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# Hyperhomocysteinaemia is associated with biochemical hyperandrogenaemia in women with reproductive age

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ABSTRACT

*Objective:* Hyperhomocysteinaemia is a well-established risk factor for cardiovascular disease. This study investigated the relationship between hyperhomocysteinaemia and factors related to polycystic ovary syndrome (PCOS).

*Study design:* Case–control study. Three hundred and thirty-nine women were included; of these, 84 had hyperhomocysteinaemia (homocysteine >12.4 μmol/l) and 255 had normal homocysteine levels. Homocysteine, high-sensitivity C-reactive protein, insulin resistance, metabolic disturbance and PCOS-related disturbance were evaluated. The clinical and biochemical characteristics of women with hyperhomocysteinaemia and normal homocysteine levels, including insulin resistance, metabolic disturbance and PCOS-related disturbance, were compared.

*Results:* Correlation was found between serum homocysteine level and serum total testosterone level and diastolic blood pressure. No correlation was found between serum homocysteine level and age, body mass index, insulin resistance and lipid profile. Women with hyperhomocysteinaemia had a significantly higher risk for biochemical hyperandrogenaemia and higher serum total testosterone levels than women with normal homocysteine levels. The prevalence rates of PCOS, oligo-amenorrhoea, polycystic ovary morphology and metabolic disturbance did not differ between the two groups. The parameters of insulin resistance and lipid profiles were similar between the two groups, and signs of clinical hyperandrogenism (hirsutism and the modified Ferriman–Gallwey score) did not differ between the two groups. Logistic regression analysis found a significant association between hyperandrogenaemia and hyperhomocysteinaemia (odds ratio 2.24, 95% confidence interval 1.26–4.01).

*Conclusions:* For women with PCOS, an elevated serum total testosterone level is the main factor associated with hyperhomocysteinaemia. The association between biochemical hyperandrogenism and hyperhomocysteinaemia may contribute to cardiovascular risk for women with PCOS.



## 1. Introduction

The relationship between cardiovascular disease (CVD) and polycystic ovary syndrome (PCOS) remains unclear [1,2]. PCOS is a heterogeneous syndrome of unknown aetiology [3]; phenotypic variation in women with PCOS influences the findings of abnormal metabolic and cardiovascular risk parameters. Studies describing the risk of CVD that have focused on isolated signs of PCOS, such as polycystic ovaries, hyperandrogenism or chronic anovulation, have reported mixed results [4]. Postmenopausal women with a history of PCOS are, however, more likely to be diabetic, obese, have metabolic syndrome and have angiographic coronary artery disease compared with women without clinical features of PCOS [5]. Hyperhomocysteinaemia has been established as an independent risk factor for thrombosis and CVD, and may partially account for the increased risk of CVD associated with insulin resistance [6–8]. Studies related to homocysteine and PCOS have reported inconsistent results [9–13]. Some have suggested that homocysteine levels did not differ between PCOS patients and controls [9,10], and others have proposed that women with PCOS had

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higher levels of homocysteine compared with controls [11,12]. Therefore, this prospective study was conducted to evaluate serum homocysteine levels in women with various PCOS-related phenotypes. Associations between the clinical and biochemical characteristics of hyperhomocysteinaemia and the signs of PCOS were also evaluated.

## 2. Materials and methods

This study was approved by the Institutional Review Board of the Wan Fang Medical Centre at Taipei Medical University, Taipei, Taiwan, and was conducted at the outpatient clinic of the Wan Fang Medical Centre at Taipei Medical University from 1 November 2010 to 31 July 2012. This study was registered at ClinicalTrial.gov (NCT01256970).

### 2.1. Study population

The study participants were recruited from the patients who visited the authors' reproductive endocrinology clinics with chief complaints of infertility, menstrual disturbance, dysmenorrhoea and hirsutism. Each patient enrolled in this study signed an informed consent form. Exclusion criteria were: (1) diagnosis of congenital adrenal hyperplasia, androgen-secreting tumour, Cushing's syndrome, disorders of the uterus or chromosomal anomalies; (2) less than 3 years since menarche; (3) age more than 45 years; and (4) taking hormones or drugs for major medical diseases. Serum homocysteine level was used for cardiovascular risk evaluation from 1 November 2010, and 400 women had been evaluated by 31 July 2012. Of these, 61 women were excluded due to hyperprolactinaemia ($n = 47$) and ovarian failure ($n = 14$). As such, 339 women were included in this study.

### 2.2. Data collection

The subjects' medical history included a detailed menstrual and medical/surgical history, anthropometric measurements (weight, height, waist circumference and hip circumference) and blood pressure. The number of menstrual cycles over the previous year was recorded. The waist-to-hip ratio was defined as waist circumference/hip circumference. The dates and assays for blood sampling have been described previously [14]. Total testosterone levels were measured using a commercial kit, Testosterone RIA DSL-4000 (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). The range of expected values was defined as the central 95% of the 168 female observations, corresponding to a range of 0.1–0.8 ng/ml. Homocysteine levels were measured by a commercial kit (Abbott Laboratories, Abbott Park, IL, USA). The range of expected values was defined as the central 95% of the 170 female observations, corresponding to a range of 4.6–12.4 μmol/l. Hyperhomocysteinaemia was defined as serum homocysteine >12.4 μmol/l.

The following components were measured and calculated: (1) total testosterone, androstenedione, dehydroepiandrosterone sulphate, 17-α-OH progesterone and free androgen index; (2) fasting insulin, fasting glucose, 2-h oral glucose tolerance test glucose level and homeostasis model assessment of the insulin resistance index (HOMA-IR); (3) serum follicle-stimulating hormone, luteinizing hormone and prolactin; (4) total cholesterol, triglycerides, and high- and low-density lipoprotein; and (5) glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and high-sensitivity C-reactive protein. The free androgen index was calculated using the formula: testosterone (nmol/l) × 100/sex-hormone-binding globulin (nmol).

PCOS was diagnosed according to the 2003 Rotterdam criteria, which required the presence of at least two of the following three criteria for a diagnosis of PCOS: polycystic ovary morphology, oligo-/amenorrhoea and hyperandrogenism. The definitions of oligo-/amenorrhoea and polycystic ovary morphology have been described in detail elsewhere [15]. Hyperandrogenism was defined as hirsutism and/or biochemical hyperandrogenaemia. Hirsutism was evaluated using the modified Ferriman–Gallwey (mF–G) method, which was performed by a single technician. Hirsutism was defined as an mF–G score ≥6. Biochemical hyperandrogenaemia was defined as total serum testosterone ≥2.78 mmol/l.

The insulin sensitivity index was evaluated by HOMA-IR using the following formula: HOMA-IR = [fasting insulin (μU/ml) × fast-fasting glucose (mg/dl)]/405.

The 2006 World Health Organization diagnostic criteria for diabetes were employed (fasting plasma glucose ≥7.0 mmol/l or 2-h plasma glucose ≥11.1 mmol/l). Impaired glucose tolerance was defined as 2-h glucose levels of 7.8–11.1 mmol/l in the 75-g oral glucose tolerance test. In women with impaired glucose tolerance, the fasting plasma glucose level should be <7 mmol/l.

Metabolic syndrome was defined (2005 National Cholesterol Education Program–Adult Treatment Panel III) as the presence of at least three of the following criteria: abdominal obesity (waist circumference >80 cm in women), serum triglycerides ≥1.7 mmol/l, serum high-density lipoprotein <1.3 mmol/l, systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg, and fasting plasma glucose ≥7.0 mmol/l.

### 2.3. Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences Version 13.0 (SPSS, Inc., Chicago, IL, USA). Correlations between serum homocysteine level and PCOS-related parameters were evaluated using Pearson's correlation coefficients with the two-tailed method. The data are presented as mean ± standard deviation in Table 2. Chi-squared test and Fisher's exact test were used to compare categorical variables, and analysis of variance was used to compare continuous variables. Logistic regression analyses and odds ratios (OR) with 95% confidence intervals (CI) were used to examine the relationship between hyperhomocysteinaemia and the associated risk factors. Differences between groups were considered significant if the $p$-value was less than 0.05.

**Table 1**
Correlation of homocysteine with clinical and biochemical parameters in all 339 study participants.

| | Homocysteine correlation | $p$-Value |
|---|---|---|
| High-sensitivity C-reactive protein | 0.022 | 0.686 |
| Age | −0.086 | 0.114 |
| Body mass index | 0.058 | 0.289 |
| Waist circumference | −0.001 | 0.979 |
| Number of menstrual cycle per years | 0.012 | 0.824 |
| Average ovarian volume | −0.016 | 0.778 |
| Total testosterone | 0.172 | 0.001* |
| Androstenedione | 0.066 | 0.223 |
| Dehydroepiandrosterone sulphate | 0.022 | 0.689 |
| Modified Ferriman–Gallwey score | 0.050 | 0.362 |
| Systolic pressure | 0.106 | 0.054 |
| Diastolic pressure | 0.134 | 0.015* |
| Fasting insulin | −0.046 | 0.401 |
| Fasting glucose | −0.023 | 0.681 |
| HOMA-IR | −0.031 | 0.568 |
| Total cholesterol | 0.053 | 0.335 |
| High-density lipoprotein | 0.007 | 0.903 |
| Low-density lipoprotein | 0.060 | 0.272 |
| Sex-hormone-binding globulin | −0.082 | 0.130 |

HOMA-IR, homeostasis model assessment insulin resistance index.
* $p < 0.05$.

## 3. Results

Table 1 shows the correlation of homocysteine with the clinical and biochemical parameters. Serum homocysteine level was found to be correlated with serum total testosterone level and diastolic pressure.

One hundred and eighty-eight of the 339 women in the study had PCOS. Serum homocysteine levels did not differ between women with PCOS and women without PCOS (11.1 ± 3.0 vs 10.8 ± 2.7 μmol/l; $p = 0.292$).

According to the three diagnostic components of PCOS, the 339 women were classified into eight subgroups: normal control

**Table 2**
Biochemical and clinical characteristics of women with hyperhomocysteinaemia and normal homocysteine levels.

| | Total | Hyper-homocystinemia | Normal homocystine levels | *p*-Value |
|---|---|---|---|---|
| Case number | 339 | 84 | 255 | |
| Homocysteine (μmol/l) | 10.9 ± 2.8 | 14.9 ± 2.2 | 9.7 ± 1.5 | <0.001* |
| Age (years) | 27.3 ± 6.7 | 26.3 ± 5.8 | 27.6 ± 7.0 | 0.107 |
| Menarche (age in years) | 12.6 ± 1.5 | 12.4 ± 1.4 | 12.6 ± 1.5 | 0.193 |
| Body mass index (kg/m$^2$) | 24.8 ± 6.3 | 25.0 ± 6.4 | 24.7 ± 6.2 | 0.729 |
| hsCRP (nmol/l) | 2.29 ± 3.69 | 2.34 ± 3.43 | 2.27 ± 3.77 | 0.878 |
| SHBG (nmol/l) | 43.8 ± 29.4 | 45.5 ± 31.7 | 43.3 ± 28.7 | 0.543 |
| Systolic pressure (mmHg) | 112.9 ± 17.1 | 115.5 ± 19.2 | 112.0 ± 16.3 | 0.110 |
| Diastolic pressure (mmHg) | 76.8 ± 13.8 | 79.6 ± 15.1 | 76.0 ± 13.3 | 0.043* |
| Number of cycles per year | 7.5 ± 4.0 | 8.0 ± 3.9 | 7.3 ± 4.0 | 0.183 |
| Polycystic ovarysyndrome | 55% | 56% | 55% | 0.916 |
| Oligo-/amenorrhoea | 63% | 56% | 66% | 0.102 |
| Polycystic ovary morphology | 57% | 61% | 56% | 0.421 |
| Hyperandrogenism | 46% | 55% | 43% | 0.055 |
| Biochemical hyperandrogenaemia | 22% | 32% | 18% | 0.006* |
| Hirsutism | 34% | 35% | 34% | 0.875 |
| Anthropometric measures | | | | |
| Height (cm) | 160.2 ± 5.2 | 159.8 ± 5.0 | 160.3 ± 5.2 | 0.421 |
| Weight (kg) | 63.7 ± 16.8 | 63.9 ± 17.2 | 63.6 ± 16.7 | 0.865 |
| Waist (cm) | 83.2 ± 14.7 | 82.5 ± 15.1 | 83.4 ± 14.6 | 0.641 |
| Hip (cm) | 8.70 ± 11.5 | 99.2 ± 11.3 | 98.5 ± 11.6 | 0.664 |
| Waist:hip ratio | 0.84 ± 0.09 | 0.83 ± 0.08 | 0.84 ± 0.09 | 0.142 |
| Androgens | | | | |
| Total testosterone (nmol/l) | 2.00 ± 0.96 | 2.19 ± 1.06 | 1.93 ± 0.92 | 0.030* |
| Androstenedione (nmol/l) | 9.03 ± 4.47 | 9.37 ± 4.50 | 8.92 ± 4.46 | 0.425 |
| Free androgen index | 7.44 ± 7.16 | 8.04 ± 7.50 | 7.25 ± 7.04 | 0.381 |
| DHEA-S (nmol/l) | 5137 ± 2656 | 5022 ± 2644 | 5175 ± 2663 | 0.647 |
| 17-OH PRG (nmol/l) | 3.07 ± 2.26 | 3.41 ± 2.39 | 2.93 ± 2.17 | 0.110 |
| m-FG score | 4.09 ± 2.67 | 4.24 ± 2.70 | 4.05 ± 2.66 | 0.562 |
| Insulin sensitivity and glucose tolerance | | | | |
| Fasting insulin (pmol/l) | 107.6 ± 99.5 | 94.0 ± 78.1 | 112.1 ± 105.3 | 0.150 |
| Fasting glucose (mmol/l) | 5.13 ± 1.06 | 5.10 ± 0.82 | 5.13 ± 1.13 | 0.827 |
| 2-h glucose (mmol/l) | 6.37 ± 2.59 | 6.32 ± 2.76 | 6.39 ± 2.54 | 0.855 |
| HOMA-IR | 3.56 ± 3.76 | 3.19 ± 3.17 | 3.68 ± 3.93 | 0.307 |
| Impaired glucose tolerance | 14% | 18% | 13% | 0.244 |
| Diabetes mellitus | 5% | 5% | 6% | 0.803 |
| Hormonal components | | | | |
| TSH (mIU/ml) | 2.06 ± 1.29 | 2.01 ± 1.33 | 2.08 ± 1.27 | 0.685 |
| LH (mIU/ml) | 10.14 ± 10.44 | 9.73 ± 6.36 | 10.27 ± 11.48 | 0.677 |
| FSH (mIU/ml) | 6.46 ± 2.17 | 6.10 ± 1.87 | 6.58 ± 2.25 | 0.083 |
| Prolactin (pmol/l) | 592.6 ± 227.7 | 576.5 ± 231.8 | 597.9 ± 226.6 | 0.457 |
| Liver function | | | | |
| GOT (IU/l) | 25.1 ± 12.4 | 25.6 ± 12.4 | 24.9 ± 12.4 | 0.677 |
| GPT (IU/l) | 26.4 ± 23.8 | 27.3 ± 25.4 | 26.2 ± 23.3 | 0.719 |
| Lipid profiles and blood pressure | | | | |
| Cholesterol (mmol/l) | 4.87 ± 0.92 | 4.91 ± 0.96 | 4.86 ± 0.91 | 0.664 |
| Triglycerides (mmol/l) | 1.07 ± 1.00 | 1.03 ± 0.73 | 1.08 ± 1.08 | 0.738 |
| HDL (mmol/l) | 1.42 ± 0.42 | 1.44 ± 0.43 | 1.41 ± 0.42 | 0.612 |
| LDL (mmol/l) | 2.90 ± 0.82 | 2.94 ± 0.91 | 2.89 ± 0.79 | 0.676 |
| Metabolism | | | | |
| Metabolic syndrome | 26% | 26% | 26% | 0.988 |
| Hypertension | 28% | 31% | 27% | 0.538 |
| HDL <1.3 mmol/l | 44% | 45% | 44% | 0.889 |
| Triglycerides >1.7 mmol/l | 12% | 12% | 12% | 0.954 |
| Waist >80 cm | 50% | 46% | 52% | 0.361 |

hsCRP, high-sensitivity C-reactive protein; SHBG, sex-hormone-binding globulin; DHEA-S, dehydroepiandrosterone sulphate; 17-OH PRG, 17-α-OH progesterone; m-FG, modified Ferriman–Gallwey score; HOMA-IR, homeostasis model assessment insulin resistance index; TSH, thyroid-stimulating hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data are either mean ± standard deviation or percentages.

* $p < 0.05$.

($n = 44$; $10.7 \pm 2.9$ μmol/l), polycystic ovary morphology alone ($n = 33$; $11.5 \pm 3.0$ μmol/l), oligo-/amenorrhoea alone ($n = 49$; $10.2 \pm 2.4$ μmol/l), hyperandrogenism alone ($n = 25$; $11.0 \pm 2.4$ μmol/l), polycystic ovary morphology + oligo-/amenorrhoea ($n = 58$; $10.4 \pm 2.2$ μmol/l), polycystic ovary morphology + hyperandrogenism ($n = 22$; $11.4 \pm 2.8$ μmol/l), oligo-/amenorrhoea + hyperandrogenism ($n = 28$; $11.3 \pm 3.0$ μmol/l) and polycystic ovary morphology + oligo-/amenorrhoea + hyperandrogenism ($n = 80$; $11.4 \pm 3.4$ μmol/l). Serum homocysteine levels did not differ significantly between the women with these eight PCOS-related phenotypes ($p = 0.166$).

Significantly elevated serum homocysteine levels were found, however, for women with biochemical hyperandrogenaemia (serum total testosterone $\geq$2.78 mmol/l) compared with women with normal serum total testosterone levels ($11.9 \pm 3.4$ vs $10.7 \pm 2.6$ μmol/l; $P = 0.001$).

Table 2 shows the clinical and biochemical characteristics of women with hyperhomocysteinaemia and normal homocysteine levels. Women with hyperhomocysteinaemia had a significantly higher risk for biochemical hyperandrogenaemia and higher serum total testosterone levels than women with normal homocysteine levels. The prevalence rates of PCOS, polycystic ovary morphology, oligo-/amenorrhoea and metabolic disturbance did not differ between the two groups. The parameters of insulin resistance and lipid profiles were similar between the two groups, and signs of clinical hyperandrogenism (hirsutism and m-FG score) did not differ between women with and without hyperhomocysteinaemia.

Logistic regression analyses were used to examine the relationships between hyperhomocysteinaemia and the associated risk factors for all 339 subjects. The initial variables entered in this model included age, BMI and PCOS. Results showed that none of these parameters was associated with hyperhomocysteinaemia. When age, BMI, polycystic ovary morphology, oligo-/amenorrhoea, biochemical hyperandrogenaemia and hirsutism were entered into the model as individual variables, however, hyperandrogenaemia was found to be the only parameter that was significantly associated with hyperhomocysteinaemia (OR 2.22, 95% CI 1.19–4.00). Polycystic ovary morphology (OR 1.09, 95% CI 0.63–1.88), oligo-/amenorrhoea (OR 0.60, 95% CI 0.40–1.05) and hirsutism (OR 0.87, 95% CI 0.50–1.54) were not associated with the risk of hyperhomocysteinaemia.

## 4. Comments

Women with PCOS are often assumed, a priori, to be at an increased risk for CVD. The underlying physiological mechanism of this increased vascular risk remains unexplained, but it may be related to worsening of endothelial dysfunction and/or structural vessel properties induced by oxidative stress [16]. An association between PCOS and CVD has not been established [4]. Possible associations have been reported between PCOS and diabetes, lipid abnormalities and other cardiovascular risk factors [1,17]. At long-term follow-up, however, morbidity and mortality from coronary heart disease among women with PCOS is not as high as predicted previously [18]. Menstrual cycle irregularity may be a marker of metabolic abnormalities predisposing women to increased risk for CVD [19], but there is little evidence for an association between hyperandrogenism per se and cardiovascular events [4].

Hyperhomocysteinaemia is a well-established risk factor for CVD. Homocysteine, a sulphur-containing amino acid formed during the metabolism of methionine, exerts cytotoxic effects on the vascular endothelium [8]. Elevated total plasma homocysteine has been established as an independent risk factor for thrombosis and CVD. A strong relationship between plasma homocysteine level and mortality has been reported in patients with angiographically confirmed coronary artery disease [7]. Serum homocysteine level was found to be correlated with serum total testosterone level in this study, but no association was found between serum homocysteine level and insulin resistance, and a previous report found no correlation between serum homocysteine level and insulin level, body mass, type of obesity or diabetes status [16,20,8].

Hyperandrogenism is usually defined as clinical and/or biochemical. This study, however, found that hyperhomocysteinaemia was associated with biochemical hyperandrogenaemia but not with hirsutism. Phenotypic variations in hyperandrogenic women may influence the findings of abnormal metabolic and cardiovascular risk parameters. Carmina et al. found that homocysteine level was elevated in cases of classic hyperandrogenism with chronic anovulatory PCOS, but not in cases of hyperandrogenism and polycystic ovaries in women with normal ovulatory cycles [13]. The present study found that hyperhomocysteinaemia was associated with biochemical hyperandrogenism but not with clinical hyperandrogenism (hirsutism). Although hirsutism was found more often in women with confirmed coronary artery disease [21], the present study did not find abnormal serum homocysteine levels in women with hirsutism. This might imply different pathways of metabolic disturbance in clinical and biochemical hyperandrogenism.

Several studies, including some population-based studies, have linked plasma homocysteine levels to blood pressure [22]. An association between homocysteine level and diastolic pressure was also found in this study. Mechanisms that could explain the relationship between homocysteine level and blood pressure include increased arterial stiffness, endothelial dysfunction with decreased availability of nitric oxide, low folate status and insulin resistance [22]. The correlation between homocysteine level, total testosterone level and blood pressure might explain why hyperandrogenaemic women with PCOS had elevated blood pressure independent of age, insulin resistance, obesity or dyslipidaemia [23]. The women evaluated in the present study were recruited from the outpatient clinic of a tertiary care centre, and this sample is not representative of the general population. Therefore, the results should be applied to the general population with caution. The study population consisted entirely of Chinese Taiwanese women. The authors previously published findings indicating that the prevalence of hirsutism was less common in Chinese Taiwanese women [24]. As such, the results of this study should be verified in different ethnic populations.

## 5. Conclusion

For women with PCOS, elevated serum total testosterone level is the main factor associated with hyperhomocysteinaemia. The association between biochemical hyperandrogenism and hyperhomocysteinaemia may contribute to cardiovascular risk for women with PCOS.

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