

Insulin Resistance in the Sisters of Women with Polycystic Ovary Syndrome: Association with Hyperandrogenemia Rather Than Menstrual Irregularity

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This study was performed to determine whether the sisters of women with polycystic ovary syndrome (PCOS) have evidence for insulin resistance. Three hundred and thirty-six women with PCOS, 307 sisters of these probands, and 47 control women were studied. The sisters were grouped by phenotypes: PCOS [hyperandrogenemia (HA) with chronic oligo- or amenorrhea, $n = 39$], HA with regular menses ($n = 36$), unaffected (UA; $n = 122$), and unknown ($n = 110$). The analyses were adjusted for age and body mass index. PCOS and HA sisters of women with PCOS had similar and significantly elevated fasting insulin levels ($P = 0.001$) as well as similar and significantly decreased fasting glucose/insulin ratios ($P < 0.001$) suggestive of insulin resistance compared with UA sisters and control women. Markers of insulin resistance were associated with hyperandrogenemia and not with menstrual

irregularity. PCOS sisters also had decreased levels of SHBG ($P = 0.02$) suggestive of higher ambient insulin levels. PCOS sisters had increased levels of proinsulin ($P = 0.04$) compared with control women, which suggested pancreatic β -cell dysfunction in this group of sisters. The magnitude of obesity also differed significantly among the groups of sisters. The PCOS sisters were significantly more obese than all the other groups, and the HA sisters were more obese than the UA sisters. We conclude that markers of insulin resistance are associated with hyperandrogenemia rather than menstrual irregularity in the sisters of women with PCOS. Menstrual irregularity may be related to the magnitude of insulin sensitivity or insulin secretion or to other factors associated with obesity. (*J Clin Endocrinol Metab* 87: 2128–2133, 2002)

POLYCYSTIC OVARY syndrome (PCOS) is a disorder of unknown etiology characterized by chronic anovulation and hyperandrogenism (1). It remains a diagnosis of exclusion of specific disorders of the ovaries, adrenals, and pituitary gland. PCOS is frequently associated with insulin resistance (2). There is now convincing evidence from studies in which insulin levels have been lowered by several modalities that hyperinsulinemia contributes to the hyperandrogenemia (HA) and anovulation (3–7).

Familial clustering of PCOS consistent with a genetic susceptibility to the disorder has been well documented (8–12). Recently, we have shown that there are two reproductive phenotypes in the sisters of women with PCOS: 1) chronic anovulation and HA consistent with typical PCOS, and 2) HA with regular menses and apparently normal fertility (13). Previous studies have suggested that women with HA and regular cycles were not insulin resistant (14–16). Family studies have suggested that HA in PCOS sisters is common and may be a monogenic trait (13, 17). A twin study of PCOS women showed high correlation within twin pairs for both circulating androgen and fasting insulin levels (18). Defects in insulin action persist in cultured skin fibroblasts, and increased androgen production persists in passaged thecal

cells that have been removed from the *in vivo* environment for generations consistent with genetic traits (19, 20).

It was our hypothesis that insulin resistance produced anovulation in sisters with the PCOS phenotype. Recent evidence to support this hypothesis comes from mice with insulin resistance secondary to targeted disruption of insulin receptor substrate-2 (21) or the brain insulin receptor that develop obesity, insulin resistance, and anovulation (22). According to our hypothesis, only sisters with PCOS should be insulin resistant; those with HA alone should have normal insulin sensitivity.

Subjects and Methods

Subjects

Women with PCOS or women with 6 or fewer menses per yr were recruited by advertisements and from the practices of the co-authors. This study was approved by the institutional review boards of Pennsylvania State University, University of Pennsylvania Medical Center, and Brigham and Women's Hospital, and all women gave written informed consent. To qualify as a PCOS index case, a woman had either a total T level above 58 ng/dl or unbound T (uT) greater than 15 ng/dl and 6 or fewer menses per yr (13). Secondary causes of HA and anovulation, such as nonclassical 21-hydroxylase deficiency, hyperprolactinemia, or androgen-secreting tumors, were excluded by appropriate tests. Only non-Hispanic white PCOS probands were studied to control for ethnic and racial differences in insulin sensitivity (23, 24). Three hundred and thirty-six non-Hispanic white women with PCOS fulfilled these criteria. There were 81 women with

Abbreviations: ANCOVA, Analysis of covariance; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; HA, hyperandrogenemia; PCOS, polycystic ovary syndrome; UA, unaffected; uT, unbound T.

PCOS who had no sisters and 36 PCOS women who had sisters who were not willing to be studied. This left 219 PCOS probands who had at least 1 sister studied. There were 370 sisters among these probands, and 307 were willing to be studied. Reproductive hormone data for 147 sisters has been previously reported (13). The 307 sisters were phenotyped as follows: PCOS, as defined for probands; HA, elevated T and/or uT with menses every 27–35 d; unaffected (UA), normal androgens and menses every 27–35 d; and phenotype unknown. Sisters were assigned phenotype unknown because they did not fulfill the phenotyping criteria or because of confounding medications or conditions (Table 1). Confounding medications that affect the metabolic criteria examined include oral contraceptive agents (25), hypertensive medications (26), and insulin-sensitizing medications (6, 27). Other sisters were assigned unknown status because they had confounding reproductive conditions, including premenarche, pregnancy, lactation, hysterectomy, or menopause.

To control for the impact of multiple sisters with the same phenotype in a family, the sisters' data were averaged to yield 1 value/phenotype/family. Of the 35 families with sisters with PCOS, 31 families had 1 PCOS sister each and 4 families had 2 PCOS phenotype sisters/family, whose data were averaged. Of the 34 families with HA sisters, 32 had 1 HA sister each, and 2 families had 2 HA phenotype sisters/family whose data were averaged. Of the 99 families with UA sisters, 77 families had 1 UA sister each, 21 families had 2 UA phenotype sisters/family whose data were averaged, and 1 family had 3 UA phenotype sisters whose data were averaged.

Sisters who participated in the study were either evaluated on-site at one of the three study centers (n = 122 of those analyzed) or off-site in a local hospital or clinical laboratory with phlebotomy and laboratory processing capability (n = 185 of those analyzed). All had morning blood samples obtained after an overnight fast. Those who were seen on-site also had blood pressure and waist/hip girth ratios determined. Height and weight in the subjects studied off-site were self-reported. We have performed a validation study of self-reported height and weight in 166 individuals and determined that self-reported and on-site measurements were very similar. There were no differences between groups by unpaired *t* test. There was a clinically insignificant paired difference in height and weight that did not result in a significant difference in body mass index (BMI; data not shown). Plasma and serum were shipped on ice packs and stored at –20 C or below before analysis in a central laboratory. Testing was performed to document that there was no deterioration of the samples with shipping.

Forty-seven reproductively normal, control women with 27- to 35-d menstrual cycles and comparable age, weight, and ethnicity were studied. They were recruited to be comparable with PCOS probands (and not to match sisters or specific phenotypes of PCOS sisters). These women

did not have a history of hypertension or diabetes mellitus, personally or in a first degree relative. All control women were examined by one of the study investigators and had no hirsutism (Ferriman-Gallwey score, <8) (28). Control women had normal glucose tolerance based on a 75-g, 2-h, oral glucose tolerance test applying WHO criteria (29).

Assays

All assays were determined from a single fasting sample. Plasma glucose levels were determined by the glucose oxidase technique (5). Insulin, gonadotropin, T, uT, and dehydroepiandrosterone sulfate (DHEAS) levels were determined as previously reported (13). Human proinsulin was determined with a double antibody method using reagents obtained from Linco Research, Inc. (St. Charles, MO). The proinsulin antibody used shows less than 0.1% cross-reactivity with human insulin and human C peptide. SHBG was determined with a two-site immunoradiometric assay using reagents obtained from Diagnostics Systems Laboratories, Inc. (Webster, TX). All hormone and SHBG assays had interassay coefficients of variation below 10%.

Data analysis

Because there were significant differences in age and BMI after the sisters were grouped by phenotype, data were analyzed by using an analysis of covariance (ANCOVA) adjusted for age and BMI for each of the continuous response variables. All of the data were log-transformed before the ANCOVA, except age and BMI. For each ANCOVA, a least significant difference *post-hoc* test of differences between each subject pair of groups was performed. The statistical programs used in analysis were Data Desk 6.1 (Data Description, Ithaca, NY) and S-plus (MathSoft, Seattle, WA). Data are presented as the untransformed mean \pm sd. The sample sizes shown reflect the effect of averaging multiple sisters within the same phenotype per family. Averaging sisters of the same phenotype within the same family was performed to avoid skewing the data from families with multiple affected individuals. Linear correlations for metabolic parameters were performed between PCOS probands and sisters. We also examined bimodality for all of the parameters, which suggests the presence of two populations. A bimodal distribution is suggestive of a potential genetically determined trait (30). Using custom software written in S-plus, we ran Silverman's critical bandwidth bootstrap test (31, 32).

Results

Clinical and reproductive features (Tables 1 and 2)

The 307 sisters were phenotyped as follows (Table 1): PCOS (n = 39), HA (n = 36), UA (n = 122), and phenotype unknown (n = 110). HA sisters were significantly younger than UA sisters and control women (Table 2). The PCOS sisters were significantly more obese than HA and UA sisters ($P < 0.001$). Control women had similar BMIs as the PCOS sisters, as they were selected to be similar in BMI to PCOS probands, but UA sisters were also significantly less obese than the control women ($P = 0.001$). Systolic blood pressures tended to be higher in PCOS sisters ($P = 0.08$). PCOS and HA sisters had similar and significantly elevated levels of T, uT, DHEAS, and LH levels compared with UA sisters and with control women, consistent with our previous report (13).

Metabolic features (Table 3)

Fasting glucose levels were significantly higher in PCOS sisters than in HA sisters and control women ($P < 0.001$). Fasting glucose levels were also significantly higher in UA sisters than in control women ($P < 0.001$). Fasting insulin levels were significantly higher in PCOS sisters than in UA sisters and control women ($P < 0.001$). Fasting insulin levels were significantly higher in HA sisters than in control

TABLE 1. Prevalence of phenotypes among sisters

Phenotype	No. of sisters affected	% of sister population
PCOS	39	13%
HA	36	12%
UA	122	40%
Unknown (reasons)	110	35%
Normal T and uT levels and ≤ 6 menses/yr	22	
Normal T and uT levels and menses every 35–60 d	8	
DHEAS elevated with menses 27–35 d	13	
DHEAS elevated with ≤ 6 menses/yr	2	
T elevated with menses every 35–60 d	6	
PRL > 25 ng/ml	1	
Menopausal	2	
Premenarchal	3	
Breastfeeding during study	1	
Pregnant within 1 month of study	1	
Hysterectomy	6	
Oral contraceptive use	39	
Hypertensive medication use	5	
Insulin sensitizer use	1	

TABLE 2. Clinical features, androgen, and gonadotropin levels in sisters of PCOS probands

	PCOS (n = 35)	HA (n = 34)	UA (n = 99)	Control (n = 47)	P-ANCOVA
Age (yr)	29 ± 6	25 ± 6 ^a	31 ± 9	31 ± 7	0.0012
BMI (kg/m ²)	32.4 ± 6.9 ^b	27.9 ± 5.4	26.9 ± 6.7 ^c	31.0 ± 9.2	<0.001
Systolic (mm Hg)	120 ± 15	116 ± 12	114 ± 14	113 ± 10	0.08
Diastolic (mm Hg)	73 ± 9	71 ± 8	70 ± 10	71 ± 9	0.60
T (ng/dl)	83 ± 40 ^a	74 ± 19 ^a	30 ± 11	31 ± 11	<0.001
uT (ng/dl)	26 ± 13 ^a	22 ± 10 ^a	6 ± 3	7 ± 4	<0.001
DHEAS (ng/ml)	2400 ± 1390 ^a	2800 ± 1420 ^a	1460 ± 589	1530 ± 685	<0.001
LH (mIU/ml)	18 ± 23 ^d	22 ± 11 ^a	11 ± 14 ^c	5 ± 4	<0.001
FSH (mIU/ml)	10 ± 3	9 ± 4	11 ± 5	10 ± 3	0.71

Unadjusted mean ± SD.

^a Significant vs. UA and control.^b Significant vs. HA and UA.^c Significant vs. control.^d Significant vs. HA, UA, and control. Conversion factors to SI units T and uT × 3.467 (nmol/liter), DHEAS × 0.002714 (μmol/liter).**TABLE 3.** Metabolic parameters in sisters

	PCOS (n = 35)	HA (n = 34)	UA (n = 99)	Control (n = 47)	P-ANCOVA
Glucose (mg/dl)	89 ± 9 ^a	83 ± 8	87 ± 8 ^a	84 ± 8	<0.001
Insulin (μU/ml)	24 ± 11 ^b	19 ± 12 ^b	14 ± 7 ^c	14 ± 8	<0.001
Glucose; insulin ratio	4.6 ± 2.3 ^b	5.6 ± 2.6 ^b	7.3 ± 2.6 ^c	7.5 ± 3.5	<0.001
Proinsulin (pmol/liter)	17 ± 11 ^d	13 ± 11	10 ± 5	13 ± 10	0.04
SHBG (nmol/liter)	89 ± 79 ^d	123 ± 82	138 ± 82	92 ± 42	0.02

Unadjusted mean ± SD.

^a Significant vs. HA and control.^b Significant vs. UA and control.^c Significant vs. control.^d Significant vs. UA. Conversion to SI units, glucose × 0.0551 (mmol/liter), insulin × 7.175 (pmol/liter).

women ($P < 0.001$), whereas these levels were lower in UA sisters compared with control women ($P < 0.01$) after adjustment for age and BMI. Proinsulin levels were significantly increased in PCOS sisters compared with UA sisters ($P < 0.01$). Glucose/insulin ratios were similarly and significantly decreased in PCOS and HA sisters compared with UA sisters and control women ($P < 0.001$).

Correlations between PCOS probands and their sisters

Linear correlations were performed between PCOS proband and their sisters (using mean values from multiple sisters of the same phenotype within a family) for glucose, insulin, and proinsulin values. There was a significant correlation observed in fasting glucose ($r = 0.251$; $P < 0.01$), but the correlations coefficients for insulin levels ($r = 0.158$; $P = 0.06$) and proinsulin levels ($r = 0.237$; $P = 0.06$) approached, but did not achieve, statistical significance.

Comparisons of PCOS probands (Table 4)

To determine whether PCOS probands with sisters differed from those without sisters, we compared clinical, biochemical, and metabolic parameters in PCOS probands without sisters ($n = 81$) to those who had sisters ($n = 219$) and to those who had sisters who were not willing to be studied ($n = 36$). There were no differences for the variables studied. In addition, because our study population was recruited from across the nation, we examined whether there was a difference between the sisters who were studied in one of the three institutions and those who had their blood drawn and

processed in local facilities and shipped to us for assay. Again, there were no significant differences observed in any of the metabolic parameters across the sister populations (data not shown).

Modality analysis

Modality analysis was performed to determine whether the distributions of the various characteristics were unimodal or bimodal in the sisters and control women. When individual sisters of phenotype PCOS, HA, or UA were examined, insulin ($P = 0.01$) levels had statistically significant bimodality; there was no significant bimodal relationship in the control women ($P = 0.21$). Statistically significant bimodality was noted for both sisters and control women for fasting glucose levels (sisters, $P = 0.03$; control women, $P = 0.02$). The distribution of proinsulin levels approached statistical significance for bimodality in the sisters ($P = 0.07$), but not in the control women ($P = 0.84$). These results should be interpreted with caution, however, because many of the significant results were due to only a few points in the right tail of the distribution. We also noted significant modality for T ($P = 0.002$) and uT ($P = 0.02$), as reported in our prior study (13). There was no significant bimodality noted for T values in control women ($P > 0.50$).

Discussion

Sisters who fulfill criteria for the diagnosis of PCOS and those with the second reproductive phenotype of elevated androgen levels with regular menses (HA) had elevated in-

TABLE 4. Comparison of PCOS probands

	PCOS probands with no sisters (n = 81)	PCOS probands with sisters studied (n = 219)	PCOS probands with sisters not studied (n = 36)	P-ANCOVA
T (ng/dl)	79 ± 41	78 ± 32	85 ± 35	0.37
uT (ng/dl)	28 ± 13	28 ± 19	27 ± 16	0.72
Insulin (μU/ml)	26 ± 18	26 ± 16	25 ± 16	0.60
Glucose: insulin ratio	5.1 ± 3.4	4.6 ± 2.6	4.7 ± 2.1	0.59

Unadjusted mean ± SD. Conversion factors to SI units T and uT × 4.467 (nmol/liter), insulin × 7.175 (pmol/liter).

sulin levels and decreased glucose/insulin ratios suggestive of insulin resistance (33). Sisters of women with PCOS who were assigned unaffected status on the basis of androgen levels and menstrual cycles had normal parameters of insulin action. These observations suggest that HA and the metabolic abnormalities are associated in PCOS families. Insulin resistance *per se* cannot account for the differences in menstrual cyclicity between PCOS and HA sisters. However, HA *per se* identifies sisters at risk for insulin resistance.

This study demonstrates that insulin resistance clusters in PCOS families, consistent with a genetic susceptibility to this trait. Previous studies have shown that defects in insulin action persist in cultured skin fibroblasts from PCOS women (19), suggesting that these abnormalities may have a genetic basis. There is evidence of heritability of insulin levels in monozygotic twins sharing polycystic ovary morphology (18). Further, male and female first degree relatives of PCOS probands have been reported with elevated insulin levels (34). The significant correlations between PCOS probands and their sisters for glucose levels is consistent with a heritable component to this trait (35). One of the possible interpretations of the significant bimodal distribution of insulin levels among the sisters is that this may be a monogenic trait controlled by two alleles at an autosomal locus (13, 30). Further study is needed to support this view. Elevated proinsulin levels in PCOS sisters suggest that they also had pancreatic β-cell dysfunction (36), and this may place them at greater risk to develop diabetes mellitus (37). Heritability of β-cell dysfunction has been identified in first degree relatives of PCOS probands (38) and has been postulated by some to be the primary defect in families with type 2 diabetes (39).

Elevated insulin levels tracked with HA rather than with menstrual irregularity. Although we have not confirmed that HA sisters are ovulatory, they are significantly more fecund than PCOS sisters, consistent with normal ovulation (13). These observations argue against our hypothesis that insulin resistance *per se* produces anovulation in PCOS. It may be that the severity of insulin resistance determines anovulation. PCOS sisters were the most obese, and obesity is associated with worsening insulin resistance (40). Glucose/insulin ratios, fasting parameters of insulin sensitivity that correlate well with dynamic measures of insulin action in PCOS (33, 41), were lowest in the PCOS sister group, followed by the HA group, but this did not achieve statistical significance. PCOS sisters had the lowest SHBG levels, and as insulin is a potent regulator of circulating SHBG levels (42), this suggests that the PCOS sisters had more profound

hyperinsulinemia. In contrast, SHBG levels in HA and unaffected sisters were comparable. Pharmacological interventions to improve insulin sensitivity can restore ovulation without normalizing insulin action (7, 27, 43), so it may be the magnitude, rather than the presence, of insulin resistance that determines ovulatory status.

The mechanisms by which defects in insulin action modulate ovulation are of considerable interest. There is now a large body of evidence indicating that hyperinsulinemia can affect gonadal function through a variety of mechanisms, including direct actions on the hypothalamic-pituitary axis (2, 44). PCOS and HA groups of sisters had similarly elevated levels of LH compared with both UA sisters and controls, suggesting inappropriate gonadotropin secretion in both HA groups (13, 45). This observation suggests that factors in addition to inappropriate gonadotropin secretion are required to produce anovulation. For example, recent studies in mice with targeted disruption of the brain insulin receptor that develop anovulation and obesity suggest that insulin signaling in the brain may modulate reproduction (22). It is also possible that other neuroendocrine factors associated with obesity may have accounted for the putative differences in ovulatory status between PCOS and HA sisters. A candidate factor is leptin, a hormone produced by fat cells with well documented reproductive effects in rodents (46). Leptin levels were not measured in this study, but we have previously shown that circulating leptin levels do not differ in PCOS and weight-comparable control women (47). Our results suggest that obesity modifies the phenotype in PCOS families. Whether this is environmental or reflects the interaction of additional genes will require further investigation.

In summary, the present study demonstrates clustering of markers of insulin resistance in the sisters of PCOS probands consistent with genetic traits. These abnormalities are associated with hyperandrogenemia, rather than menstrual irregularity. Accordingly genetic linkage and analyses in PCOS families using the hyperandrogenemia phenotype may also identify susceptibility genes for the metabolic phenotype. Insulin resistance *per se* does not account for the differences in menstrual cyclicity in affected sisters, and it remains possible that the magnitude of insulin resistance and/or additional factors are necessary to produce anovulation. Phenotyping sisters of women with PCOS on the basis of circulating T levels also identifies those at risk for insulin resistance and, accordingly, for the long-term health consequences of PCOS: diabetes mellitus (48) and cardiovascular disease (49).

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