Insulin Resistance Variability in Women with Anovulatory and Ovulatory Polycystic Ovary Syndrome, and Normal Controls

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Key words
- ovulation
- insulin resistance
- polycystic ovary syndrome
- cardiovascular risk

Abstract

Women with polycystic ovary syndrome (PCOS) were found to have a higher biological variability in insulin resistance (IR) compared to controls, but it is unknown whether this variability in IR differs between PCOS who are anovulatory compared to those who have an ovulatory cycle. The primary aim of this study was to compare and contrast the variability of IR in women with ovulatory and anovulatory PCOS, in comparison to normal subjects. 53 Caucasian women with PCOS and 22 normal ovulating women were recruited. Fasting blood was collected each day on 10 consecutive occasions at 3–4 day intervals for analysis of insulin, glucose, progesterone, and testosterone. Analysis of progesterone levels showed 22 of 53 women with PCOS to have had an ovulatory cycle. Insulin resistance was calculated by HOMA method. Women with anovulatory PCOS had higher mean and variability of IR compared to those having an ovulatory cycle, and both were significantly higher than controls (mean ± SEM; HOMA-IR 4.14 ± 0.14 vs. 3.65 ± 0.15 vs. 2.21 ± 0.16, respectively) after adjustment or BMI. The mean BMI for individual PCOS patients correlated with mean HOMA-IR ($p = 0.009$). Insulin resistance in women with anovulatory PCOS is both higher and more variable than in ovulatory PCOS. Since anovulatory PCOS therefore mimics the IR features of type 2 diabetes more closely, anovulation may be particularly associated with a higher cardiovascular risk compared to PCOS patients who ovulate.

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder with a prevalence of 6–7% of women in the reproductive age [1–4], though this may be an underestimate as it was based on criteria before the Rotterdam consensus. PCOS is characterised by chronic anovulatory infertility and hyperandrogenism with the clinical manifestation of oligomenorrhoea, hirsutism, and acne [5]. Insulin resistance (IR) and its biological variability have been shown to be increased in women with PCOS [6], but there has not been any studies on its variability. While variability in IR has not been proven to add to the risk already present in patients with high mean IR values, glucose variability has certainly been found to be associated with increased free radical damage independently of mean glucose in type 2 diabetes (T2DM) [7]. The introduction of Rotterdam criteria has created a new entity of ovulatory subgroups of women with PCOS and there had been suggestions that these groups have different cardiometabolic characteristics. We aim to study whether a difference exists between variability of IR among the 2 subgroups of ovulatory and anovulatory women with PCOS.

Patients and Methods

The data presented here are combination data from previous studies [8,9]. 53 Caucasian women with PCOS were recruited from the endocrinology clinic at Hull Royal Infirmary, United Kingdom. All subjects were diagnosed to have PCOS by the ESHRE/ASRM Rotterdam criteria [10], where all patients had evidence of biochemical hyperandrogenism and hirsutism (Ferriman-Gallwey score >8), chronic anovulation (cycle length <21 days or >35 days and <8 cycles per year), and polycystic ovaries on transvaginal ultrasound. Nonclassical 21-hydroxylase deficiency, hyperprolactinaemia, and androgen

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secreting tumours were excluded by appropriate tests before the diagnosis of PCOS was made. Diabetes was excluded in all subjects by a 75 g oral glucose tolerance test. No subjects were on oral contraceptive pills or taking any medications that could alter their insulin resistance at that time, or for the preceding 3 months before trial entry. 22 Caucasian women with normal menstrual cycle and no evidence of clinical or biochemical hyperandrogenism were recruited as controls. All subjects gave their informed written consent prior to entering the study that had been approved by the local research ethics committee.

Fasting venous blood was collected into serum gel tubes (Becton Dickinson, Cowley, Oxfordshire, UK) at the same time each day (08:00–09:00) on 10 consecutive occasions at 3–4 day intervals to include measurements over a period of 5 weeks that would have included a complete menstrual cycle. Ovulation was defined by a progesterone level greater than 20 nmol/l [11]. Samples were separated by centrifugation at 2 000 *g* for 15 min at 4 °C, and serum was stored at −20 °C within 1 h of collection to be batch analysed at the end of study. Plasma glucose was analysed within 4 h of collection.

Reagents

Serum insulin was assayed using a competitive chemiluminescent immunoassay, supplied by Euro/DPC, Llanberis, UK. The assay was performed on a DPC Immulite 2000 analyser (Euro/DPC, Llanberis, UK), using the manufacturer's recommended protocol. There was no stated cross-reactivity with proinsulin. Plasma glucose was measured using a Synchron LX 20 analyser (Beckman-Coulter, High Wycombe, UK), using the manufacturer's recommended protocol. The coefficient of variation for this assay was 1.2 % at a mean glucose value of 5.3 mmol/l (94.6 mg/dl). The IR was calculated using the homeostasis model assessment (HOMA) method where HOMA-IR = (insulin × glucose)/22.5 [12]. Serum testosterone was measured by isotope dilution liquid chromatography-tandem mass spectrometry (Waters Corporation, Manchester, UK) and SHBG was measured by immunometric assay with fluorescence detection on the DPC Immulite 2000 analyser using the manufacturer's recommended protocol. The free androgen index was obtained as the quotient 100 T/SHBG. Before analysis, all the serum samples were thawed and thoroughly mixed.

Statistical analysis

Statistical analysis was performed using SPSS for Windows, version 14.0. In data where distribution between individuals violated the assumptions of normality when tested using the Kolmogorov-Smirnov test, differences were calculated by Mann-Whitney U method. These parameters include HOMA-IR and insulin. Correlation was calculated by Pearson's method and non-Gaussian data was log transformed prior to analysis. Biovariability data was analysed by calculating analytical, within subject, and between subject variances (SD_A^2, SD_I^2, SD_G^2, respectively) according to the methods of Fraser and co-workers [13, 14]. Using this technique, analytical variance (SD_A^2) was calculated from the difference between duplicate results for each specimen ($SD_A^2 = \Sigma d^2/2N$, where d is the difference between duplicates, and N is the number of paired results). The variance of the first set of duplicate results for each subject on the 10 assessment days was used to calculate the average biological intra-individual variance (SD_I^2) by subtraction of SD_A^2 from the observed dispersion. For all analysis, a 2-tailed $p < 0.05$ was considered to indicate statistical significance.

Results

Analysis of progesterone levels showed 22 of 53 women with PCOS to have had an ovulatory cycle (42 %). We defined an ovulatory cycle as the presence of a progesterone surge of above 20 nmol/ within the 5 weeks of study period [11]. Biochemical parameters of patients are shown in **Table 1** and all data presented as mean ± SEM for consistency. BMI and waist circumference were nonsignificantly different between the ovulatory and anovulatory groups. One outlier (patient 19) from the ovulatory group was excluded from calculations as the HOMA-IR was above 2 standard deviations from the overall mean in the group. Women with anovulatory PCOS were found to be significantly more insulin resistant compared to ovulatory PCOS, and both were significantly higher than controls (mean ± SEM; HOMA-IR 4.14 ± 0.14 vs. 3.65 ± 0.15 vs. 2.21 ± 0.16, respectively) after adjustment or BMI. This was reflected in a significantly lower SHBG in the anovulatory compared to the ovulatory group, and both were lower than controls. The biological variability of IR

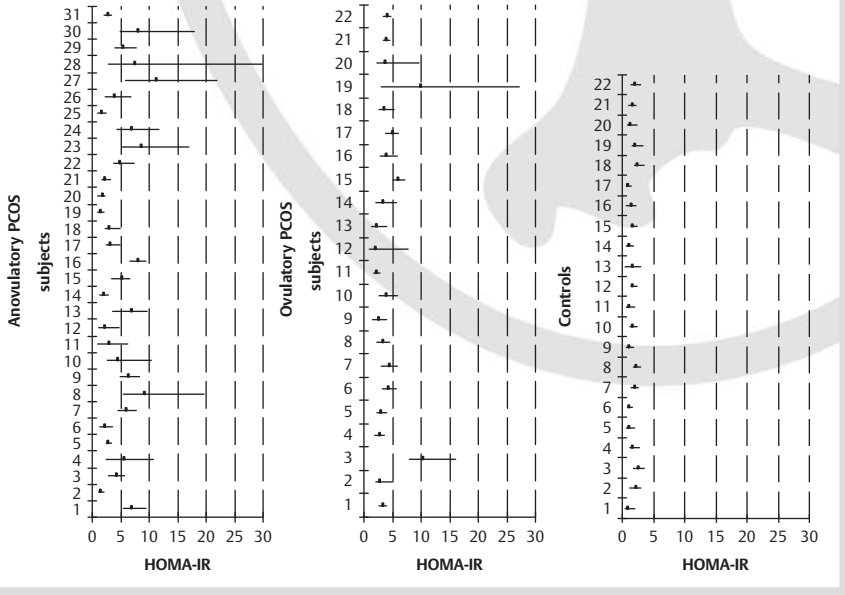

Fig. 1 Mean and range of HOMA-IR in subjects with anovulatory PCOS, ovulatory PCOS, and controls.

Table 1 Biochemical parameters of subjects with anovulatory PCOS, ovulatory PCOS, and controls

	Anovulatory PCOS (n = 31)	Ovulatory PCOS (n = 21)	Controls (n = 22)	p-Value for anovulatory vs. ovulatory PCOS	p-Value for anovulatory PCOS vs. controls	p-Value for ovulatory PCOS vs. controls
Insulin (μU/ml)	21.3 ± 0.7	17.6 ± 0.5	7.8 ± 0.2	0.12	< 0.01	< 0.01
HOMA-IR*	4.14 ± 0.14	3.65 ± 0.15	2.21 ± 0.16	< 0.01	< 0.01	< 0.01
BV HOMA	5.9 ± 4.8	1.0 ± 0.4	0.2 ± 0.02	0.01	0.01	0.03
SHBG (nmol/l) (range 35–100)	24.6 ± 0.7	26.9 ± 0.5	59.5 ± 1.8	< 0.01	< 0.01	< 0.01
Glucose (mmol/l)	4.9 ± 0.02	4.9 ± 0.03	4.7 ± 0.02	0.25	0.01	0.01
BMI (kg/m^3)	34.3 ± 1.3	33.7 ± 1.1	29.5 ± 1.2	0.96	< 0.01	< 0.01
Waist circumference (cm)	106.6 ± 2.7	105.0 ± 3.4	81.8 ± 6.5	0.72	0.02	0.03
Hs-CRP (mg/l)	7.19 ± 0.56	4.56 ± 0.37	not done	0.14	NA	NA
Total cholesterol (mmol/l)	4.77 ± 0.06	5.09 ± 0.08	not done	< 0.01	NA	NA
FAI (%) (range 0–3.0)	8.25 ± 0.34	5.84 ± 0.19	1.91 ± 0.10	< 0.01	< 0.01	< 0.01
Total testosterone (nmol/l)	1.69 ± 0.05	1.47 ± 0.04	1.05 ± 0.04	0.02	< 0.01	< 0.01
Age (years)	26.3 ± 1.3	27.0 ± 1.6	35.8 ± 1.1	0.71	< 0.01	< 0.01
Progesterone (nmol/l)	1.3 ± 0.1	7.6 ± 0.8	11.6 ± 1.1	< 0.01	< 0.01	< 0.01

Data are presented as mean ± SEM and local laboratory reference range is given in brackets. All serum results are obtained from fasting variables. BV HOMA: biological variation of HOMA; NA: not applicable

To convert values for insulin to picomoles per liter, multiply by 6. To convert values for SHBG to micrograms per deciliter, divide by 34.7

* HOMA values adjusted for BMI

was greater in anovulatory PCOS compared to ovulatory PCOS and both were higher than that in controls (**Fig. 1**). The mean BMI for individual PCOS patients correlated with mean HOMA-IR ($p = 0.009$). Mean progesterone is significantly higher in controls compared to ovulatory PCOS and both higher than anovulatory PCOS (**Table 1**).

Discussion

Disturbances in insulin efficacy and insulin secretion are major features of the metabolic syndrome and might precede the development of diabetes and atherosclerosis by decades [15]. This may present initially with endothelial dysfunction mainly through chronic low grade inflammation via actions of TNF-α that induces production of IL-6 [16]. IL-6 inhibits liproprotein lipase activity, enhances aromatase activity, and increases the hepatic production of triglycerides [17]. IL-6 is stimulated by TNF-α, and TNF-α further stimulates C-reactive protein that has been found to be correlated with obesity, insulin resistance, endothelial dysfunction, and therefore is a cardiovascular risk. Level of IL-6 had been found to be higher in obese women with PCOS[18]. Type 2 diabetes subjects exhibit significantly higher expression levels of visfatin/PBEF/NAMpt in monocytes compared to control that is suggested to contribute to the increased risk for cardiovascular disease [19]. Visfatin is also increased in women with PCOS [20]. However, the role of resistin in patients with PCOS and thus cardiovascular risk in this group is still debatable. In one study, serum resistin level in patients with PCOS were no different to matched controls but resistin mRNA levels were 2-fold higher in omental adipocytes from PCOS patients [21]. Others have reported that there is an increase in serum resistin level in patients with PCOS [22], although it has been suggested that this increase is dependent on BMI [23]. Against this argument is that in BMI matched patients, serum resistin was found not to be different compared to controls [24,25]. Women with PCOS were also found to have higher advanced glycation end products (AGEs), an oxidative stress marker, compared with women having the isolated characteristics of the syndrome [26]. When assessing whether PCOS is associated with increased cardiovascular risk, we currently lack end point studies and the 2 available at present showed conflicting results. Longitudinal data from the Nurses Health Study for subjects followed through for cardiovascular events found that a history of menstrual cycle irregularity was associated with an increased risk of nonfatal and fatal coronary heart disease [27], whereas, another study showed that a history of coronary heart disease was not significantly more common in women with PCOS [28]. It is possible that the differences observed may be accountable to the heterogeneity of the different ovulatory subgroups of patients with PCOS. To date, there is limited understanding of the relative prevalence of risk factors across the different reproductive phenotypes of PCOS and few studies have adequately examined the nature history of metabolic disorders in the different ovulatory phenotypes of PCOS. Clarification of this will enable better stratification of patients. Our group of women with ovulatory PCOS represents a different subgroup of patients to the most commonly reported group who have normal menses, as our patients have menstrual irregularities and obesity.

We have previously shown that women with PCOS have a higher and more variable IR compared to controls [9]. It was also shown that the classical hyperandrogenic PCOS is more insulin resistant than control group [29]. This is the first study to compare the biological variability of IR in anovulatory and ovulatory women with PCOS and demonstrated that women with anovulatory PCOS have a higher biological variability compared to ovulatory PCOS, and both were higher than normal menstruating women. While biological variability in IR has not been proven to add to the risk already present in patients with high mean IR values, glucose variability has recently been found to be associated with increased risk of free radical damage, independent of mean glucose in patients with type 2 diabetes [7]. It remained possible that an increase in biological variability of IR may be associated to or in fact is an independent risk for cardiovascular risk. Whether the association between a high biological variability in IR is the cause or effect of the state of anovulation remains undetermined. However, if it is true that the difference in biological variability of IR is the key to the differences in the ovulatory status in this group of clinically oligo-ovulatory woman with PCOS, then this would be a target of therapeutic modification. Further research is necessary to confirm this postulation.

Several reports have shown that ovulatory PCOS patients have a milder form of PCOS that include a milder insulin resistance [30–32]. Our data showed that 42% of women recruited with PCOS had an ovulatory cycle at the time of study. Women with anovulatory PCOS had higher mean and biological variability of IR compared to those having an ovulatory cycle, and both were higher than women without PCOS. However, it is unknown whether this is a transient feature associated with an ovulatory cycle or a feature of this group of ovulatory patients. As there were no differences between the numbers of menses between groups, it suggests that the former is more likely. Should this be true then it would also suggest that women with PCOS with a longer duration of anovulation may have a higher cardiovascular risk. The variation in the spectrum of risk depending on the frequency of ovulatory cycles may help explain why the data on cardiovascular events in PCOS remains inconclusive as this variable has not been accounted for previously.

Carmina et al. have reported that ovulatory women with hyperandrogenaemia exhibit a mild insulin resistance and altered lipid profile, similar to that of patients with classic (anovulatory) PCOS [33]. The same group suggested that women with anovulatory PCOS were more insulin resistant [34] and had a higher central fat distribution compared to ovulatory and matched controls, which was associated with a lower level of adiponectin [35]. Others reported that ovulatory women with PCOS have lower levels of progesterone in the early luteal phase and that may have resulted in reduced fertility [36]. Diamanti-Kandarakis et al. reported that anti-Mullerian hormone (AMH) values were statistically significantly higher in women with anovulatory PCOS compared to ovulatory PCOS and these were significantly higher than anovulatory women without PCOS and controls. A significant correlation between AMH and advanced glycosylated end products (AGEs), an oxidative marker was observed suggesting that AGEs, and AMH, may interact in the anovulatory mechanisms in women with PCOS [37]. These studies are difficult to compare with our findings as they used subjects with ovulatory PCOS who clinically had a normal menstrual cycle that differed to our study where our subjects had clinical oligomenorrhoea.

Our data showed no significant difference in BMI and waist circumference between the anovulatory and ovulatory groups, but HOMA-IR and its biological variability were significantly different between the 2 groups. This suggests that the difference observed were unlikely to be due to obesity but rather the ovulatory state of the subjects.

In conclusion, the mean and biological variability of IR were higher in women with anovulatory PCOS compared to ovulatory PCOS, and both were higher than controls. If variability of IR is related to the risk of cardiovascular disease then it suggests that those PCOS patients who are anovulatory may be at particularly high risk compared to those who do ovulate.

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