The Egyptian Journal of Medical Human Genetics (2015) **16**, 367–374

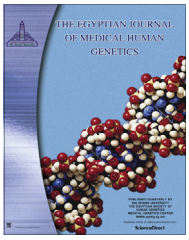
HOSTED BY

ELSEVIER

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com



ORIGINAL ARTICLE

Metabolic abnormalities in young Egyptian women with polycystic ovary syndrome and their relation to *ADIPOQ* gene variants and body fat phenotype

Moushira Zaki [a,*], Shams Kholoussi [b], Somaia Ismail [c], Haiam Abdel Raouf [b], Iman Helwa [b], Naglaa Hassan [a], Eman Youness [d], Nadia A. Mohamed [d], Sanaa Kamal [a], Walaa Yousef [a], Mohamed Shaker [a], Wafaa Ezzat [e], Yasser A. Elhosary [e], Omnia M. Saleh [e], Mona El Gammal [g], HalaT. El-Bassyouni [f], Samira Ismail [f], Mamdouh Bibars [g], Osama Azmy [g]

[a] *Biological Anthropology Department, Egypt*
[b] *Immunogenetics Department, National Research Centre, Giza, Egypt*
[c] *Medical Molecular Genetics Department, National Research Centre, Giza, Egypt*
[d] *Medical Biochemistry Department, National Research Centre, Giza, Egypt*
[e] *Internal Medicine Department, National Research Centre, Giza, Egypt*
[f] *Clinical Genetics Department, National Research Centre, Giza, Egypt*
[g] *Reproductive Health Research Department, National Research Centre, Giza, Egypt*

Received 4 May 2015; accepted 24 May 2015
Available online 25 June 2015

KEYWORDS

ADIPOQ variants;
PCOS;
Metabolic syndrome;
Insulin resistance;
Central obesity;
Young women

Abstract *Background:* Polycystic ovary syndrome (PCOS) is the most common endocrine disorder. It is associated with high prevalence of metabolic risk factors, but little is known about the prevalence of metabolic syndrome (MS) and its components among Egyptian PCOS women. The objective of the study was to determine the metabolic abnormalities among young Egyptian women with PCOS and evaluate their relation with *adiponectin* gene (*ADIPOQ*) variants and body fat adiposity pattern.

Materials and methods: The present study included 80 PCOS women and 80 healthy women with similar age and body mass index. All participants underwent clinical, anthropometric, biochemical, ultrasonographic and adiponectin (*ADIPOQ*) gene 11391G > A (rs17300539) examinations.

Insulin resistance was assessed by the Homeostatic model assessment for insulin resistance (HOMA-IR).

Results: MS was identified in 22.5% of PCOS women. The most prevalent MS components in PCOS women were central obesity, decreased high-density lipoprotein cholesterol (HDL-C), and

Abbreviations: PCOS, Polycystic Ovarian Syndrome; BMI, Body Mass Index; BP, Blood Pressure; MUAC, Mid Upper Arm Circumference; WC, Waist Circumference; HC, Hip Circumference; WHR, Waist to Hip Ratio; SF, Skin Fold; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HDL-C, High Density Lipoprotein Cholesterol; LDL-C, Low Density Lipoprotein Cholesterol; TG, Triglycerides

* Corresponding author.

Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2015.05.007

increased triglycerides (TG), blood pressure (BP) and fasting glucose levels. The study found association between *ADIPOQ* promoter variants −11391G > A and MS in PCOS women. Moreover, multivariate logistic regression analysis showed association between abdominal fat accumulation and IR in PCOS.

Conclusion: The prevalence of MS was significantly higher in PCOS women than controls, and central obesity and hypertension are risk factors for insulin resistance. Moreover, obesity plays a key role in the development of PCOS and *ADIPOQ* −11391G > A gene variants showed association with MS.

1. Introduction

Polycystic ovary syndrome (PCOS) as the most common endocrinopathy among reproductive aged women is a major health and economic burden. Depending on the criteria used for its definition, the method used to define each criterion and the study population, the prevalence of PCOS ranges between 2.2% and 26% in various countries [1]. The ovarian dysfunction syndrome encompasses a broad spectrum of clinical signs and symptoms. Clinical manifestations include menstrual irregularities, hyperandrogenism and infertility [2]. According to previous reports, insulin resistance, obesity and dyslipidemia have commonly been described as associated with PCOS [3]. These disorders are also the features of the so-called metabolic syndrome (MS) or syndrome X, another cluster of endocrine disturbances defined by the World Health Organization, the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) and the International Diabetes Federation (IDF) guidelines [4].

Metabolic syndrome (MS) which is a common disorder related to visceral obesity and insulin resistance (IR) is associated with atherosclerosis and cardiovascular (CV) disease [5,6]. The prevalence of MS is high, occurring in 23.7% of the USA population over 20 years [7]. The prevalence also increases with age, from 6.7% in the third decade to 43.5% in the 7th decade [8,9]. The overall prevalence of MBS appears to be similar between the USA and European countries with reported rates of 23.5% in Spain and 23.9% in Portugal [10].

In Egypt, a cross-sectional observational study was conducted on 1450 women visiting the outpatient clinic of Minia University Maternity Hospital [11], included 620 middle-aged fertile women with an intrauterine contraceptive device and gave birth more than 2 years previously and 830 with primary or secondary infertility reported that the prevalence of PCOS in the fertile women was 14% (87/620) and 37.5% (311/830) in the secondary infertile women.

MS is a combination of cardiovascular risk factors, including dyslipidemia, impaired fasting glucose levels, abdominal obesity and high blood pressure. Insulin resistance, as a major defect in MS, appears to be a common linkage between these coexisting abnormalities [12]. Since the anthropometric and metabolic abnormalities found in PCOS overlap with the components of MS [13,14], the issue regarding MS in women with PCOS has generated tremendous interest. Diagnosis of MS requires clinical and laboratory information that is grouped into criteria. However, each institute defines the cut-off for each criterion differently. Such difference would affect the prevalence of MS, even within the same population [15]. Recent studies have found a much higher prevalence of MS in women with PCOS than in those without PCOS [13,16,17]. According to estimates based on the US population, the prevalence of MS in women with PCOS is approximately 43–46% [18,19].

The aim of the present study was to assess the metabolic abnormalities among young Egyptian women with PCOS and evaluate the influence of body fat adiposity pattern and *ADIPOQ* gene variants and metabolic abnormalities.

2. Subjects and methods

All the procedures used in this study were in accordance with the guidelines of the Helsinki Declaration on Human Experimentations. The study was approved by local ethics committee of the National Research Centre (No: 13176); the purpose of the protocol was explained to both the patients and control women, and written informed consent was obtained from them before beginning the study.

This prospective case-control study included eighty Egyptian women with PCOS between ages 20 and 35 years. They were referred from different Obstetrics and Gynecology centers to the National Research Centre Clinics between 2013 and 2014. All participated in the project entitled "Body adiposity phenotypes, dietary intake, adiponectin gene variants, metabolic markers and their significance in obesity-related diseases."

Eighty women controls of similar age and BMI of patients were selected. All subjects were sedentary and were not participating in any specific diet plan. The mean age of attaining menarche in PCOS patients was 12.83 ± 1.11 years, and for controls was 12.73 ± 1.35 years.

The diagnosis of PCOS was based on Rotterdam-PCOS criteria [20].

According to these criteria, PCOS were diagnosed if at least two of the following criteria were present: oligoamenorrhoea, clinical or biochemical hyperandrogenism and PCO on ultrasonography. Exclusion criteria: women with congenital adrenal hyperplasia, androgen-secreting neoplasms, androgenic/anabolic drug use or abuse, Cushing's syndrome, syndromes of severe insulin resistance, thyroid dysfunction, and hyperprolactinemia.

All control subjects underwent an ultrasonographic examination by a gynecologist, and women who had any pathologic findings or polycystic ovaries were excluded from the study. HOMA-IR cut-off was = 3.46 as insulin resistant [21].

MS was defined using the definition of the 2001 National Cholesterol Education Program Adult Treatment Panel III

(*NCEP ATP III*) [22] with the presence of three or more of the following abnormalities: waist circumference (WC) $\geqslant$88 cm; fasting serum glucose of at least 110 mg/dL; fasting serum triglycerides (TG) $\geqslant$150 mg/dL; serum high-density lipoprotein cholesterol (HDL-C) $<$50 mg/dL, and blood pressure $\geqslant$130/85 mmHg. All subjects underwent a clinical examination where body weight, height, waist and hip circumferences, and blood pressure were measured. All women met the inclusion criteria, infertility $\geqslant$1 year and age $<$37 years.

2.1. Anthropometry and blood pressure

Anthropometric measurements included body weight, height, mid upper arm, thigh waist and hip circumferences and skinfold thickness biceps, triceps and subscapular, suprailiac and abdominal skin fold thickness were measured. All measurements were taken 3 times on the left side of the body and the mean of the 3 values was used. Body weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 cm. Height was measured with the patients standing with their backs leaning against the stadiometer of the same scale. BMI was calculated as weight in kilograms divided by height in meters square (kg/m2). Waist circumference (WC) and hip circumference (HC) were measured in cm using a plastic, non-stretchable tailor's tape. WC was measured with light clothing at a level midway between the lower rib margin and the iliac crest standing and breathing normally. HC was measured at the level at the widest circumference over the buttocks (at the greater trochanter). Subsequently the waist hip ratio (WHR) was calculated as WC divided by HC. Skin-fold thickness was measured to the nearest mm, except for low values (usually 5 mm or less) when it was taken to the nearest 0.5 mm. These readings were made at six sites on all subjects, at the biceps, triceps, subscapular and supra-iliac areas, using Holtain caliper (Ltd, Bryberian, Crymmych, Pembrokeshire). The subscapular skinfold was taken at an angle of about 45'' to the vertical. Biceps was measured at the level of the mid-point between the acromion (lateral edge of the acromion process) and the radius (proximal and lateral border of the radius bone) on the mid-line of the anterior surface of the arm, triceps (vertical fold, midway between acromion, and olecranon processes on the posterior surface of the arm), and the position of the suprailiac skinfold was the diagonal fold just above the iliac crest even with the anterior axillary line, abdominal skin fold was at 5 cm adjacent to the umbilicus to the right side. Anthropometric measurements were obtained according to standardized equipment and following the recommendations of the International Biological Program [23].

Systolic and diastolic blood pressures (SBP and DBP) were measured twice in the right arm in a sitting position after a 10 min rest period; using a mercury sphygmomanometer the average of the two measurements was used for analysis. Blood pressure was measured according to a standardized operating procedure using a calibrated sphygmomanometer and brachial inflation cuff (HEM-7200 M3, Omron Healthcare, Kyoto, Japan). Hypertension was defined according to the guidelines set by the Joint National Committee on Prevention, Evaluation and Treatment of High Blood Pressure [24]: systolic blood pressure (SBP) > 140 mmHg or diastolic blood pressure (DBP) > 90 mmHg or taking antihypertensive medication.

3. Laboratory measurements

Venous blood samples were collected by direct venipuncture after an overnight (minimum 12 h) fast. Blood samples were analyzed for glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and insulin. Serum lipids (enzymatic methods) and plasma glucose (glucose oxidase method) were assayed using the Hitachi 7060 C automatic biochemistry analysis system. HDL-C and LDL-C were measured directly.

Serum insulin was measured by monoclonal antibody-based sandwich enzyme-linked immunosorbent assays (ELISA) (Linco Research Inc., St. Charles, MO).

Insulin resistance index was calculated through surrogate marker HOMA-IR. Insulin resistance (IR) was estimated using the homeostasis model assessment index-insulin resistance (HOMA-IR). With the determination of insulin and glucose, it was possible to determine HOMA-IR using the following formula: [serum insulin $\times$ serum glucose/22.5].

3.1. Transvaginal ultrasonography

A single transvaginal ultrasound scan was performed at a random time (during the menstrual cycles) in subjects reporting absent, irregular or regular periods. Scans were performed by a single ultrasonographer using an Ultra Sonix RP ultrasound scanner equipped with a 9-MHz transvaginal transducer (UltraSonix, Version 2.3.5, Vancouver, BC). Each ovary was visualized and anatomic orientation with respect to the utero-ovarian ligament was established. Ovaries were scanned from the inner to outer margins in both the transverse and sagittal planes.

4. Molecular genotyping

Genomic DNA was extracted from peripheral blood leucocytes using the salting out method. The Adiponectin (ADIPOQ) gene promoter $-11391G>A$ (rs17300539) single nucleotide polymorphism (SNP) was genotyped [24]. The polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) genotyping procedure was carried out to the extracted genomic DNA. The following primers were used (G-11391A): 5′-CATC AGAA TGTG TGGC TTGC-3′ as forward and 5′-AGAAGCAG CCTG GAGA ACTG-3′ as a reverse primer.

Each PCR reaction contained 25 μl final volume consisting of the following 250 ng genomic DNA, 200 uM dNTPS, 0.5 unit of DNA polymerase (DyNAZyme II, FINZYMES) and 20 pmol of each primer.

The thermocycling conditions consisted of initial denaturation at 94 °C for 10 min, followed by 40 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, then the final extension at 72 °C for 10 min. The PCR products were digested with MspI restriction endonuclease (Fermentas, Germany) at 37 °C for 15 min. The products of the digest were then visualized on a 2.5% agarose gel stained with ethidium bromide, showing DNA fragments of 137 and 26 bps when the GG genotype was detected and 163 bps of undigested PCR product

when AA was detected. A pattern of 163, 137 and 26 bps was obtained when GA genotype was detected.

5. Statistical analysis

All analyses were performed by using Statistical Product and Service Solutions (SPSS) (version 13.0, spss Inc., Chicago, IL, USA). Continuous variables were presented as mean ± SD and analyzed using independent sample *t*-test for normally distributed data or Mann–Whitney *U*-test for skewed data. Categorical variables were expressed as proportion (percentage) and analyzed by the chi-square test or Fisher's exact tests as appropriate. Multivariate logistic regression was used to examine independent predictors of MS and IR in PCOS. The results were expressed as odds ratio (OR) with 95% confidence interval (CI) and evaluated by two sided *p* value. Baseline characteristics of the PCOS and controls were evaluated by analysis of variance.

Pearson's partial correlation coefficients for body adiposity indices and each measure of metabolic parameters were calculated, adjusted for BMI and age. Normal distribution was tested using the Kolmogorov–Smirnov test. $P < 0.05$ was considered statistically significant. Chi-square goodness of fit test was then utilized to evaluate Hardy–Weinberg equilibrium in patient and control groups.

6. Results

The mean age of women in the control group (25.1 ± 3.4 year) was similar to the mean age of the PCOS group (24.9 ± 2.4 year). The clinical and anthropometric characteristics of women with PCOS and controls are given in Table 1. The mean BMI of the two groups ranged between 25 and 30 kg/m^2, and there was no significant difference between the two means. But different patterns of fat distribution were seen between the two groups.

Patients with PCOS had higher waist circumference, waist to hip ratio, abdominal skin-fold thickness and blood pressure level compared to normal controls of the same age and BMI ($P < 0.05$).

Metabolic features of PCOS patients were compared with the controls (Table 2). The PCOS patients showed significantly higher levels of serum fasting glucose, insulin, HOMA-IR, TG, and LDL and lower levels of HDL-C than the matched age and BMI controls.

In PCOS women, increased waist circumference (> 88 cm) was found in 96.25% of women, reduced HDL-C (< 50 mg/dl) was found in 75% of women and increased TG level was found in women (63.75%) and increased blood glucose was found in 33.75% of PCOS women while in BMI-matched controls, increased waist circumference was found in 37.5% of women, reduced HDL-C was found in 25% of women, increased TG level was found in women (12.5%) and increased blood glucose in 13.75% ($p < .05$) (Fig. 1).

Pearson's partial correlation coefficients for body adiposity indices, adjusted for age and BMI and the metabolic parameters were evaluated (Table 3). Although there was no statistically significant correlation between body fat % and metabolic markers, the WC, WHR and abdominal skin fold thickness showed a significant positive correlation with metabolic parameters including the systolic and diastolic blood pressure, fasting glucose, TG and negative correlation with HDL-C levels.

Table 4 shows multivariate logistic regression performed to examine the predictors of IR in women with PCOS. For

Table 1 Clinical and anthropometric characteristics of PCOS and controls.

Parameters	Group	Mean ± SD	*P* values
Age (years)	PCOS	24.9 ± 2.4	.07
	Controls	25.1 ± 3.4	
BMI (kg/m^2)	PCOS	27.48 ± 4.30	.06
	Controls	26.90 ± 3.75	
Age at menarche (year)	PCOS	12.83 ± 1.11	.07
	Controls	12.73 ± 1.35	
Systolic BP (mmHg)	PCOS	135.71 ± 11.65	.05
	Controls	114.74 ± 10.11	
Diastolic BP(mmHg)	PCOS	84.76 ± 9.28	.05
	Controls	57.00 ± 73.07	
MUAC (cm)	PCOS	35.65 ± 6.37	.06
	Controls	35.95 ± 6.67	
WC (cm)	PCOS	110.63 ± 17.43	.05
	Controls	99.89 ± 17.63	
HC (cm)	PCOS	122.23 ± 17.00	.06
	Controls	119.92 ± 17.29	
WHR	PCOS	.82 ± .06	.04
	Controls	.83 ± .12	
Biceps SF (mm)	PCOS	22.67 ± 11.90	.07
	Controls	24.66 ± 11.01	
Triceps SF(mm)	PCOS	29.82 ± 7.61	.08
	Controls	31.12 ± 9.31	
Subscapular SF(mm)	PCOS	35.21 ± 9.37	.06
	Controls	33.19 ± 10.29	
Abdominal SF(mm)	PCOS	37.95 ± 7.83	.02
	Controls	22.54 ± 8.65	
Suprailiac SF(mm)	PCOS	21.40 ± 9.79	.06
	Controls	22.95 ± 7.74	

PCOS, Polycystic Ovarian Syndrome; BMI, Body Mass Index; BP, Blood Pressure; MUAC, Mid Upper Arm Circumference; WC, Waist Circumference; HC; Hip Circumference; WHR, Waist to Hip Ratio; SF, Skin Fold.

Table 2 Metabolic features in PCOS women and controls.

Parameters	Group	Mean ± SD	*P* values
Glucose (mg/dl)	PCOS	125.71 ± 68.85	.05
	Controls	98.70 ± 52.95	
Insulin (μU/ml)	PCOS	23.03 ± 1.5	.03
	Controls	13.06 ± 1.2	
HOMA-IR	PCOS	5.6 ± 1.4	.04
	Controls	2.2 ± 1.2	
Cholesterol (mg/dl)	PCOS	208.70 ± 51.04	.05
	Controls	188.38 ± 48.01	
Triglycerides(mg/dl)	PCOS	140.09 ± 93.31	.01
	Controls	104.05 ± 48.03	
HDL-C (mg/dl)	PCOS	43.22 ± 14.93	.02
	Controls	49.57 ± 14.65	
LDL-C (mg/dl)	PCOS	137.30 ± 28.44	.03
	Controls	119.10 ± 29.45	

PCOS, Polycystic Ovarian Syndrome; HOMA-IR Homeostatic Model Assessment for Insulin Resistance; HDL-C, High Density Lipoprotein Cholesterol; LDL-C, Low Density Lipoprotein Cholesterol.

women with PCOS, WHR ratio > 0.85 cm (OR: 1.620, 95% CI 1.437–1.618) and WC ⩾ 88 cm (OR: 1.72, 95% CI 1.558–1.948) and increased blood pressures (>130/>85 mm Hg) (OR: 1.52, 95% CI 1.658–1.977) were risk factors for IR in PCOS women, independent of obesity level and age.

The frequency of the *ADIPOQ*−11391GOA variants GG, GA and AA was not deviated from the expected proportions of genotypes in the population predicted by the Hardy–Weinberg equilibrium (Table 5), the genotype distributions at position −11391 were significantly different between the PCOS groups with and without MS.

Among PCOS with MS, the frequency of −11391GG was significantly lower compared to those without MS (55.55 vs. 72.58%) (p = .02). Moreover, frequency of the −11391A alleles was significantly different between the two groups (p = 0.04) and the carriers of the −11391A allele were more frequent among PCOS with MS (27.77 vs. 13.11%).

7. Discussion

The results of our study show the frequency of MS in reproductive age women with PCOS to be 20.5% which is similar to the prevalence of MS in other ethnicities and races diagnosed with PCOS [17]. However in some ethnic groups, such as the American population, the prevalence is higher than our study, at about 40% [19,25]. These differences are possibly related to factors such as age, diet and lifestyle that cause increased waist circumferences, hypertriglyceridemia and reduced HDL cholesterol levels as important components for MS.

The prevalence of MS in women with PCOS is less common in southern Italy compared with women in the USA, using the most used method to assess the presence of metabolic syndrome (the ATP-III criteria), the prevalence of MS was 8.2% in Italy [18] than in 43–46% of patients in the USA [19,25], and all diagnostic criteria were less common in Italian PCOS, the main difference was in the lower prevalence of hypertriglyceridemia (increased triglycerides were present in only 9.3% of patients).

In another study, the quantity of saturated fat in the diets of USA women was almost double than that of Italian women [26].

The prevalence of MS in the Egyptian PCOS was 22.5%. In the current study women with PCOS had markedly higher levels of HOMA-IR score, serum triglycerides and blood pressure and lower HDL-C levels as compared to age-BMI-matched healthy women.

The prevalence of MS in obese women with PCOS was approximately twofold increase compared with non-obese women. Since most of the women with PCOS (38–88%) are overweight or obese, therefore there is little doubt that adiposity plays an important role in development and maintenance of PCOS and strongly influences the severity of both its clinical and endocrine characteristics in numerous women [27]. Obesity appears to be an independent factor for MS abnormalities [28,29] and our results are in accordance with the idea that as BMI increases the prevalence of high WC and hypertension increases. Nevertheless, fasting serum glucose levels and the rates of dyslipidemia showed no statistically significant difference among the BMI groups, an important finding corroborating the relevance of screening for MS and other cardiovascular risk factors in all women with PCOS, regardless of obesity. The results of this study have confirmed the high frequency of MS and its components, in particular a decreased HDL-C level and an increased TG level in women with PCOS.

Thus, these women are at increased risk of diabetes mellitus and cardiovascular disease (CVD). Therefore, it is important to screen all women with PCOS for cardiovascular risk factors. Recognition and clinical management of this high-risk group are important to prevent CVD and associated mortality in the population.

Polycystic ovaries were diagnosed by pelvic or intravaginal sonography according to the Rotterdam conference criteria [30].

The consequences of the polycystic ovary syndrome extended beyond the reproductive axis; as women with PCOS are at substantial risk for the metabolic syndrome.

Also, previous studies indicated that 30–40% of women with PCOS have impaired glucose tolerance, and as many as 10% have type 2 diabetes by their fourth decade [31,32].

A meta-analysis supported a greater prevalence of glucose intolerance (IGT), type 2 diabetes (DM2) and the metabolic syndrome in women with PCOS as compared with women without PCOS. The odds of metabolic disturbance were two to four times as high in PCOS women [33]. The predisposition of PCOS women to various metabolic disturbances, including obesity, IGT, atherogenic dyslipidaemia and hypertension, increased in the long-term risk of DM2 and cardiovascular disease (CVD), which indicated that PCOS carried significant public health implication. Recent evidence also indicated more frequent CVD death in women with PCOS [34].

An economic evaluation estimated that 40% of the economic costs of PCOS can be attributed to DM2 in the USA, highlighting the need for prevention of long term complications through appropriate screening diagnosis and intervention for PCO [34].

Clinical hyperandrogenism was defined as the presence of hirsutism (Ferriman–Gallwey) score ⩾6 with no oral contraceptive pills were used within three months. Biochemical hyperandrogenism was present if testosterone >2.8 nmol/L or androgen >10.8 nmol/L, which were the normal range of 95% percentile in the population in our laboratory. PCO was defined as the presence of at least one ovary with 12 or more follicles measuring 2–9 mm in diameter. The relationship between hyperandrogenemia and metabolic abnormalities is controversial. Apridonidze et al. [19], described a higher prevalence of hyperandrogenemia in women with concomitant PCOS and MS, other study concluded that DHEAS correlated inversely with arterial structure, suggesting possible cardioprotective effects of endogenous DHEAS in women with PCOS [35].

However, other studies from Dokras et al. [13], and Cheung et al. [17], all failed to demonstrate any significant differences in serum concentrations in total testosterone and androgen between those PCOS women with or without MS. Therefore, it appeared that hyperandrogenemia, by itself, may not directly contribute to the development of MS in women with PCOS.

Homeostatic Model Assessment score (HOMA), is a good indicator of insulin resistance (IR). This HOMA score was significantly higher in the PCOS subgroups compared to controls, similar to the findings of Chae et al. [36]. The increase of IR was explained by higher central obesity in the present PCO group similarly as reported by Cosar et al. [37]. Dyslipidemia

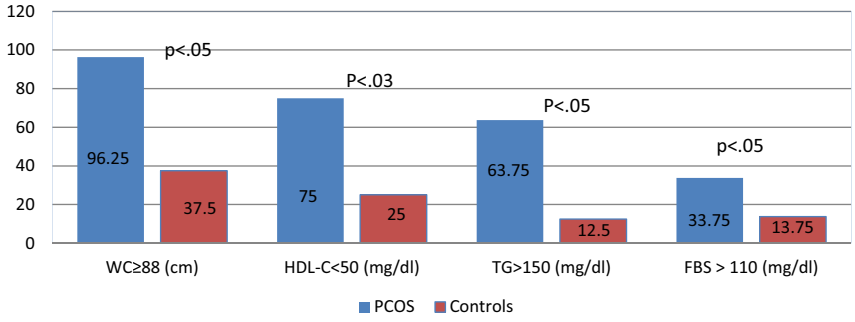


Figure 1 Frequency distribution of the metabolic syndrome components in women with PCOS and normal controls.

Table 3 Pearson's partial correlation coefficient for measures of adiposity and metabolic parameters in PCOS women.

	WC	WHR	Abdominal skin fold thickness	Body fat %
HOMA-IR	0.35 (0.02)	0.34 (0.03)	0.35 (0.05)	0.055 (0.45)
FB glucose	0.65 (0.05)	0.51 (0.05)	0.38 (0.01)	0.065 (0.55)
Systolic BP	0.65 (0.04)	0.35 (0.03)	0.45 (0.05)	0.155 (0.06)
Diastolic BP	0.65 (0.04)	0.35 (0.03)	0.45 (0.05)	0.175 (0.07)
TG	0.65 (0.04)	0.35 (0.03)	0.45 (0.05)	0.153 (0.53)
HDL-C	−0.65 (0.04)	−0.35 (0.03)	−0.45 (0.05)	0.075 (0.63)

Data are presented with correlation coefficient (*P* value).
PCOS, Polycystic Ovarian Syndrome; FBG, Fasting Blood Glucose; BP, Blood Pressure; TG, Triglycerides; HDL-C, High Density Lipoprotein Cholesterol; WC, Waist Circumference; WHR, Waist to Hip Ratio.

Table 4 Predictors of IR in Egyptian PCOS by using multivariate logistic regression analysis.

	Risk factor	OR*	95.0% CI	*P*
IR	WHR > 0.85 cm	1.62	1.437–1.618	.001
	WC ⩾ 88 cm	1.72	1.558–1.948	.001
	High-blood pressure (>130/>85 mmHg)	1.52	1.658–1.977	.001

IR, Insulin Resistance; WHR, Waist to Hip Ratio; WC, Waist Circumference.
* Age and BMI adjusted odds ratio.

is one of the common metabolic abnormalities in PCOS, which is associated with IR and hyperinsulinemia, which was also noted in the present study.

Previous studies reported that in women with obesity and infertility as well as anovulatory women have significantly more subcutaneous abdominal fat and higher fasting insulin levels and the same amount of intra-abdominal fat compared with ovulatory women with the same BMI [38]. A possible explanation for an increased volume of subcutaneous abdominal fat in anovulatory women with obesity could be provided by the concept of a critical intra-abdominal fat threshold. This concept suggests that during constant high calorie food consumption, storage of fat in intra-abdominal fat reaches a point of saturation, after which fat is shunted to the subcutaneous fat compartments [39]. With increased accumulation of subcutaneous abdominal fat, inflammatory changes and increase in adipocyte size occur. Fat is then shunted from subcutaneous abdominal fat to the liver and skeletal muscle which contributes to an increase of IR [40,41]. In this study the prevalence of different elements of the metabolic syndrome in Egyptian PCOS women with fat accumulation was significantly higher than those without abdominal fat accumulation (waist to hip ratio >.85).

It has been demonstrated that a high percentage of PCOS patients even without general obesity have excessive visceral fat accumulation [42]. It has been suggested that increased accumulation of fat in the abdominal site of PCOS women may induce adipose tissue dysfunction [43] and increase IR [44,45] and hyperandrogenism [46], both of which are common features of PCOS.

In addition, other studies reported that resumption of ovulation during weight loss is associated with a decrease in fasting insulin and free testosterone levels [47,48] and associated with improvement in IR, and lower insulin levels which leads to less androgen [49]. Lower free androgen levels in the long term, limits the amount of abdominal fat accumulation [50,51].

Although the presence of MS has been linked to decreased adiponectin values, the association of *ADIPOQ* variants with MS and its components remains vague [52]. The effect of the *ADIPOQ* gene on the risk of obesity and MS may vary

Table 5 Distribution of the *ADIPOQ* −11391G>A genotypes and alleles in PCOS with and without MS.

Genotype/allele	MS(+) (*n* = 18)	MS(−) (*n* = 62)	*P*
	n (%)	*n* (%)	
GG	10 (55.55)	45 (72.58)	.02
GA	6 (33.33)	17 (27.41)	
AA	2 (11.11	0 (0)	
G	26 (72.22)	107 (86.29)	.04
A	10 (27.77)	17 (13.71)	

MS(+): with metabolic syndrome.
MS(−): without metabolic syndrome.

according to ethnicity, age and the degree of obesity across populations [53]. Some studies indicated that *ADIPOQ* variants may contribute to MS and its components [54], whereas other reports point to the limited impact of *ADIPOQ* gene on MS parameters [55]. In this study we found association between *ADIPOQ* promoter variants −11391G > A and MS in young Egyptian women with PCOS.

In conclusion, the prevalence of MS was significantly higher in PCOS women than controls, and central obesity and hypertension are risk factors for insulin resistance. Moreover, obesity plays an important role in the development of PCOS and *ADIPOQ*−11391G > A gene variants is associated with MS among young Egyptian women with PCOS.

Conflict of interest

There is no conflict of interest.

Acknowledgment

Authors are greatly thankful to the National Research Centre for funding the project entitled "Body adiposity phenotypes, dietary intake, adiponectin gene variants, metabolic markers and their significance in obesity-related diseases"(10010007) that enabled us to use the data to establish this work.

References

[1] Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med 2010;8:41.
[2] Scarpitta AM, Sinagra D. Polycystic ovary syndrome: an endocrine and metabolic disease. Gynecol Endocrinol 2000; 14(5):392–5.
[3] Sam S, Dunaif A. Polycystic ovary syndrome: syndrome XX? Trends Endocrinol Metab 2003;14(8):365–70.
[4] Korhonen S, Hippelainen M, Niskanen L, Vanhala M, Saarikoski S. Relationship of the metabolic syndrome and obesity to polycystic ovary syndrome: a controlled, population-based study. Am J Obstet Gynecol 2001;184:289–96.
[5] Doelle B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001;24:683–9.
[6] Doelle GC. The clinical picture of metabolic syndrome. Postgrad Med 2004;116:35–8.
[7] Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. J Am Med Assoc 2002;287:356–9.
[8] Alvarez Cosmea A, Lopez Hernandez V, Suarez Garcia S, Aria Garcia T, Preto Diaz MA, Diaz Gonzalez L. Differences in the prevalence of metabolic syndrome according to the ATP-III and WHO definitions. Med Clin 2005;124:368–70.
[9] Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the third National Health and Nutrition Examination Survey, 1988–1994. Arch Intern Med 2003;163:427–36.
[10] Santos AC, Lopes C, Barros H. Prevalence of metabolic syndrome in the city of Porto. Rev Port Cardiol (Lisboa) 2004;23:45–52.
[11] Sanad A S. Prevalence of polycystic ovary syndrome among fertile and infertile women in Minia 2014; International Federation of Gynecology and Obstetrics. Published by Elsevier Ireland Ltd., All rights reserved.doi: http://dx.doi.org/10.1016/j.ijgo.2013.11.004 Governorate, Egypt.
[12] Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech 2009;2:231–7.
[13] Dokras A, Bochner M, Hollinrake E, Markham S, Vanvoorhis B, Jagasia DH. Screening women with polycystic ovary syndrome for metabolic syndrome. Obstet Gynecol 2005;106:131–7.
[14] Moran L, Teede H. Metabolic features of the reproductive phenotypes of polycystic ovary syndrome. Hum Reprod Update 2009;15:477–88.
[15] Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005;365(9468):1415–28.
[16] Vural B, Caliskan E, Turkoz E, Kilic T, Demirci A. Evaluation of metabolic syndrome frequency and premature carotid atherosclerosis in young women with polycystic ovary syndrome. Hum Reprod 2005;20:2409–13.
[17] Cheung LP, Ma RC, Lam PM, Lok IH, Haines CJ, So WY, et al. Cardiovascular risks and metabolic syndrome in Hong Kong Chinese women with polycystic ovary syndrome. Hum Reprod 2008;23:1431–8.
[18] Glueck CJ, Papanna R, Wang P, Goldenberg N, Sieve-Smith L. Incidence and treatment of metabolic syndrome in newly referred women with confirmed polycystic ovarian syndrome. Metabolism 2003;52:908–15.
[19] Apridonidze T, Essah PA, Iuorno MJ, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005;90:1929–35.
[20] Welt CK, Gudmundsson JA, Arason G, Adams J, Palsdottir H, Gud laugsdottir G, et al. Characterizing discrete subsets of polycystic ovary syndrome as defined by the Rotterdam criteria: the impact of weight on phenotype and metabolic features. J Clin Endocrinol Metab 2006;91:4842–8.
[21] Gayoso-Diz P, Otero-González A, Rodriguez-Alvarez MX, Gude F, Cadarso-Suarez C, García F, et al. IR index (HOMA-IR) levels in a general adult population: curves percentile by gender and age. The EPIRCE study. Diabetes Res Clin Pract 2011;94: 146–55.
[22] Executive Summary of the Third Report of the National Cholesterol Education Program (NECP), Expert Panel on Detection. Evaluation and Treatment of High Blood Cholesterol in Adults. (Adult Treatment Panel III). J Am Med Assoc 2001; 285:2486–2497.
[23] Hiernaux J and Tanner JM. Growth and physical studies. In: Human Biology: A Guide to Field Methods. Weiner JS, Lourie SA, editors. Oxford. U.K: IBP. London, Blackwell Scientific Publications; 1969.
[24] Tabatabaei-Malazy O, Ranjbar SH, Amoli MM, et al. Gender-specific differences in the association of adiponectin gene polymorphisms with body mass index. Rev Diabet Stud 2010;7:241–6.
[25] Soares EM, Azevedo GD, Gadelha RG, Lemos TM, Maranhao TM. Prevalence of the metabolic syndrome and its components in Brazilian women with polycystic ovary syndrome. Fertil Steril 2008;89:649–55.
[26] Carmina E, Napoli N, Longo RA, Rini GB. Lobo RA Metabolic syndrome in polycystic ovary syndrome (PCOS): lower prevalence in southern Italy than in the USA and the influence of criteria for the diagnosis of PCOS. Eur J Endocrinol 2006;154:141–5.
[27] Carmina E, Legro R, Stamets K, Lowell J, Lobo RA. Differences in body weight between American and Italian women with the polycystic ovary syndrome: influence of the diet. Hum Reprod 2003;11:2289–93.
[28] Barber TM, McCarthy MI, Wass JA, Franks S. Obesity and polycystic ovary syndrome. Clin Endocrinol (Oxf) 2006;65(2): 137–45.
[29] Faloia E, Canibus P, Gatti C, Frezza F, Santangelo M, Garrapa GG, et al. Body composition, fat distribution and metabolic characteristics in lean and obese women with polycystic ovary syndrome. J Endocrinol Invest 2004;27:424–9.

[30] Ehrmann DA, Liljenquist DR, Kasza K, Azziz R, Legro RS, Ghazzi MN. Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91:48–53.
[31] Balen AH, Laven JS, Tan SL, Dewailly D. Ultrasound assessment of the polycystic ovary: international consensus definitions. Hum Reprod Update 2003;9:505–14.
[32] 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;22:141–6.
[33] 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. J Clin Endocrinol Metab 1999;84:165–9.
[34] Moran LJ, Misso ML, Wild RA, Norman RJ. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update 2010;16:347–63.
[35] Azziz R, Marin C, Hoq L, Badamgarav E, Song P. Health care-related economic burden of the polycystic ovary syndrome during the reproductive life span. J Clin Endocrinol Metab 2005;90:4650–8.
[36] Meyer C, McGrath BP, Cameron J, Kotsopoulos D, Teede HJ. Vascular dysfunction and metabolic parameters in polycystic ovary syndrome. J Clin Endocrinol Metab 2005;90:4630–5.
[37] Chae SJ, Kim JJ, Choi YM, Hwang KR, Jee BC, Ku SY, et al. Clinical and biochemical characteristics of polycystic ovary syndrome in Korean women. Hum Reprod 2008;23:1924–31.
[38] Cosar E, Ucok K, Akgun L, Koken G, Sahin FK, Arioz DT, et al. Body fat composition and distribution in women with polycystic ova ry syndrome. Gynecol Endocrinol 2008;24:428–32.
[39] Kuchenbecker WK, Groen H, Zijlstra TM, Bolster JH, Slart RH, van derJagt EJ, et al. The subcutaneous abdominal fat and not the intraabdominal fat compartment is associated with anovulation in women with obesity and infertility. J Clin Endocrinol Metab 2010;95:2107–12.
[40] Freedland ES. Role of a critical visceral adipose tissue threshold (CVATT) in metabolic syndrome: implications for controlling dietary carbohydrates: a review. Nutr Metab (Lond) 2004;1:12.
[41] Weiss R. Fat distribution and storage: how much, where, and how? Eur J Endocrinol 2007;157:S39–45.
[42] Koska J, Stefan N, Permana PA, Weyer C, Sonoda M, Bogardus C, et al. Increased fat accumulation in liver may link insulin resistance with subcutaneous abdominal adipocyte enlargement, visceral adiposity, and hypoadiponectinemia in obese individuals. Am J Clin Nutr 2008;87:295–302.
[43] Kirchengast S, Peterson B, Hauser G, Knogler W. Body composition characteristics are associated with the bone density of the proximal femur end in middle- and old-aged women and men. Maturitas 2001;39:133–45.
[44] Escobar-Morreale HF, Villuendas G, Botella-Carretero JI, Alvarez-Blasco F, Sanchón R, Luque-Ramírez M, et al. Adiponectin and resistin in PCOS: a clinical, biochemical and molecular genetic study. Hum Reprod 2006;21:2257–65.
[45] Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. Nat Rev Endocrinol 2011;7:219–31.
[46] Gulcelik NE, Aral Y, Serter R, Demir Y, Culha C. Adiponectin is an independent determinant of insulin resistance in women with polycystic ovary syndrome. Gynecol Endocrinol 2006;22:511–5.
[47] Kirschner MA, Samojlik E, Drejka M, Szmal E, Schneider G, Ertel N. Androgen–estrogen metabolism in women with upper body versus lower body obesity. J Clin Endocrinol Metab 1990;70:473–9.
[48] Guzick DS, Wing R, Smith D, Berga SL, Winters SJ. Endocrine consequences of weight loss in obese, hyperandrogenic, anovulatory women. Fertil Steril 1994;61:598–604.
[49] Pasquali R, Gambineri A, Pagotto U. The impact of obesity on reproduction in women with polycystic ovary syndrome. BJOG 2006;113:1148–59.
[50] Poretsky L. On the paradox of insulin-induced hyperandrogenism in insulin-resistant states. Endocr Rev 1991;12:3–13.
[51] Bohler Jr H, Mokshagundam S, Winters SJ. Adipose tissue and reproduction in women. Fertil Steril 2010;94:795–825.
[52] Heid IM, Wagner SA, Gohlke H, et al. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1727 healthy Caucasians. Diabetes 2006;55:375–84.
[53] Gong M, Long J, Liu Q, Deng HC. Association of the ADIPOQ rs17360539 and rs266729 polymorphisms with type 2 diabetes: a meta-analysis. Mol Cell Endocrinol 2010;325:78–83.
[54] Park JW, Park J, Jee SH. ADIPOQ gene variants associated with susceptibility to obesity and low serum adiponectin levels in healthy Koreans. Epidemiol Health 2011;33:1–7.
[55] Heid IM, Henneman P, Hicks A, et al. Clear detection of ADIPOQ locus as the major gene for plasma adiponectin: results of genome wide association analyses including 4659 European individuals. Atherosclerosis 2010;208:412–20.