertained.

As demonstrated in the present analysis, development of hirsutism is clearly multifactorial and, given this complexity, may often be present without elevated circulating levels of T. In fact, it would appear that given the poor correlation between hirsutism and androgen levels, hirsutism clearly cannot be used as a surrogate for elevated androgen levels. This suggests that both hirsutism, reflecting clinical hyperandrogenism, and androgen levels, reflecting biochemical hyperandrogenism, should be sought in the diagnosis of PCOS. Polycystic ovary syndrome has many metabolic defects, including hyperinsulinemia, that are likely involved in the development of hirsutism, allowing hirsutism to retain its value as a diagnostic criterion for PCOS, even without significant correlation to androgen levels. Additionally, considering the health implications and morbidity implied by a diagnosis of PCOS, we have shown previously that individuals with hirsutism and ovulatory dysfunction alone have similar degrees of insulin resistance as individuals with hyperandrogenemia and ovulatory dysfunction alone (3). Therefore, hirsutism, even in the absence of proven hyperandrogenemia, is a valuable and necessary diagnostic criterion for PCOS and should remain so.

In conclusion, the present data suggest that the development and progression of hirsutism in patients with PCOS is associated with the circulating levels of INS and, to a lesser degree, TT and 17OH-P. The relationship of TT and INS appears to be synergistic although only at the higher INS levels. Alternatively, at lower INS levels 17OH-P demonstrates a more significant association. Most notably, over 90% of the variation in the mFG score was not related to the factors studied, likely reflecting intrinsic factors related to PSU function or sensitivity and other circulating factors not yet assessed.

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FIGURE 1.

Graph depicting the simultaneous relation of all predictors and possible confounders assessed using the multivariate classification and regression tree (CART) analysis.

TABLE 1

Univariate associations of race with mFG score

Race	n (%)	mFG score, mean $\pm$ SD
White non-Hispanic	617 (82.5%)	8.04 $\pm$ 4.88
Black non-Hispanic	114 (15.2%)	9.04 $\pm$ 5.05
Other	17 (2.3%)	10.00 $\pm$ 4.87

Note: One individual did not have race recorded and was therefore not analyzed. mFG = modified Ferriman-Gallwey.

TABLE 2

Univariate correlation coefficients for each independent variable with mFG score

Variable	Mean	SD	n	Spearman correlation	Spearman <i>P</i> value
Total testosterone, ng/dL	91.2	61.2	748	−0.020	.5906
Free testosterone, ng/dL	0.9	0.6	746	−0.005	.8907
DHEAS, ng/mL	2331.5	1290.3	748	−0.039	.2849
Fasting insulin, mIU/mL	26.9	25.3	383	0.175	.0006
Fasting glucose, mg/dL	91.1	22.3	399	−0.039	.4372
HOMA-IR	114.7	143.8	380	0.152	.003
SHBG, nmol/L	180.3	69.7	746	−0.043	.246
17-Hydroxyprogesterone, ng/mL	1.4	1.1	736	0.093	.0118
Age, yrs	27.5	7.4	749	0.017	.6428
Body mass index, kg/m ²	33.6	9.3	749	0.121	.0009

Note: HOMA-IR = homeostatic model assessment for insulin resistance; mFG = modified Ferriman-Gallwey.