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Insulin resistance in patients with polycystic ovary syndrome is associated with elevated plasma homocysteine

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BACKGROUND: Elevated levels of plasma homocysteine have recently been implicated as a significant risk factor for cardiovascular disease, pre-eclampsia, and recurrent pregnancy loss, and have been found to be associated with insulin resistance in a number of clinical situations. We examined the relationship between plasma homocysteine and insulin resistance in patients with polycystic ovary syndrome (PCOS). METHODS: A total of 155 infertile patients with PCOS as defined by clinical, biochemical and ultrasound criteria were screened for insulin resistance utilizing single-sample fasting insulin and glucose measurement, calculated by glucose:insulin ratio or homeostasis model assessment (HOMA) index. Total plasma homocysteine was measured by fluorescence polarization immunoassay. One hundred normo-ovulatory women with normal ovaries being treated for other infertility diagnoses served as a control group. RESULTS: Insulin resistance was found in the majority of PCOS patients: –53.5% (83/155), 60.6% (94/155) and 65.8% (102/155), when defined by fasting insulin, glucose:insulin ratio, or logHOMA respectively. Mean plasma homocysteine in the PCOS group was significantly higher than in the normal ovary group (11.5 ± 7.4 versus 7.4 ± 2.1 μmol/l, $P < 0.001$). Insulin-resistant PCOS patients had significantly higher plasma homocysteine (12.4 ± 8.4 μmol/l) than non-insulin-resistant PCOS patients (9.6 ± 4.4 μmol/l) regardless of body mass index ($P = 0.003$ by groups, $P = 0.005$ by correlation of single samples). Thirty-four per cent (53/155) of the PCO patients had homocysteine values >95th percentile of the controls (11.0 μmol/l, $P < 0.0001$). Statistically significant correlations were found between all insulin resistance indices and homocysteine levels. Multiple logistic regression defined insulin resistance as the major factor examined that influenced homocysteine levels. CONCLUSIONS: Insulin resistance and hyperinsulinaemia in patients with PCOS is associated with elevated plasma homocysteine, regardless of body weight. This finding may have important implications in the short term regarding reproductive performance, and in the long term regarding cardiovascular complications associated with insulin-resistant PCOS.

Key words: homocysteine/insulin resistance/metabolic syndrome/polycystic ovary syndrome

Introduction

Homocysteine (Hcy) is an intermediate formed during the breakdown of the amino acid methionine, and may undergo remethylation to methionine, or trans-sulphuration to cystathione and cysteine. Classic homocysteinaemia has been characterized as the accumulation of Hcy due to defects in enzymatic pathways. Recent research has pointed to many non-enzymatic factors which may influence Hcy levels—including age, gender, or sex-steroid environment (Giltay *et al*., 1998a; Van Baal *et al*., 1999), nutrition, smoking, chronic inflammation (McCarty 2000a) and coffee consumption, and physical activity (Nygard *et al*., 1998; Schneede *et al*., 2000). Insulin levels have also been implicated as a modulating factor of Hcy, in that insulin inhibits hepatic cystathione β synthase activity (House *et al*., 1999; McCarty, 2000b). Increased levels of Hcy have been positively associated with insulin levels in a number of clinical situations.

Insulin resistance (IR) or ingestion of a diet with a high insulinaemic index will tend to increase plasma Hcy (Giltay *et al*., 1998b; Nygard *et al*., 1998; Bar-On *et al*., 2000; Meigs *et al*., 2001).

Patients with non-insulin-dependent diabetes mellitus (NIDDM), or hypertension and IR, especially when associated with nephropathy, have increased Hcy levels (Henning *et al*., 1999; Stabler *et al*., 1999; Sheu *et al*., 2000). Insulin sensitivity was found to be inversely related to plasma homocysteine in pregnant women with pre-eclampsia (Rajkovic *et al*., 1997; Laivuori *et al*., 1999). Elevated levels of plasma Hcy have been

shown to be a significant long-term risk factor for atherogenesis and chronic vascular damage (Nygard *et al*., 1995; El-Khairy *et al*., 1999), especially in situations where insulin levels are increased (Das *et al*., 1999; Knekt *et al*., 2001; McFarlane *et al*., 2001).

Polycystic ovary syndrome (PCOS) is a common endocrinopathy involving ovulatory disturbances, hyperandrogenism, infertility and an increased miscarriage rate. Obesity and hyperinsulinaemia are also frequently encountered in PCOS (Dunaif *et al*., 1992; Gennarelli *et al*., 2000; Iuorno and Nestler, 2001). Late complications of PCOS commonly include NIDDM, hypertension, dyslipidaemia, atherosclerosis and vascular disease (Talbott *et al*., 1995; Robinson *et al*., 1996; Dahlgren and Janson, 2000). Thus it seems logical to hypothesize that elevated Hcy levels could be another feature of PCOS, being both associated with IR, reproductive failure (Craig *et al*., 2002) and late vascular complications. This study was designed to examine the relationship between Hcy and IR in infertile patients with or without PCOS.

Materials and methods

Subjects

All patients presenting to the Infertility and IVF Unit at the Assaf Harofeh Medical Center are routinely screened by pelvic ultrasound (Logic 200á, General Electric, 5 MHz probe) to rule out ovarian pathologies and to assess ovarian morphology. A total of 156 patients attending the unit over a 1 year period were diagnosed with PCOS, using menstrual, laboratory and ultrasound criteria. Menstrual criteria included oligo- or amenorrhoea (cycle length irregular, >45 days or <6 periods per year). Biochemical criteria included an elevated menstrual LH:FSH ratio (>2.5), or elevated (>2.8 nmol/l) total serum testosterone (>95th percentile of control women with normal ovaries and regular menstruation). Ultrasound criteria used for diagnosis were those described by Adams *et al*. (1985, 1986), i.e. an enlarged ovary with ⩾10 peripherally arranged small follicular cysts and a hyperechogenic central stroma. These criteria are widely accepted as diagnostic of PCOS (Zawadzki and Dunaif, 1992; Dunaif, 1997; Eldar-Geva *et al*., 2001; Koivunen *et al*., 2001; Kirchengast and Huber, 2001).

Ninety of these patients were candidates for treatment by ovulation induction with gonadotrophins, and 66 were candidates for IVF/ICSI treatment, due to additional infertility diagnoses.

Serum prolactin, thyroid hormone and 17-hydroxy-progesterone determinations were done on all patients and were within normal limits.

One hundred patients attending our infertility unit for treatment during the same time period, due to isolated male factor or tubal factor infertility, were randomly chosen during the 7-month recruitment period as a control group. These patients had regular menstrual periods, normal ovulatory cycles, had no ultrasound or clinical signs of PCOS, and were not selected by any other clinical sign including body weight. These patients had the same height, weight and menstrual blood tests as the PCOS group.

Laboratory tests

All patients were weighed on two separate days and had height measured. Baseline day 3 LH and FSH were measured in a spontaneous or progestogen-induced menses before treatment was initiated. Plasma glucose, insulin and homocysteine were measured after an overnight fast, during menses. Patients treated with ovulation induction (n = 66) had no medical treatment at this time, whereas patients entering an IVF cycle (n = 90) were either initiating at that point injection of Triptorelin 3.75 mg i.m. (Decapeptyl CR™ 3.75; Ferring, Germany) or had already been treated for 8–10 days with intranasal Nafarelin 600 µg/day (Synarel™; Delpharm, France). Patients had not started folic acid supplementation or metformin treatment at the time of homocysteine testing. Patients then went on to stimulation with gonadotrophin injections and folic acid supplementation (800 µg/day) to ovulation or IVF after oocyte retrieval, fertilization and embryo transfer. One patient in the IVF group was dropped from the study at this point as she was receiving phenytoin for epilepsy (known to interfere with plasma homocysteine determination).

Glucose and insulin were measured from plasma separated and frozen immediately after venipuncture, and analysed in blocks of 25. Plasma glucose was measured in duplicate by the glucose oxidase method (Beckman, USA). Plasma immunoreactive insulin was measured using the Phadeseph RIA kit (Pharmacia, Sweden). The intra- and inter-assay coefficients of variation (CV) for glucose and insulin were 2.2 and 3.5% (glucose) and 4.7 and 6.2% (insulin) respectively.

Homocysteine was measured as total homocysteine from plasma separated and frozen immediately after venipuncture, and analysed in triplicates in blocks of 25. Homocysteine exists in plasma mostly as oxidized homocysteine bound to albumin, although reduced homocysteine and disulphide homocysteine forms also exist, therefore in this study total L-homocysteine was determined using Fluorescence Polarization Immunoassay (FPIA) by IMX analysis (Abbott Diagnostics, Axis-Shield, Norway). Briefly, bound oxidized and disulphide homocysteine is reduced, then enzymatically converted to *S*-adenosyl-L-homocysteine, which is then labelled and measured by immunoassay. The intra-assay CV was 2.7% or lower, and the inter-assay CV was 3.7–5.2% over the spectrum of concentrations determined in our population.

IR may be determined by a number of different methods (Del Prato, 1999; Katz *et al*., 2000; Stumvoll *et al*., 2000; McAuley *et al*., 2001); we chose to use those which utilize static fasting glucose and insulin measurements, which are highly correlated to dynamic measurements after glucose loading (Matthews *et al*., 1985; Legro *et al*., 1998; Bonora *et al*., 2000; Brun *et al*., 2000; Fukushima *et al*., 2000; Hanson *et al*., 2000; Mather *et al*., 2001). These include fasting insulin, the glucose:insulin ratio [glucose (mmol/l)/insulin (mIU/l)], the HOMA (homeostasis model assessment) index [glucose (mmol/l)*insulin (mIU/l)/22.5], or the logarithmic transformation of the HOMA index ($\log_{10}$HOMA). Correlations between clinical or biochemical parameters and IR were calculated using all of the above-mentioned indices. The upper limit of normal was constructed by calculating the mean + 2SD of the normal control group, for each variable. These calculations accurately reflected the accepted values in the above-mentioned reference publications.

Statistics

Statistics were calculated with the aid of the SPSS™ computerized statistical package (SPSS for Windows, Version 6.1, SPSS Inc. USA), utilizing Fisher's exact test to compare differences in rates, and Student's t-test to compare differences between parametric data sets. Pearson's correlation coefficients were used to calculate correlation between paired data sets. Significance of correlation and the relative contribution of each variable were calculated by single or multiple regression analyses respectively. Significance was set as $P < 0.05$.

Results

Insulin resistance: definitions

IR was defined as an abnormal result in fasting insulin, glucose:insulin ratio, or logHOMA value, determined by calculating threshold values >95th percentile of the normal ovary control group. These were: fasting insulin >19.1 mIU/l, glucose:insulin ratio <0.24, and logHOMA value >0.59. Thus, IR was found in 53.5% (83/155) of PCOS patients by fasting insulin, 60.6% (94/155) of PCOS patients by glucose:insulin ratio and 65.8% (102/155) of PCO patients by logHOMA, all highly statistically significant as compared with normal controls ($P < 0.0005$, χ^2, all groups). Further calculations concerning IR were done using logHOMA and the 0.59 threshold, as this was the most inclusive variable.

The PCOS group was stratified by logHOMA: 102 patients had a logHOMA ⩾0.59, (designated PCOS-IR) and 53 had a logHOMA <0.59 (designated PCOS-NIR). Elevated logHOMA was found to be significantly associated with a higher body mass index (BMI), a higher fasting glucose level, all IR indices, and elevated plasma homocysteine (Table I).

All of the static IR indices were highly inter-correlated. Pearson correlation coefficients of 0.79–0.97 were found between insulin and the other indices, 0.74–0.92 between glucose:insulin ratio and the other indices, and 0.91–0.93 between logHOMA and the other indices.

Body weight and other variables

PCOS patients were found to be more obese than our control (normal ovary) patients. When patients were stratified by BMI, 45.2% (70/155) of the PCOS patients had a BMI ⩾27 kg/m^2, whereas only 22% (22/100) of the control group had a BMI ⩾27 ($\chi^2 = 14.1$, $P = 0.002$) (Table II). Elevated BMI was significantly associated with IR in PCOS patients (BMI in PCOS-IR: 28.6 ± 6.4; BMI in PCOS-NIR: 23.9 ± 3.4; $P = 0.002$) (Table I).

IR indices were also significantly elevated in obese PCOS patients as opposed to lean PCOS patients (mean logHOMA 0.77 versus 0.52), although both lean and obese PCOS patients were more insulin resistant than their normal ovary counterparts (logHOMA 0.77 and 0.52 versus 0.37 and 0.26 respectively) (Table II). Elevated LH/FSH ratio was significantly associated with the PCOS state, whether obese or lean, insulin-resistant or non-insulin-resistant (Tables I and II). Conversely, patients' age was not associated with any other clinical or biochemical variable.

Homocysteine as related to other variables

Homocysteine levels were significantly elevated in PCOS patients [11.5 ± 7.4 μmol/l (all PCOS), 12.4 ± 8.4 μmol/l (PCOS-IR) versus 7.4 ± 2.1 μmol/l (controls), $P < 0.001$] (Table I). Elevated homocysteine was noted in both lean and

Table I. Clinical and biochemical data of all patients; polycystic ovary syndrome (PCOS) patients stratified by insulin resistance

Group	Age (years)	BMI (kg/m^2)	LH:FSH	Insulin (mIU/l)	Glucose:insulin ratio	LogHOMA	Homocysteine (μmol/l)
Reference range[a]		20–25	<2	<15	>0.33	<0.5	5–11
95th % normals[b]		<30.6	<1.78	<19.1	>0.24	<0.59	<11.0
PCOS: all patients (n = 155)	28.7 4.3[c]	27.1 ± 6.1	2.12 ± 1.1	22.7 ± 12.1	0.29 ± 0.18	0.63 ± 0.27	11.5 ± 7.4
PCOS-IR, LogHOMA >0.59 (n = 102)	28.8 ± 4.5	28.6 ± 6.4	2.14 ± 1.2	28.5 ± 10.8	0.20 ± 0.06	0.78 ± 0.16	12.4 ± 8.4
PCOS-NIR, logHOMA <0.59 (n = 53)	28.4 ± 3.9	23.9 ± 3.4	2.06 ± 1.0	11.6 ± 4.4	0.47 ± 0.2	0.34 ± 0.19	9.6 ± 4.4
P-value: PCOS-IR versus PCOS-NIR	NS	0.002	NS	< 0.001	< 0.001	< 0.001	0.02
Control: normal ovary (n = 100)	29.6 ± 4.3	24.5 ± 3.1	1.15 ± 0.3	10.3 ± 4.4	0.53 ± 0.17	0.28 ± 0.2	7.41 ± 2.1
P-value: PCOS-IR versus control	NS	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001
P-value: PCOS-NIR versus Control	NS	NS	0.001	NS	NS	NS	0.001

Values are mean ± SD.
[a]References in literature.
[b]Upper 95th percentile of normal ovary (control group).
[c]BMI = body mass index; IR = insulin-resistant; NIR = non-insulin-resistant; LogHOMA = logarithmic transformation of the homeostasis model analysis index of insulin resistance.
NS = not significant.

Table II. Comparison of clinical and biochemical parameters of patients, as stratified by body mass index (BMI)

Group	Age (years)	BMI (kg/m^2)	LH/FSH	Insulin (mIU/l)	Glucose:insulin ratio	LogHOMA	Homocysteine (μmol/l)
Reference range (literature)		20–25	<2	<15	<0.33	<0.5	5–11
PCOS lean (BMI <27), n = 85 (85/155) (54.8%)	28.1 ± 3.7	22.7 ± 2.5	2.14 ± 1.3	17.8 ± 9.2	0.35 ± 0.21	0.52 ± 0.25	11.1 ± 7.0
PCOS obese (BMI ⩾27), n = 70 (70/155) (45.2%)	29.6 ± 4.8	32.4 ± 4.6	2.08 ± 1.0	28.7 ± 12.7	0.21 ± 0.1	0.77 ± 0.22	12.1 ± 7.9
Normal lean (BMI <27), n = 78 (78/100) (78%)	29.2 ± 4.3	23.2 ± 1.8	1.16 ± 0.3	9.8 ± 4.1	0.55 ± 0.31	0.26 ± 0.2	7.17 ± 1.9
Normal obese (BMI ⩾27), n = 22 (22/100) (22%)	31 ± 4.0	29.1 ± 2.2	1.09 ± 0.29	12.2 ± 5.0	0.45 ± 0.19	0.37 ± 0.18	8.25 ± 2.3
Statistical significance (P)							
PCOS lean versus PCOS obese	NS	< 0.001	NS	< 0.001	< 0.001	< 0.001	NS
PCOS lean versus normal lean	NS	NS	0.01	< 0.001	< 0.01	< 0.001	0.02
PCOS obese versus normal obese	NS	0.014	0.001	< 0.001	< 0.001	< 0.001	0.02

PCOS = polycystic ovary syndrome; LogHOMA = logarithmic transformation of the homeostasis model analysis index of insulin resistance.

obese PCOS, as opposed to normal ovary subjects (11.1 and 12.1 versus 7.17 and 8.25 µmol/l for lean and obese PCOS versus lean and obese controls respectively) (Table II).

The 95th percentile for homocysteine in our control group was 11.0 µmol/l: the normal range is stated to be 5–11 µmol/l (Bar-On *et al*., 2000; Chambers *et al*., 2001). When a level of 11.0 µmol/l was used as a threshold, 34.1% (53/155) of the PCOS patients had Hcy values >95th percentile of the controls ($P < 0.0001$) (Table III). Of the 53 NIR-PCOS patients, only 13 (24.5%) had an elevated (>11 µmol/l) Hcy level, whereas 43 of the 102 (42%) IR-PCOS patients had an elevated Hcy level ($\chi^2 = 4.69$, $P = 0.04$) (Table III), demonstrating the differential effect of IR on homocysteine.

Comparing lean normal ovary controls with lean PCOS patients, these two groups were found to have insignificantly different BMI, but highly significant differences in IR and homocysteine levels. Lean PCOS patients had significantly higher homocysteine levels than lean controls, 11.1 ± 7.0 versus 7.17 ± 1.9 µmol/l, $P = 0.02$) (Table II), thus, body weight was not found to be predictive of Hcy, rather, IR regardless of body weight was correlated with Hcy levels.

Correlation between variables

Correlation between clinical or biochemical criteria and homocysteine was examined on a paired-data basis. No correlation was found between age and PCOS, age and IR, or age and homocysteine levels. No correlation between LH or LH:FSH ratios and IR, or homocysteine levels, was found. However, all indices of IR (insulin alone, glucose:insulin ratio, HOMA index, and logHOMA) were significantly correlated to homocysteine levels. Figure 1 shows paired-data analysis of the correlation between homocysteine levels and the logarithmic transformation of the HOMA index in all patient groups: $r = 0.30$, $R^2 = 0.091$, $P = 0.005$ (ANOVA linear regression: mean

Table III. Clinical and biochemical data for all patients using normal[a] homocysteine (Hcy) as a threshold

Group (n = 255)	N (%)	BMI (kg/m^2)	LH/FSH	Insulin (mIU/l)	Glucose:insulin ratio	LogHOMA	Homocysteine (µmol/l)
Hcy <11.0[a], normal ovaries	n = 96 (96%)	24.5 ± 3.1	1.14 ± 0.3	10.5 ± 4.4	0.52 ± 0.29	0.28 ± 0.2	7.16 ± 1.7
Hcy <11.0, PCOS	n = 102 (65.8%)	27.1 ± 5.8	2.02 ± 1.02	21.4 ± 12.2	0.32 ± 0.2	0.6 ± 0.28	8.36 ± 1.7
Hcy ⩾11.0, normal ovaries	n = 4 (4%)	23.1 ± 3.4	1.15 ± 0.05	7.45 ± 2.2	0.65 ± 0.2	0.16 ± 0.12	13.3 ± 1.7
Hcy ⩾11.0, PCOS	n = 53 (34.1%)	27.2 ± 6.5	2.31 ± 1.39	25.1 ± 10.8	0.24 ± 0.12	0.7 ± 0.22	17.5 ± 10.1
P-value		NS	NS	0.001	0.004	0.001	< 0.0001

[a]Homocysteine levels of 11 ìmol/l were the 95th percentile of the normal control group.
PCOS-NIR group, n = 53, Hcy <11.0 = 40/53, Hcy ⩾11.0 = 13/53.
PCOS-IR group, n = 102, Hcy <11.0 = 59/102, Hcy ⩾11.0 = 43/102, $P = 0.04$, $\chi^2 = 4.69$.
BMI = body mass index; LogHOMA = logarithmic transformation of the homeostasis model analysis index of insulin resistance; PCOS = polycystic ovary patients; IR = insulin-resistant; NIR = non-insulin-resistant.

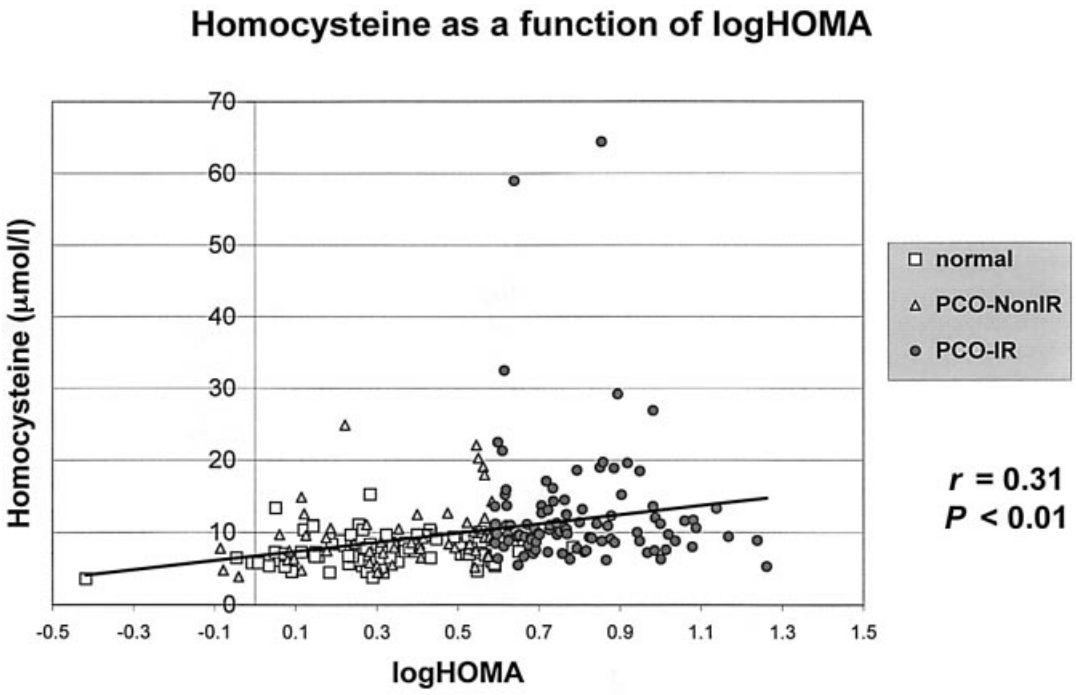

Figure 1. Plasma homocysteine as a function of the logarithmic transformation of the HOMA index (homeostasis model assessment of insulin resistance). Each point represents paired data from one patient. Normal = normal ovary controls; PCO-NonIR = non-insulin-resistant polycystic ovaries; PCO-IR = insulin-resistant polycystic ovaries.

square = 0.71, F = 7.98). All other IR indices, i.e. fasting insulin, HOMA index and glucose:insulin ratio were also similarly significantly correlated on single (paired) regression analysis with Hcy levels (r = 0.26, r = 0.24, r = –0.29, all $P <$ 0.01 respectively).

Multiple regression testing was carried out to examine the relative contribution of the tested variables (age, BMI, LH/FSH, insulin, glucose, glucose:insulin ratio and logHOMA) on homocysteine levels in all patients—normal and PCOS included. Stepwise linear regression analysis was carried out with homocysteine as the dependent variable, resulting in a multiple R of 0.33, with a mean square for the regression of 931.6, F = 26.0, $P <$ 0.0001. Homocysteine levels were significantly affected by logHOMA (T = 5.1, $P <$ 0.0001), with HOMA and insulin following (T = 1.74, P = 0.08, T = 1.70, P = 0.09 respectively), whereas the PCOS state or BMI were not statistically significant independent factors affecting homocysteine levels.

Discussion

Infertility associated with PCOS has been attributed to numerous factors, including oligo-anovulation, dysfunctional gonadotrophin secretion, elevated systemic and/or local ovarian androgen levels, and dysfunction of any or several ovarian growth factors and their binding proteins. Recently, research has focused on systemic and local effects of IR and its secondary effects—systemic, metabolic and ovarian.

Evidence has accrued to indicate that hyperinsulinaemia and/or any or all of the phenotypes of the IR syndromes in the general population may have various deleterious metabolic effects, including causing an increase of plasma Hcy (Meigs *et al*., 2001). We examined this association in our specific population of patients with PCOS and infertility, by determining the correlation between IR and elevated Hcy. IR was found to be extremely common in this patient group, regardless of the defining criteria for IR, and was practically universal in the obese PCOS subgroup. Three previous studies have found an association between IR and elevated homocysteine in specific patient groups in women of reproductive age. Laivuori *et al*. (1999) examined the association between IR and elevated Hcy in pregnant women with pre-eclampsia. Elevated Hcy was related to IR in women with pre-eclampsia, but not in normal controls. It is of interest to note that, as in our study, elevated Hcy was best correlated to fasting insulin (single sample) and not to insulin and/or glucose increase after glucose loading. It is possible that the study group included patients with PCOS, although this was not specified. Another study (Yarali *et al*., 2000, 2001) looked at the cardiac function of PCOS patients, as a function of IR and Hcy levels. Hcy was significantly elevated in both lean and obese PCOS patients, and was related to IR and not necessarily to body weight. As in our study, Hcy levels were even higher in lean PCOS than obese PCOS patients, and to a degree highly similar to that found in our study (lean PCOS: 12.6 ± 5.6 μmol/l; obese PCOS: 10.0 ± 1.3 μmol/l; control: 8.7 ± 2.7 μmol/l). Sills *et al*. (2001) did not find any association between the finding of PCO and plasma Hcy and insulin levels were insignificantly higher with polycystic ovaries. This study differentiated between patients on the basis of ultrasound morphology only, not incorporating other components of the syndrome.

Homocysteine levels are positively correlated to risk of cardiovascular disease and complications, by increasing oxidative stress in vascular endothelium, activation of platelets (Stamler *et al*., 1993; Coppola *et al*., 2000; Mujumdar *et al*., 2002), impairment of blood flow (Chambers *et al*., 2001), stimulation of vascular smooth muscle proliferation (Van Guldener and Stehouwer, 2000) and may be one of the signals inducing apoptosis in vascular endothelial cells by activating unfolded protein response (Zhang, 2001). Thus, elevated Hcy may impair implantation by interfering with endometrial blood flow and vascular integrity, and has been documented to increase the probability of early pregnancy loss (Nelen *et al*., 2000; Quere *et al*., 2001). Both impaired implantation and increased rates of miscarriage are more frequent in PCOS, even after controlling for ovulatory abnormalities, increased LH, and hyperandrogenism, which might be due in part to elevated Hcy in these patients.

IR (or the IR syndromes, IRS) itself is a risk factor for cardiovascular disease, diabetes, hypertension nephropathy, and dyslipidaemia (Goldstein *et al*., 2001); all of the long-term implications of these facets of the 'metabolic syndromes' can be aggravated by elevated Hcy. Thus, in metabolic terms, PCOS may possibly be considered another variant of the IRS, or at the very least be considered an early 'marker' of the IRS. As such, the infertility treatment provider should actively search for signs of metabolic dysfunction, including elevated Hcy levels, and endeavour to optimize metabolic factors in order to achieve the best results in the short term (reproductive function) and in the long term (cardiovascular and metabolic functions).

Homocysteine levels are influenced by a number of variables which were not examined in this study, including smoking, renal function, vitamin B status and enzyme dysfunction states. All of the patients in our infertility practice are actively discouraged from smoking; the proportion of smokers among our patients was 9.0% (23/255), divided proportionately between study and control groups; these numbers did not allow for adequate statistical testing. Although renal status was not examined, all of these young women entered treatment in good general health and none had hypertension or oedema. Methyltetrahydrofolate reductase (MTHFR) enzyme deficiencies and vitamin levels were not screened in this patient group, as in the study by Laivuori *et al*. (1999) and Tsanadis *et al*. (2001). Vitamin B_{12} levels and folic acid levels were examined in the study by Yarali *et al*., and no significant differences were found between PCOS and controls. As the frequency of this mutation is in the order of 5–10% in unselected populations, it seems unlikely that MTHFR status could be responsible for the differences between homocysteine in PCOS and controls in this study, although this is currently being determined.

The vascular aspect of PCOS has already been implicated in previous studies on plasminogen activator inhibitor-1 (PAI-1), with its attendant effects on blood flow; the effects of elevated PAI-1 may also be aggravated by elevated homocysteine (Atiomo *et al*., 1998). Endothelial dysfunction in PCOS was

documented both by decreased response to vasodilation (Paradisi *et al*., 2001) and by the finding of increased levels of endothelin-1 in insulin-resistant PCOS patients (Diamanti-Kandarakis, 2001) and increased oxidative stress markers (Sabuncu *et al*., 2001). It is possible that these findings are due to—in part—to increased Hcy levels.

We suggest that the vascular–endothelial aspect of insulin-resistant PCOS—including the additional complicating factor of hyperhomocysteinaemia—has important diagnostic and treatment implications, and warrants further clinical and laboratory investigation. Furthermore, actively treating elevated homocysteine in these patients could increase implantation and decrease pregnancy loss. Prospective studies could address the practical implications of our findings in terms of improved treatment success.

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