RESEARCH

Can dysglycemia in OGTT be predicted by baseline parameters in patients with PCOS?

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Abstract

Background: Polycystic ovary syndrome (PCOS) is considered a risk factor for the development of type 2 diabetes mellitus (T2DM). However, which is the most appropriate way to evaluate dysglycemia in women with PCOS and who are at increased risk are as yet unclear.
Aim of the study: To determine the prevalence of T2DM, impaired glucose tolerance (IGT), and impaired fasting glucose (IFG) in PCOS women and potential factors to identify those at risk.
Subjects and methods: The oral glucose tolerance test (OGTT), biochemical/hormonal profile, and ovarian ultrasound data from 1614 Caucasian women with PCOS and 362 controls were analyzed in this cross-sectional multicenter study. The data were categorized according to age and BMI.
Results: Dysglycemia (T2DM, IGT, and IFG according to World Health Organization criteria) was more frequent in the PCOS group compared to controls: 2.2% vs 0.8%, $P = 0.04$; 9.5% vs 7.4%, $P = 0.038$; 14.2% vs 9.1%, $P = 0.002$, respectively. OGTT was essential for T2DM diagnosis, since in 88% of them basal glucose values were inconclusive for diagnosis. The presence of either T2DM or IFG was irrespective of age ($P = 0.54$) and BMI ($P = 0.32$), although the latter was associated with IGT ($P = 0.021$). There was no impact of age and BMI status on the prevalence of T2DM or IFG. Regression analysis revealed a role for age, BMI, fat deposition, androgens, and insulin resistance for dysglycemia. However, none of the factors prevailed as a useful marker employed in clinical practice.
Conclusions: One-third of our cohort of PCOS women with either T2DM or IGT displayed normal fasting glucose values but without confirming any specific predictor for dysglycemic condition. Hence, the evaluation of glycemic status using OGTT in all women with PCOS is strongly supported.

Key Words
- polycystic ovary syndrome
- diabetes
- insulin resistance
- androgens
- age
- impaired glucose tolerance

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Introduction

Polycystic ovary syndrome (PCOS), a multifaceted, ever-changing disease, constitutes a common disorder in women of reproductive age. The standard view is that during the first decades of reproductive life, hyperandrogenic symptoms, accompanied by oligomenorrhea, dominate the clinical picture followed by metabolic disorders including dysglycemia and dyslipidemia later in life (1). Moreover, progression of normoglycemia to either intermediate hyperglycemia or diabetes mellitus (T2DM) has been observed in prospective studies of large PCOS cohorts of mostly obese women (2, 3), since the two preconditions for T2DM development are encountered in PCOS. Specifically, profound insulin resistance (IR) constitutes a key player underlying PCOS and has been implicated in syndrome pathophysiology (4, 5). Meanwhile, the prevalence of pancreatic ß-cell dysfunction is much higher in women suffering from PCOS by comparison to their normal ovulating, non-hyperandrogenic peers (6).

However, there is controversy as to whether PCOS in itself is a risk factor for T2DM, as suggested in some studies, or whether, as proposed by others, T2DM predominantly occurs in the context of obesity (7, 8, 9). A recent meta-analysis of genetic studies suggests that there is no inherent T2DM risk in PCOS and that, instead, it is a result of either increased BMI, of androgens, and/or of low sex hormone-binding globulin (SHBG) values (10). However, PCOS constitutes a polygenic trait and the clusters of genes leading to metabolic disturbances including T2DM are different to those associated with overt hyperandrogenic signs (11).

Several guidelines suggest regular assessment of glycemic status in PCOS populations but without consensus on the optimal method to detect potential dysglycemia (12). Moreover, the most accurate means for testing hyperglycemia including fasting plasma glucose (FPG), oral glucose tolerance test (OGTT), or hemoglobin A1c (HbA1c) has to be clarified (13). Several studies have been carried out on this issue over the last decades with conclusions compromised by a number of methodological limitations. The current state of uncertainty could be attributed to the different diagnostic criteria for PCOS, small number of studied subjects, lack of control groups, and racial or ethnic differences (14). Moreover, a diversity of IR values in PCOS women has been observed over the years appearing to improve in lean subjects but to worsen in obese (15, 16).

In an attempt to overcome these obstacles and elucidate the issue, we carried out a large cross-sectional study of glycemic status in 1614 PCOS women and 362 normally ovulating, non-hyperandrogenic women, who served as controls. To the best of our knowledge, this is the largest cohort of women of Caucasian origin from Europe recruited for the purpose of evaluating glycemic status in PCOS. Our aim was to report the prevalence of dysglycemia in this cohort and to pinpoint potential factors that could improve differentiation between PCOS women likely to develop T2DM and those with minimal risk.

Research design and methods

Subjects

The subjects comprised all women diagnosed with PCOS between 2008 and 2019 at the following centers: (i) the Gynecological Endocrinology Infirmary of the Second Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Greece; (ii) the Clinic of Endocrinology, Diabetes, and Metabolic Diseases, Faculty of Medicine, University of Belgrade, Serbia; (iii) the Department of Endocrinology and Diabetes, Hellenic Red Cross Hospital, Athens, Greece; (iv) the Department of Endocrinology, Diabetology, and Isotope Therapy, University of Medicine, Wrocław, Poland; (v) the Endocrine Unit, Metropolitan Hospital, Athens, Greece; (vi) the Department of Endocrinology, Ushate 'acad. IV. Penchev', Sofia, Bulgaria. In total, data from 1614 women with PCOS were collected. Diagnosis of PCOS was based on the Rotterdam criteria. The control group was composed of 362 normally ovulating, non-hyperandrogenic women. The women in the control group were healthy volunteers with normal ovulating cycles (28 ± 2 days, blood progesterone levels >10 ng/mL in the luteal phase of two consecutive cycles), no signs of hyperandrogenism or hyperandrogenemia, and normal sonographic appearance of the ovaries.

We excluded women with galactorrhea or any other endocrine or systemic disease that could possibly affect the reproductive physiology from the total study group. We also excluded women with reported use during the previous semester of any medication that could interfere with the normal function of the hypothalamic–pituitary–gonadal axis, including metformin.

In all subjects, BMI, waist-to-hip ratio (WHR), and degree of hirsutism (Ferriman–Gallwey score (FG)) were evaluated, and glucose, lipids (HDL, LDL, triglycerides, and cholesterol), insulin, gonadotropins (luteinizing hormone (LH) and follicle-stimulating hormone (FSH)),

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estradiol (E2), and androgen levels were measured. Menstrual cyclicity was recorded via a detailed history of the menstrual cycles of the previous year. Baseline blood samples were taken and an OGTT was performed in the same morning after an overnight fast. Insulin and blood glucose levels were measured at baseline and at 30, 60, and 120 min after oral ingestion of a glucose load of 75 g anhydrous glucose dissolved in water. On the same day, abdominal or transvaginal ultrasonography was performed and ovarian morphology as well as ovarian volume was evaluated. HbA1c values were obtained in 442 women with PCOS.

Diagnosis of T2DM was based on the American Diabetes Association (ADA) criteria (17). Namely, on the basis of FPG levels, levels <100 mg/dL (5.6 mmol/L) were normal, values ranging from 100 to 125 mg/dL were impaired fasting glucose (IFG), and values of 126 mg/dL or higher than 126 mg/dL (7.0 mmol/L) were T2DM. An additional categorization of IFG according to WHO criteria (110–125 mg/dL, 6.1–7 mmol/L) was added (18). Considering OGTT, as normal values post-glucose load was defined as values below 140 mg/dL (7.8 mmol/L), IGT as values between 140 199 mg/dL, and DM as values of 200 mg/dL (11.1 mmol/L) or higher. Regarding HbA1c results, subjects were classified as 'normal' if HbA1c was <5.7%, 'increased risk for diabetes' if HbA1c was 5.7–6.4%, or 'diabetic if HbA1c was >6.5% based on the ADA criteria (17).

To define the distinctive characteristics of the patients of interest according to age group, all subjects were divided into three different age subgroups. Group A represented adolescent and young women, with the age limits being 17–25 years of age ($n = 628$); Group B included women of reproductive years, namely, from 26 to 35 years of age ($n = 650$); and Group C corresponded to women of late reproductive age, specifically, from 36 years to menopause ($n = 36$). Regarding obesity status, patients were stratified according to their BMI, including normal weight (NW), overweight (OW), and obese (OB), the corresponding BMI values ranging from 20 to 24.9, from 25 to 29.9, and >30 kg/m^2, respectively.

Biochemical measurements

Blood samples were collected from all patients and healthy controls between 08:00 h and 10:00 h after an overnight fast. All samples were obtained during the early follicular phase of their menstrual cycle or at any time in amenorrheic subjects with progesterone levels <5 ng/mL. Samples were immediately centrifuged and serum was stored at −80°C until assayed. Hormonal samples were evaluated in the same lab of each center. The assays and ultrasound measurements employed have been described in detail elsewhere (16). Briefly, plasma fasting glucose was determined by the glucose oxidase color method (Glucose LR, GOD-PAP; Linear Chemicals, Barcelona, Spain). HbA1C was measured in venous blood by the D10 Hemoglobin testing system (Bio-Rad laboratories) that is based on cation exchange HPLC. Measurements of serum total cholesterol, HDL, and triglycerides were performed using the Siemens Advia 1650 Clinical Chemistry System (Siemens Medical Solutions). Insulin was measured using a solid-phase enzyme-amplified sensitivity immunoassay (INS-EASIA; Biosource Technologies, Nivelles, Belgium). Total testosterone was measured by ELISA (testosterone enzyme immunoassay test kit, LI7603; Linear Chemicals). SHBG serum levels were measured by ELISA (SHBG ELISA, MX 520 11; IBL, Hamburg, Germany). Androstenedione (Δ4A) was measured by RIA using active androstenedione-coated tube RIA kit DSL 3800 (Diagnostic Systems Laboratories, Webster, TX, USA). DHEAS serum levels were measured by DSL DHEAS RIA kit (Diagnostic Systems Laboratories). LH and FSH were measured using the LHsp and FSH IRMA kits from Biosource Technologies. E2 was measured by RIA (Immunometrics, London, UK).

The intra- and inter-assay coefficients of variation for low and high levels, respectively, were (i) 3.0 and 5.3% and 4.5 and 9.5% for insulin, (ii) 5.0 and 6.4% and 4.4 and 8.4% for total testosterone, (iii) 3.0 and 5.3% and 7.2 and 8.4% for SHBG, (iv) 6.5 and 8.8% and 3.5 and 4.5% for LH, (v) 2.7 and 5.3% and 1.6 and 3.6% for FSH, (vi) 9.4 and 6.3% and 9.6 and 9.9% for DHEAS, and (vii) 5.6 and 2.8% and 9.8 and 7.0% for Δ4A. Free androgen index (FAI) was determined as follows: testosterone (nmol/L) × 100/SHBG (nmol/L). The homeostasis model assessment of IR index (HOMA-IR) was calculated as follows: fasting insulin (mIU/L) × glucose (mg/dL)/405. In centers using different assays for hormonal evaluation, standardization of measurements was carried out among labs and a 5% variation in results was observed. In cases of uncertainty, these results were not included in the analysis.

Ovarian ultrasonography

Studies were performed during the follicular phase in ovulatory subjects. Three-dimensional ovarian morphology and size were determined and recorded, in each case by the same operator at each center, and all sonographic records were reviewed and scored by a third sonographer for the statistical analysis assessment according to the Rotterdam criteria.

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Ethics

Informed consent was obtained from all women and the institutional review board of all participating hospitals approved the study. The study met the requirements of the 1975 Declaration of Helsinki.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows (Released 2017, Version 25.0., IBM Corp). Variables were assessed for normality by evaluation of histograms and by the Kolmogorov–Smirnov test. Differences between groups were assessed using Fisher's exact test for categorical variables, the Mann–Whitney *U*-test for quantitative non-normally distributed variables, and Student's *t*-test for normally distributed variables. Overall, comparisons of continuous variables between groups were carried out by using ANOVA or the Kruskal–Wallis test. Correlations were assessed by Pearson's or Spearman's correlation coefficient (r). Associations of outcomes with continuous or dichotomic variables were assessed in linear and logistic regression models, respectively. A probability value of $P < 0.05$ was considered statistically significant for all tests. To visualize the prognostic value of the different anthropometric, biochemical, and hormonal parameters, 120-min glucose receiver–operator characteristic (ROC) curves were created using the 'ROC' application of the SPSS, and the respective under the curve areas (AUC) were calculated.

Results

The prevalence of dysglycemia was significantly higher in the PCOS group compared to controls. Specifically, for T2DM it was 2.2% vs 0.8%, $P=0.04$; for IGT it was 9.5% vs 7.4%, $P=0.038$; and for IFG it was 14.2% vs 9.1%, $P=0.002$. Women with PCOS were younger but more OB than controls and exhibited a higher degree of dyslipidemia, IR, and hyperandrogenism (clinical and biochemical), as well as increased ovarian volume compared to controls. Pertinent findings of the two groups are depicted in Table 1. Regarding PCOS characteristics, anovulation was reported in 47.3%, hirsutism in 56.2%, acne in 23.7%, and PCOS morphology on ultrasound in 87.5% of the PCOS group.

Table 1 Prevalence of dysglycemia, metabolic and hormonal profiles, and ovarian volume in women with PCOS and controls.

	PCOS (*n* = 1614)	**Controls** (*n* = 362)	***P***
Age (years)	25.14 ± 5.56	30.36 ± 5.96	0.027
BMI (kg/m^2)	27.34 ± 7.09	25.49 ± 5.97	<0.001
WHR	0.79 ± 0.08	0.78 ± 0.06	0.052
FG score	10.34 ± 3.76	4.24 ± 1.23	<0.001
IFG >100 mg/dL (%)	31.0	30.6	0.035
IFG >110 mg/dL (%)	14.2	9.11	0.002
IGT (%)	10.1	7.45	0.038
T2DM (%)	1.2	0.82	0.041
Glucose 0′ (mg/dL)	96.02 ± 11.89	94.19 ± 12.58	0.011
Insulin (pmol/L)	12.96 ± 8.36	10.32 ± 9.17	0.002
HOMA-IR	3.17 ± 2.20	2.51 ± 1.27	<0.001
Glucose 120′ (mg/dL)	106.77 ± 29.32	104.48 ± 29.66	0.200
Cholesterol (mmol/L)	4.34 ± 0.73	4.05 ± 0.424	0.002
LDL (mmol/L)	2.91 ± 0.93	2.16 ± 0.62	0.001
HDL (mmol/L)	1.11 ± 0.29	0.95 ± 0.26	0.001
Triglycerides (mmol/L)	1.16 ± 0.31	0.98 ± 0.23	0.001
LH (IU/L)	8.62 ± 5.42	6.18 ± 3.43	0.001
FSH (IU/L)	5.89 ± 2.0.5	6.82 ± 2.44	<0.001
E2 (pmol/L)	229.02 ± 157.11	213.13 ± 124.28	0.032
Testosterone (nmol/L)	2.74 ± 1.38	0.36 ± 0.13	<0.001
SHBG (nmol/L)	43.47 ± 26.44	64.01 ± 33.70	<0.001
FAI	7.80 ± 6.32	2.51 ± 1.59	<0.001
DHEAS (nmol/L)	4.32 ± 1.34	2.18 ± 0.67	<0.001
Δ4A (nmol/L)	1.96 ± 0.78	0.94 ± 0.41	<0.001
Ovarian volume (cm^3)	12.81 ± 3.42	6.24 ± 2.12	<0.001

E2, estradiol; FG, Ferriman–Gallway; FAI, free androgen index; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; IFG, Impaired fasting glucose; IGT, Impaired glucose tolerance; LH, luteinizing hormone; LDL, low-density lipoprotein; SHBG, sex hormone-binding globulin; T2DMM, diabetes mellitus type 2; WHR, waist-to-hip ratio; Δ4A, androstenedione.

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HbA1c values were available in a subset of women with PCOS (27%) with a mean value of 5.32 ± 0.45%.

Women suffering from PCOS with either T2DM or IGT were more OB and more insulin resistant. They displayed a higher degree of dyslipidemia and WHR compared to those women with either IFG or normal glucose tolerance, as shown in Table 2. Of interest, BMI, HOMA-IR, insulin, and androgen levels were significantly higher in PCOS women with IGT compared to those with T2DM. When subjects with T2DM were compared to all other women with PCOS as a whole, it was found that WHR (0.83 ± 0.08 vs 0.78 ± 0.08, $P = 0.04$), HbA1C (7.2 ± 0.54 vs 5.35 ± 0.44), LDL (3.44 ± 0.72 vs 2.90 ± 0.87, $P = 0.03$), and FAI (10.68 ± 9.72 vs 7.76 ± 6.25, $P = 0.04$) were significantly higher in those with T2DM.

The stratification of available data intra-PCOS according to age showed that the younger subjects were more hyperandrogenemic than their older counterparts, but the degree of dyslipidemia and IR was similar among groups. However, dysglycemia presenting either as IFG, IGT, and HbA1C was significantly increased in the older subgroup, although this was not the case for T2DM, as illustrated in Table 3. When the same type of analysis was carried out based on obesity status, it was observed that OB women with PCOS showed worse hormonal and metabolic profile than their normal-weight peers, and they had a higher prevalence of either IFG or IGT, but not of T2DM, in comparison to the other subgroups (Table 4). Age stratification of glycemic status in controls did not reach statistical significance among subgroups.

When the prevalence of T2DM was stratified according to age and BMI simultaneously, no difference was found among age and BMI subgroups. Specifically, in patients aged 17–22 years, T2DM was detected in three lean and two OB subjects. The corresponding distribution for patients aged 22–30 years was four lean, one OW, and two OB, whereas in those older than 31 years, two OW and five OB suffered from T2DM (Fig. 1A), and a similar result was obtained when the same type of analysis was conducted regarding IFG prevalence (Fig. 1C). However, regarding IGT the highest prevalence was found in those women in the highest third tile of both age and BMI (Fig. 1B). In control groups T2DM, IGT, and IFG prevalence was higher in older and more OB subjects.

OGTT was essential for accurate T2DM diagnosis since 15 of 17 subjects with T2DM had basal glucose values lower than the cut-off value of 124 mg/dL. As depicted in Fig. 2, FPG values were not sufficiently diagnostically accurate to identify subjects with either IGT or T2DM. In fact, FPG

Table 2 Comparison of PCOS characteristics among women with PCOS classified according to their glycemic status.

Variable	T2DM	IGT	IFG (ADA)	NGT
Age (years)	26.68 ± 6.58	26.23 ± 5.97	25.28 ± 5.44	25.04 ± 5.60
BMI (kg/m^2)	28.91 ± 3.65^{a,b,c}	31.04 ± 9.25^{d,e}	26.96 ± 6.69	26.53 ± 6.64
WHR	0.84 ± 0.08^{a,b,c}	0.82 ± 0.08^{d,e}	0.79 ± 0.07 ^{f}	0.76 ± 0.07
FG score	10.32 ± 4.36	10.81 ± 2.27	11.15 ± 4.56	11.23 ± 3.68
Glucose 0′ (mg/dL)	105.57 ± 18.44^{c}	103.64 ± 11.22^{e}	108.46 ± 6.40^{f}	88.68 ± 7.17
Glucose 120′ (mg/dL)	217.37 ± 18.5^{a,b,c}	156.97 ± 14.27^{d,e}	120.33 ± 31.43^{f}	95.25 ± 20.55
Insulin (pmol/L)	14.82 ± 5.36^{a}	15.11 ± 8.52^{d,e}	13.39 ± 10.13	14.31 ± 10.31
HOMA-IR	3.88 ± 2.48^{a,b,c}	4.49 ± 2.09^{d,e}	3.26 ± 1.89^{f}	2.79 ± 1.87
HbA1C (%)	7.2 ± 0.54^{a,b,c}	6.1 ± 0.67^{e}	5.9 ± 0.48^{f}	5.2 ± 0.73
Cholesterol (mmol/L)	5.23 ± 0.85^{a,b,c}	4.89 ± 0.84^{d,e}	4.63 ± 0.92	4.68 ± 0.90
LDL (mmol/L)	3.44 ± 0.72^{a,b,c}	3.06 ± 0.77^{e}	3.11 ± 0.90^{f}	2.80 ± 0.85
HDL (mmol/L)	1.49 ± 1.14^{a,b}	1.34 ± 0.51	1.32 ± 0.50	1.25 ± 0.69
Triglycerides (mmol/L)	1.15 ± 0.65	1.18 ± 0.64	1.17 ± 0.69	1.01 ± 0.54
LH (IU/L)	10.17 ± 5.32	11.31 ± 5.23	9.11 ± 7.35	8.91 ± 5.33
FSH (IU/L)	7.24 ± 2.37	6.80 ± 3.43	6.46 ± 3.44	6.51 ± 3.22
E2 (pmol/L)	234.67 ± 132.4	221.133 ± 144.3	231.52 ± 138.3	223.13 ± 114.9
Testosterone (nmol/L)	2.50 ± 0.99^{a}	2.94 ± 1.33^{d,e}	2.38 ± 0.12	2.41 ± 1.43
SHBG (nmol/L)	35.42 ± 22.93^{b,c}	34.52 ± 18.29^{d,e}	63.30 ± 34.81	46.01 ± 29.08
FAI	2.67 ± 0.49^{a,c}	3.46 ± 2.47^{d,e}	2.81 ± 1.86^{f}	1.04 ± 1.30
DHEAS (nmol/L)	3.00 ± 1.64^{a,b,c}	4.15 ± 2.32	3.78 ± 2.01^{f}	4.61 ± 4.06
Δ4A (nmol/L)	1.04 ± 0.53^{b}	0.93 ± 0.35^{d}	1.74 ± 0.47^{f}	0.95 ± 0.43
Ovarian volume (cm^3)	13.31 ± 4.41	12.11 ± 5.42	11.92 ± 4.53	12.44 ± 4.89

[a]Statistical significance, $P < 0.05$ between T2DM vs IGT; [b]Statistical significance, $P < 0.05$ between T2DM vs IFG; [c]Statistical significance, $P < 0.05$ between T2DM vs NGT; [d]Statistical significance, $P < 0.05$ between IGT vs IFG; [e]Statistical significance, $P < 0.05$ between IGT vs NGT; [f]Statistical significance, $P < 0.05$ between IFG vs NGT.

E2, estradiol; FG, Ferriman–Gallway; FAI, free androgen index; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; IFG, Impaired fasting glucose; IGT, Impaired glucose tolerance; LH, luteinizing hormone; LDL, low-density lipoprotein; SHBG, sex hormone-binding globulin; T2DMM, diabetes mellitus type 2; WHR, waist-to-hip ratio; Δ4A, androstenedione.

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Table 3 Comparison of dysglycemia prevalence and PCOS characteristics among women with PCOS classified according to age subgroups.

Variable	**Group A** (17–25 years, *n* = 628)	**Group B** (26–35 years, *n* = 650)	**Group C** (>35 years, *n* = 336)
AGE (years)	19.91 ± 1.53[a,b]	25.75 ± 1.96	33.74 ± 3.26
BMI (kg/m^2)	26.51 ± 6.70[b]	26.69 ± 6.842[c]	30.16 ± 7.56
WHR	0.78 ± 0.09[b]	0.78 ± 0.06[c]	0.82 ± 0.08
FG score	14.154 ± 4.82[a,b]	12.54 ± 2.45[c]	8.21 ± 2.31
IFG ADA (%)	35.5[b]	36.0[c]	41.4
IFG WHO (%)	11.6[b]	11.1[c]	16.8
IGT (%)	7.5[b]	8.1[c]	16.4
T2DM (%)	1.1	0.8	2.1
Glucose 0′ (mg/dL)	95.46 ± 12.02	95.83 ± 11.95	97.47 ± 11.48
HbA1C (%)	5.2 ± 0.76[b]	5.4 ± 0.81[c]	5.6 ± 0.32
Glucose 120′ (mg/dL)	105.91 ± 27.25[b]	105.28 ± 28.55[c]	111.30 ± 33.88
Insulin (pmol/L)	13.14 ± 8.21	12.67 ± 8.59	13.16 ± 8.21
HOMA-IR	3.14 ± 2.04	3.13 ± 2.31	3.29 ± 2.27
Cholesterol (mmol/L)	4.74 ± 0.92	4.75 ± 0.90	4.87 ± 1.05
LDL (mmol/L)	2.9 5 ± 0.82	2.86 ± 0.92	2.92 ± 0.85
HDL (mmol/L)	1.45 ± 0.59	1.45 ± 0.64	1.52 ± 0.71
Triglycerides (mmol/L)	1.06 ± 0.55	1.05 ± 0.52	1.09 ± 0.79
LH (IU/L)	10.22 ± 7.23[a,b]	7.91 ± 5.25	6.95 ± 4.69
FSH (IU/L)	5.82 ± 1.94	5.86 ± 1.78	6.10 ± 2.65
E2 (pmol/L)	214.46 ± 121.15	218.19 ± 131.54	221.42 ± 147.12
Testosterone (nmol/L)	2.75 ± 1.34[b]	2.84 ± 1.42[c]	2.52 ± 1.34
SHBG (nmol/L)	38.30 ± 20.93[a,b]	47.09 ± 29.67	46.66 ± 27.98
FAI	8.65 ± 6.65[a,b]	7.51 ± 6.28	6.72 ± 5.48
DHEAS (nmol/L)	4.30 ± 3.58[b]	4.55 ± 3.88[c]	3.59 ± 3.18
Δ4A (nmol/L)	0.9 6 ±0.41[b]	0.98 ± 0.41[c]	0.81 ± 0.39
Ovarian volume (cm^3)	13.22 ± 6.53	12.82 ± 5.67	11.67 ± 4.32

[a]Statistical significance, $P < 0.05$ between group A vs Group B; [b]Statistical significance, $P < 0.05$ between group A vs Group C; [c]Statistical significance, $P < 0.05$ between group B vs Group C.

missed more than one-third of women with dysglycemia. Specifically, among 163 patients with IGT, 108 of them (66.26%) had IFG, and the corresponding value for the 19 patients with T2DM was 12 (63.16%). Overall, among 182 patients with abnormal responses to OGTT, 62 had normal FPG (34.07%). Although, HbA1C values were not available in all women with PCOS, among those with IGT HbA1c was indicative of increased risk for diabetes in the vast majority of them (63.45%), but not for those with IFG (44.21%). Finally, regarding T2DM, data were insufficient for analysis

Considering the factors related to dysglycemia in PCOS, as reflected in glucose values post OGTT load, the univariate analysis demonstrated a significant positive association with BMI (r = 0.215, $P < 0.001$), WHR (r = 0.126, $P < 0.001$), fasting glucose (r = 0.377, $P < 0.001$), HbA1C (r = 0.301, $P < 0.001$), insulin (r = 0.119, $P < 0.001$), HOMA-IR (r = 0.238, $P < 0.001$), cholesterol (r = 0.086, $P < 0.001$), LDL (r = 0.156, $P < 0.001$), triglycerides (r = 0.106, $P < 0.001$), testosterone (r = 0.090, $P < 0.001$), and FAI (r = 0.198, $P < 0.001$). On the contrary a negative association was noticed for HDL (r = –0.089, $P < 0.001$) and SHBG values (r = –0.166, $P < 0.001$),

From multivariate analysis, it was shown in three different models that the degree of dysglycemia was significantly associated in Model 1 with BMI, LDL, and testosterone (R^2 0.079), in Model 2 with BMI, HOMA-IR, and FAI (R^2 0.073) and Model 3 with WHR, LDL, and SHBG (R^2 0.68) (Table 5). Regarding factors specifically related to the diagnosis of T2DM, IGT, and IFG, ROC curves evaluating the effect of several parameters were assessed (Table 6). However, due to low AUC values and at least one time point per parameter, none of the tested characteristics was further evaluated as a good classifier.

Discussion

The present study evaluated a large cohort of women with PCOS and confirmed a number of important metabolic facts. First, the prevalence of dysglycemia defined either as T2DM, IGT, or IFG is significantly increased in women with PCOS compared to controls. However, age and BMI, alone or in combination, could not conclusively identify those women at increased risk for either T2DM or IGT. Furthermore, several parameters that were analyzed, such

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Table 4 Comparison of dysglycemia prevalence and PCOS characteristics among women with PCOS classified according to BMI subgroups.

Variable	Normal weight	Overweight	Obese
AGE (years)	24.32 ± 4.89	25.34 ± 5.64	26.40 ± 6.29
BMI (kg/m^2)	21.75 ± 2.12^{a,b}	27.88± 1.40^{c}	36.66 ± 4.92
WHR	0.75 ± 0.07^{a,b}	0.80 ± 0.07	0.81 ± 0.07
FG score	12.21 ± 3.23^{a,b}	11.24 ± 3.13	10.33 ± 3.42
IFG ADA (%)	33^{a,b}	38.6^{c}	42.7
IFG WHO (%)	10.4^{b}	11.7^{c}	16.4
IGT (%)	6.7^{a,b}	9.4^{c}	16.7
T2DM (%)	0.9	0.9	2
Glucose 0′ (mg/dL)	94.58 ± 11.36	96.53 ± 11.20	97.96 ± 11.92
HbA1C (%)	5.3 ± 0.44^{a,b}	5.6 ± 0.42^{c}	5.9 ± 0.73
Glucose 120′ (mg/dL)	101.63 ± 26.79	107.83 ± 27.10	115.07 ± 33.16
Insulin (pmol/L)	9.70 ± 6.25^{a,b}	13.43 ± 7.58^{c}	18.29 ± 9.29
HOMA-IR	2.27 ± 1.44^{a,b}	3.31 ± 1.98^{c}	4.64 ± 2.61
Cholesterol (mmol/L)	4.67 ± 0.86^{b}	4.79 ± 0.83	4.90 ± 1.08
LDL (mmol/L)	2.41 ± 0.83^{a,b}	2.91 ± 0.75^{c}	3.11 ± 0.73
HDL (mmol/L)	1.51 ± 0.56	1.50 ± 0.72	1.39 ± 0.67
Triglycerides (mmol/L)	0.96 ± 0.61^{b}	1.08 ± 0.55^{c}	1.51 ± 0.56
LH (IU/L)	9.97 ± 6.19^{a,b}	7.41 ± 4.63	7.01 ± 5.02
FSH (IU/L)	6.03 ± 1.92	5.80 ± 1.81	5.72 ± 2.41
E2 (pmol/L)	238.62 ± 125.21	223.34 ± 124.52	218.78 ± 128.76
Testosterone (nmol/L)	2.57 ± 1.20^{a,b}	2.87 ± 1.50	2.93 ± 1.53
SHBG (nmol/L)	53.23 ± 28.77^{a,b}	37.13 ± 20.25^{c}	27.98 ± 13.82
FAI	5.78 ± 4.15^{a,b}	8.83 ± 6.20^{c}	11.26 ± 8.23
DHEAS (nmol/L)	3.70 ± 2.88^{a,b}	4.65 ± 3.90	4.91 ± 4.42
Δ4A (nmol/L)	0.95 ± 0.40	0.94 ± 0.43	0.91 ± 0.43
Ovarian volume (cm^3)	12.32 ± 8.61	13.12 ± 5.62	11.87 ± 4.89

[a]Statistical significance, $P < 0.05$ between NW vs OW; [b]Statistical significance, $P < 0.05$ between NW vs OB; [c]Statistical significance, $P < 0.05$ between OW vs OB.

as lipids, androgens, IR, and ovarian volume, did not provide sufficient accuracy as potential discriminators of glycemic status within the PCOS population. Finally, since one-third of women with PCOS with either T2DM or IGT displayed normal fasting glucose values, there is a clear need for the evaluation of glycemic status by OGTT in this population.

The prevalence of dysglycemia in the present study was significantly higher in the PCOS group compared to controls (T2DM 2.2% vs 0.82%, $P=0.04$; IGT 9.5% vs 7.45%; and $P=0.038$, IFG 14.2% vs 9.11%, $P=0.002$). The average prevalence of T2DM was 4.5% with the highest Europid prevalence in Nordic women in the fifth decade of life (19). This represents an exemption from the standard description of increased prevalence in the third decade of life. Explanation could be in the lack of longitudinal studies on metabolic profiles in PCOS women during the whole reproductive period or the impact of obesity. Finally, there are significant ethnic variations recently noticed (20, 21). The prevalence of IGT of 10.3% was below an average while the prevalence of IFG of 14.2% exceeded an average for the respective category for PCOS and healthy women of even older age (22). The heterogeneity of dysglycemia in these categories has not been fully elucidated. Various criteria applied for PCOS diagnosis in different ethnic groups analyzed, IFG definition used, as well as age and BMI distribution are likely to play a substantial role (23, 24).

One of the major tasks undertaken in the present study was to identify potential discriminators in women with PCOS prone to dysglycemia. However, regarding T2DM development age and BMI did not have any discriminatory role in our group, a finding not reported from other research groups (25, 26). Besides a smaller sample size in other studies, differences in ethnic origin and weight distribution should be considered as well. Namely, in the aforementioned study, NW PCOS patients were present in one-third while in our group those ones comprised almost half of the total groups, respectively (26).

Our older and OB PCOS patients had more prevalent IFG or IGT that is in line with other studies linking increasing age and BMI with prediabetes among women with PCOS (27, 28). However, considering these two factors together, they remained significant only in subjects with IGT, but not in IFG or T2DM (Fig. 1). This finding is compatible with that of several studies, showing that the prevalence of

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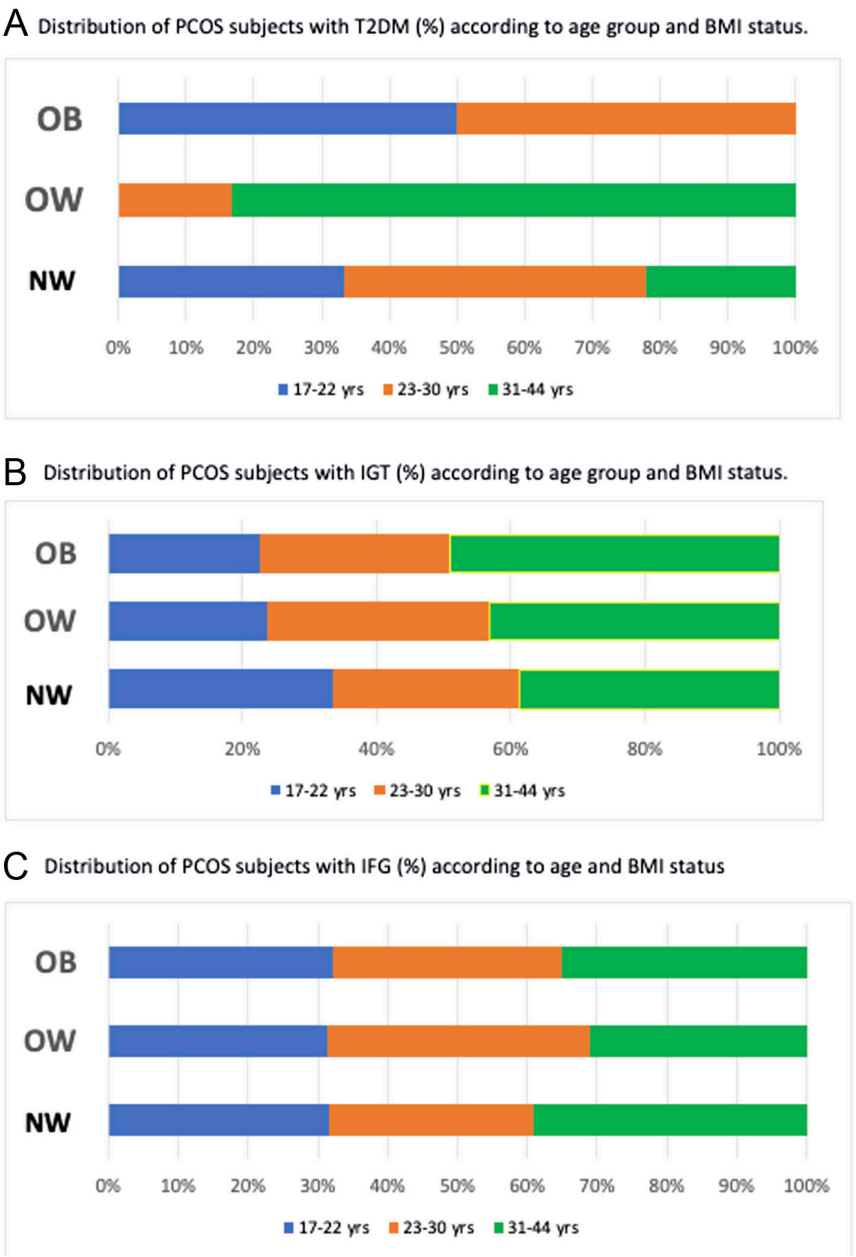

Figure 1
(A) Distribution of PCOS subjects with T2DM (%) according to age group and BMI status. (B) Distribution of PCOS subjects with IGT (%) according to age group and BMI status. (C) Distribution of PCOS subjects with IFG (%) according to age and BMI status.

IGT was lower in lean compared to OB women with PCOS (29). Of interest, women with IGT were more OB, exhibited a worse metabolic hormonal profile, and were more insulin resistant than their peers with either T2DM or IFG (Table 2). Whether women with IGT represent a specific group or are in the process of developing T2DM cannot as yet be determined.

It could be hypothesized that PCOS women with IFG may represent a different population from those with IGT. In fact, isolated IFG is usually observed in subjects with predominantly hepatic IR and normal muscle insulin sensitivity, whereas individuals with isolated IGT have normal to slightly reduced hepatic insulin sensitivity and moderate to severe muscle IR (30). Consequently, subjects with IFG may be prone to T2DM development, whereas subjects with IGT may comprise patients in whom dysglycemia occurs as a consequence of hyperandrogenemia. Indeed, the detrimental effect of androgens in muscle insulin sensitivity in lean women with PCOS has been documented (31). The above hypothesis is further supported by the observation that women with IGT tend to be more hyperandrogenic and OB than their peers with IFG (Table 2).

The role of obesity should not be neglected given that increased adiposity exerts a synergistic but independent, adverse effect on glucose metabolism and intrinsic IR in

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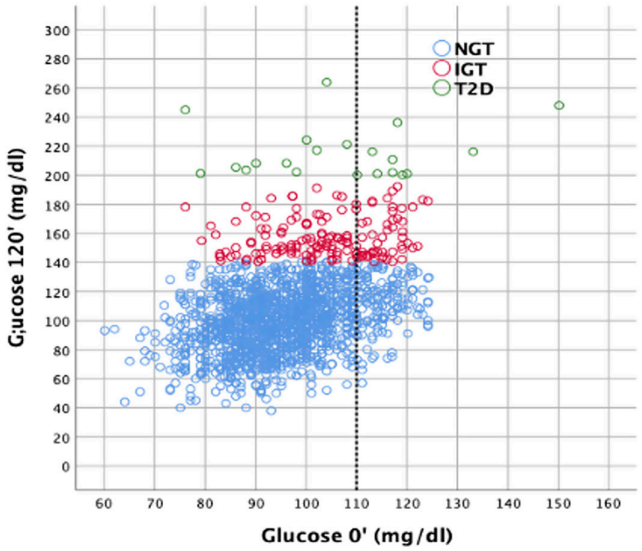

Figure 2
Classification of glycemic status according to glucose basal (0') and post OGTT (120') values. Vertical axis at IFG value of 110 mg/dL. Blue, Red and Green signs stand for subjects with NGT, IGT, and T2DM, respectively.

women with PCOS. In fact, it has been estimated via clamp techniques that obesity accounts for about 30% of the degree of IR in PCOS women (4). HOMA-IR was recognized as a potential marker for the diagnosis of dysglycemia in PCOS women. It must be emphasized that in the present study, HOMA-IR was associated with dysglycemia in both uni- and multivariate analysis, but we were unable to define a cut-off point with a high prognostic value in contrast to others (32). It could be speculated that ethnicity as well as variation among different insulin assays could influence this HOMA-IR cut-off value. Similarly, an increased ratio of truncal/lower body fat constitutes an established risk factor for adverse carbohydrate metabolism (33).

Hyperandrogenemia has been directly linked to impaired glycemic status (34), while testosterone, SHBG or FAI represent strong indicators of dysglycemia (35, 36, 37, 38). Lower SHBG contributes to T2DM and metabolic syndrome in the general population (39). Moreover, SHBG production is associated with insulin concentrations, and translated into causative relation of hyperandrogenemia with IR in PCOS. Higher quartile of FAI was associated with an increased risk of glucose intolerance even after adjustment for age, BMI, waist circumference, insulin, and family history of diabetes. However, a single cut-off point for FAI has not to date been defined. Moreover, the definition of biochemical hyperandrogenism in PCOS needs detailed steroid profiling (40), otherwise patients with the disorder may be overlooked.

Considering the ovarian volume, we did not observe any association with glycemic status in our cohort. This finding was to a degree anticipated given that polycystic ovarian morphology does not appear to confer metabolic risk in women with PCOS (41). Investigating the impact of various PCOS phenotypes did not show differences in the incidence of dysglycemia (42) that was observed in our study as well.

A role for FPG could be evolved from the hypothesis on being a part of glucose intolerance continuum. Namely, it is proposed that the higher the FPG the greater the risk for IGT/T2DM (43). However, FPG was not able to accurately predict dysglycemia regardless of age, BMI, IR, and hyperandrogenemia in the present cohort, a finding corroborating that of a previous study (44). Therefore, diagnostic un-accuracy with FPG determination only led to continuous debate regarding the use of OGTT in the diagnosis of dysglycemia in women with PCOS. Therefore, the findings of the present study strongly support the notion that OGTT should be performed routinely in all women with PCOS, irrespective of age and BMI. Furthermore, It should not be neglected that primary prevention of T2DM in normal subjects has been demonstrated only in those with IGT, regardless of their FPG or HbA1c values (45). Finally, we should bear in mind that the 3–6% variation reported in most commercially available glucose assays may easily characterize the same patient as either NGT or IFG.

Table 5 Models of multivariate analysis related to glucose values post OGTT load.

Parameters	Model 1		Model 2		Model 3	
	BMI, LDL, Testosterone		BMI, HOMA-IR, FAI		WHR, LDL, SHBG	
	P	Beta ± SE	*P*	Beta ± SE	*P*	Beta ± SE
BMI	<0.001	1.27 ± 0.17	<0.001	0.61 ± 0.14		
WHR					0.009	45.82 ± 17.51
HOMA-IR			0.009	1.27 ± 0.48		
LDL	0.004	4.12 ± 1.42			0.010	4.09 ± 1.58
Testosterone	<0.001	5.71 ± 1.14				
SHBG					<0.001	−0.21 ± .05
FAI			<0.001	0.59 ± 0.14		
R^2	**0.079**		**0.073**		**0.68**	

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Table 6 ROC curves evaluating parameters associated with T2DM, IGT, and IFG.

	T2DM		IGT		IFG	
	Area ± SE	CI	Area ± SE	CI	Area ± SE	CI
Age	0.59 ± 0.07	0.44–0.75	0.53 ± 0.02	0.47–0.59	0.51 ± 0.01	0.48–0.55
BMI	0.61 ± 0.07	0.46–0.74	0.64 ± 0.02	0.59–0.69	0.57 ± 0.01	0.53–0.60
WHR	0.69 ± 0.07	0.54–0.83	0.61 ± 0.02	0.55–0.66	0.52 ± 0.01	0.48–0.55
Fasting glucose	0.66 ± 0.07	0.51–0.80	0.70 ± 0.02	0.66–0.74	1	1
HbA1C (%)	0.63 ± 0.03	0.41–0.87	0.59 ± 0.04	0.48–0.63	0.53 ± 0.05	0.41–0.67
Insulin	0.56 ± 0.07	0.41–0.70	0.59 ± 0.02	0.54–0.64	0.54 ± 0.01	0.51–0.57
HOMA-IR	0.59 ± 0.06	0.45–0.72	0.64 ± 0.02	0.59–0.69	0.61 ± 0.01	0.58–0.64
Cholesterol	0.65 ± 0.07	0.49–0.80	0.52 ± 0.03	0.46–0.59	0.53 ± 0.02	0.49–0.58
LDL	0.6 9 ± 0.06	0.55–0.82	0.55 ± 0.03	0.48–0.62	0.58 ± 0.02	0.53–0.63
HDL	0.40 ± 0.08	0.23–0.57	0.43 ± 0.03	0.36–0.50	0.36 ± 0.02	0.32–0.41
Triglycerides	0.51 ± 0.09	0.33–0.70	0.53 ± 0.03	0.46–0.60	0.57 ± 0.02	0.53–0.62
Testosterone	0.5 0 ± 0.06	0.36–0.63	0.54 ± 0.02	0.49–0.59	0.50 ± 0.01	0.47–0.54
SHBG	0.40 ± 0.07	0.26–0.55	0.38 ± 0.02	0.33–0.43	0.45 ± 0.01	0.42–0.49
FAI	0.58 ± 0.07	0.44–0.71	0.61 ± 0.02	0.55–0.66	0.53 ± 0.02	0.50–0.57
DHEAS	0.47 ± 0.06	0.34–0.60	0.53 ± 0.02	0.48–0.58	0.47 ± 0.02	0.43–0.50
Δ4A	0.54 ± 0.07	0.40–0.68	0.48 ± 0.02	0.43–0.54	0.46 ± 0.01	0.43–0.50

For all the above reasons, OGTT was proposed as an essential step in the evaluation of any woman with PCOS (36).

Regarding $HbA1_C$ levels, ADA has approved its use as a surrogate index of dysglycemia. Indeed, in the present study, Hba1C values seemed to correlate adequately with glycemic status as shown in Tables 2, 3, 4, and 6. HbA1C undisputedly constitutes a very useful tool in everyday practice in comparison to OGTT since it is less time-consuming, reduced variability, and more convenient for both the patient and health providers (46). However, we are reluctant to strongly support the use of HbA1C as an index of dysglycemia in women with PCOS, due to several reasons. First, in the present study data on HbA1c were only available in a subset of women with PCOS. Furthermore, due to the irregular menstrual pattern, significant modifications of either hematocrit and/or ferritin levels frequently coexist in these women, making HbA1C evaluation unreliable as a predictor of glycemic status (47). Additionally, in smaller studies (48, 49), HbA1c levels did not appear to improve the diagnostic performance of dysglycemia in women with PCOS, while the role of HbA1c in the diagnosis of dysglycemia has been questioned in OW and OB subjects, who compose the vast majority of the PCOS population (50).

One major disadvantage of the present study was that assessment of either steroids or IR was not carried out with the gold standard methods such as mass spectrometry and clamp techniques. In fact, these methods might facilitate spotting those PCOS women with a higher risk of developing T2DMM, as shown previously (51). However, these methods are time-consuming and extremely expensive to be performed in this very large cohort. In addition, the inclusion of subjects younger than the age of 20 years, but at least 3 years post menarche, may have an impact on our findings, given the uncertainty of mechanisms governing PCOS in adolescence.

In conclusion, although the prevalence of dysglycemia is significantly increased in women with PCOS, we were unable to identify an anthropometric/biochemical/hormonal marker as a potent indicator of those women at risk. This outcome may be partly attributed to the fact that PCOS constitutes a polygenic trait. Thus, it is most likely that some of the above-mentioned factors need to be considered in combination with a molecular marker in order to develop an accurate prognostic model. Until the latter advance has been made, based on our analysis of this large cohort of European women of Caucasian origin, we strongly advocate that OGTT should be performed in all patients with PCOS regardless of their age, BMI, body composition, fasting glucose levels, degree of IR, and hyperandrogenemia. In other words, a significant number of PCOS women with dysglycemia may be overlooked with the use of other surrogate methods of glycemic status assessment. Therefore, a cascading process should be implemented, including early identification of women at risk, followed by OGTT, and tailored strategies for loss of weight and fat deposition.

We suggest that PCOS is a risk factor for T2DM. However, since hyperandrogenemia and IR are gradually improved through time in lean women suffering from the syndrome and that the risk of T2DM development in this subgroup of PCOS women is comparable to that in controls (52), a significant reduction in risk for dysglycemia and development of T2DM would be expected with the above approach. The evidence strongly indicates that the risk for developing T2DM is elevated in overweight and OB women

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with PCOS, whereas in NW women with PCOS, this risk appears to decrease through time.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

1 Torchen LC. Cardiometabolic risk in PCOS: more than a reproductive disorder. *Current Diabetes Reports* 2017 **17** 137. (https://doi.org/10.1007/s11892-017-0956-2)
2 Rubin KH, Glintborg D, Nybo M, Abrahamsen B & Andersen M. Development and risk factors of type 2 diabetes in a nationwide population of women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2017 **102** 3848–3857. (https://doi.org/10.1210/jc.2017-01354)
3 Ollila MM, West S, Keinänen-Kiukaanniemi S, Jokelainen J, Auvinen J, Puukka K, Ruokonen A, Järvelin MR, Tapanainen JS, Franks S, *et al*. Overweight and obese but not normal weight women with PCOS are at increased risk of type 2 diabetes mellitus – a prospective, population-based cohort study. *Human Reproduction* 2017 **32** 423–431. (https://doi.org/10.1093/humrep/dew329)
4 Cassar S, Misso ML, Hopkins WG, Shaw CS, Teede HJ & Stepto NK. Insulin resistance in polycystic ovary syndrome: a systematic review and meta-analysis of euglycaemic-hyperinsulinaemic clamp studies. *Human Reproduction* 2016 **31** 2619–2631. (https://doi.org/10.1093/humrep/dew243)
5 Diamanti-Kandarakis E & Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocrine Reviews* 2012 **33** 981–1030.
6 Dunaif A & Finegood DT. Beta-cell dysfunction independent of obesity and glucose intolerance in the polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 942–947. (https://doi.org/10.1210/jcem.81.3.8772555)
7 Barber TM & Franks S. Obesity and polycystic ovary syndrome. *Clinical Endocrinology* 2021 **40** 37–51. (https://doi.org/10.1111/cen.14421)
8 Forslund M, Landin-Wilhelmsen K, Trimpou P, Schmidt J, Brännström M & Dahlgren E. Type 2 diabetes mellitus in women with polycystic ovary syndrome during a 24-year period: importance of obesity and abdominal fat distribution. *Human Reproduction Open* 2020 **2020** hoz042. (https://doi.org/10.1093/hropen/hoz042)
9 Gambineri A, Patton L, Altieri P, Pagotto U, Pizzi C, Manzoli L & Pasquali R. Polycystic ovary syndrome is a risk factor for type 2 diabetes: results from a long-term prospective study. *Diabetes* 2012 **61** 2369–2374. (https://doi.org/10.2337/db11-1360)
10 Zhu T, Cui J & Goodarzi MO. Polycystic ovary syndrome and risk of type 2 diabetes, coronary heart disease, and stroke. *Diabetes* 2021 **70** 627–637. (https://doi.org/10.2337/db20-0800)
11 Dapas M, Lin FTJ, Nadkarni GN, Sisk R, Legro RS, Urbanek M, Hayes MG & Dunaif A. Distinct subtypes of polycystic ovary syndrome with novel genetic associations: an unsupervised, phenotypic clustering analysis. *PLoS Medicine* 2020 **17** e1003132. (https://doi.org/10.1371/journal.pmed.1003132)
12 Andersen M & Glintborg D. Diagnosis and follow-up of type 2 diabetes in women with PCOS: a role for OGTT? *European Journal of Endocrinology* 2018 **179** D1–D14. (https://doi.org/10.1530/EJE-18-0237)
13 Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ & International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Human Reproduction* 2018 **33** 1602–1618. (https://doi.org/10.1093/humrep/dey256)
14 Ezeh U, Ida Chen YD & Azziz R. Racial and ethnic differences in the metabolic response of polycystic ovary syndrome. *Clinical Endocrinology* 2020 **93** 163–172. (https://doi.org/10.1111/cen.14193)
15 Livadas S, Kollias A, Panidis D & Diamanti-Kandarakis E. Diverse impacts of aging on insulin resistance in lean and obese women with polycystic ovary syndrome: evidence from 1345 women with the syndrome. *European Journal of Endocrinology* 2014 **171** 301–309. (https://doi.org/10.1530/EJE-13-1007)
16 Livadas S, Macut D, Bothou C, Kuliczkowska-Płaksej J, Vryonidou A, Bjekic-Macut J, Mouslech Z, Milewicz A & Panidis D. Insulin resistance, androgens, and lipids are gradually improved in an age-dependent manner in lean women with polycystic ovary syndrome: insights from a large Caucasian cohort. *Hormones* 2020 **19** 531–539. (https://doi.org/10.1007/s42000-020-00211-z)
17 American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes – 2021. *Diabetes Care* 2021 **44** S15–S33. (https://doi.org/10.2337/dc21-S002)
18 World Health Organization & International Diabetes Federation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF Consultation, 2006.
19 Eades CE, France EF & Evans JMM. Prevalence of impaired glucose regulation in Europe: a meta-analysis. *European Journal of Public Health* 2016 **26** 699–706. (https://doi.org/10.1093/eurpub/ckw085)
20 Ganie MA, Dhingra A, Nisar S, Sreenivas V, Shah ZA, Rashid A, Masoodi S & Gupta N. Oral glucose tolerance test significantly impacts the prevalence of abnormal glucose tolerance among Indian women with polycystic ovary syndrome: lessons from a large database of two tertiary care centers on the Indian subcontinent. *Fertility and Sterility* 2016 **105** 194–201.e1. (https://doi.org/10.1016/j.fertnstert.2015.09.005)
21 Dabadghao P, Roberts BJ, Wang J, Davies MJ & Norman RJ. Glucose tolerance abnormalities in Australian women with polycystic ovary syndrome. *Medical Journal of Australia* 2007 **187** 328–331. (https://doi.org/10.5694/j.1326-5377.2007.tb01273.x)
22 Kakoly NS, Khomami MB, Joham AE, Cooray SD, Misso ML, Norman RJ, Harrison CL, Ranasinha S, Teede HJ & Moran LJ. Ethnicity, obesity and the prevalence of impaired glucose tolerance and type 2 diabetes in PCOS: a systematic review and meta-regression. *Human Reproduction Update* 2018 **24** 455–467. (https://doi.org/10.1093/humupd/dmy007)
23 Stovall DW, Bailey AP & Pastore LM. Assessment of insulin resistance and impaired glucose tolerance in lean women with polycystic ovary syndrome. *Journal of Women's Health* 2011 **20** 37–43. (https://doi.org/10.1089/jwh.2010.2053)
24 Zhang B, Wang J, Shen S, Liu J, Sun J, Gu T, Ye X, Zhu D & Bi Y. Association of androgen excess with glucose intolerance in women with polycystic ovary syndrome. *BioMed Research International* 2018 **2018** 6869705. (https://doi.org/10.1155/2018/6869705)
25 Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK & Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care* 1999 **22** 141–146. (https://doi.org/10.2337/diacare.22.1.141)
26 Pelanis R, Mellembakken JR, Sundström-Poromaa I, Ravn P, Morin-Papunen L, Tapanainen JS, Piltonen T, Puurunen J, Hirschberg AL, Fedorcsak P, *et al*. The prevalence of type 2 diabetes is not increased in normal-weight women with PCOS. *Human Reproduction* 2017 **32** 2279–2286. (https://doi.org/10.1093/humrep/dex294)
27 Lee H, Oh JY, Sung YA, Chung H & Cho WY. The prevalence and risk factors for glucose intolerance in young Korean women with

polycystic ovary syndrome. *Endocrine* 2009 **36** 326–332. (https://doi.org/10.1007/s12020-009-9226-7)
28 Wei HJ, Young R, Kuo IL, Liaw CM, Chiang HS & Yeh CY. Prevalence of insulin resistance and determination of risk factors for glucose intolerance in polycystic ovary syndrome: a cross-sectional study of Chinese infertility patients. *Fertility and Sterility* 2009 **91** 1864–1868. (https://doi.org/10.1016/j.fertnstert.2008.02.168)
29 Legro RS, Kunselman AR, Dodson WC & Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 165–169. (https://doi.org/10.1210/jcem.84.1.5393)
29 Ciampelli M, Leoni F, Cucinelli F, Mancuso S, Panunzi S, De Gaetano A & Lanzone A. Assessment of insulin sensitivity from measurements in the fasting state and during an oral glucose tolerance test in polycystic ovary syndrome and menopausal patients. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 1398–1406. (https://doi.org/10.1210/jc.2004-0410)
30 Abdul-Ghani MA, Tripathy D & DeFronzo RA. Contributions of β-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. *Diabetes Care* 2006 **29** 1130–1139. (https://doi.org/10.2337/diacare.2951130)
31 Hansen SL, Svendsen PF, Jeppesen JF, Hoeg LD, Andersen NR, Kristensen JM, Nilas L, Lundsgaard AM, Wojtaszewski JFP, Madsbad S, *et al*. Molecular mechanisms in skeletal muscle underlying insulin resistance in women who are lean with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2019 **104** 1841–1854. (https://doi.org/10.1210/jc.2018-01771)
32 Möhlig M, Flöter A, Spranger J, Weickert MO, Schill T, Schlösser HW, Brabant G, Pfeiffer AFH, Selbig J & Schöfl C. Predicting impaired glucose metabolism in women with polycystic ovary syndrome by decision tree modelling. *Diabetologia* 2006 **49** 2572–2579. (https://doi.org/10.1007/s00125-006-0395-0)
33 Zhao X, Zhong J, Mo Y, Chen X, Chen Y & Yang D. Association of biochemical hyperandrogenism with type 2 diabetes and obesity in Chinese women with polycystic ovary syndrome. *International Journal of Gynaecology and Obstetrics* 2010 **108** 148–151. (https://doi.org/10.1016/j.ijgo.2009.09.021)
34 Chen X, Yang D, Li L, Feng S & Wang L. Abnormal glucose tolerance in Chinese women with polycystic ovary syndrome. *Human Reproduction* 2006 **21** 2027–2032. (https://doi.org/10.1093/humrep/del142)
35 Espinós-Gómez JJ, Corcoy R & Calaf J. Prevalence and predictors of abnormal glucose metabolism in Mediterranean women with polycystic ovary syndrome. *Gynecological Endocrinology* 2009 **25** 199–204. (https://doi.org/10.1080/09513590802585597)
36 Bhattacharya SM. Polycystic ovary syndrome and abnormalities in glucose tolerance. *International Journal of Gynaecology and Obstetrics* 2009 **105** 29–31. (https://doi.org/10.1016/j.ijgo.2008.11.031)
37 Amato MC, Magistro A, Gambino G, Vesco R & Giordano C. Visceral adiposity index and DHEAS are useful markers of diabetes risk in women with polycystic ovary syndrome. *European Journal of Endocrinology* 2015 **172** 79–88. (https://doi.org/10.1530/EJE-14-0600)
38 Gracelyn LJ & Pushpagiri N. Prevalence of glucose tolerance test abnormalities in women with polycystic ovarian syndrome. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology* 2017 **4** 1739–1745. (https://doi.org/10.18203/2320-1770.ijrcog20151215)
39 Ding EL, Song Y, Manson JE, Hunter DJ, Lee CC, Rifai N, Buring JE, Gaziano JM & Liu S. Sex hormone-binding globulin and risk of type 2 diabetes in women and men. *New England Journal of Medicine* 2009 **361** 1152–1163. (https://doi.org/10.1056/NEJMoa0804381)
40 Livadas S, Pappas C, Karachalios A, Marinakis E, Tolia N, Drakou M, Kaldrymides P, Panidis D & Diamanti-Kandarakis E. Prevalence and impact of hyperandrogenemia in 1,218 women with polycystic ovary syndrome. *Endocrine* 2014 **47** 631–638. (https://doi.org/10.1007/s12020-014-0200-7)
41 Dewailly D, Lujan ME, Carmina E, Cedars MI, Laven J, Norman RJ & Escobar-Morreale HF. Definition and significance of polycystic ovarian morphology: a task force report from the Androgen Excess and Polycystic Ovary Syndrome Society. *Human Reproduction Update* 2014 **20** 334–352. (https://doi.org/10.1093/humupd/dmt061)
42 Li H, Li L, Gu J, Li Y, Chen X & Yang D. Should all women with polycystic ovary syndrome be screened for metabolic parameters? A hospital-based observational study. *PLoS ONE* 2016 **11** e0167036. (https://doi.org/10.1371/journal.pone.0167036)
43 Nichols GA, Hillier TA & Brown JB. Progression from newly acquired impaired fasting glusose to type 2 diabetes. *Diabetes Care* 2007 **30** 228–233. (https://doi.org/10.2337/dc06-1392)
44 Ortiz-Flores AE, Luque-Ramírez M, Fernández-Durán E, Alvarez-Blasco F & Escobar-Morreale HF. Diagnosis of disorders of glucose tolerance in women with polycystic ovary syndrome (PCOS) at a tertiary care center: fasting plasma glucose or oral glucose tolerance test? *Metabolism: Clinical and Experimental* 2019 **93** 86–92. (https://doi.org/10.1016/j.metabol.2019.01.015)
45 Stevens JW, Harvey RC, Johnson M & Khunti K. Preventing the progression to type 2 diabetes mellitus in adults at high risk: a systematic review and network meta-analysis of lifestyle, pharmacological and surgical interventions. *Value in Health* 2014 **17** A335. (https://doi.org/10.1016/j.jval.2014.08.643)
46 Gooding HC, Milliren C, St Paul M, Mansfield MJ & DiVasta A. Diagnosing dysglycemia in adolescents with polycystic ovary syndrome. *Journal of Adolescent Health* 2014 **55** 79–84. (doi:10.1016/j.jadohealth.2013.12.020.)
47 Solomon A, Hussein M, Negash M, Ahmed A, Bekele F & Kahase D. Effect of iron deficiency anemia on HbA1c in diabetic patients at Tikur Anbessa specialized teaching hospital, Addis Ababa Ethiopia. *BMC Hematology* 2019 **19** 2. (https://doi.org/10.1186/s12878-018-0132-1)
48 Celik C, Abali R, Bastu E, Tasdemir N, Tasdemir UG & Gul A. Assessment of impaired glucose tolerance prevalence with hemoglobin A_1c and oral glucose tolerance test in 252 Turkish women with polycystic ovary syndrome: a prospective, controlled study. *Human Reproduction* 2013 **28** 1062–1068. (https://doi.org/10.1093/humrep/det002)
49 Velling Magnussen L, Mumm H, Andersen M & Glintborg D. Hemoglobin A1c as a tool for the diagnosis of type 2 diabetes in 208 premenopausal women with polycystic ovary syndrome. *Fertility and Sterility* 2011 **96** 1275–1280. (https://doi.org/10.1016/j.fertnstert.2011.08.035)
50 Chatzianagnostou K, Vigna L, Di Piazza S, Tirelli AS, Napolitano F, Tomaino L, Bamonti F, Traghella I & Vassalle C. Low concordance between HbA1c and OGTT to diagnose prediabetes and diabetes in overweight or obesity. *Clinical Endocrinology* 2019 **91** 411–416. (https://doi.org/10.1111/cen.14043)
51 Moghetti P, Tosi F, Bonin C, Di Sarra D, Fiers T, Kaufman JM, Giagulli VA, Signori C, Zambotti F, Dall'Alda M, *et al*. Divergences in insulin resistance between the different phenotypes of the polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** E628–E637. (https://doi.org/10.1210/jc.2012-3908)
52 Anagnostis P, Paparodis RD, Bosdou JK, Bothou C, Macut D, Goulis DG & Livadas S. Risk of type 2 diabetes mellitus in polycystic ovary syndrome is associated with obesity: a meta-analys is of observational studies. *Endocrine* 2021 **74** 245–253. (https://doi.org/10.1007/s12020-021-02801-2)

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