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ORIGINAL ARTICLE *Reproductive endocrinology*

Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic–hyperinsulaemic clamp

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STUDY QUESTION: What is the prevalence of insulin resistance (IR) and the contributions of intrinsic and extrinsic IR in women diagnosed with polycystic ovary syndrome (PCOS) according to the Rotterdam criteria?

SUMMARY ANSWER: We report novel clamp data in Rotterdam diagnosed PCOS women, using World Health Organization criteria for IR showing that women with PCOS have a high prevalence of IR, strengthening the evidence for an aetiological role of IR in both National Institutes of Health (NIH) and Rotterdam diagnosed PCOS in lean and overweight women.

WHAT IS KNOWN ALREADY: PCOS is a complex endocrine condition with a significant increased risk of gestational diabetes and type 2 diabetes.

STUDY DESIGN, SIZE, DURATION: Using a cross-sectional study design, 20 overweight and 20 lean PCOS (Rotterdam criteria), 14 overweight and 19 lean body mass index (BMI)-matched control non-PCOS women underwent clinical measures of IR after a 3-month withdrawal of insulin sensitizers and the oral contraceptive pill.

MATERIALS, SETTING, METHODS: In an academic clinic setting, glucose infusion rate (GIR) on euglycaemic–hyperinsulinaemic clamp was investigated as a marker of insulin sensitivity.

MAIN RESULTS AND THE ROLE OF CHANCE: PCOS women were more IR than BMI-matched controls (main effect for BMI and PCOS; $P < 0.001$). IR was present in 75% of lean PCOS, 62% of overweight controls and 95% of overweight PCOS. Lean controls (mean $\pm$ SD; GIR 339 $\pm$ 76 mg min^{-1} m^{-2}) were less IR than lean PCOS (270 $\pm$ 66 mg min^{-1} m^{-2}), overweight controls (264 $\pm$ 66 mg min^{-1} m^{-2}) and overweight PCOS (175 $\pm$ 96 mg min^{-1} m^{-2}). The negative relationship between BMI and IR reflected by GIR was more marked in PCOS ($y = 445.1 - 7.7x$, $R^2 = 0.42$ ($P < 0.0001$) than controls ($y = 435.5 - 4.6x$, $R^2 = 0.04$ ($P < 0.01$)).

LIMITATIONS, REASONS FOR CAUTION: The study did not use glucose tracer techniques to completely characterize the IR, as well as the lack of matching for body composition and age.

WIDER IMPLICATIONS OF THE FINDINGS: IR is exacerbated by increased BMI, supporting intrinsic IR in PCOS. BMI impact on IR is greater in PCOS, than in controls, irrespective of visceral fat, prioritizing lifestyle intervention and the need for effective therapeutic interventions to address intrinsic IR and prevent diabetes in this high-risk population.

STUDY FUNDING/COMPETING INTEREST(S): This investigator-initiated trial was supported by grants from the National Health & Medical Research Council (NHMRC) Grant number 606553 (H.J.T., N.K.S. and S.K.H.) as well as Monash University and The Jean Hailes

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CLINICAL TRIAL REGISTRATION: ISRCTN84763265.

Key words: prevalence of insulin resistance / BMI / visceral fat / hyperandrogenism

Introduction

Polycystic ovary syndrome (PCOS) affects 12–21% of reproductive-aged women (March *et al.*, 2010; Boyle *et al.*, 2012) and has major reproductive (leading cause of anovulatory infertility) (Teede *et al.*, 2011), psychological (anxiety and depression) (Deeks *et al.*, 2010) and metabolic (increased type 2 diabetes mellitus and cardiovascular risk factors) (Moran *et al.*, 2010) impacts, representing a substantial health burden (Fig. 1). On meta-analysis the risk of type 2 diabetes in PCOS is increased to 4.43-fold (OR, 95% CI 4.06–4.82; Moran *et al.*, 2010, 2011) even after correcting for body mass index (BMI). Despite PCOS prevalence and health implications, the aetiology and ideal therapies for PCOS remain unclear. Insulin resistance (IR) is a central characteristic in the majority of affected women (Teede *et al.*, 2007), driving both hyperandrogenism and clinical features. Underlying mechanisms of IR remain ill-defined (Teede *et al.*, 2011), contributing to controversy over diagnostic criteria, and a lack of optimal therapies. Therapeutic strategies in PCOS include medical therapy (metformin) (Meyer *et al.*, 2005), exercise (Hutchison *et al.*, 2011; Harrison *et al.*, 2012) and diet-induced weight loss, which all reduce, yet do not reverse IR and fail to optimally treat PCOS. In this context, greater insight into the aetiology of IR in PCOS is needed.

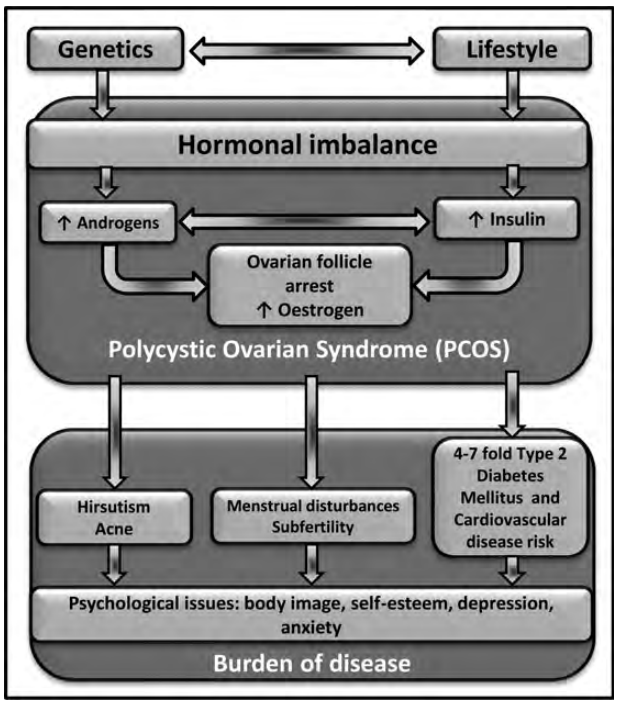


Figure 1. Schema of the aetiology, clinical features and health burden of PCOS (reproduced from Teede *et al.*, 2011 with permission).

Since the sentinel publication by Dunaif *et al.* (1989) noting increased IR in PCOS, reported prevalence of IR in PCOS has varied widely, attributable to the arbitrary and inconsistent definition of IR, the variable and often inaccurate methodologies, the heterogeneity of PCOS and the evolving diagnostic criteria. The Rotterdam criteria include women with milder reproductive and metabolic features of PCOS and while theoretically IR may be less prevalent in women diagnosed via Rotterdam criteria, the prevalence of IR on clamps studies has not been reported (Moran and Teede, 2009).

While not useful in the clinical setting, euglycaemic–hyperinsulinaemic clamps remain the gold standard for research-based assessment of IR. Based on non-clamp data, prevalence of IR has been reported to range from 50 to 70% in women with PCOS (Carmina *et al.*, 1992; Legro *et al.*, 1998). Traditionally, this IR was attributed to obesity in PCOS (Rachon and Teede, 2010), yet it has been hypothesized that intrinsic or unique PCOS-related IR is present and is compounded by separate extrinsic or BMI-related IR (Dunaif *et al.*, 1989; Diamanti-Kandarakis and Papavassiliou 2006; Teede *et al.*, 2007). The concept of intrinsic IR remains controversial in the setting of conflicting literature, with inadequate sample size and application of inaccurate methods to test IR (Dunaif *et al.*, 1989; Mancini *et al.*, 2009; Rabøl *et al.*, 2011). Intrinsic IR has been supported by recent mechanistic PCOS studies including evidence of insulin signalling abnormalities with both unique PCOS- and common BMI-related abnormalities (Corbould *et al.*, 2005, 2006; Diamanti-Kandarakis and Papavassiliou, 2006). Prior work by our group suggests that intrinsic IR in PCOS may in part be related to selectively increased visceral fat deposition in overweight women with National Institutes of Health (NIH)-diagnosed PCOS. To progress understanding on aetiology of PCOS, IR in PCOS needs to be examined in larger studies, using gold standard clamp methods, comprehensive analysis of visceral fat and needs to include women diagnosed by Rotterdam criteria and women across the BMI range.

In this context, we hypothesize that the majority of women with PCOS diagnosed via Rotterdam criteria will be IR and that PCOS involves both intrinsic PCOS-specific IR seen in lean women, compounded by extrinsic BMI-related IR in overweight women. We aimed to comprehensively examine both IR prevalence and impact of BMI across four groups: lean non-PCOS controls, lean PCOS (intrinsic IR), obese non-PCOS controls (extrinsic IR) and obese PCOS women (intrinsic + extrinsic IR), using gold standard insulin clamps.

Materials and Methods

Participants

Seventy-three premenopausal women with and without PCOS were recruited through community advertisements. The women were categorized according to PCOS status and matched for BMI. Categorization into BMI groups was based on the threshold BMI of 27 kg m^{-2}, as an *a priori* decision, as this is the inflexion point in the relationship between BMI and IR (Garca-Estevez *et al*., 2004) and as previously published by our group (Hutchison *et al*., 2011, 2012; Harrison *et al*., 2012). Diagnosis of PCOS was undertaken by expert endocrinologists (S.K.H., A.E.J. and H.J.T.) based on Rotterdam criteria with two of (i) irregular menstrual cycles (<21 or >35 days), (ii) clinical (hirsutism, acne) or biochemical (elevation of at least one circulating ovarian androgen) hyperandrogenism and (iii) PCO on ultrasound (Group, 2004). As this work expands on a previous smaller overweight PCOS study, the exclusion criteria and screening for other causes of hyperandrogenism have been previously described (Hutchison *et al*., 2011). The Southern Health Research Advisory and Ethics Committee approved the study and participants gave written informed consent. The clinical trial registration number is ISRCTN84763265.

Study design

At screening (3 months prior to testing), standard diet and lifestyle advice were delivered (Heart Foundation recommendations (www.heartfoundation.org.au)) and medications affecting end-points including insulin sensitizers, anti-androgens and hormonal contraceptives were ceased. Data were collected in the follicular phase of the menstrual cycle where feasible.

Clinical and biochemical measurements

Participants anthropometric assessments including body weight, height, waist and hip circumference and computed axial tomography (CT) scans for visceral fat assessments were conducted as previously reported (Hutchison *et al*., 2011).

Insulin sensitivity was assessed by the euglycaemic–hyperinsulinaemic clamp technique as previously reported (Hutchison *et al*., 2011). Briefly, the clamp was performed 72 h after a standardized high-carbohydrate diet prior to an overnight fast. Venous fasting blood samples were collected, analysed and stored as appropriate after arterialization. Insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was infused at 40 mU m^{-2} min^{-1} for 120 min generating an elevated, stable insulin concentration from 10 to 120 min, with plasma glucose maintained at ~5 mmol/l, using variable infusion. Glucose was assessed every 5 min and the glucose infusion rate (GIR) was calculated during last 30 min of the insulin-stimulated period and expressed as glucose (mg) per body surface area (m^2) per min.

Stored blood samples were batch analysed for serum fasting glucose, total cholesterol, high-density lipoprotein(HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, insulin and testosterone and glycosylated haemoglobin (HbA1c) as previously reported (Meyer *et al*., 2005). LDL and the homeostatic model IR assessment (HOMA) were calculated as previously described (Meyer *et al*., 2005).

Statistics

All data are presented as mean ± SD. Results are presented for 73 participants. Two-tailed statistical analysis was performed using SPSS for Windows 20.0 software (SPSS, Inc., Chicago, IL, USA) with statistical significance was accepted when $P \leq 0.05$. Data were assessed for normality and log transformed where appropriate and analysed using univariate analysis of variance (ANOVA) (PCOS status × body weight status) using age as a covariate. Correlations of BMI and GIR with the lipid profile parameters, and GIR with free androgen index (FAI) were determined using the Pearson's product moment correlation coefficient (r). Hierarchical linear regression was used to investigate the influence of visceral fat on GIR and to account for the significant age contributions to the accumulated visceral fat in all women. Split linear regressions were used to demonstrate the *a priori* distinction of lean and obese groups based on a BMI threshold of 27 kg m^{-2} for the exacerbation of IR in the whole group.

Results

We confirmed the *a priori* BMI categorization into lean and overweight/obese women, based on a BMI cut-off of 27 kg m^{-2}, demonstrating a stronger impact of BMI on GIR equal to or above a BMI of 27 kg m^{-2} across all groups (Fig. 2A). Specifically, all women with a BMI <27 kg m^{-2} demonstrated that for every 1 BMI unit increase, GIR was 2.6 units lower ($R^2 = 0.005$ ($P = 0.7$)) compared with the 7.0 units lower for every BMI unit increase in women with a BMI ≥27 kg m^{-2} ($R^2 = 0.212$ ($P = 0.007$); Fig. 2A).

We analysed 34 overweight women ($n = 20$ PCOS and $n = 14$ controls with a BMI ≥27 kg m^{-2}) and 39 lean women ($n = 20$ PCOS and $n = 19$ controls with a BMI <27 kg m^{-2}) with characteristics reported in Table I. The lean women with and without PCOS, and overweight women with PCOS were well matched for age (~28 years). Overweight control women were older than other groups ($P < 0.001$). Using age as a covariate, we noted that age did not influence outcome variables measured ($P > 0.05$) except visceral fat ($P < 0.001$).

Women were primarily Caucasian (68%), but the cohort also included women with a European (14%), Asian/Indian (12%) and a mixed race (6%) background. BMI, body weight, waist and hip circumference, fasting glucose, HOMA, HbA1c, triglycerides, HDL, LDL, LDL:HDL ratio, abdominal subcutaneous and visceral fat were significantly different between the combined groups of lean and obese women (main effect of BMI, $P < 0.05$; Table I) and were not clearly related to PCOS status. Overall, BMI and GIR correlated with triglycerides ($r = 0.39$ ($P = 0.001$) and $r = -0.39$ ($P = 0.001$)), HDL ($r = -0.61$ ($P < 0.001$) and $r = 0.56$ ($P < 0.001$)) and the LDL/HDL ratio ($r = 0.53$($P < 0.001$) and $r = -0.55$ ($P < 0.001$)), respectively.

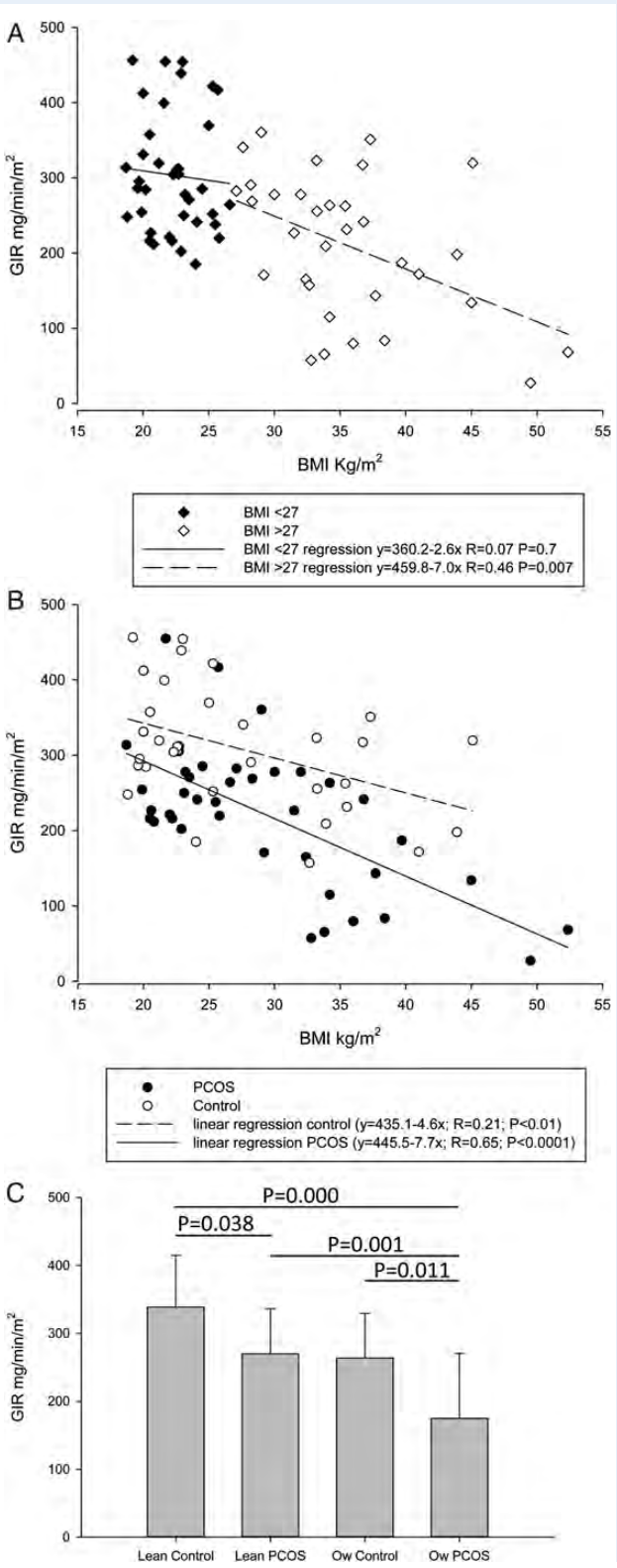
Testosterone was different between lean and overweight women with PCOS (main effect of PCOS, $P = 0.001$ and $P = 0.04$ respectively; Table I), and fasting insulin was different for lean and overweight women with and without PCOS (main effect PCOS, $P = 0.04$; main effect BMI, $P < 0.001$; Table I). Both BMI and PCOS were related to FAI (Table I, PCOS and BMI, $P < 0.001$, PCOS × BMI $P < 0.05$). IR was correlated to androgen status (FAI) where $r = -0.44$ ($P < 0.001$) and $r = -0.52$ ($P < 0.001$) for all women and women with PCOS, respectively.

IR is a continuous measure and is defined arbitrarily. We defined IR on clamp-derived GIR levels as less than the 25th centile of lean matched controls (non-PCOS specific World Health Organization (WHO) criteria) (Grundy *et al*., 2004). IR as determined by GIR normalized to body surface area showed that overall PCOS women were more IR than BMI-matched controls, even after correction for age (main effect for PCOS and BMI $P < 0.001$; Fig. 2B).

Specifically, lean controls (339 ± 76 mg min^{-1} m^{-2}) were less IR than lean PCOS (269 ± 66 mg min^{-1} m^{-2}), overweight controls (264 ± 66 mg min^{-1} m^{-2}) and overweight PCOS (175 ± 96 mg min^{-1} m^{-2}), respectively (Fig. 2C). There was no significant difference in IR between lean PCOS women and overweight controls. Also, overweight women with PCOS were significantly more IR than all groups including overweight controls (Fig. 2C). IR was present in 75% of lean PCOS, 62% of overweight controls and 95% of overweight PCOS (Fig. 3A). The increased IR in PCOS is highlighted by the frequency distribution curve for GIR which is shifted to the left in PCOS (Fig. 3B).

Lean PCOS phenotypes in this community-recruited study included 5/19 with NIH PCOS and 14/19 with Rotterdam PCOS only who did not meet NIH criteria. In the overweight women, 17/20 had NIH PCOS and 3/20 had Rotterdam criteria alone. All participants diagnosed with PCOS according to the Rotterdam criteria in both the lean and overweight groups had irregular menstrual cycles and PCO on ultrasound, with none having hyperandrogenism clinically or biochemically. Overall 53% of PCOS women met NIH criteria. IR was present in 70% of lean Rotterdam, non-NIH PCOS and 80% of lean NIH PCOS with both of these lean subgroups demonstrating lower GIR's of 279 ± 74 and 248 ± 41 mg min^{-1} m^{-2} compared with lean controls (339 ± 76 mg m^{-2} min^{-1}), respectively ($P < 0.05$). Once corrected for BMI, we noted insulin sensitivity for all women was different between controls (301 ± 89 mg min^{-1} m^{-2}) and both NIH (195 ± 91 mg min^{-1} m^{-2}, $P < 0.005$) and Rotterdam only (PCOS + irregular cycles) PCOS phenotypes (260 ± 89 mg min^{-1} m^{-2}, $P < 0.04$).

There was a negative relationship between BMI and IR (GIR; Fig. 2B), which is more marked in women with PCOS (PCOS $R^2 = 0.42$ ($P < 0.0001$) versus controls $R^2 = 0.04$ ($P < 0.01$)), with every 1 unit increase in BMI associated with 7.7 unit lower GIR versus the 4.6 units in control women (Fig. 2B). Visceral fat, a known major contributor to IR and assessed here via visceral fat area on CT, was negatively related to GIR, whereby after accounting for the unequal variance and age, visceral fat accounted for 39, 31 and 39% of the GIR variance overall (adjusted $r^2 = 0.390$; $P < 0.001$), in controls (adjusted $r^2 = 0.312$; $P = 0.002$) and in PCOS (adjusted $r^2 = 0.392$; $P < 0.001$) women, respectively.



Discussion

Here using gold standard clamp techniques, we confirm that PCOS women, irrespective of BMI, are more IR (Dunaif *et al.*, 1989, Ovalle and Azziz, 2002) and report novel data that the prevalence of IR in PCOS based on the WHO definition (<25th centile of GIR in healthy lean controls) is 75% in lean PCOS, 62% in overweight controls and 95% in overweight PCOS in a largely Caucasian population. Overall, we show significantly higher IR in lean PCOS women versus lean controls, supporting the hypothesis that a unique 'intrinsic-related

Figure 2. The relationship between BMI and IR as determined by the GIR in the last 30 min of the 120 min hyperinsulinaemic–euglycaemic clamp. (**A**) Scatterplot of GIR versus BMI where women are separated by BMI at the threshold of 27 kg m^{-2} and associated regressions lines. (**B**) Scatterplot of GIR versus BMI where women are separated by PCOS status, with associated regression lines. (**C**) Mean GIR ± SD data for lean control ($n = 19$), lean PCOS ($n = 20$), overweight/obese (ow) control ($n = 14$) and ow PCOS ($n = 20$) women. Groups defined by the ends of the horizontal bars were significantly different from each other (univariate ANOVA).

Table I. Clinical characteristics of lean (BMI <27 kg m^{-2}) and overweight (BMI >27 kg m^{-2}) women with and without PCOS.

Clinical feature	Lean controls (n = 19)	Lean PCOS (n = 20)	Overweight controls (n = 14)	Overweight PCOS (n = 20)	P value main effect of PCOS	P value main effect of BMI
General characteristics						
Age (years)	28 ± 6	27 ± 4	35 ± 4	30 ± 6	0.028	<0.001
Height (cm)[a]	165 ± 7	166 ± 7	164 ± 4	164 ± 5	0.627	0.221
Body weight (kg)[a]	59 ± 7	63 ± 8	94 ± 16	95 ± 18	0.316	<0.001
BMI (kg m^{-2})[a]	22 ± 2	23 ± 2	35 ± 6	36 ± 7	0.349	<0.001
Waist (cm)[a]	71 ± 5	74 ± 7	102 ± 14	101 ± 11	0.157	<0.001
Hip (cm)[a]	85 ± 7	88 ± 9	119 ± 15	120 ± 14	0.329	<0.001
WHR[a]	0.83 ± 0.04	0.85 ± 0.04	0.85 ± 0.10	0.85 ± 0.06	0.591	0.538
Insulin sensitivity						
Fasting glucose (mmol l^{-1})[a]	4.6 ± 0.3	4.5 ± 0.3	4.9 ± 0.4	4.8 ± 0.6	0.788	0.015
Fasting insulin (pmol l^{-1})[a,b]	24 ± 9	26 ± 10	120 ± 60	172 ± 83	0.043	<0.001
HOMA[a,b]	0.8 ± 0.3	0.8 ± 0.3	4.4 ± 2.6	6.3 ± 3.2	0.143	0.044
HbA1c (%)[a]	4.7 ± 1.2	5.0 ± 0.1	5.4 ± 0.3	5.4 ± 0.4	0.439	0.002
Body composition						
CT abdominal visceral fat (cm^2)[d]	32 ± 20	35 ± 10	122 ± 35	118 ± 59		
Log CT abdominal visceral fat[a]	1.45 ± 0.20	1.53 ± 0.15	2.07 ± 0.13	2.01 ± 0.25	0.257	<0.001
CT abdominal subcutaneous fat (cm^2)[a]	183 ± 69	234 ± 71	550 ± 169	535 ± 175	0.635	<0.001
Hormonal status						
Testosterone (nmol l^{-1})[a]	1.7 ± 0.5	2.1 ± 0.8	1.5 ± 0.8	2.6 ± 0.8	0.001	0.060
SHBG (nmol l^{-1})[a]	79 ± 19	69 ± 34	46 ± 29	32 ± 11	0.070	<0.001
FAI[a,c]	2.3 ± 1.0	3.5 ± 1.8	4.4 ± 3.5	9.2 ± 4.5	<0.001	<0.001
Lipid profile						
Cholesterol (mmol l^{-1})[a]	4.7 ± 0.6	4.9 ± 0.7	4.8 ± 0.9	4.9 ± 1.1	0.382	0.915
Triglycerides (mmol l^{-1})[a]	0.8 ± 0.6	0.8 ± 0.7	1.1 ± 0.3	1.4 ± 0.9	0.350	0.015
HDL (mmol l^{-1})[a]	1.7 ± 0.4	1.7 ± 0.4	1.3 ± 0.3	1.1 ± 0.3	0.596	0.001
LDL (mmol l^{-1})[a]	2.6 ± 0.5	2.9 ± 0.6	3.1 ± 0.7	3.2 ± 0.9	0.299	0.075
LDL:HDL ratio[a]	1.7 ± 0.6	1.7 ± 0.5	2.5 ± 0.7	3.1 ± 1.4	0.086	<0.001

Data are mean ± SD.
BMI, body mass index; CT, computed axial tomography; FAI, free androgen index (([testosterone]/[SHBG]) × 100); HbA1c, glycosylated haemoglobin; HDL, high-density lipoprotein; HOMA, homeostatic model assessment of IR; LDL, low-density lipoprotein; SHBG, steroid hormone binding globulin; WHR, waist-to-hip ratio.
[a]Data analysis used age as covariate due to the significant difference between groups.
[b]Statistical analysis reported for the log-transformed data due to unequal variance.
[c]PCOS × BMI interaction $P < 0.05$.
[d]Unequal variance of data was log transformed for statistical analysis.

IR exists in women with PCOS'. We also confirm that extrinsic BMI-related IR occurs in both control and PCOS women and demonstrate that BMI has a more potent extrinsic IR impact than is seen in controls. On phenotypic subgroup analysis, we also demonstrated that 14/19 lean Rotterdam diagnosed PCOS women who had the PCO and irregular cycle phenotype without hyperandrogenism, and did not meet NIH diagnostic criteria, still greater IR on insulin clamps than did lean controls. Finally, we report that unlike IR, lipid abnormalities appear to be primarily related to BMI and are not significantly related to PCOS status *per se*.

IR is defined as an impaired biological response to exogenous or endogenous insulin, reflecting disturbed metabolic and mitogenic

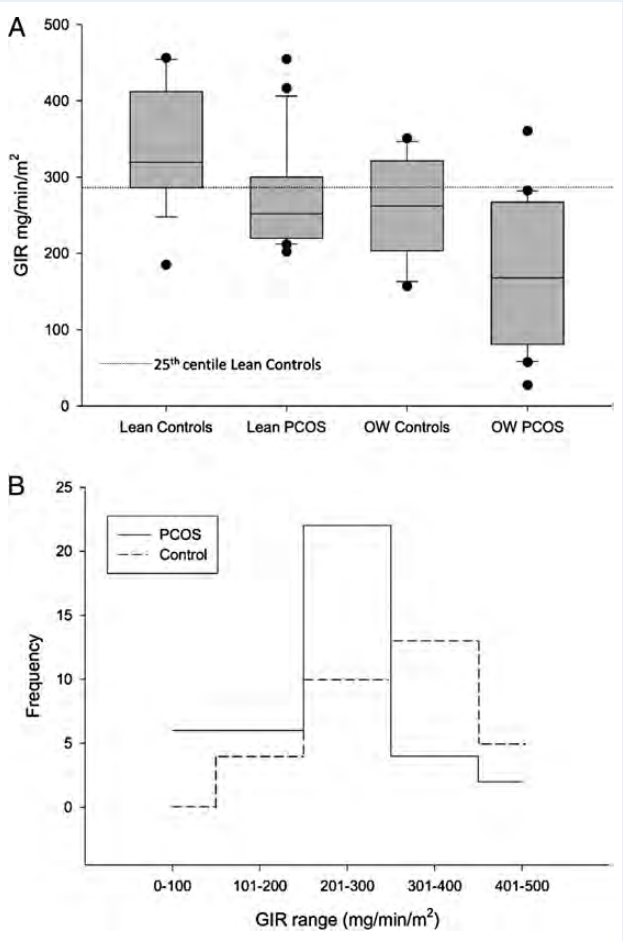


Figure 3. IR prevalence demonstrated by (**A**) box and whisker plots showing the median (central line), range (whiskers), 25th to 75th centiles (box) and individual outliers (dots) of the GIRs for lean control ($n = 19$), lean PCOS ($n = 20$), overweight/obese (ow) control ($n = 14$) and ow PCOS ($n = 20$) women with thresholds for IR in lean and ow PCOS women (WHO defined as below the 25th centiles of the lean control group) and (**B**) the shift in frequency to lower GIR in women with PCOS independent of BMI.

processes (Consensus Development Conference on Insulin Resistance (1998)). IR is a continuous variable measured with a range of different methodologies and defined based on controversial cut-off values. Studies on IR in PCOS rarely use gold standard clamp techniques and do not conventionally include a control group to define IR based on cut-offs in healthy controls, in a given population (Grundy *et al.*, 2004). Given the important role that IR plays in PCOS and the high risk of type 2 diabetes, we have studied the prevalence of IR in lean and overweight PCOS women recruited from the community, using gold standard clamp methods and defined IR using WHO criteria as a GIR below the lowest quartile for the appropriate control population (Grundy *et al.*, 2004). We also used an age-appropriate lean healthy group of women as the control group. In this context we present novel data demonstrating that overall 85% of women with PCOS were IR, with 75% of lean and 95% of obese women having WHO-defined IR. Overall our data show a higher prevalence of IR in PCOS compared with other studies using clamps (Dunaif *et al.*, 1989; Ovalle and Azziz, 2002; Rabøl *et al.*, 2011), the insulin tolerance test (68–76%; Carmina *et al.*, 1992) or frequently sample intravenous glucose tolerance test (53%; Legro *et al.*, 1998) or indeed the ethnicity independent consensus of 50–70% prevalence (Ovalle and Azziz, 2002). These discrepancies in reported IR prevalence in PCOS across the BMI range cannot only be attributed methodological differences but also the lack of a consistent definition of IR and the variable use of control populations. Given the current data, in the context of previous literature, we conclude that IR is present in the large majority of women with PCOS independent of BMI. Understanding of the high prevalence of IR in this condition arguably reduces the heterogeneity of hormonal abnormalities that contribute to metabolic and reproductive consequences of PCOS and highlights the need for greater research into the mechanistic underpinnings of IR to progress the understanding of PCOS aetiology.

Conflicting results on the prevalence of IR in PCOS also stem from the evolution of the diagnosis of PCOS, from NIH to the Rotterdam criteria. Clamp data on IR in Rotterdam-diagnosed PCOS women compared with controls across the BMI range have not been published to date. Rotterdam criteria remain controversial, with the additional diagnostic criteria of PCO on ultrasound resulting in more women diagnosed with PCOS and in the inclusion of women with milder reproductive and metabolic PCOS features compared with those diagnosed by NIH criteria (Moran *et al.*, 2011). However, we have previously demonstrated that Rotterdam, non-NIH PCOS cases still have metabolic abnormalities compared with controls (Moran and Teede, 2009). Here we advance knowledge in this area further by demonstrating for the first time that 70% of lean women diagnosed with PCOS on Rotterdam criteria, most of whom do not meet NIH criteria and who represent a milder reproductive PCOS phenotype, are still IR compared with BMI-matched controls and have a more severe metabolic phenotype than controls. Indeed subgroup analysis of the PCO and irregular cycle phenotype without hyperandrogenism (non-NIH PCOS), corrected for BMI, still had higher IR lean controls in the current study. Consistent with this finding, prior studies using less accurate measures of IR have shown that metabolic and endocrine differences including increased IR are present in women with irregular cycles and PCO (Welt *et al.*, 2006), regardless of the androgen status, although these features may be milder compared with women with hyperandrogenic phenotypes (Dewailly *et al.*, 2006). Another study using HOMA scores did not demonstrate a difference in IR between control and PCOS based on irregular cycles and PCO on ultrasound (Barber *et al.*, 2007); however, insulin clamps used in the current study are a more accurate reflection of IR than HOMA scores. It appears that the more controversial Rotterdam phenotype of PCO and irregular cycles does have elevated IR when measured using accurate methods. As controversy over PCOS diagnostic criteria persists, this finding in lean women is important and suggests that even reproductively milder subgroups with PCOS do have IR and metabolic abnormalities independent of obesity. Clinical implications of this include the need to screen for metabolic complications in both NIH and Rotterdam-diagnosed women, across the BMI range (Teede *et al.*, 2011); however,when to start and how often to screen using which tests still require further research including a better understanding of the natural history of PCOS including the different phenotypes of the condition.

PCOS-associated (intrinsic) IR has been proposed as a contributor to PCOS aetiology for over two decades, where significant IR was noted to occur independent of BMI (Dunaif *et al.*, 1989). Others have suggested that there is a significant IR in lean PCOS women compared with lean controls (Li and Li, 2012). However, intrinsic IR in PCOS has been contentious with a lack of consistent results, potentially related to limited quality of the data including variable use of inaccurate methods to assess and define IR in PCOS (Mancini *et al.*, 2009). The current study, using gold standard methodology and an internationally accepted definition of IR, demonstrates significantly higher IR in lean PCOS women versus lean controls, supporting the hypothesis that a unique 'intrinsic IR' exists in women with PCOS. In this setting, greater understanding of the underlying mechanisms and genetic basis for intrinsic PCOS-related IR is needed. Limited mechanistic IR research in PCOS suggests aberrant peripheral insulin signalling through insulin receptor substrate 1 in PCOS, compared with controls (Corbould *et al.*, 2005, 2006; Diamanti-Kandarakis and Papavassiliou, 2006). Other proposed mechanisms of intrinsic IR may include reduced mitochondrial biogenesis (Skov *et al.*, 2007) and/or function (Rabøl *et al.*, 2011), but the results thus to date are not supportive of this hypothesis (Hutchison *et al.*, 2012). Further investigation into potential mechanisms is warranted to progress understanding of PCOS aetiology and to identify potential future therapeutic targets in this common condition. Indeed, current literature suggests that metformin, an insulin sensitizer, may be more effective in non-obese women with PCOS (Misso *et al.*, 2013), suggesting that therapies may selectively target intrinsic and extrinsic IR differentially. Likewise, the impact of lifestyle intervention may primarily target extrinsic BMI-related IR in PCOS, with further research needed to clarify mechanisms of therapeutic action in PCOS.

Obesity is well known to increase extrinsic IR in the general population, with the impact of BMI on IR being more marked once BMI increases beyond 27 $kg\,m^{-2}$ (Garca-Estevez *et al.*, 2004). As we confirm here, obesity exacerbates IR in PCOS (Teede *et al.*, 2007) with overweight women with PCOS having higher IR (Dunaif *et al.*, 1989; Mancini *et al.*, 2009; Hutchison *et al.*, 2011). Our current data also highlight the novel finding that there is an increased impact of BMI on IR, in women with PCOS, compared with in BMI-matched controls. As visceral fat has been implicated in the aetiology of IR in in PCOS (Lord *et al.*, 2006; Hutchison *et al.*, 2011), we investigated if visceral fat accounted for the differences in IR between PCOS and controls. Our data demonstrated that visceral fat makes similar contributions to IR in PCOS as it does in control women, indicating that visceral fat is more likely a contributor to extrinsic IR and also showing that visceral fat is not the only driver of differences in IR between PCOS and controls. The impact of BMI and visceral fat on the interaction between extrinsic and intrinsic IR in PCOS is not yet well understood and warrants further research. Overall, increased BMI and increased visceral fat in PCOS reflect a significant health concern and the current data strengthen the argument for aggressive lifestyle intervention to prevent weight gain and induce weight loss to minimize associated extrinsic IR (Teede *et al.*, 2011). Notably, the similar degree of IR in lean PCOS and overweight control women is consistent with the high risk of diabetes in PCOS, independent of BMI and reinforces the need for screening for glucose intolerance even in lean PCOS women (Meyer *et al.*, 2005; Moran *et al.*, 2010; Teede *et al.*, 2011). In contrast, we did not observe a significant relationship between lipids and PCOS status, with lipids primarily related to BMI status, again highlighting the need for aggressive weight management.

The strengths of the current study include a community-recruited cohort of PCOS women, the extension of PCOS diagnostic criteria to include those with Rotterdam-diagnosed PCOS, the use of the hyperinsulinaemic–euglycaemic clamp methodology with pre-clamp dietary control and the inclusion of healthy controls who were matched for BMI and were not taking any medication. Limitations include not using glucose tracer techniques to completely characterize the IR, and the lack of matching for body composition and age. Also there were proportionately more women diagnosed by Rotterdam, but not NIH criteria, in the lean compared with in the overweight PCOS group.

We report for the first time the prevalence of IR on clamp studies in women with Rotterdam-diagnosed PCOS, where 75% of lean and 95% of overweight women with PCOS are IR, based on WHO criteria, using age-appropriate lean healthy control women. We show that the overwhelming majority of women with PCOS are IR including those who are lean and those who meet Rotterdam criteria but not NIH diagnostic criteria for PCOS, specifically those with the PCO and irregular cycle, non-hyperandrogenic PCOS phenotype. Additionally, we confirm that IR is higher in women with PCOS in the presence of an inherent, intrinsic IR that is further worsened with increasing BMI and demonstrate a more potent extrinsic IR impact of BMI in PCOS compared with controls. Given the clinical implications of IR including a high risk of type 2 diabetes, future research is needed into mechanisms of intrinsic and extrinsic IR in PCOS and into novel targeted therapies. Potentially, lifestyle change may best manage extrinsic IR (Hutchison *et al.*, 2011; Harrison *et al.*, 2012) and pharmacological interventions, such as metformin, may best target intrinsic PCOS-related IR; however, more research is needed.

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Authors' roles

N.K.S. and H.J.T. were involved with conception and design, analysis and interpretation of data. N.K.S. and S.C. analysed and interpreted data and wrote the manuscript. S.C., A.E.J., S.K.H., C.L.H. and R.F.G. researched the data. All authors undertook the critical revision for important intellectual content and approved the final version for publication.

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Conflict of interest

None declared.

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