Diabetes Care Publish Ahead of Print, published online March 10, 2008

Metabolic Phenotype in the Brothers of Women with Polycystic Ovary Syndrome

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Received for publication 16 November 2007 and accepted in revised form 3 March 2008.

ABSTRACT

Objective: Hyperandrogenemia, insulin resistance, and dyslipidemia demonstrate familial aggregation in the female first-degree relatives of women with PCOS suggesting that these defects are heritable. Hyperandrogenemia also appears to be the male reproductive phenotype. We performed this study to test the hypothesis that brothers of women with PCOS have similar metabolic defects to their proband sisters.

Research Design and Methods: This was a prospective **c**ase-control study performed at four Academic Medical Centers in United States. Fasting blood was obtained from 196 Non-Hispanic White brothers of women with PCOS and 169 control men of comparable age, body mass index (BMI), and ethnicity to brothers. A separate analysis was performed by study site to assess potential regional variations in metabolic parameters.

*Results***:** Overall, brothers of women with PCOS had significantly higher total (P=0.001) and LDL cholesterol (P=0.01) as well as triglyceride levels (P=0.01) compared to control men, although there were regional variations in these differences. There were significant positive correlations between brothers and their sisters with PCOS for total (ρ=0.2, P=0.009) and LDL cholesterol (ρ=0.3, P=0.001) and triglyceride (ρ=0.2, P=0.05) levels. Brothers also had significantly higher fasting insulin levels and homeostatic index of insulin resistance (P=0.02 for both comparisons) compared to control men.

Conclusions: Brothers of women with PCOS have dyslipidemia as well as evidence for insulin resistance similar to their proband sisters with PCOS. These findings are consistent with the hypothesis that some metabolic abnormalities in PCOS are heritable and are not sex-specific.

Polycystic ovary syndrome (PCOS) is a complex genetic disorder that affects ~7% of premenopausal women (1). It is the leading cause of anovulatory infertility and an important risk factor for type 2 diabetes mellitus and metabolic syndrome in young adult women as well as in adolescent girls (1). The major reproductive phenotype is hyperandrogenemia and up to 40% of premenopausal sisters are affected (2). Moreover, mothers (3) and brothers (4) also have elevated mean androgen levels when compared to control subjects of comparable sex, age, weight and ethnicity. We have identified one PCOS susceptibility allele within a dinucleotide repeat (D19S884) in intron 55 of the fibrillin-3 gene on chromosome 19p13.2 that is linked and associated with hyperandrogenemia (5).

Abnormalities in insulin action, secretion, glucose tolerance and lipid levels also demonstrate familial aggregation in first-degree female relatives of women with PCOS (3,6-9). Elevated LDL cholesterol levels are the most consistent lipid abnormality in affected women as well as in their sisters and mothers (3,8-10). In addition, the prevalence of metabolic syndrome is increased in these groups (3,9). Further, increased LDL levels and metabolic syndrome are significantly associated with hyperandrogenemia suggesting that the cardinal reproductive feature of the syndrome plays a direct role in the etiology of the associated metabolic abnormalities (3,9). Consistent with this hypothesis, the PCOS susceptibility allele is associated with markers of insulin resistance in women with PCOS (5). Features of the metabolic phenotype, however, have been less well characterized in male than in female relatives. These studies have also been constrained by lack of control data (11) and/or relatively small sample sizes (7,12). The most comprehensive studies have come from Chile and Turkey (7,8,13) and have suggested that male first-degree relatives have insulin resistance (7,13) and dyslipidemia (8). However, the relevance of these latter studies to a US-based population is unclear since there can be ethnic and racial differences in insulin action and lipid metabolism (14,15). Moreover, none of these studies investigated predictors of male metabolic abnormalities. Accordingly, we performed this study of anthropometric and metabolic parameters in a large cohort of brothers of women with PCOS from the US compared to simultaneously recruited control men of comparable age, BMI, ethnicity and location. Further, we examined whether the male metabolic phenotypes were correlated with those in the PCOS probands suggesting heritability or were sex-specific.

RESEARCH DESIGN AND METHODS

Study population. We prospectively enrolled 196 brothers aged 18-55 years of 158 Caucasian women of European origin with PCOS and 169 unrelated control men of comparable age, body mass index (BMI) and

ethnicity between January 1995 and October 2005. The study was conducted at the Pennsylvania State University College of Medicine-Hershey Medical Center (HMC), University of Pennsylvania Medical Center, Brigham and Women's Hospital (BWH) and Northwestern University Feinberg School of Medicine (NU) after approval by each site's Institutional Review Board. Written informed consent was obtained from all subjects prior to their participation in the study. Data on reproductive hormone levels from 119 of these brothers have previously been reported (4). The diagnosis of PCOS was made in the probands by an elevation of circulating testosterone (T) and/or non-sex hormone binding globulin bound (unbound [uT]) levels associated with chronic oligomenorrhea (2). Women with non-classical 21-hydroxylase deficiency, hyperprolactinemia and androgen-secreting tumors were excluded by appropriate tests (2). Two families had four brothers, four families had three brothers, 24 families had two brothers and 128 families had one brother. The clinical and biochemical features on the probands have been reported as part of previous studies (2,4).

The selection criteria for control men were: 1) no major medical or psychiatric illnesses, 2) no personal history of hypertension, and no personal or first-degree family history of diabetes and 3) normal glucose tolerance according to the WHO criteria (16). Excluding the diagnosis of PCOS, based on family history is problematic because it frequently remains undiagnosed, particularly the variants of the syndrome with regular menstrual cycles. Further, if there are no female first-degree relatives of reproductive age, the presence of PCOS cannot be assessed. Finally, it has been our experience that men rarely know the reproductive histories of their female first-degree relatives. Thus, we do not screen our control subjects for first-degree relatives with PCOS, rather we assume in power calculations that ~7% (prevalence of PCOS in general population) of male controls will have a first-degree relative with PCOS compared to 100% of brothers. Inclusion of control men with a first-degree relative with PCOS would bias the study towards the null hypothesis. Neither brothers nor control men were taking any medications known to alter sex hormone metabolism or glucose homeostasis for at least one month prior to study.

Data collection. Brothers were evaluated at one of the four study sites (on-site, n=55) or in a local laboratory (off-site, n=141). The majority of on-site brothers were studied at HMC (n=53). All control men were studied on-site: 95 at HMC, 53 at NU and 21 at BWH. Height, weight, blood pressure and waist measurements were obtained as previously reported for the on-site subjects (2,4,9). For the off-site subjects, the height and weight were self-reported as previously validated (2,4,9). Waist circumference was self-reported for off-site brothers who were provided with a calibrated tape measure for this determination (n=52). Self-measured waist circumference correlates well with

measurements performed by a trained technician (3). There were no differences in height, weight or waist circumference between brothers who were studied on-site and had these parameters measured by study personnel compared to those brothers studied off-site in whom these parameters were self-reported. Information on tobacco, alcohol and exercise history was obtained by questionnaire in 95 brothers and 112 control men. A morning blood sample was obtained after an overnight fast from all subjects as previously reported (2,4,9). All control men and 27 of brothers (14%) underwent 75-g oral glucose tolerance test with fasting and 2 h post-challenge blood sampling for glucose and insulin levels.

Assays. Plasma glucose, insulin, proinsulin, T, uT, dehydroepiandrosterone sulfate (DHEAS), sex hormone binding globulin (SHBG), total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride levels were measured as previously reported (2,4).

Data Analyses. The homeostatic index of insulin resistance (HOMA IR) was calculated according to the HOMA model, a structural computer model of the glucose-insulin feedback system in the homeostatic state (www.dtu.ox.ac.uk/homa/index.html). For analysis of the data, the family unit was the case; in families with multiple brothers, brothers' data were averaged to yield one mean value per family for brothers (4). Log transformation of the data was performed when necessary to achieve homogeneity of variance. Continuous variables were compared using analysis of covariance adjusted for age. Analyses were repeated: 1) after exclusion of brothers with a first-degree relative with type 2 diabetes (n=46); 2) after adjustment for tobacco use, alcohol intake and exercise history; 3) after exclusion of brothers with history of hypertension (n=8); and 4) after exclusion of brothers with glucose intolerance defined as fasting glucose >100 mg/dl (n=17) or 2 h post-challenge glucose ≥140 mg/dl (impaired glucose tolerance n=2, diabetes n=1) (16). Associations between PCOS probands and their brothers for reproductive and metabolic hormones were assessed using Pearson or Spearman correlation coefficients. Predictors of HOMA IR, LDL cholesterol and triglyceride levels in brothers were determined using general linear model. The relationship between SHBG levels and HOMA IR was examined by Spearman correlation coefficient and was further adjusted for age and BMI. Dichotomous variables were compared using *chi-square* analysis.

Our previous studies have suggested that subjects from central Pennsylvania area have an increased prevalence of dyslipidemia (9,10), thus we investigated the impact of study site on outcomes. We had a sufficient number of subjects to perform the following comparisons: brothers studied on-site at HMC (HMC brothers) vs. control men studied at HMC; HMC brothers vs. brothers studied off-site or at other sites (non-HMC brothers); non-HMC brothers vs. control men studied at NU; and control men studied at HMC vs.

control men studied at NU. Since the study was initiated in 1995, we also examined the impact of study time by comparing a subgroup of brothers and control men studied in 2001 or later. Analyses were performed using the 11.0 PC package of SPSS statistical software (SPSS, Inc., Chicago, IL). A $P < 0.05$ was considered significant. Data are reported as the geometric mean + SD.

RESULTS

Brothers were slightly but significantly younger than control men despite recruitment of both groups in the same age range (Table 1). BMI and waist circumference were similar in both groups (Table 1)**.** Overall, 71% of brothers and the same percentage of control men were overweight or obese as defined by a BMI $\geq$25 kg/m^2. DHEAS levels were significantly higher in brothers than control men (brothers, 8.21 ± 3.34 μmol/l vs. control men, 6.38 ± 3.00 μmol/l; $P<0.001$), as previously reported (4), but there were no significant differences in either total T, uT or SHBG levels (data not shown). Systolic blood pressure was similar but diastolic blood pressure was significantly higher in brothers compared to control men (Table 1). Fasting insulin and proinsulin levels and HOMA IR were significantly higher while fasting proinsulin/insulin ratios did not differ in brothers compared to control men (Table 1). Cholesterol, LDL cholesterol and triglyceride levels were significantly higher in brothers compared to control men, whereas HDL cholesterol levels were similar in the two groups (Figure 1). The differences in total and LDL cholesterol and fasting insulin levels and HOMA IR remained significant after exclusion of brothers with a first-degree relative with diabetes ($P<0.05$). The differences in total and LDL cholesterol also remained significant after excluding brothers with glucose intolerance or hypertension as well as after adjustment for alcohol intake, tobacco use and exercise history ($P<0.05$). Furthermore, the changes in total and LDL cholesterol levels remained significant in the subgroup analysis of brothers and control men recruited in 2001 and later ($P<0.01$ for both comparisons). According to the Framingham study data, overall death increases by 5% and cardiovascular death by 9% for each 0.26 mmol/l increase in cholesterol levels at levels greater than 4.62 mmol/l (17). The mean cholesterol level for brothers of 4.89 mmol/l was above this threshold and significantly more brothers (58%) compared to control men (47%) had total cholesterol levels above the 4.62 mmol/l threshold ($P<0.05$ by *chi-square* analysis).

There were significant positive correlations between PCOS probands and their brothers for DHEAS ($\rho=0.4$, $P<0.001$), SHBG ($\rho=0.2$, $P=0.008$), cholesterol ($\rho=0.2$, $P=0.009$), LDL cholesterol ($\rho=0.3$, $P=0.001$), triglyceride ($\rho=0.2$, $P=0.05$), fasting insulin ($\rho=0.2$, $P=0.009$) and proinsulin levels ($\rho=0.3$, $P=0.002$). BMI (B coeff. =0.1, $P<0.001$) was the only predictor of HOMA IR. HOMA IR was the strongest predictor of LDL cholesterol levels with an increase of` 0.21

mmol/l in LDL for each unit increase in HOMA IR after adjustment for age, BMI, T, SHBG and DHEAS levels (Table 2). Age was also a predictor of LDL levels after adjustment for BMI, HOMA IR, DHEAS, T and SHBG levels. Age, SHBG and HOMA IR were predictors of triglyceride levels, independent of BMI (Table 2). SHBG levels were significantly negatively correlated with HOMA IR (ρ=-0.23, P=0.005) but the correlation was no longer significant after adjustment for BMI (ρ=-0.14, P=0.09).

We evaluated the impact of study site on our findings. HMC brothers did not differ from non-HMC brothers, except that HDL cholesterol levels were significantly lower in HMC brothers (0.98 ± 0.26 mmol/l vs. 1.13 ± 0.03 mmol/l respectively, P=0.002). In contrast, total and LDL cholesterol levels as well as triglyceride levels were significantly higher in HMC control men compared to NU control men (P<0.01). When brothers were compared to control men at HMC, triglyceride levels remained significantly higher (P=0.04) but total and LDL cholesterol did not differ. HMC brothers also had significantly lower HDL cholesterol levels than HMC control men (0.98 ± 0.26 mmol/l vs. 1.11 ± 0.28 mmol/l respectively, P=0.02). The findings in the non-HMC brothers compared to the NU control men were similar to the findings for the overall group; total and LDL cholesterol and triglyceride levels were significantly higher (P<0.01 for all 3 comparisons), while HDL cholesterol levels were similar.

CONCLUSIONS

We found that brothers of women with PCOS have dyslipidemia and evidence for insulin resistance. In the overall group of brothers, there were significant increases in total and LDL cholesterol levels and in triglyceride levels compared to control men. These metabolic abnormalities were similar to those reported in women with PCOS (10) and their first-degree female relatives (3,9). The LDL cholesterol elevations in brothers were independent of glucose intolerance and obesity and persisted after adjustment for lifestyle factors such as tobacco use, alcohol intake or exercise. There were significant positive correlations between insulin, LDL cholesterol and triglyceride levels in brothers and their proband sisters. Our findings support the hypothesis that some metabolic features of PCOS are heritable and are not sex-specific.

Fasting insulin levels and HOMA IR were higher in brothers compared to control men suggesting that brothers were insulin resistant. In contrast, there was no significant differences in insulin sensitivity assessed by frequently sampled intravenous glucose tolerance test in our recent study of 23 brothers compared to matched control men (12). However, this study lacked adequate power for the observed differences in insulin sensitivity to achieve statistical significance. Further, decreases in insulin clearance could have contributed to the differences in fasting insulin levels and HOMA IR in brothers in

the present study (18). We were not able to evaluate the prevalence of glucose intolerance in brothers since only a small number (n=27) had oral glucose tolerance testing and brothers who were treated with antidiabetic medications were excluded from the study. We were also not able to compare the prevalence of metabolic syndrome in brothers to that in control men since, by design, features of metabolic syndrome were specifically excluded as part of the selection criteria for the control men.

In brothers, HOMA IR, independent of BMI, was the strongest predictor of LDL elevations suggesting that insulin resistance is an important factor in the pathogenesis of LDL cholesterol abnormalities in the male members of PCOS families. Neither DHEAS nor testosterone levels predicted LDL levels in brothers. In contrast to brothers, hyperandrogenemia rather than insulin resistance was the strongest predictor of LDL cholesterol levels in women with PCOS and their first-degree female relatives (3,9,10). Our findings are consistent with prior reports that, in contrast to women, endogenous sex hormone levels may have a lesser impact on circulating LDL levels in men (19). However, insulin resistance also usually does not alter circulating LDL levels (20), although it can influence LDL particle size (20). Thus, the relationship between markers of insulin resistance and LDL levels in brothers may reflect the association of both of these parameters with another factor not accounted for in our analysis. In addition to higher LDL cholesterol levels, brothers also had higher triglyceride levels compared to control men. Triglyceride levels were elevated only in obese women with PCOS and their obese first-degree relatives (9,10). HOMA IR, independent of BMI, was a predictor of triglyceride levels in brothers, suggesting that insulin resistance *per se* was an important determinant of triglyceride elevations in brothers. SHBG levels also predicted triglyceride levels in brothers but were most likely a proxy for insulin resistance since hepatic SHBG production is down-regulated by hyperinsulinemia (14). Consistent with this hypothesis, SHBG levels were significantly correlated with HOMA IR in brothers. Metabolic abnormalities were a consistent finding in brothers across study sites. However, there were regional differences in control subjects such that some of metabolic abnormalities noted in the brothers were attenuated in comparison to HMC control men. The HMC brothers and controls are from a fairly homogenous Caucasian population of German and Dutch ancestry. Our findings in the present as well as previous studies suggest that this population has an increased prevalence of dyslipidemia (9,10). Regional variations in the environment and ethnicity represent an important potential confounder of case-control studies and likely contributes to conflicting reports of PCOS phenotypes (14). Nevertheless, there were metabolic abnormalities when brothers were compared to controls of comparable location supporting

our hypothesis that brothers have a metabolic phenotype.

Brothers had a mean increase of 0.26 mmol/l in total cholesterol levels compared to control men. Cholesterol levels are directly related with 30-year overall and cardiovascular mortality, especially in men under the age of 50 years (17). The mean cholesterol level for brothers of 4.89 mmol/l was above the threshold of 4.62 mmol/l at which overall death increases by 5% and cardiovascular death by 9% for each 0.26 mmol/l increase in cholesterol levels (17). Further, significantly more brothers than control men had total cholesterol levels above this threshold. In addition, insulin resistance is an independent predictor of cardiovascular disease risk in men (21).

In summary, brothers of women with PCOS have a metabolic phenotype consisting of dyslipidemia and insulin resistance. The familial aggregation of these metabolic abnormalities is consistent with heritable traits. Given the high prevalence of PCOS, being a male first-degree relative may represent an important new risk factor for cardiovascular disease in men.

ACKNOWLEDGEMENTS

Grant Support: NIH grants U54 HD34449, R01 DK40605, P50 HD044405, M01 RR00048, M01 RR10732, K12 RR017707, and M01 RR02635.

The authors wish to thank the women with PCOS and their brothers for participating in this study, Barbara Scheetz, Jennifer Schlinder, Elizabeth Podczerwinski, and Jennifer Steel for coordinating the study and the staff of the General Clinical Research Centers at the Pennsylvania State University College of Medicine, Brigham and Women's Hospital and Northwestern University's Feinberg School of Medicine for their care of the study participants.

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TABLE 1. Clinical and Metabolic Features

Variables	**Brothers***	**Control Men**	**P-Value†**
Age (years)	30 ± 8	33 ± 10	0.004
BMI (kg/m^2)	28.4 ± 5.7	28.0 ± 4.9	0.44
Waist circumference (cm)	93 ± 15	96 ± 14	0.40
Systolic blood pressure (mm Hg)	125 ± 15	123 ± 12	0.15
Diastolic blood pressure (mm Hg)	77 ± 10	75 ± 9	0.02
Fasting glucose (mmol/l)	4.9 ± 0.5	5.1 ± 0.4	0.14
Fasting insulin (pmol/l)	90 ± 60	78 ± 42	0.02
Fasting proinsulin (pmol/l)	13 ± 17	11 ± 7	0.02
Fasting proinsulin/insulin	0.13 ± 0.11	0.12 ± 0.08	0.70
HOMA IR	1.9 ± 1.2	1.7 ± 0.9	0.02

Data are presented as geometric mean ± SD; *Data have been averaged for brothers from the same family. †Analysis of covariance adjusted for age.

TABLE 2. Multivariate analysis for predictors of LDL and triglyceride levels in brothers

Variable	**Unit Δ**	**LDL (mmol/l)**	**CI (mmol/l)**	**Triglyceride (mmol/l)**	**CI (mmol/l)**
Age	1 year	0.024	0.005-0.043	0.031	0.002-0.060
			P=0.02		P=0.04
BMI	1 kg/m^2	0.003	-0.025-0.030	0.0001	-0.056-0.025
			P=0.86		P=0.44
HOMA IR	1	0.210	0.057-0.363	0.658	0.460-0.856
			P=0.008		P<0.001
T	1 nmol/l	0.0003	-0.001-0.001	0.0007	-0.0005-0.002
			P=0.35		P=0.23
SHBG	1 nmol/l	-0.003	-0.008-0.002	-0.014	-0.021-(-0.006)
			P=0.29		P=0.001
DHEAS	1 μmol/l	0.0003	0.00003-0.0003	0.0001	-0.0002-0.0002
			P=0.18		P=0.84

FIGURE 1. Data are presented as mean ± SE; *P=0.001 brothers vs. controls, **P=0.01 brothers vs controls