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Plasma Homocysteine, Fasting Insulin, and Androgen Patterns among Women with Polycystic Ovaries and Infertility

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Abstract

Objective: To measure plasma homocysteine, androgen, and insulin concentrations in women with normal and polycystic-appearing ovaries in an infertility setting.

Methods: Among women referred for infertility evaluation ($n = 54$), homocysteine, androstenedione, DHEAS, total testosterone, fasting insulin/glucose and methyltetrahydrofolate reductase (MTHFR) polymorphism status (C677T mutation) were studied. Ovaries were examined via transvaginal sonogram by one observer and scored as either normal ($n = 18$) or polycystic ($n = 36$).

Results: When polycystic ovaries were identified, mean total testosterone was significantly higher than when non-polycystic ovaries were present ($p = 0.01$), although no measured androgen was outside the normal reference range in either group. Average BMI was higher in the polycystic group, but the difference was not significant ($p = 0.10$). We observed a trend toward higher mean fasting insulin levels in women with polycystic ovaries, but this increase did not reach statistical significance ($p = 0.07$). Median plasma homocysteine was identical (7.0 mmol/*l*) in both populations, and no study subject exceeded the current recommended maximum reference value.

Conclusions: In this population, the presence of polycystic ovaries was associated with higher serum androgens (especially total testosterone) although none of the measured androgens were above the normal range. While fasting insulin levels were also higher in this group, median plasma homocysteine levels were similar irrespective of ovarian morphology. Concomitant plasma homocysteine derangements in this population of young, lean patients with polycystic-appearing ovaries seem unlikely. Further studies are needed to clarify the role(s) of homocysteine in human reproductive physiology.

Key words: homocysteine, polycystic ovary, hyperandrogenism, risk factors

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Introduction

Increased risk for spontaneous abortion and infertility have long been identified as unwelcome companions of the polycystic ovary syndrome (PCOS), a chronic multisystem endocrine disorder characterized by irregular menses, hirsutism, obesity, hyperlipidemia, androgenization, insulin resistance, subfertility, and enlarged, polycystic ovaries. As the most common endocrine disorder in reproductive-age women,[1] PCOS represents an important clinical entity for both general gynecology and subspecialty infertility practice. Although uniform and specific diagnostic criteria for this multisystem endocrinopathy are still evolving,[2] the adverse reproductive consequences of PCOS are well-documented.[3,4] As epidemiologic studies of PCOS in other disciplines have gained traction, the diagnosis of PCOS increasingly is seen as an important marker of disease independent of a woman's wish to conceive or avoid miscarriage.

Ultrasound advancements have provided new data supporting the classic observations of ovarian features originally made at surgery. Indeed, the PCOS phenotype has been refined to include a characteristic ovarian morphology by both standard B-mode and color Doppler sonography. What causes the PCOS ovary to develop in this distinctive and abnormal way for some women, and not others? It is not known whether such changes are primary signs of genetically-programmed ovarian dysfunction, or if they represent secondary manifestations of a physiologic compensatory mechanism perhaps in response to inadequate ovarian and/or follicular oxygenation. Clinical data from *in vitro* fertilization have shown that periovulatory follicles from PCOS ovaries are not well-oxygenated, and that dissolved oxygen content in follicular fluid retrieved from such follicles is markedly low.[5] Cytoplasmic and chromosomal disorders have been linked to follicular hypoxia in these experimental cases.

Homocysteine has been identified as a novel clinical marker for vascular disease and thromboembolism. In this pilot study we sought to measure androgens and plasma homocysteine concentrations in women with polycystic and normal appearing ovaries, to elucidate a possible association between homocysteine and ovarian morphology. Methyltetrahydrofolate reductase (MTHFR) polymorphisms which are known to influence homocysteine metabolism were also studied in our patients.

Materials and Methods

After approval by the Atlanta Medical Center institutional review board, healthy female volunteers ($n = 54$) were studied between August and December 1999. Well-nourished nonsmoking women on a regular diet and not yet taking prenatal vitamins (or folate supplements) were eligible to participate. Exclusion criteria included pregnancy, lactation, known or suspected coagulopathy, renal disease, alcoholism or substance abuse, digestive disorder, diabetes mellitus or other endocrinopathy. Informed consent was obtained from all study subjects before enrollment.

The following parameters were measured: age, BMI, menstrual cycle day (if applicable), duration of infertility, BUN/Cr, plasma homocysteine, fasting insulin and glucose, androstenedione, total testosterone, DHEAS and MTHFR polymorphism status. For the latter test, genotype was registered either as homozygous (aa), heterozygous (Aa), or "no mutation" (AA) for the C677T gene polymorphism.

All regularly menstruating women were evaluated in the early follicular phase (< CD 7). Serum specimens were collected in pressurized ethylenediamine tetra-acetate (EDTA) tubes from patients by peripheral venipuncture between 7:00–9:30 a.m. following an overnight fast. Samples (approx. 5 m*l*) were centrifuged within 20 min at 3000 $g \times 15$ min with separated plasma immediately stored at – 20°C for batch analysis. Homocysteine was measured by fluorescence polarization immunoassay method, using the Abbott IMx system following standard calibration and control procedures (sensitivity < 0.50 mmol/*l*, CV = 4.1%). Fasting (serum) insulin measurements were obtained by double antibody radioimmunoassay (sensitivity = 2 mU, CV = 6.2%). Serum total testosterone, androstenedione, and DHEAS were measured by conventional direct chemiluminescent competitive immunoassay.

Allele-specific PCR/restriction fragment length polymorphism (RFLP) analysis was performed on peripheral leukocytes via restriction endonuclase *HinfI*, for identification of the C677T polymorphism in the methyltetrahydrofolate reductase gene located on chromosome 1p36.3 [nt 653–675 and 807–832].

Evaluation of ovarian morphology was by sonographic assessment using a 3.5 mHz transvaginal probe (SDU400+, Shimadzu Corporation; Kyoto, Japan). All pelvic images were recorded and scored using a binary scale (0 = non-polycystic, 1 = polycystic). The polycystic

designation was assigned when ovaries demonstrated 10 or more small (< 10 mm) peripheral cysts with central stromal sparing observed in a monoplanar view.

Statistical comparison between polycystic and non-cystic groups was by Student's *t*-test with unequal variances or by chi-square test, as appropriate, performed by computer assisted data program (S-PLUS, MathSoft Corporation, Cambridge, MA). All data were reported as mean and interquartile range (25th, 75th percentile).

Results

Principal demographic and clinical characteristics of the study population are depicted in Table 1. When stratified by ovarian morphology, no statistically important difference in age or BMI was evident among study subjects. However, women with polycystic ovaries tended to be heavier than those with non-polycystic ovaries. Mean infertility duration among women with polycystic ($n = 36$) and non-polycystic ($n = 18$) ovaries was 25.2 and 30.0 months, respectively ($p = 0.57$). Baseline renal function as estimated by serum BUN and creatinine measurement at enrollment was also the same for both groups.

The polycystic ovarian morphology was associated with significantly higher mean levels of total testosterone when compared to the reference group (60.6 vs 42.7 ng/dl, $p = 0.01$), although other measured androgens were not similarly elevated. However, the non-cystic and polycystic ovary groups did not differ in their mean total plasma homocysteine levels (7 mmol/*l*), although the observed measurement range (data spread) for homocysteine was more narrow in the non-cystic ovary group (Fig. 1).

Overall prevalence of methyltetrahydrofolate reductase gene mutations in this group was low, as only 3 and 18 women were identified with homozygous and heterozygous mutations, respectively. This degree of polymorphism represents a general frequency distribution of 5.6 and 33% among study subjects. When genotype was tabulated according to ovarian morphology, no significant differences in MTHFR gene polymorphism as a function of phenotype were found, but the subsets resulting from sample partitioning limited the accuracy of statistical testing.

All study patients had a normal fasting plasma glucose (Table 2). Although mean fasting insulin level was somewhat higher among the polycystic ovary women compared to non-cystic controls, this difference was not significant ($p = 0.45$). When hyperinsulinemia was defined as fasting insulin > 25 mU, only 11 women (20.4%) with "high" fasting values were identified. Among these 11 patients, all but two had polycystic-appearing ovaries. Formal 2-hour insulin-glucose tolerance testing was normal for these 11 women.

Discussion

This pilot study describes for the first time the relationships among androgens, insulin, plasma homocysteine and the polycystic ovary phenotype. Our preliminary data suggest that normal or low homocysteine levels are common in young women with polycystic ovaries, and adverse

Table 1. Demographic, clinical and genotypic characteristics of 54 study patients referred for infertility evaluation as a function of general sonographic ovarian appearance

	Ovarian morphology	
	Non-cystic ($n = 18$)	Polycystic ($n = 36$)
Age (yr)*	32.0 (28.0; 34.8)	29.6 (26.0; 33.0)
BMI	27.5 (22.3; 33.6)	33.5 (25.5; 37.5)
Infertility duration (months)	30.0 (12.0; 48.0)	25.2 (0; 24.0)
BUN (mg/dl)	11.8 (10.0; 14.8)	12.3 (10.0; 13.3)
Cr (mg/dl)	0.83 (0.73; 0.90)	0.85 (0.80; 0.30)
MTHFR C677T gene status**		
aa (homozygous)	1	2
Aa (heterozygous)	9	9
AA (no mutation)	8	25

BMI: body mass index (kg/m^2), MTHFR: methyltetrahydrofolate reductase

* Summary data reported as mean [IQR (25; 75)]. There were no significant differences in the 2 groups ($p > 0.05$, by Student's *t*-test with unequal variances).

** Presented as number of patients. Polymorphism status was independent of genotype for all subsets (morphology vs. aa/Aa/AA $p = 0.18$; morphology vs any mutation, $p = 0.08$), by chi-square test.

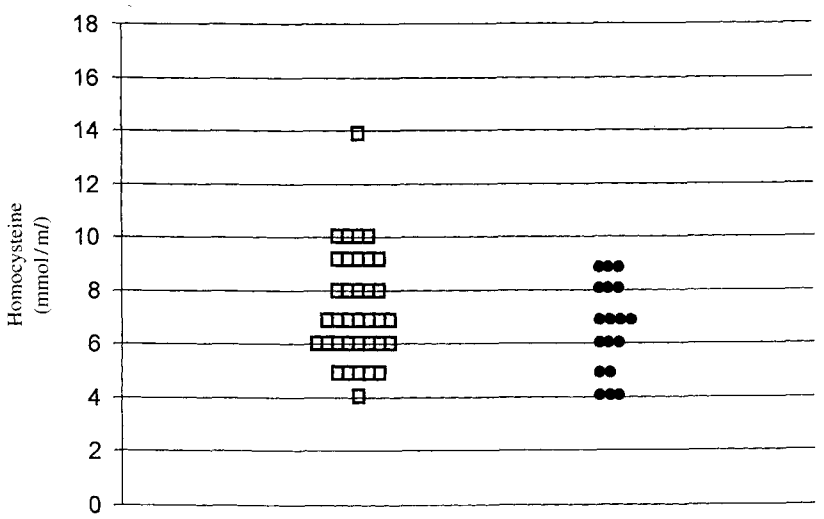

Fig. 1. Distribution of plasma homocysteine values as measured by fluorescence polarization immunoassay method in 54 women with polycystic (□) and non-cystic (●) ovaries. Median plasma levels were identical in both groups (7 mmol/ml).

Table 2. Serum androgen, insulin, and plasma homocysteine comparisons between women with sonographically normal and polycystic-appearing ovaries

	Ovarian morphology		p*
	Non-cystic ($n = 18$)	Polycystic ($n = 36$)	
Fasting insulin	14.7 (7.3; 14.8)	17.6 (9.0; 23.8)	0.45
# ≥ 10 mU**	11	26	0.07
# > 25 mU**	2	9	0.61
Fasting glucose (mg/dl)	86.4 (85.0; 89.0)	87.0 (81.0; 92.0)	0.71
Androstenedione (ng/dl)	177 (132; 249)	213 (149; 249)	0.12
DHEAS (mcg/ml)	1.7 (1.0; 2.3)	1.9 (1.2; 2.4)	0.28
Total testosterone (ng/dl)	42.7 (31.3; 51.8)	60.6 (40.8; 75.0)	0.01
Plasma homocysteine (mmol/l)	6.6 (5.2; 8.0)	7.4 (6.0; 9.0)	0.16

Summary data reported as mean [IQR (25; 75)]. DHEAS: dehydroepiandrostenedione sulfate

* by Student's *t*-test with unequal variances.

** Reported as number of patients; sample partitioning limited the accuracy of statistical comparisons (p-values calculated by contingency table method).

health consequences are probably not mediated by homocysteine derangements when PCOS is defined solely by ultrasound parameters. Furthermore, we conclude that both insulin and androgen levels tend to be relatively higher when polycystic ovaries are observed.

Since the anovulatory or oligoovulatory status frequently seen in PCOS has been regarded by some investigators as an independent marker for increased risk of coronary heart disease,[6] the absence of any description of homocysteine among such women was intriguing. Furthermore, as oxygen tension is considered critical to optimal oocyte health, even subtle perturbations in oxygenation might be developmentally relevant even during early preovulatory stages. The finding that persistent follicular oxygen starvation may antagonize oocyte metabolism[7] led to our hypothesis that homocysteine might contribute to the poor reproductive milieu when polycystic ovaries were present. It was for this reason that homocysteine was identified as a potential marker to study specifically in a population of women with polycystic ovaries referred for infertility evaluation.

We were careful not to label these women as PCOS, since our study patients were classified only by sonographic findings. As polycystic ovaries can exist in some women as a normal morphological variant without any endocrine or reproductive dysfunction,[8] our methodology would overestimate the actual prevalence of PCOS by relying exclusively on sonographic features. In this report, we elected to use a simplified

assessment schema confined to ovarian appearance alone. While a diagnostic approach to PCOS based on sonography has been reported by others,[9,10] insistence on a specific endocrine, sonographic or clinical criteria to diagnose PCOS results in the inclusion of women representing a "focused segment isolated from the broad clinical spectrum in which these patients really belong."[11] Our patients therefore had polycystic-appearing ovaries, but not necessarily PCOS.

Fasting insulin criteria for patients with PCOS are lower than those used for insulinoma (the clinical entity for which the fasting insulin test was originally developed), so we also analyzed our patients against a lower insulin breakpoint (> 10 mU) as described elsewhere.[12,13] This approach identified 37 women (68.5%) as hyperinsulinemic (Table 2). Using this ultrastrict criterion, women with polycystic ovaries accounted for 70.3% of the elevated insulin values while women with non-cystic ovaries comprised the remainder (29.7%, $p = 0.07$). Such a trend towards higher fasting insulin levels among women just with polycystic-appearing ovaries and not classical PCOS warrants additional study, and forms the basis of ongoing research.

Our laboratory's assessment of MTHFR reductase polymorphism status was supported by a recent investigation that showed abnormal genotypes to be associated with the extent of coronary atherosclerosis in high-risk patients.[14] Homozygous deficiency of MTHFR has been linked to severe hyperhomocysteinemia, and affected individuals often exhibit serious atherothrombotic complications.[15] Although the current report included young women with no identifiable risk factors for atherosclerosis (other than polycystic ovaries), we incorporated the same genetic testing for our patients. This was done because the risk of vascular disease and homocysteine in the setting of polycystic ovaries alone has not been quantified. Renal clearance is also an important factor in homocysteine kinetics,[16] and our estimate of this parameter showed the 2 study groups to be comparable. In any case, the highest plasma homocysteine value obtained in our sample was only 14 mmol/*l* (measured in a polycystic ovary patient), which is still within the normal homocysteine reference range (5–15 mmol/*l*).[15]

While these preliminary results suggest that the sole finding of polycystic ovaries is not associated with harmful elevations of plasma homocysteine or hyperandrogenism in a young infertility population, the role of homocysteine as a marker for reproductive health among older women with polycystic ovaries and/or PCOS deserves additional study. Cardiovascular and epidemiologic reports have suggested a dose-response effect for homocysteine,[17,18] and these studies underscore the need for large-scale testing and calculation of relative risk parameters for plasma homocysteine unique to women with polycystic ovaries and/or PCOS. Additionally, how plasma homocysteine may change with aging in such women is an important unanswered question remaining from this preliminary work, and should be addressed by longitudinal studies.

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