Diverse impacts of aging on insulin resistance in lean and obese women with polycystic ovary syndrome: evidence from 1345 women with the syndrome

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Abstract

Background: Polycystic ovary syndrome (PCOS) represents a moving spectrum of hormonal to metabolic abnormalities, as women with the syndrome are aging. Hormonal abnormalities, anovulation, and hyperandrogenic signs were predominant during the early years of PCOS and fade away with the years. Metabolic abnormalities and insulin resistance (IR) remain throughout the PCOS life cycle; however, it is unclear as to how they change, as women with the syndrome are aging.

Objective: To evaluate the changes in IR and its associations with clinical, biochemical, hormonal, and ultrasound findings in a large cohort of women with PCOS and controls, as they are aging.

Design: A cross-sectional study was carried out to evaluate the diverse impacts of aging on IR.

Setting: An outpatient clinic was chosen for the study.

Participants: A total of 1345 women with PCOS (Rotterdam criteria) and 302 controls of Caucasian origin and Greek ethnicity comprised the study group.

Main outcome and measures: The impact of age on IR, as calculated using homeostasis model assessment of IR (HOMA-IR) index, and several PCOS characteristics were evaluated.

Results: In PCOS, age (-0.045 ± 0.008) was negatively, and BMI positively (0.18 ± 0.007) associated with HOMA-IR ($R^2 = 0.36$). When data were stratified with regard to the BMI status, a negative association of age with HOMA-IR was found in lean, normal, and overweight patients ($r: -0.266, -0.233, -0.192, P < 0.001$), which was neutralized in obese patients ($r: -0.009, P: NS$). Free androgen index and BMI were positively associated with HOMA-IR in all age quartiles. When mean HOMA-IR values were plotted according to BMI subgroups at different age quartiles, a significant gradual decrease in HOMA-IR was observed in normal ($P < 0.001$) and overweight ($P: 0.004$), but not obese, women ($P: 0.202$) across age quartiles.

Conclusions: Aging increases IR in obese but not in lean and overweight women with PCOS. As BMI and androgens are positively associated with HOMA-IR and androgens decline through time, it appears that if women with PCOS do not become obese they may exhibit a better metabolic profile during their reproductive years.

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Introduction

Polycystic ovary syndrome (PCOS) is a multifaceted disorder affecting 6–15% of women of reproductive age, depending on the criteria used for diagnosis, and a broad

spectrum of phenotypes is incurred in the syndrome (1, 2). Hyperandrogenemia and insulin resistance (IR) constitute the cardinal hormonal defects encountered in the vast

majority of patients, irrespective of race and ethnicity. The central role of IR in the pathophysiology of PCOS was elucidated from the elegant work by Dunaif and coworkers in the 1990s (3, 4). Specifically, robust data have shown that IR enhances hyperandrogenemia, by induction of ovarian and adrenal steroidogenesis and by decreasing hepatic liver SHBG synthesis. Furthermore, IR has been linked to oxidative stress, subclinical inflammation, and endothelial dysfunction, usually encountered in patients with PCOS. IR constitutes a prerequisite for diabetes mellitus (DM) and/or metabolic syndrome development, and consequently the increased incidence of these two morbidities in women with PCOS, compared with their BMI-matched peers, is expected (5, 6, 7, 8, 9). However, the available prospective studies reporting the significantly higher incidence of DM in women with PCOS compared with general population are few and DM is found mainly in obese women with the syndrome (10, 11, 12).

During aging in normal subjects of either sex, a gradual increase in body weight is observed, which is associated with an unfavorable impact on metabolic profile and IR has been considered the main pathophysiological link between obesity and metabolic derangements (13, 14). Moreover, in a normal population, the aging process is associated with a gradual increase in IR and β -cell decompensation, leading to DM development (15). Nevertheless, the molecular pathways regulating IR are completely different in patients with DM from those with PCOS, and a different degree of IR in different tissues has been identified in patients (16). Furthermore, there are scanty data reporting IR progression through time in PCOS. Accordingly, the notion that DM evolution is the outcome of IR rise has not been substantiated in patients with the syndrome.

Although it is known that, in PCOS, androgens gradually decline through time, the natural history of IR in women with the syndrome has not been unveiled yet. However, on clinical grounds, this question is of great importance, as it will probably identify protective mechanisms to reduce the occurrence of DM in PCOS. Therefore, we designed this study with the aim to evaluate the impact of age on IR in women of reproductive age with PCOS diagnosed using the Rotterdam criteria. Anthropometric, clinical, metabolic, hormonal, and ultrasound data from a large cohort of women with PCOS ($n=1345$) were analyzed according to age in a cross-sectional manner. In order to assess the impact of obesity on IR, all available data were analyzed according to the BMI. Additionally, data from 302 control women were analyzed in parallel. As an index of IR, HOMA-IR was employed, which has universally been considered as an appropriate

tool for the evaluation of IR and progression to DM in population studies (17).

Subjects and methods

Subjects

All women diagnosed with PCOS between 2004 and 2012 at the PCOS Clinic, Evgenideio Hospital, Medical School, University of Athens, and the Gynecological Endocrinology Infirmary of the Second Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, were included in this study. Diagnosis of PCOS was based on the Rotterdam criteria (18). A total of 1508 women consecutively diagnosed with PCOS were evaluated. From the initial sample, women younger than 15 years of age and older than 40 years of age, significantly obese (BMI $> 50 \text{ kg/m}^2$), and with fasting glucose higher than 7 mmol/l were excluded from the analysis. Accordingly, data from 1345 women with PCOS were included in this study.

None of the women studied had galactorrhea or any endocrine or systemic disease that could possibly affect the reproductive physiology. None of them reported use of any medication that could interfere with the normal function of the hypothalamic–pituitary–gonadal axis, including metformin, during the last semester. When basic 17α -hydroxyprogesterone (17α -OHP) levels were $> 1.5 \text{ ng/ml}$, the Synacthen test (Synacthen 0.25 mg/1 ml; Novartis Pharma) was performed to rule out congenital adrenal hyperplasia. Other causes of hyperandrogenism, including prolactinoma, Cushing's syndrome, and androgen-secreting tumors, were also excluded.

The control group comprised 302 normally ovulating, non-hyperandrogenic women. Women of the control group were healthy volunteers with normal ovulating cycles (28 ± 2 days, blood progesterone levels $> 10 \text{ ng/ml}$ in two consecutive cycles), no signs of hyperandrogenism, or hyperandrogenemia and normal sonographic appearance of the ovaries.

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

In all subjects, BMI, waist-to-hip ratio (WHR), systolic and diastolic blood pressure, Ferriman–Gallwey score (FG), glucose, lipids (HDL, LDL, triglycerides, and cholesterol), insulin, liver function tests, gonadotropins (luteinizing hormone (LH) and follicle-stimulating hormone (FSH)), androgens, ovarian volume, and total follicle count were analyzed. Baseline blood samples were collected between

days 3 and 7 of the menstrual cycle in the control group and between 3 and 7 days after a spontaneous bleeding episode in patients with PCOS after an overnight fast. On the same day, abdominal or transvaginal ultrasonography was performed and the volume of each ovary, as well as the number of follicles in each ovary, was determined.

Assays

Plasma fasting glucose (mg/dl) was determined by the glucose oxidase color method (Glucose LR, GOD-PAP; Linear Chemicals, Barcelona, Spain). Total cholesterol (TC; mg/dl) was determined by the enzymatic Cobas Mira method (Cholesterol LR, CHOD-PAP; Linear Chemicals). Insulin (μ U/ml) was measured by solid-phase enzyme-amplified sensitivity immunoassay (INS-EASIA; Biosource Technologies, Nivelles, Belgium). Total testosterone (ng/dl) was measured by ELISA (testosterone enzyme immunoassay test kit, LI7603; Linear Chemicals). SHBG serum levels (nmol/l) were measured by ELISA (SHBG ELISA, MX 520 11; IBL, Hamburg, Germany). DHEAS (ng/ml) serum levels were measured using a DSL DHEAS RIA kit (Diagnostic Systems Laboratories, Webster, TX, USA). LH (IU/l) and FSH (IU/l) were measured using the LHsp and FSH IRMA kits from Biosource Technologies, and Δ 4A (ng/ml) was measured by RIA using active androstenedione-coated tube RIA kit DSL 3800 (Diagnostic Systems Laboratories). The intra- and inter-assay coefficient of variation (CV) values 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 Δ 4A.

Free androgen index (FAI) was determined as follows: $T \text{ (nmol/l)} \times 100 / \text{SHBG (nmol/l)}$ (19). The homeostasis model assessment of IR (HOMA-IR) index was calculated using the following formula: $\text{fasting insulin (mIU/l)} \times \text{glucose (mg/dl)} / 405$ (20).

Ovarian ultrasonography

Studies were carried out during the follicular phase in ovulatory subjects. Three-dimensional ovarian morphology and size were determined and recorded (on film) by transvaginal ultrasound for all subjects, except for the case of virginity. In each case, by the same operator at each center, all sonographic records were reviewed and scored by a third sonographer for the statistical analysis assessment according to the Rotterdam criteria.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences software (IBM SPSS Statistics for Windows, Version 20.0.; IBM Corp., Armonk, NY, USA). Results are expressed as mean $\pm$ s.d. The Kolmogorov–Smirnov statistic was performed to test continuous variables for normality. Spearman's or Pearson's correlation coefficients (r) were determined for the assessment of the relationship between the examined variables. Age quartiles were defined by cut off points that divide the total population in groups containing 25% of the total observations each. In order to demonstrate a significant coefficient in the range of 0.20 with the power of 85%, a total sample of 177 subjects would be required, which was taken into account in the subgroup analyses according to BMI and age status. Comparisons of parameters between two groups were performed by t -test or Mann–Whitney U test, depending on the distribution (normal or not) of the examined variables. Similarly, comparisons among the three groups were performed by ANOVA or the Kruskal–Wallis test. Stepwise multivariate regression analysis was performed in order to define the independent predictors of the HOMA-IR. Independent variables included age, BMI, FAI, androstenedione, and SHBG. A probability value of $P < 0.05$ was considered statistically significant.

Results

Data from 1345 women with PCOS (Rotterdam criteria) and 302 controls were analyzed. The mean age of the PCOS group was 24.49 ± 5.59 years (range: 15–49 years) and the mean BMI $26.57 \pm 6.77 \text{ kg/m}^2$ (range: 16.2–49 kg/m^2). The mean age of the control group was 32.21 ± 14.40 years (range: 17.5–41 years) with a mean BMI of $25.28 \pm 6.14 \text{ kg/m}^2$ (range: 18–32 kg/m^2) respectively. Pertinent data of the studied population and comparisons between the studied groups are given in Table 1. In a subset of PCOS and control subjects older than 30 years of age, it was shown that HOMA-IR was significantly higher in women with PCOS, in both lean (1.66 ± 0.08 vs 1.39 ± 0.07 , $P: 0.015$) or obese (3.37 ± 0.16 vs 2.64 ± 0.23 , $P: 0.015$) subjects.

Associations in PCOS and controls

In the PCOS group, age was positively correlated with BMI ($r: 0.143$), WHR ($r: 0.175$), glucose ($r: 0.82$), and FSH ($r: 0.139$) and negatively with FG score ($r: -0.119$), testosterone ($r: -0.76$), Δ 4 ($r: -0.93$), DHEAS ($r: -0.165$), 17-OHP ($r: -0.187$), LH/FSH ratio ($r: -0.094$), prolactin ($r: -0.098$),

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Table 1 Pertinent data of studied population with PCOS.

	PCOS		Controls		P
	n	Mean $\pm$ S.D.	n	Mean $\pm$ S.D.	
Age (years)	1345	24.49 $\pm$ 5.59	302	32.21 $\pm$ 14.40	<0.001
BMI (kg/m ²)	1345	26.57 $\pm$ 6.77	302	25.28 $\pm$ 6.14	<0.001
WHR	1225	0.79 $\pm$ 0.19	302	0.78 $\pm$ 0.06	0.200
Systolic BP (mmHg)	419	108.08 $\pm$ 13.50	302	98.03 $\pm$ 10.27	NS
Diastolic BP (mmHg)	419	68.82 $\pm$ 11.22	302	71.47 $\pm$ 9.47	NS
FG score	1345	8.35 $\pm$ 4.68	302	3.04 $\pm$ 2.64	<0.001
Glucose (mmol/l)	1323	5.23 $\pm$ 0.67	302	5.25 $\pm$ 0.63	NS
Insulin (pmol/l)	1317	83.62 $\pm$ 56.19	302	68.20 $\pm$ 30.00	<0.001
HOMA-IR	1317	2.80 $\pm$ 1.98	302	2.24 $\pm$ 0.92	<0.001
Cholesterol (mmol/l)	419	485 $\pm$ 0.95	112	4.69 $\pm$ 0.85	0.04
Triglycerides (mmol/l)	419	1.09 $\pm$ 0.52	112	0.78 $\pm$ 0.35	<0.01
HDL (mmol/l)	419	1.22 $\pm$ 0.29	112	1.43 $\pm$ 0.32	<0.01
LDL (mmol/l)	419	3.11 $\pm$ 0.85	112	2.55 $\pm$ 0.48	<0.01
ALT (IU/l)	419	19.31 $\pm$ 7.19	112	17.34 $\pm$ 4.91	NS
AST (IU/l)	419	23.51 $\pm$ 15.03	112	21.28 $\pm$ 6.73	NS
FSH (IU/l)	1345	5.88 $\pm$ 2.08	302	6.83 $\pm$ 2.43	<0.001
LH (IU/l)	1345	7.65 $\pm$ 5.59	302	6.02 $\pm$ 3.43	<0.001
LH/FSH	1345	1.31 $\pm$ 1.02	302	1.02 $\pm$ 1.12	<0.001
PRL (pmol/l)	1345	618 $\pm$ 318	259	573 $\pm$ 266	0.012
Testosterone (nmol/l)	1345	2.60 $\pm$ 1.06	302	1.36 $\pm$ 0.47	<0.001
SHBG (nmol/l)	1327	41.93 $\pm$ 24.72	302	62.24 $\pm$ 34.28	<0.001
FAI	1327	7.80 $\pm$ 3.08	302	2.84 $\pm$ 1.86	<0.001
$\Delta 4$ (nmol/l)	1345	0.10 $\pm$ 0.04	302	0.05 $\pm$ 0.03	<0.001
DHEAS (nmol/l)	1345	10.03 $\pm$ 5.21	302	6.56 $\pm$ 2.71	<0.001
17-OHP (nmol/l)	1345	3.40 $\pm$ 1.37	302	2.51 $\pm$ 1.21	<0.001
TSH (mIU/l)	1345	2.12 $\pm$ 1.09	302	2.10 $\pm$ 1.26	NS
FT ₄ (pmol/l)	971	157.27 $\pm$ 20.19	302	155.98 $\pm$ 14.16	NS
Volume of the right ovary (ml)	1114	8.22 $\pm$ 4.46	302	5.21 $\pm$ 2.09	<0.001
Volume of the left ovary (ml)	1114	7.88 $\pm$ 4.10	302	4.68 $\pm$ 1.78	<0.001
Number of follicles in the right ovary	1114	11.14 $\pm$ 5.09	302	6.27 $\pm$ 2.79	<0.001
Number of follicles in the left ovary	1114	11.11 $\pm$ 5.25	302	5.94 $\pm$ 2.38	<0.001

insulin (r : -0.080), HOMA-IR (r : -0.06), FAI (r : -0.182), and follicle number (r : -0.125) ($P < 0.001$ for all comparisons). In the control group, age was positively correlated with BMI (r : 0.135) and WHR (r : 0.159), and negatively with testosterone (r : -0.213), DHEAS (r : -0.211), and FAI (r : -0.278) ($P < 0.05$ for all comparisons), whereas the association of age with HOMA-IR was not significant. In stepwise multivariate regression analysis with HOMA-IR as a dependent variable and age, BMI, FAI androstenedione, and SHBG as independent variables, HOMA-IR was found to be independently associated ($R^2 = 0.60$) with age (β -coefficient $\pm$ S.E.M.: -0.047 ± 0.008) and BMI (0.18 ± 0.007).

Correlations according to age quartiles

When PCOS data were stratified according to age quartiles, it was found that age was negatively associated with

HOMA-IR for the age groups < 20 (r : -0.168 , $P < 0.01$) and $20-24$ years (r : -0.139 , $P < 0.01$), but not with BMI and FAI. However, in the age group older than 28 years, a weak positive association was disclosed between age and HOMA-IR (r : 0.133 , $P < 0.05$), but a stronger association of age with BMI was found (r : 0.255 , $P < 0.01$). Regarding the correlation of HOMA-IR with BMI, a strong positive association was disclosed in all age subgroups (r : 0.480 , 0.431 , 0.531 , and 0.665 respectively; $P < 0.001$ for all comparisons) (Fig. 1). A similar finding was made regarding the association between HOMA-IR and FAI (r : 0.227 , 0.325 , 0.295 , and 0.270 respectively; $P < 0.001$ for all comparisons).

Correlations according to BMI subgroups

When an analogous stratification of data was carried out regarding BMI status, it was shown that in lean, normal,

Figure 1

Correlation of HOMA-IR with BMI at different age quartiles in PCOS group.

and overweight patients a negative association of age with HOMA-IR existed ($r: -0.266, -0.233, \text{ and } -0.192$, $P < 0.001$ for all comparisons), which was neutralized in obese patients ($r: -0.009$, P : NS), as illustrated in Fig. 2. Regarding the association of FAI with age, a negative association was documented in all BMI subgroups, except lean PCOS (Fig. 3). Furthermore, when mean HOMA-IR values were plotted according to BMI subgroups at different age quartiles, a significant gradual decrease in HOMA-IR was observed in lean, normal, and overweight women across age quartiles (Fig. 4). A similar analysis was performed between hyperandrogenemic and non-hyperandrogenemic PCOS and analogous findings were observed (data not shown). Investigation of correlations between age, lipid parameters, and blood pressure levels was carried out according to the BMI status. Age was inversely correlated with systolic blood pressure ($r = -0.21$, $P = 0.01$) for BMI category 20–25 kg/m² and inversely correlated with triglycerides ($r = -0.25$, $P = 0.02$) for BMI category 25–30 kg/m². Power analyses of all analyses performed strongly suggest that our findings are accurate.

Discussion

In this study, in a very large cohort of women with PCOS with same ethnicity and race, it is shown for the first time that aging affects IR in a distinctly different way between obese and lean women with the syndrome. IR is increased, as they are getting older, in obese PCOS, but not in lean ones diagnosed with Rotterdam criteria. Specifically, HOMA-IR was negatively associated with age in the total

PCOS group as well as in different BMI subgroups, namely lean, normal, and overweight subjects (Fig. 2). Additionally, when available data were stratified according to age quartiles in different BMI subgroups, a significant decrease in HOMA-IR through age was noted in non-obese patients (Fig. 3). Furthermore, multivariate regression analysis demonstrated an independent association of HOMA-IR with age (inverse) as well as BMI.

This finding is unexpected as, according to current knowledge, PCOS constitutes a prediabetic state and through time DM development is expected, due to IR increment. However, this concept is based on studies conducted in women diagnosed using the NIH and not the Rotterdam criteria, which were applied in this study. Furthermore, a careful evaluation of available literature data revealed that the group of patients developing DM were significantly obese at initial evaluation and/or gained weight through the period of observation, whereas the percentage of women who kept their BMI stable did not develop DM in the long term (21). This finding is noteworthy and is especially emphasized in the studies by Norman *et al.* (12) and Gambineri *et al.* (22), where lean patients or those who did not gain weight did not progress to DM. Specifically, in the study by Gambineri *et al.* in 249 patients followed up for a mean period of 16 years, the relative risk of DM evolution in lean subjects was 0.6, whereas the corresponding number in obese patients exceeded 2.

The prospective studies in which increased susceptibility to impaired glucose tolerance (IGT) and/or DM has been reported were based on significantly obese patients with a mean BMI ranging from 35 to 41 kg/m² (10, 12, 23).

Figure 2

Correlation of HOMA-IR with age according to the BMI status in the PCOS group.

Figure 3

Correlation of FAI with age according to the BMI status in PCOS group.

On the other hand, the studies conducted in overweight patients did not report increased evolution from normoglycemia to DM followed up for 5.5–21 years (24, 25). Only one study reported progression of 2% from normal glucose tolerance to DM in lean patients with PCOS followed up for 15 years (26). Only one early study reported increase in insulin levels (11), a finding not corroborated by the other available reports (23, 24, 25, 26, 27, 28). The existing prospective studies assessing progression to DM in women with PCOS are summarized in Table 2 and emphasize the role of BMI in DM development.

The role of obesity in IR increase, a prerequisite for progression to DM, is highlighted in this study. Specifically, a strong and positive association between HOMA-IR and BMI is found among all age subgroups, suggesting that BMI exerts an impact on IR irrespective of age (Fig. 1). Additionally, it has been demonstrated that in patients younger than 24 years of age, age is negatively correlated with HOMA-IR and only in patients older than 28 years of age is a weak-positive association between age and HOMA-IR revealed. However, in this age group, HOMA-IR is strongly correlated with BMI and this positive association should be attributed mainly to BMI increment. The fact that regression analysis showed that only age and BMI affect significantly and in opposite directions the degree of IR in PCOS highlights the impact of obesity on IR in the aging process of women with PCOS.

There are data suggesting that women with PCOS display a higher degree of intrinsic IR compared with age- and BMI-matched peers (3, 7, 29). Additionally, in this study, women with PCOS display higher HOMA-IR values

compared with their control peers, even in those older than 30 years of age, irrespective of the BMI (Table 1). Nevertheless, this study for the first time provides ample evidence that in women with PCOS (Rotterdam criteria) through time IR is not increased, but rather ameliorated, in non-obese women with PCOS (Fig. 3). Accordingly, the argument that PCOS constitutes a risk factor for DM development in non-obese women with the syndrome should be reappraised, taking into consideration, however, that the present data are the product of a cross-sectional study and not of a prospective study.

During the last two decades, it has become evident that both IR and β -cell dysfunction are essential conditions for DM development, and aging is related to both these conditions (14, 30, 31). However, if IR is improved over the years in non-obese women with PCOS, this phenomenon can counteract the diminished β -cell secretion, thus reducing DM risk. Moreover, it must be borne in mind that although lean women with PCOS display intrinsic IR, the degree of IR is comparable to their obese control peers (4, 32). Hence, obesity *per se* seems to be the critical risk factor for IR development and one may hypothesize that DM occurrence in women with PCOS may be an epiphenomenon due to an increased BMI, as obesity and PCOS usually coincide. This hypothesis was, in fact, provided by the Escobar-Morreale group, who reported a significantly higher prevalence of PCOS in overweight and obese women compared with their lean peers (28.3 vs 5.5% respectively), and this finding has been

Figure 4

Mean HOMA-IR values in different age quartiles at different BMI subgroups.

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Table 2 Evolution of diabetes and IGT in PCOS (available literature data).

Reference	n	BMI	Duration of follow-up (years)	Evolution of IGT	Evolution of DM	Insulin levels
(10)	25	41	2.4	40%	NA	NA
(11)	21	32	10		NA	Deterioration
(12)	67	35–41	6	9.3%	7.4%	NA
		27–28		0	0	NA
(26)	34	24	15	NA	2%	Stable
(23)	35	35	3	16%	NA	Stable
(24)	172	26	2.6	0%	0%	Stable
	82	26	5.5	0%	0%	Stable
(21)	84	?	14	9.5%	8.3%	NA
(25)	35	27.1	21	0%	0%	Stable
(27)	118	27	20	NA	NA	Stable
(22)	249		16.9	Total group	39%	NA
		<25		Incidence rate $\times$ 100 person-years	0.25	NA
		25–30			0.63	NA
		>30			2.02	NA

confirmed by other research groups (33, 34, 35). Besides, it has been shown that women with PCOS display a strong positive family history of DM, another fundamental predisposing factor in DM development (36).

The gradual decrease in IR through time may be partly attributed to androgen decline with aging. Indeed, a chicken-and-egg situation exists between IR and hyperandrogenemia in PCOS, and several *in vitro* and *in vivo* studies have demonstrated an improvement in IR through androgen reduction (37, 38, 39). Additionally, in either pre- or postmenopausal normal women, a direct relationship between androgen levels and IR or DM risk has been determined (40, 41). In women with PCOS, but also in controls, androgen levels gradually decrease through time, as has been shown in several studies (42, 43, 44, 45) and confirmed in the present one. However, it should be emphasized that androgens decreased irrespective of the BMI, implying that the association of androgens with age is direct and not through obesity.

There are three limitations of this study: i) the use of RIA for insulin quantification, which has become outdated nowadays, but was the method of choice during sample collection; ii) the cross-sectional, retrospective design of the study; and iii) the small control group ($n=302$). However, the main scope of this paper was to evaluate IR values of intra-PCOS group according to age and BMI. The control group was used to assure us that the women with PCOS studied were more insulin resistant than controls, and not to directly compare women with PCOS with controls.

In conclusion, we provide ample evidence that, through the reproductive years, in women with PCOS

(Rotterdam criteria) a gradual improvement in IR is observed in non-obese patients. Whether this trend should be attributed to the resolution of ovulation, as it has been recently suggested (27), to decrease in androgens, to prevention of obesity, or to a combination of these interrelated factors remains to be further elucidated. However, our data suggest that in everyday practice, the clinician should encourage lean women to maintain their body weight and insist on obese women to reduce their body weight, as this will not only benefit their present health status but may also prevent the development of metabolic sequelae later in their life.

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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Author contribution statement

S Livadas and E Diamanti-Kandarakis worked in the original idea and wrote the manuscript. A Kollias performed statistical analysis. D Panidis and E Diamanti-Kandarakis collected all data.

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