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β-Cell Glucose Sensitivity determines hyperglycaemia in Polycystic Ovary Syndrome

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ABSTRACT

Aims: To investigate the mechanism of hyperglycaemia in women with Polycystic Ovary Syndrome (PCOS) by modelling physiological insulin secretion.
Methods: 45 non-diabetic women with PCOS (defined by Rotterdam criteria) and 47 controls were studied. Insulin secretion was modelled from glucose and C-peptide concentrations during a 6-point oral glucose tolerance test (OGTT) and insulin resistance (IR) was determined by the Matsuda Insulin Sensitivity Index (ISI).
Results: (1). β-Cell Glucose Sensitivity (βCGS) is not intrinsically impaired in PCOS, but IR is increased. (2) However, women with PCOS and 2-hour hyperglycaemia (glucose >7.5 mmol/l]) were characterized by worse βCGS compared with women with 2-hour glucose <7.5 mmol/l (mean [SD]) 43.5 [23.6] versus 109.0 [68.5] pmol/min/ml/mmol; $p = 0.04$), and had higher waist circumference (116.8 [15.8] vs 93.5 [15.9] cm; $p < 0.01$) and 120 minute insulin concentrations (964.0 [579.2–1214.7] vs 328.2 [242.2–475.7] pmol/l; $p = 0.01$). (3). In contrast, lean, insulin sensitive women with PCOS were euglycemic, even in the presence of poor βCGS, and exhibited favourable cardiometabolic risk profiles.
Conclusions: βCGS is not intrinsically impaired in PCOS, but it is the critical determinant of hyperglycaemia. Women with low βCGS, who also have central adiposity and IR exhibit 2-hour hyperglycaemia, whereas women with high βCGS are able to maintain euglycaemia despite central adiposity and IR. These findings show that (i) waist circumference and 2-hour glucose identify women at higher risk of diabetes, and confirm that (ii) obesity and IR are the key reversible targets for diabetes prevention in women with PCOS.

Introduction

Polycystic Ovary Syndrome (PCOS) continues to be a relatively neglected and less well understood risk factor for type 2 diabetes (T2D). Although guidelines advocate diabetes screening in women with PCOS,[1] the mechanism of hyperglycaemia and the identification of women at risk remains ill-defined. There continues to be controversy over how best to screen for prediabetes and diabetes in women with PCOS, and there is often continuing reluctance to perform glucose tolerance tests.

Pancreatic β cell dysfunction, obesity and insulin resistance (IR) are the fundamental problems leading to T2D.[2,3] Dunaif

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demonstrated underlying IR in PCOS,[4] and several studies have suggested insulin secretory abnormalities.[5–8] However, the insulin secretory defect in PCOS remains ill-defined because of confounding effects of obesity and important methodological limitations. Previous studies have used non-physiological parenteral methods, such as the Frequently Sampled Intravenous Glucose Tolerance Test with intravenous boluses of glucose and tolbutamide[5], the hyperinsulinaemic euglycaemic clamp technique,[6,7] the Frequently Sampled Intravenous Glucose Tolerance Test with intravenous boluses of glucose, and intravenous infusions of insulin followed by graded intravenous infusion of glucose, and oscillatory intravenous infusion of glucose.[8] Although these methods have been widely used in the literature, an important limitation is that they are unphysiological, in particular because they do not elicit the physiological incretin response to oral glucose, whereas computer modelling of endogenous insulin secretion overcomes this drawback.[9]

We used modelling of insulin secretion, a method not previously applied in PCOS, to investigate whether β Cell Glucose Sensitivity (βCGS) is fundamentally impaired. The results demonstrate the interplay between insulin secretion and action in PCOS, and identify women who are at risk of diabetes. The results also suggest why the glucose tolerance test is probably the best method to screen for prediabetes and diabetes in women with PCOS.

Materials and methods

Participants

The study was approved by the National Health Service research ethics committee (Devon and Cornwall REC, reference 09/HO203/63). Participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki. Women aged 18–46 years with PCOS were recruited from primary and secondary care. The diagnosis of PCOS was based upon the presence of oligo/amenorrhoea (cycle >35 days) and clinical or biochemical hyperandrogenism (in accordance with both Rotterdam[10] and NIH[11] criteria), after excluding relevant differential diagnoses. Non-classic 21-hydroxylase deficiency, hypothyroidism and hyperprolactinemia were excluded by measurement of 17α-hydroxyprogesterone, thyrotrophin and prolactin. Healthy controls were recruited by advertisement, and PCOS was excluded. Other exclusion criteria were diabetes, pregnancy, breastfeeding, current treatment with clomiphene, any pituitary or other endocrine diseases, menopausal status, and any intestinal condition that could affect glucose absorbtion. Women receiving estrogen-containing medication were not excluded but this potential confounder was considered in the analysis. There was no significant difference between the groups in the use of combined oral contraceptives and progestogen–only contraceptive medication.

No participants had been treated with antiandrogens or aromatase inhibitors. Women receiving metformin treatment (for PCOS) were eligible subject to a 3-month washout period. Three such women with PCOS were included after washout of metformin.

Sample size and design

We examined the hypothesis that increased 2-hour glucose concentrations in PCOS would be associated with both impaired βCGS and IR, based on other findings.[12] Therefore the ability to detect differences in both IR and βCGS was important. Using HOMA-R data from Lord et al, 2006[13] for PCOS and Cascella et al, 2008[14] for controls, a mean difference of approximately 1.5 HOMA-R units was expected. Thus, 43 participants per group provided >95% power at the 5% significance level, and 50 per group allowed for 14% dropouts. Although there were no previous βCGS data in PCOS, other studies[12,15] suggested two groups of 50 would detect important differences in βCGS. To determine the influence of adiposity on IR and βCGS, women with PCOS were purposively stratified for body mass index (BMI) (18.5–24.9; 25–29.9; 30–34.9; >35 kg/m^2), with 12–13 women in each category. Controls were matched for BMI category and age. After confirmation of classification and withdrawals, complete data were available for 45 women with PCOS and 47 controls.

Clinical methods

Women attended at 08:00 after an overnight fast from 22.00 hours. Weight, height and BMI were recorded.[16] Waist circumference was measured at the umbilicus by a single observer. Blood pressure was recorded in triplicate using a manual sphygmomanometer after subjects were recumbent for 5 minutes. Clinical hyperandrogenism was defined by modified Ferriman-Gallway score[17] >6 and oligomenorrhoea as menstrual cycle >35 days. Body composition was determined by electrical bioimpedance (Tanita BC-418MA) and visceral fat by regional bioimpedance (Tanita ViScan AB-140) (Tanita Corporation, Japan). A 2-hour, 75 g oral glucose tolerance test (OGTT) was performed, with venous blood sampling at 0, 15, 30, 60, 90 and 120 minutes. Samples for insulin and C-peptide were immediately centrifuged at 3000 rpm at 4 °C for 5 minutes, separated and frozen at −80° until measurement.

Biochemical methods

Insulin was measured using the ADVIA Centaur immunoassay system (Siemens Healthcare Diagnostics, Frimley, UK) [limit of detection (LoD) 69.81 pmol/l, coefficient of variation (CV) < 3.3%] and C-peptide by chemiluminescent immunometric assay (Siemens Healthcare Diagnostics, Camberley, UK) [LoD 33.0 pmol/l, CV<3.6%]. Glucose was measured by the glucose oxidase method. Testosterone was measured by competitive immunoassay (Roche Diagnostics, Switzerland) standardised against isotope dilution-gas chromatography/mass spectrometry [LoD 0.10 nmol/l, CV 4.84% at testosterone concentration 1.7 nmol/l - the laboratory upper reference limit, and limit of quantitation (lowest concentration measureable with CV <20%) 0.40 nmol/l]. Biochemical hyperandrogenism was defined as total testosterone >1.7 nmol/l (laboratory upper limit of normal). Sex hormone binding globulin (SHBG) was measured by double antibody immunoassay (Roche Diagnostics, Switzerland), and free testosterone calculated.[18]

Assessment of β-Cell function and insulin sensitivity

The model used to reconstruct insulin secretion and its control by glucose has been described.[19] This model consists of three blocks: 1) a model for fitting the glucose concentration profile; 2) a model of C-peptide kinetics, *i.e.* the two-exponential model of Van Cauter et al,[20] in which model parameters are individually based on the subject's anthropometric data; and 3) a model describing the dependence of insulin secretion on glucose concentration. The model calculates basal and glucose-dependent insulin secretion rates (ISR). The first insulin secretion component is represented by a glucose-insulin dose response function. The characteristic parameter of the dose response is its mean slope in the individual glucose range, which is denoted as βCGS. The dose response of insulin to glucose is additionally modulated by non-glucose-mediated factors (*i.e.* non-glucose substrates, gastrointestinal hormones and other neurotransmitters), and these are collectively described by the potentiation factor ratio. The second insulin secretion component represents a dynamic dependence of insulin

secretion on the rate of change of glucose concentration, and this is denoted by rate sensitivity (RS). Insulin clearance was calculated as the ratio of the insulin secretion rate area-under-curve (AUC)/insulin concentration AUC,[21] and Insulin Sensitivity Index (ISI) was calculated from the OGTT using the method of Matsuda and DeFronzo.[22] These methods are listed in Table 1.

Statistical methods

Data were anonymised, independently cleaned, double-entered, and analysed using SPSS version 21 (IBM, USA). Parametric methods were used throughout and non-parametric data were logarithmically transformed as appropriate. Significance was defined as $2\alpha < 0.05$.

Results

Characteristics of women

The two groups were closely matched for BMI (mean [SD] PCOS 28.5 [5.8] vs. controls 29.4 [7.6] kg/m^2; p = 0.51). There was a borderline difference in age, but none in fat distribution or cardiometabolic parameters (Table 2). Hormonal contraception was used by 48% of women with PCOS and 43% of controls (chi square test; p = 0.67).

β-Cell Glucose Sensitivity

There were no differences in β-cell parameters between the groups (Table 3). When analysis was restricted to women not using hormonal contraception there were also no differences (p = 0.27–0.81) and therefore analysis included all women. Glucose-dependent insulin secretion was identical in women with and without PCOS (Fig. 1a). There was also no relationship between βCGS and BMI (Fig. 1b). However, women with higher 2-hour glucose levels generally had lower βCGS (Fig. 1c). In women with PCOS it was observed that 2-hour glucose >7.5 mmol/l was associated with mean significantly impaired mean (SD) βCGS at 43.5 (23.6) pmol/min/ml/mmol versus 109.0 (68.5) in women with 2-hour glucose <7.5 mmol/l (p = 0.04). Furthermore, compared to women with 2-hour glucose <7.5 mmol/l, these women had higher BMI (35.7 [9.8] vs. 27.9 [5.9] kg/m^2; p < 0.01), % body fat (43.3 [6.9] vs. 34.9 [7.9] %; p = 0.02), waist circumference (116.8 [15.8] vs. 93.5 [15.9] cm; p < 0.01) and visceral fat (14.6 [4.2] vs. 10.3 [4.6] a.u.; p = 0.05). Although ISI tended to be lower in women with 2-hour glucose >7.5 mmol/l compared with <7.5 mmol/l this fell short of significance (0.73 [0.72] vs. 0.97 [0.56]; p = 0.15), although insulin at 120 minutes was substantially elevated 964.0 [579.2–1214.7] vs. 328.2 [242.2–475.7] pmol/l (p = 0.01). Since there was no difference in insulin clearance (0.60 [0.27] vs. 0.67 [0.21] l/min/m^2; p = 0.18), the elevated insulin concentration indicated greater IR in women with PCOS and 2-hour glucose >7.5 mmol/l.

When analysis was restricted to women with PCOS in the lowest quartile of βCGS, 2-hour glucose was strongly associated with adiposity (BMI r = 0.63; p = 0.05; % body fat r = 0.73, p = 0.02) and especially central adiposity (waist circumference r = 0.81, p = 0.004; visceral fat r = 0.84; p = 0.002). ISI and insulin at 120 minutes also correlated with 2-hour glucose (r = −0.68; p = 0.06 and r = 0.95; p < 0.001).

Insulin Sensitivity Index

ISI tended to be lower in PCOS (Table 3). Since there were no differences in ISI between users and non-users of hormonal contraception all women were included in this analysis. In analysis of covariance, the overwhelming influence on ISI was BMI (F = 26.3; p < 0.001) whereas PCOS or control status made no difference (F = 0.04; p = 0.84). The influence of BMI on ISI is shown in Fig. 1d. In ANOVA, ISI was higher in controls with BMI <25 compared with BMI 25–29.9, 30–34.9 and > 35 kg/m^2

Table 2
Descriptive characteristics of women with PCOS and BMI-matched controls.

	PCOS (n = 45)	Control (n = 47)	P
Age [years]	32.3 (6.8)	35.3 (7.2)	0.04*
Ethnicity			
white	43	47	0.24
mixed race	2	0	
Current smoker			
yes	10 (22.2%)	8 (17.0%)	0.53
no	35 (77.8%)	39 (83.0%)	
BMI [kg/m^2]	28.2 (5.9)	29.8 (7.5)	0.27
Hormonal contraceptive use			
Combined contraceptive	15/45	8/47	0.24
Progestogen-only contraceptive	5/45	10/47	
No hormonal contraceptive	20/45	29/47	
Waist circumference [cm]	95.3 (16.8)	98.9 (17.8)	0.33
Body fat (%)	35.5 (7.9)	38.5 (8.3)	0.10
Visceral fat (a.u.)	10.6 (4.7)	11.3 (4.7)	0.52
Systolic Blood Pressure (mm Hg)	109 (100–118)	110 (100–120)	0.49
Diastolic Blood Pressure (mm Hg)	68 (60–77)	70 (61–79)	0.72
HbA1c (%)	5.3 (0.4)	5.3 (0.4)	0.32
HbA1c (mmol/mol)	34 (5)	34 (5)	
Fasting Plasma Glucose (mmol/l)	4.88 (0.52)	4.88.0 (0.27)	0.95
Glucose Tolerance			
normal	42	46	0.35
impaired	3	1	
HDL cholesterol (mmol/l))	58.0 (18.9)	63.4 (17.4)	0.17
Triglyceride (mmol/l)	1.10 (0.55–1.60)	0.80 (0.60–1.00)	0.24
C-Reactive Protein (mg/l)	1.85 (0.25–3.50)	1.70 (0.15–3.25)	0.89
Testosterone (nmol/l)	1.36 (0.72)	1.05 (0.52)	0.02*

Data are mean (SD) or median (IQR). a.u. = arbitrary units. Statistics are for T-Test or Mann–Whitney test, and Chi square or Fisher' exact test as appropriate. Significance * was p < 0.05.

Table 1
Methods used to describe β cell function, insulin sensitivity and insulin clearance.

Parameter	Definition	Reference
Basal insulin secretion	Fasting insulin secretion rate	19
Insulin secretion at 5 mmol/l glucose	Fasting insulin secretion rate standardised to glucose concentration of 5 mmol/l	19
Insulin secretion at 5 mmol/l glucose adjusted for basal potentiation	Fasting insulin secretion rate standardised to glucose concentration of 5 mmol/l and adjusted for non-glucose factors	19
B cell glucose sensitivity	Gradient of insulin secretion rate versus glucose concentration	19
Rate sensitivity	Dependence of insulin secretion on the rate of change of glucose concentration	19
Potentiation Factor Ratio	Potentiation of insulin secretion by non-glucose-substrates including incretins and neurotransmitters	19
Insulin Clearance Rate	Calculated as AUC of the insulin secretion rate/AUC of Insulin concentration	21
Matsuda Insulin Sensitivity Index	$10{,}000/(\sqrt{G_0 I_0}\ X\ (15.G_0+30.G_{30}+30.G_{90}+15.G_{120})/120)\ X\ (15.I_0+30.I_{30}+30.I_{60}+30.I_{90}+15.I_{120})/120)$	22

Table 3
Modeled indices of insulin secretion and Insulin Sensitivity Index in PCOS and controls.

	PCOS (n = 45)	Control (n = 47)	P
Basal Insulin Secretion Rate (pmol/min/m^2)	58.4 (39.9–74.7)	57.6 (46.0–94.2)	0.67
Insulin Secretion rate at 5 mmol/l glucose (pmol/min/m^2)	102.06 (82.9–162.2)	122.7 (85.8–167.3)	0.48
Insulin Secretion rate at 5 mmol/l glucose adjusted for basal potentiation (pmol/min/m^2)	65.01 (48.57–90.93)	69.73 (47.54–109.58)	0.51
β Cell Glucose Sensitivity (pmol/min/ml/mmol)	83.3 (62.2–131.9)	102.4 (64.1–145.8)	0.57
Rate Sensitivity (pmol/ml/mmol)	868.3 (179.6–1443.5)	687.6 (206.5–1595.9)	0.83
Potentiation Factor Ratio	1.22 (0.82–1.81)	1.27 (0.91–2.14)	0.44
Insulin Sensitivity Index	0.95 (0.57)	1.17 (0.79)	0.14

Data are mean (SD) or median (IQR). Statistics are for T-Test or Mann–Whitney test as appropriate. Significance was $p < 0.05$.

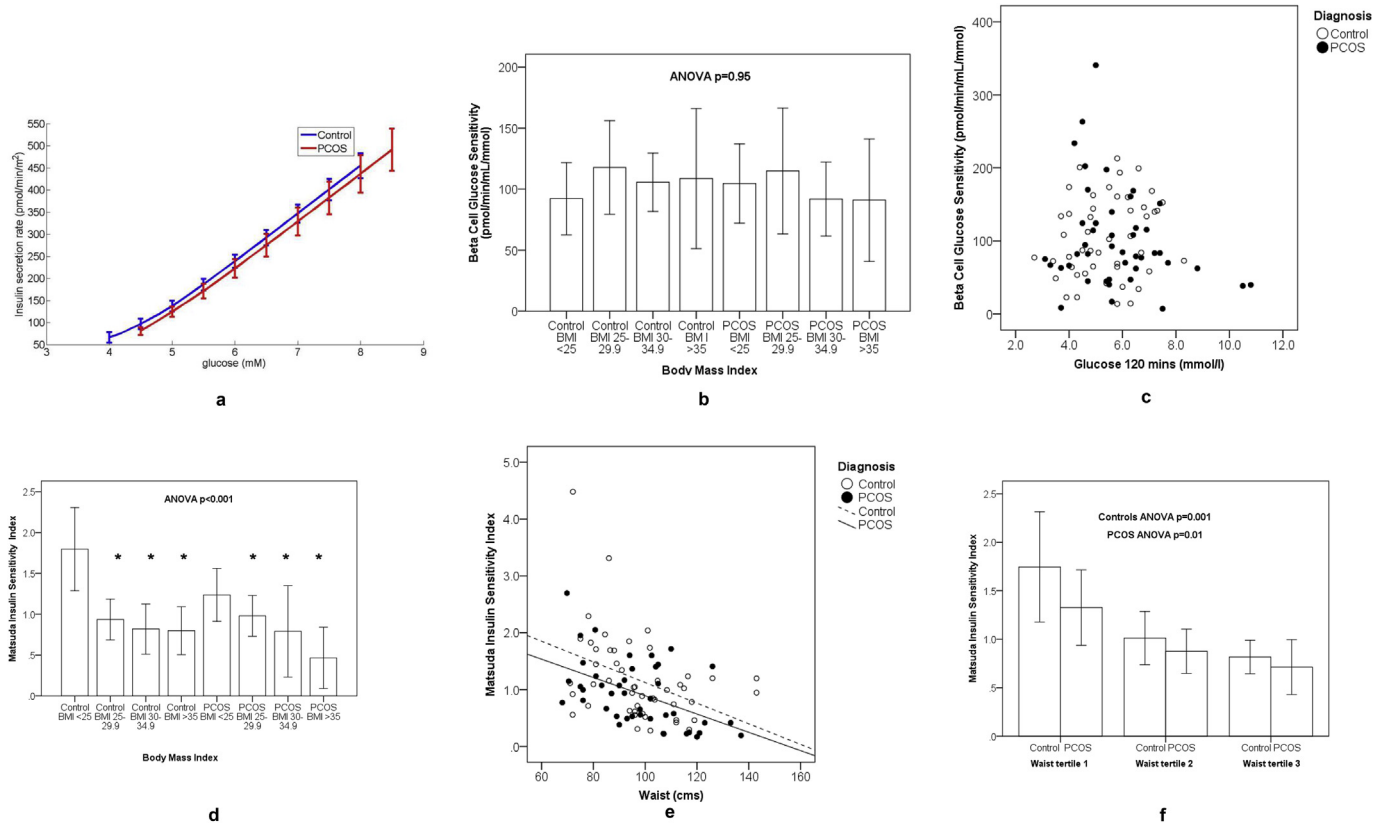

Fig. 1. Panel (a): The insulin secretion dose response relationships to glucose concentration were identical in women with and without PCOS, with no difference in β-Cell Glucose Sensitivity (i.e. the slopes of the responses). Panel (b): β-Cell Glucose Sensitivity (βCGS) by BMI category. There were no significant differences in βCGS between different BMI categories (data are mean and 95% C.I.). Panel (c): Plot of β-Cell Glucose Sensitivity (βCGS) versus 2-hour glucose in PCOS and controls. Increasing 2 hour glucose occurred only in a subset of women with low βCGS. Panel (d): Insulin Sensitivity Index (ISI) according to BMI. Compared with controls with BMI<25, ISI was significantly reduced in women in the higher BMI categories of both controls (Bonferroni's test; all $p < 0.01$) and PCOS (Bonferroni's test; respectively $p = 0.01$; $p = 0.02$; $p < 0.001$). Significant differences are denoted by asterisks (*). Data are mean (95% C.I.). Panel (e): Inverse correlations between Insulin Sensitivity Index (ISI) and waist circumference in women with PCOS ($r = 0.50$; $p < 0.001$) and controls ($r = 0.40$; $p = 0.004$). Panel (f): Insulin Sensitivity Index (ISI) versus waist circumference tertile in women with PCOS and controls.

(Bonferroni's test; all $p < 0.01$) and compared with the same BMI categories for PCOS (Bonferroni's test; respectively $p = 0.01$; $p = 0.02$; $p < 0.001$).

Moreover, in PCOS versus controls, increased 2-hour insulin (371.63 [261.14–578.07] vs. 263.91 [150.01–499.93] pmol/l ($p = 0.03$) and increased insulin AUC (59,640 (37,909–101659) vs. 43,847 (33,385–75817) pmol/l/min $p = 0.06$) were not explained by reduced insulin clearance, and so these observations confirm greater IR in women with PCOS.

ISI correlated strongly and inversely with BMI, % body fat, waist circumference and visceral fat in both PCOS, ($r = -0.50$ to -0.58; all $p \leq 0.001$) and controls ($r = -0.40$ to -0.58; all $p \leq 0.004$). The inverse relationship between ISI and central adiposity in shown in Fig. 1e. Although all four adiposity measures were strongly associated with reduced SHBG levels in PCOS and controls ($r = -0.54$-0.63; all $p < 0.001$), calculated free testosterone was unrelated to ISI in PCOS ($r = -0.13$; $p = 0.41$) or controls ($r = -0.005$; $p = 0.97$).

Interaction of β-Cell Glucose Sensitivity and insulin resistance: the role of adiposity

ISI was reduced in women in the upper versus lower waist circumference tertiles for both PCOS and controls (ANOVA with Bonferroni's test; respectively $p = 0.01$ and $p = 0.001$) (Fig. 1f). However, in PCOS, there was a significant interaction between IR and βCGS. Thus, women in the lowest βCGS quartile exhibited a steep rise in 2-hour glucose with increasing IR and waist

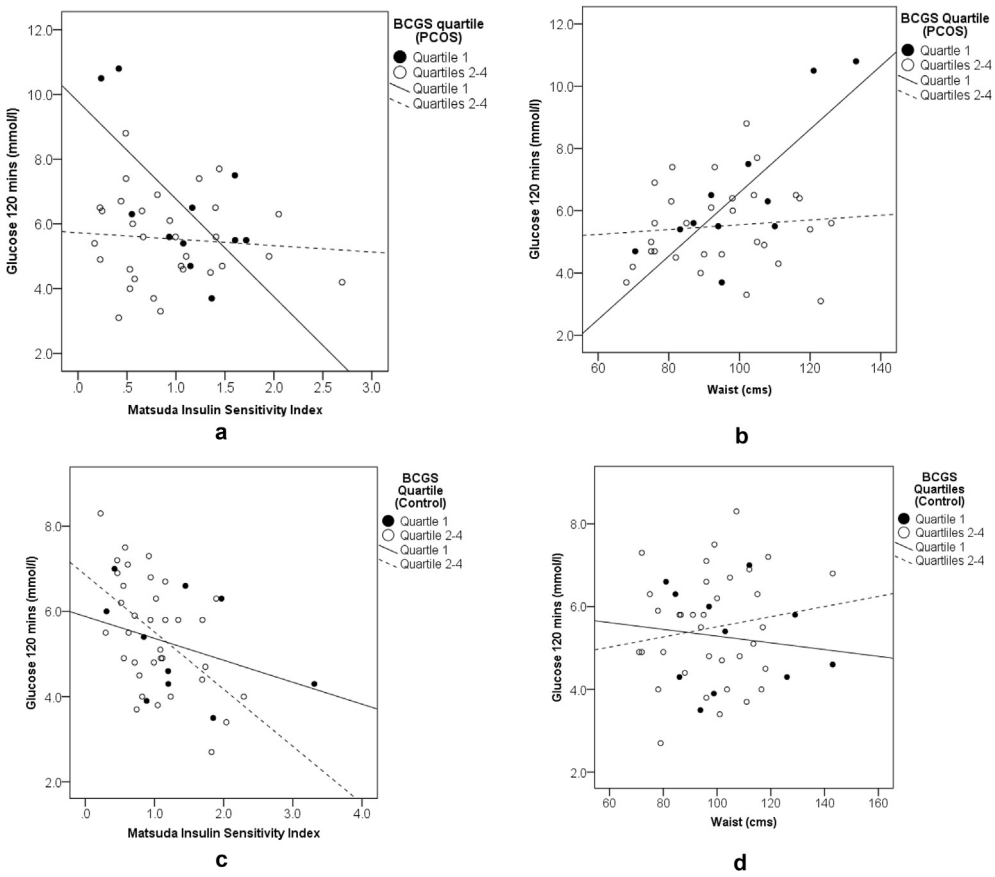

Fig. 2. Panel (a) Scatterplot of 2-hour glucose versus Insulin Sensitivity Index (ISI) in women with PCOS in the 1st[th] (lowest) quartile of β-Cell Glucose Sensitivity (βCGS) (blue markers and line) versus the upper three quartiles combined (green markers and line). ISI was inversely associated with 2-hour glucose in women in the 1st βCGS quartile ($R^2 = 0.47$) but not in quartiles 2–4 ($R^2 = 0.01$). Panel (b) Scatterplot of 2-hour glucose versus waist circumference in women with PCOS in the 1st[th] (lowest) quartile of βCGS (blue markers and line) versus the upper three quartiles combined (green markers and line). Increasing waist circumference was strongly associated with 2-hour glucose in women in the 1st βCGS quartile ($R^2 = 0.66$) but not in quartiles 2–4 ($R^2 = 0.01$). Panel (c) Scatterplot of 2-hour glucose versus ISI in control women in the 1st (lowest) quartile of βCGS (blue markers and line) versus the upper three quartiles combined (green markers and line). ISI was weakly inversely associated with 2-hour glucose in women in both the 1st βCGS quartile ($R^2 = 0.19$) and quartiles 2–4 ($R^2 = 0.22$). Panel (d) Scatterplot of 2-hour glucose versus waist circumference in control women in the 1st[th] (lowest) quartile of βCGS (blue markers and line) versus the upper three quartiles combined (green markers and line). Waist circumference showed no association with 2-hour glucose in women in either the 1st βCGS quartile ($R^2 = 0.004$) or quartiles 2–4 ($R^2 = 0.04$).

circumference compared with women in the upper three quartiles (shown combined in Fig. 2a, b). In ANOVA models with 2-hour glucose as dependent variable, βCGS quartile as a factor, and either ISI or waist circumference as covariate, there were significant interactions between βCGS and both IR and waist circumference ($F = 6.6$; $p = 0.015$ and $F = 10.2$; $p = 0.003$). In contrast, neither associations were apparent in control women (Fig. 2c, d) and neither interaction was present ($F = 1.40$; $p = 0.26$ and $F = 0.12$; $p = 0.95$). Finally, in a 2-way ANOVA, women with PCOS in the lowest tertiles of both βCGS and ISI had higher 2-hour glucoses (ANOVA $p = 0.037$). Therefore, in PCOS, elevated 2-hour glucose occurred in women with the combination of low βCGS and low ISI.

Conversely, women with lower 2-hour glucoses were more insulin sensitive. In controls, decreasing glucose tertiles were associated with increasing ISI, and decreasing insulin at 120 minutes (ANOVA; both $p < 0.001$). Similarly in PCOS, decreasing glucose tertiles were significantly associated with lower 120 minute insulin (ANOVA; $p = 0.001$), although not specifically with ISI (ANOVA; $p = 0.24$).

Finally, in women with PCOS, 2-hour glucose correlated with waist circumference ($r = 0.40$, $p = 0.008$), triglycerides ($r = 0.33$, $p = 0.03$), systolic ($r = 0.32$, $p = 0.03$), diastolic blood pressure ($r = 0.35$, $p = 0.02$) and C-reactive protein ($r = 0.31$, $p = 0.04$), and there were consistent correlations with BMI, % body fat and visceral fat, ($r = 0.35$ to 0.39; $p = 0.01$ to 0.02). In contrast, 2-hour glucose was not significantly associated with these parameters in controls ($r = 0.05–0.25$; $p = 0.10–0.90$).

Discussion

These findings provide simple insights into the origins of T2D in women with PCOS. T2D results from the interplay between IR and poor insulin secretion, and therefore conforms to the models of its pathogenesis proposed by DeFronzo[2] and Reaven.[3] The results also show that simple clinical parameters - waist circumference and 2-hour glucose - identify women at risk of developing T2D.

A central finding was that βCGS is unimpaired in PCOS compared with controls. The method used to model β-cell function has several important advantages over previous methods, because it avoids non-physiological effects of administering intravenous glucose and tolbutamide, or insulin, and generates a physiological incretin response – a key regulator of insulin secretion. Nevertheless, women with PCOS and mild hyperglycaemia at 2 hours were in the lowest quartile of βCGS. Since 2-hour glucose concentrations predict progression to diabetes,[23] these findings identify women who are probably at increased risk of T2D.

It is uncertain whether reduced βCGS results from low β-cell numbers or β-cell impairment, but both may be present. Although

hyperglycaemia itself may impair the β-cell response to glucose,[24] the finding that slim, insulin sensitive women maintain euglycaemia despite poor βCGS, suggests that reduced βCGS is the critical intrinsic characteristic predisposing certain women to diabetes. In support of this proposal, previous research found that βCGS is at least 50% inherited.[25]

The observation that IR is strongly influenced by central adiposity in PCOS confirms previous findings.[13] Although these two BMI-matched groups did not differ in ISI, central adiposity was associated with IR in both groups. However, the greater intrinsic IR of PCOS, exacerbated by central obesity, may explain the more adverse cardiometabolic effect of increasing waist circumference in PCOS compared with controls. In contrast, while poor βCGS may be the critical additional factor required for hyperglycaemia, this defect was present in the same proportion of women with and without PCOS.

The underlying mechanism for the exacerbation of IR in PCOS remains controversial although hyperandrogenism has been considered a potential mechanism. However, Dunaif found that suppression of testosterone levels did not reduce IR in PCOS.[26] We also found no correlation between free testosterone and 2-hour glucose concentrations. Nevertheless, it remains plausible that hyperandrogenism might exert some long-term influence of IR,[27] since testosterone has multiple actions on adipose tissue including insulin signalling.[28]

This study also illustrates the wide spectrum of βCGS and IR in women with PCOS. Women with PCOS in the lowest tertile of waist circumference (<87 cm) were more insulin sensitive, whereas increasing waist circumference was associated with increasing visceral fat, IR and CVD risk. Increasing levels of 2-hour glucose, even in the normal glucose tolerance range, reflect the combined influences of IR and impaired βCGS and therefore point to an increased risk of T2D. Although these relationships were not dissimilar to those seen in controls, they were stronger in PCOS.

The principal strength of this study is the rigorous method to describe insulin secretion overcoming limitations of methods using non-physiological parenteral glucose administration. The study was carefully controlled and the groups well-matched for BMI. The diagnosis of PCOS was consistent with both Rotterdam[10] and NIH criteria.[11] However, our interpretation postulates a sequence of events. Thus, IR results partly from PCOS itself and is amplified by central adiposity. In women who also have poor βCGS the increase in IR causes glucose dysregulation. A longitudinal study is required to confirm this hypothesis. The main consequence of limited sample size was that the highest risk group – women in the lowest quartile of βCGS and who were additionally centrally obese and IR was relatively small. Finally, given the homogeneous ethnic population, without further research these findings should not be extrapolated to othergroups.

Conclusions

Modelling of βCGS provides simple insights into the origins of T2D and CVD risk in women with PCOS, overcoming the limitations of methods involving parenteral glucose administration. The risks of T2D and CVD are determined by the interplay of weight gain and fat distribution, IR and intrinsic βCGS.

The findings suggest the following sequence of events: 1) Women with PCOS have intrinsically increased IR, but those who develop higher central adiposity experience the highest IR and, as a result, a more adverse CVD risk profile. 2) Of this group, women who also have poor βCGS additionally develop glucose dysregulation and increased risk of T2D, whereas those with higher βCGS are able to maintain euglycaemia despite central adiposity and IR. Therefore, central adiposity, as reflected by waist circumference, is the principal modifiable risk factor for T2D and CVD.

While βCGS is not a convenient tool in clinical practice, the 2-hour plasma glucose concentration is a simple measure identifying women with the important combination of greater IR and poor βCGS, who are the group at highest risk of diabetes.

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CRediT authorship contribution statement

Julie Tomlinson: Conceptualization, Formal analysis, Investigation, Writing - review & editing, Project administration, Funding acquisition. **Andrea Mari:** Software. **Andrea Tura:** Software, Formal analysis. **Kirsty Bond:** Investigation. **Elizabeth Stenhouse:** Supervision. **Tracy Dew:** Investigation. **Royce P. Vincent:** Investigation. **Jonathan Pinkney:** Methodology, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors have no conflicts to declare.

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