

# Atherogenic dyslipidemia, subclinical atherosclerosis, non-alcoholic fatty liver disease and insulin resistance in polycystic ovarian syndrome

[Polikistik over sendromunda insülin direnci, aterojenik dislipidemi, subklinik aterosklerozis ve non-alkolik yağlı karaciğer hastalığı]

Gamze Sinem Çağlar<sup>1</sup>,  
Mine Kiseli<sup>1</sup>,  
Rabia Seker<sup>2</sup>,  
Elif Didem Ozdemir<sup>1</sup>,  
Demet Karadag<sup>3</sup>,  
Selda Demirtas<sup>2</sup>

<sup>1</sup>University of Ufuk, Faculty of Medicine,  
Department of Obstetrics and Gynecology, Ankara  
<sup>2</sup>University of Ufuk, Faculty of Medicine,  
Department of Biochemistry, Ankara  
<sup>3</sup>University of Ufuk, Faculty of Medicine,  
Department of Radiology, Ankara

Correspondence Address  
[Yazışma Adresi]

Gamze Sinem Çağlar, MD.

Ufuk University Faculty of Medicine, Department of  
Obstetrics and Gynecology, Mevlana Bulvarı,  
No: 86-88, 06520 Balgat, Ankara, Türkiye  
Phone: +90 312 2044000  
Fax: +90 312 2847786  
E-mail: gamzesinem@hotmail.com

Registered: 09 February 2013; Accepted: 21 June 2014  
[Kayıt Tarihi: 09 Şubat 2013; Kabul Tarihi: 21 Haziran 2014]

## ABSTRACT

**Objective:** We aimed to explore the relationship between insulin resistance (IR) and small dense lipoprotein (sd-LDL) particles, carotid intima-media thickness (CIMT) and non-alcoholic fatty liver disease (NAFLD) in young normal weight PCOS cases.

**Methods:** This prospective, case-control study was designed in a University Hospital and 34 women with PCOS and 21 healthy controls were enrolled. Fasting plasma glucose, insulin, lipid (including sd-LDL particles) and hormone profiles, abdominal ultrasound and CIMT were evaluated.

**Results:** IR was present in 68% of PCOS group while in none of controls. High density lipoprotein (HDL), very low density lipoprotein (VLDL), triglycerides (TG), and sd-LDL were higher in patients with IR ( $p<0.05$ ). A positive correlation of sd-LDL with IR, VLDL and TG was found. A significantly higher rate of NAFLD and CIMT was found in PCOS. Total-testosterone levels were weakly and positively correlated with CIMT ( $r=0.277$ ,  $p=0.041$ ).

**Conclusion:** Insulin resistance and NAFLD are highly prevalent among young normal weight PCOS patients. When compared to controls levels of sd-LDL and CIMT are increased in PCOS. Insulin resistance is the key parameter for NAFLD and atherogenic dyslipidemia in PCOS. Hence, screening for NAFLD may be valuable for detection and prevention of liver disease. Higher levels of sd-LDL in insulin resistant PCOS cases necessitates treating PCOS for IR.

**Key Words:** Polycystic ovarian syndrome, non-alcoholic fatty liver disease, carotid intima-media thickness, metabolic syndrome

**Conflict of Interest:** The authors have no conflict of interest.

## ÖZET

**Amaç:** Bu çalışmada, genç, normal kilolu PKOS hastalarında insülin direncinin ve küçük dansiteli lipoprotein (sd-LDL) partiküllerinin, karotis intima-media kalınlığı ve non-alkolik yağlı karaciğer hastalığı ile ilişkisinin araştırılmasını hedefledik.

**Metod:** Otuzdört PKOS'lu hasta ile 21 sağlıklı kontrol hastasını içeren bu prospektif vaka-kontrollü çalışma bir üniversite hastanesinde yürütüldü. Hastaların açlık plazma glukoz, insülin düzeyleri, lipid (sd-LDL partiküllerini de içeren) ve hormon profilleri, abdominal ultrasonografi ve karotis intima-media kalınlığı değerlendirildi.

**Bulgular:** İnsülin direnci, PKOS'lu hastaların %68'inde mevcut iken kontrol grubunda hiçbir hastada saptanmadı. Yüksek dansiteli lipoprotein (HDL), çok düşük dansiteli lipoprotein (VLDL), trigliserid (TG) ve sd-LDL insülin direnci olan hastalarda yüksekti ( $p<0.05$ ). sd-LDL ile insülin direnci, VLDL ve TG arasında pozitif korelasyon saptandı. PKOS'da belirgin daha yüksek oranda non-alkolik yağlı karaciğer hastalığı ve artmış karotis intima-media kalınlığı izlendi. Total testosteron seviyeleri karotis intima-media kalınlığı ile zayıf pozitif korele idi ( $r=0.277$ ,  $p=0.041$ ).

**Sonuç:** İnsülin direnci ve non-alkolik yağlı karaciğer hastalığı normal ağırlıklı PKOS hastalarında yaygındır. Kontrollerle kıyaslandığında, sd-LDL ve karotis intima-media kalınlığı PKOS'da artmıştır. İnsülin direnci, non-alkolik yağlı karaciğer hastalığı ve aterojenik dislipidemi için anahtar noktadır. Bu nedenle, karaciğer yönünden tarama karaciğer hastalığının tanınmasında ve önlenmesinde değerlidir. İnsülin dirençli PKOS vakalarında yüksek sd-LDL bu konuda tedaviyi gerekli kılmaktadır.

**Anahtar Kelimeler:** Polikistik over sendromu, non-alkolik yağlı karaciğer, karotis intima-media kalınlığı, insülin direnci

**Çıkar Çatışması:** Yazarların çıkar çatışması yoktur.

## Introduction

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder affecting up to 10% of women in reproductive age [1]. Characteristic features are chronic oligoanovulation, hyperandrogenemia and/or morphologically polycystic ovaries. Recently, PCOS has gained attention due to crucial metabolic aspects which are important for long-term sequelae of the condition.

In majority of women with PCOS, the components of metabolic syndrome are present and predispose to atherosclerotic cardiovascular disease and type-2 diabetes. Additionally, non alcoholic fatty liver disease (NAFLD) which is the hepatic manifestation of metabolic syndrome [2] is more common (41%) in women with PCOS than in controls and tend to occur in early ages [3]. Various histopathological degrees of progressive steatosis, lobular inflammation and fibrosis of the liver are known and severe form is non alcoholic steatohepatitis (NASH) which presents risk for advanced fibrosis, cirrhosis and hepatocellular carcinoma [4].

In obese PCOS patients, elevated plasma TG and reduced high density lipoprotein-cholesterol (HDL-C) concentrations are seen [5]. Irrespective of obesity, low density lipoprotein cholesterol (LDL-C) levels are often modestly elevated in PCOS [6]. Among the subpopulations of particles of LDL-C, small and dense LDL particles (sd-LDL) are known to be more atherogenic than larger LDL species. When compared to body mass index (BMI) matched control subjects the elevated concentrations and proportions of sd-LDL particles in patients with PCOS [6], increase the risk of coronary heart disease and type 2 diabetes [7]. Besides, in young PCOS women precocious atherosclerosis has also been shown by increased intima-media thickness of the common carotid artery (CIMT) [8].

Atherogenic dyslipidemia, excess weight and IR all play dominant roles in developing metabolic problems in PCOS. However, screening strategies for metabolic comorbidities in PCOS are still lacking. Therefore, this study is designed to explore the relationship between IR and sd-LDL particles, CIMT and NAFLD in young normal weight PCOS cases. In addition, the predictive factors of NAFLD in PCOS are evaluated.

## Materials and Methods

The patients admitted an Out-patient Clinic to Obstetrics and Gynecology Department of University were enrolled in the study. Thirty four women with PCOS constituted the study group. The diagnosis of PCOS was made as proposed at the Rotterdam Consensus Meeting [9]. The controls (n=21) were healthy volunteers without any features of clinical or biochemical hyperandrogenism who had regular menstrual cycles. The study was approved by the Institutional Ethics Committee and informed consent was obtained from all patients and controls.

Exclusion criteria were hyperprolactinemia, thyroid dysfunction, adrenal dysfunction, diabetes mellitus, pregnancy, alcohol consumption, history of chronic viral hepatitis, hemochromatosis, autoimmune liver disease, other chronic liver diseases, history of hepatotoxic, antihypertensive, lipid lowering or antiinflammatory agent usage. None of the patients had received any drugs known to interfere with hormone levels for at least 3 months before the study. All of the subjects were nonsmokers. Anthropometric measurements, including body weight, height and waist-to-hip ratio (WHR) were measured by the same observer. Waist circumference was measured in a hospital gown at the narrowest level between the costal margin and the iliac crest and hip circumference was measured at the widest level over buttocks. Body mass index (BMI) was calculated by the formula:  $\text{weight(kg)/height(m)}^2$ . Hirsutism was determined by a modified Ferriman-Gallwey score  $>7$  [10]. Systolic (SBP) and diastolic (DBP) blood pressure were measured twice in the right arm in relaxed sitting position. The average of two measurements were used. The features of metabolic syndrome were identified [11].

Blood samples were obtained on 2<sup>nd</sup> or 3<sup>rd</sup> days of menstruation, after overnight fasting for at least 12 hours. In cases with oligoanovulation, blood was taken after progesterone withdrawal bleeding. Levels of glucose, insulin, hormone profile [follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin (PRL), total and free testosterone (Total-T and Free-T, respectively), dehydroepiandrosterone sulfate (DHEAS), 17-OHprogesterone (17-OHP) and thyroid-stimulating hormone (TSH)], and lipid profile [Total blood cholesterol (Total-C), HDL-C, LDL-C, VLDL-C and triglycerides (TG)] were determined. Plasma glucose was determined with the glucose hexokinase method, lipid profile and serum alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) levels were determined with the enzymatic method (Cobas Integra 400 Plus, Roche Diagnostics, Mannheim Germany). VLDL-C was calculated with TG/5 formula (Fridewald Formula) [12]. Small dense lipoprotein particles were measured by precipitation method [13]. After mixing with heparin-magnesium (containing 150 U/mL heparin-sodium salt and 90 mmol/L  $\text{MgCl}_2$ ), each serum sample was incubated for 10 minutes at 37°C. The supernatant was removed after centrifuging at 15000 rpm (21885 g) for 15 min; LDL-cholesterol was measured by the automatic colorimetric method by Cobas Integra.

Fasting insulin and glucose (FPG) levels were used for calculating homeostatic model assesment (HOMA-IR),  $(\text{insulin} \times \text{glycemia in } \mu\text{mol/L}/22.5)$  and quantitative insulin sensitivity check index (QUICKI)  $(1/\log \text{insulin} + \log \text{glycemia in mg/dL})$ . Insulin resistance was defined as  $\text{HOMA-IR} \geq 2.1$ . HOMA-B scores, showing pancreatic  $\beta$  cell function were assessed by the following formula:  $(\text{fasting insulin in } \mu\text{U/ml}) \times 3.33 / (\text{fasting glucose in mg/dl} - 3.5)$ . Levels of FSH, LH, E2, PRL, DHEAS, Total-T, insulin and TSH were measured with electrochemilumi-

nescence assays (ELECYS 2010 HITACHI, Roche Diagnostic, Germany). Levels of 17-OHP and Free-T were measured by radioimmunoassay. The intra- and interassay coefficients of variation (CV) were <1.9% and <4%, respectively, for all assays performed.

Fatty liver was diagnosed by abdominal ultrasound using accepted criteria which includes a bright hepatic echo pattern, increased attenuation of the ultrasound beam, and loss of intrahepatic architectural details [14]. Ultrasonographic diagnosis of fatty liver in the absence of alcohol intake or use of any medication was defined as NAFLD [14].

The measurements of CIMT was conducted using a high resolution ultrasound machine (Logic Q7, General Electric, USA) in all cases. The posterior carotid wall at 1 cm below the bifurcation of the common carotid artery was imaged in B-mode and CIMT was estimated by visual

assessment of the distance between the lumen-intima and intima-adventitia interfaces in longitudinal frames acquired during arterial diastole [15]. The mean of measurements of CIMT made at the greatest thickness on both sides was used for statistical analyses. Ultrasonographic measurements were performed by the same experienced radiologist.

### Statistical analysis

Data analysis was performed by using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, United States). Whether the distributions of continuous variables were normal or not was determined by Shapiro Wilk test. Descriptive statistics for continuous variables were shown as mean  $\pm$  SD and median (min-max), the categorical variables were shown as case number and (%). The median differences between groups were analyzed by

**Table 1.** Demographic, hormone and insulin resistance parameters of PCOS and control groups

Parameters	Control group n=21	PCOS Group n=34	p
FSH <sup>†</sup> (mIU/mL)			
Mean $\pm$ SD	5.3 $\pm$ 1.3	5.5 $\pm$ 1.6	0.655
LH <sup>‡</sup> (mIU/mL)			
Mean $\pm$ SD	6.5 $\pm$ 2.4	8.6 $\pm$ 6.3	0.897
E2 <sup>§</sup> (pg/mL)			
Median (min-max)	59(14-230)	33(5-146)	0.120
Total-T <sup>¶</sup> (ng/dL)			
Median (min-max)	0.20(0.10-0.60)	0.30(0.03-1.30)	0.216
Free-T <sup>¶</sup> (pg/dL)			
Mean $\pm$ SD	2.2 $\pm$ 0.85	2.2 $\pm$ 0.93	0.100
17-OH P <sup>b</sup> (ng/dL)			
Mean $\pm$ SD	1.6 $\pm$ 0.41	1.5 $\pm$ 0.53	0.728
DHEAS <sup>¶</sup> ( $\mu$ g/dL)			
Mean $\pm$ SD	265 $\pm$ 111	262 $\pm$ 135	0.494
TSH <sup>¶</sup> ( $\mu$ IU/mL)			
Median (min-max)	1.4(0.4-4.0)	1.9(0.5-4.3)	0.310
PRL <sup>¶</sup> (ng/mL)			
Mean $\pm$ SD	14.0 $\pm$ 5.8	12.6 $\pm$ 5.7	0.390
Fasting insulin ( $\mu$ IU/mL)			
Median (min-max)	7.9 (2.5-11.5)	9.3 (3.9-57.4)	0.052
FPG <sup>¶</sup> (mg/dL)			
Mean $\pm$ SD	85.8 $\pm$ 7.7	88.3 $\pm$ 6.2	0.188
Homa-IR <sup>¶</sup>			
Median (min-max)	1.8 (0.5-2.4)	2.0 (0.8-14.0)	0.025*
QUICKI <sup>¶</sup>			
Mean $\pm$ SD	0.36 $\pm$ 0.03	0.34 $\pm$ 0.03	0.024*
HOMA-B <sup>¶</sup>			
Mean $\pm$ SD	139.3 $\pm$ 78.5	170.5 $\pm$ 102.4	0.239

<sup>†</sup>follicle-stimulating hormone; <sup>‡</sup>luteinizing hormone; <sup>§</sup>estradiol; <sup>¶</sup>total testosterone; <sup>¶</sup>free testosterone; <sup>b</sup>17 OH-progesterone; <sup>¶</sup>dehydroepiandrosterone sulfate; <sup>¶</sup>thyroid-stimulating hormone; <sup>¶</sup>prolactin; <sup>¶</sup>fasting plasma glucose; <sup>¶</sup>homeostatic model assesment-insulin resistance; <sup>¶</sup>quantitative insulin sensitivity check index; <sup>¶</sup>homeostatic model assesment-pancreatic; <sup>¶</sup>cell function; \*statistically significant.

Mann Whitney U test. For nominal variables Pearson's Chi-Square or Fisher's Exact test were used. Strength of association between continuous variables were calculated by Spearman's Rank correlation test. Multiple Logistic Regression Backward method was used to determine the independent predictors which mostly affect NAFLD. Any variable whose univariable test had a p value <0.25 was accepted as a candidate for the multivariable model along with all variables of known clinical importance. Odds ratio (OR) and 95% confidence intervals (CI) for each independent variable were also calculated. A p value less than 0.05 was considered statistically significant.

## Results

The mean age and BMI did not differ between patients and controls (26.0±2.5 vs 26.1±2.8, p=0.981 and 22.0±1.1 vs 22.1±1.9, p=0.816; respectively). No significant difference was found in PCOS and controls regarding the WHR [median (min-max) 0.74(0.61-0.97) in PCOS and 0.73 (0.62-0.87) in controls]. Oligomenorrhea and anovulation was present in 82% of patients with PCOS. Hirsutism and acne was observed in 44.1% and 38.2% of PCOS patients, respectively. The mean SBP and DBP were similar between groups (SBP: 105±10 vs 100±12 mmHg and DBP: 65±8 vs 60±9 mmHg; p>0.05). The results of hormone profiles of PCOS patients and controls are given in Table 1. Fasting glucose, insulin levels and HOMA-B were similar between groups whereas insulin resistance calculated by QUICKI and HOMA-IR was significantly higher in patients with PCOS (Table 1). Insulin resistance defined as HOMA-IR≥2.1 was present in 20 cases with PCOS (68%). None of the controls had insulin resistance. When all the demographic characteristics of the participants were compared in cases with (n=20) and without insulin resistance (n=35), BMI and WHR were significantly high-

er in insulin resistant cases (22.7±1.4 vs 21.6±1.5 kg/m<sup>2</sup>, p=0.011; 0.77±0.06 vs 0.71±0.05, p=0.000; respectively).

There was no significant difference in parameters of lipid profile (Total-C, LDL-C and HDL-C, VLDL, TG) between cases with PCOS and controls (p>0.05, Table 2). Although not statistically significant, sd-LDL were higher in PCOS patients (p=0.077, Table 2). Levels of VLDL and sdLDL were parallel to each other. Among the lipid parameters HDL, VLDL, TG, and sd-LDL were significantly higher in insulin resistant cases (p<0.05, Table 2). According to the Spearman's Rank Correlation analyses neither the demographic characteristics (age, BMI, WHR) nor hormone profiles were correlated with sd-LDL levels. However, fasting insulin levels, HOMA-B and insulin resistance parameters were correlated with sd-LDL levels (Table 3). Moreover, a positive correlation of sd-LDL with VLDL, and TG was also found (Table 3). Two cases had PCOS and metabolic syndrome.

Carotid intima-media thickness was significantly higher in PCOS patients (p>0.05, Table 2). In addition, CIMT was significantly higher in insulin resistant PCOS cases when compared with non-insulin resistant participants (0.61 vs 0.56 mm, p=0.047). The Spearman's Rank Correlation analyses of CIMT showed no correlation with demographic characteristics (age, BMI, WHR), lipid profiles including sd-LDL levels or insulin resistance parameters (p>0.05). A positive but weak correlation was found between total-T and CIMT (r=0.277, p=0.041) (Table 3).

The results of the liver function tests (ALT, GGT) are given in Table 4. When patients with or without insulin resistance were compared with respect to their liver function tests, GGT levels were found to be significantly higher in insulin resistant cases (13.8±5.8 vs 10.9±3.4, p=0.040). A statistically significantly higher rate of NAFLD was found in PCOS cases when compared with controls

**Table 2.** Lipid profile, sd-LDL and CIMT in PCOS, controls, insulin resistance and NAFLD groups

Parameter	Control group n=21	PCOS group n=34	p	IR <sup>o</sup> (-) n=35	IR <sup>o</sup> (+) n=20	p	NAFLD <sup>c</sup> (-) n=38	NAFLD <sup>c</sup> (+) n=17	p
HDL-C <sup>†</sup> (mg/dL)	59	58	0.585	62	53	0.015*	58	62	0.806
Median (min-max)	(37-86)	(34-116)		(35-86)	(34-116)		(37-86)	(34-116)	
LDL-C <sup>‡</sup> (mg/dL)	93	101	0.824	93	104	0.942	93	103	0.540
Median (min-max)	(46-146)	(56-154)		(56-154)	(46-141)		(46-154)	(61-142)	
TG <sup>§</sup> (mg/dL)	67	80	0.066	68	86	0.019*	69	95	0.018*
Median (min-max)	(33-137)	(34-233)		(33-137)	(41-233)		(33-137)	(41-233)	
VLDL <sup>¶</sup> (mg/dL)	13	16	0.068	13	17	0.018*	13	19	0.017*
Median (min-max)	(6-27)	(7-46)		(6-27)	(8-46)		(6-27)	(8-46)	
sd-LDL <sup>**</sup> (mg/dL)									
Mean±SD	13.7±6.8	17.6±8.5	0.077	14.3±7.6	19.3±7.8	0.024*	15.5±7.5	17.5±9.0	0.378
CIMT <sup>b</sup> (mm)									
Mean±SD	0.51±0.04	0.62±0.11	0.000*	0.56±0.10	0.61±0.10	0.047*	0.55±0.09	0.64±0.12	0.005*

<sup>†</sup>high density lipoprotein cholesterol; <sup>‡</sup>low density lipoprotein cholesterol; <sup>§</sup>triglycerides; <sup>¶</sup>very low density lipoprotein cholesterol; <sup>\*\*</sup>small dense low density lipoprotein; <sup>b</sup>carotid intima media thicknesses; <sup>o</sup>Insulin resistance; <sup>c</sup>nonalcoholic fatty liver disease; \*statistically significant.

**Table 3.** The results of Spearman's Rank Correlation analyses for sd-LDL and CIMT

	sd-LDL <sup>B</sup>		CIMT <sup>A</sup>	
	r	p	r	p
Total-T <sup>†</sup>	0.103	0.453	0.277	0.041*
Free-T <sup>‡</sup>	0.097	0.480	-0.038	0.786
Fasting insulin	0.379	0.004*	0.184	0.179
FPG <sup>§</sup>	0.018	0.897	0.147	0.283
HOMA-B <sup>  </sup>	0.373	0.005*	0.152	0.269
Homa-IR <sup>¶</sup>	0.302	0.025*	0.172	0.210
QUICKI <sup>   </sup>	-0.357	0.008*	-0.142	0.302
Total-C <sup>^</sup>	0.020	0.886	0.052	0.705
HDL-C <sup>#</sup>	-0.208	0.127	0.084	0.543
LDL-C <sup>°</sup>	0.079	0.566	-0.038	0.785
VLDL <sup>□</sup>	0.436	0.001*	-0.061	0.660
TG <sup>^</sup>	0.438	0.001*	-0.059	0.669
sd-LDL <sup>B</sup>			-0.110	0.424
CIMT <sup>A</sup>	-0.110	0.424		

<sup>†</sup>total testosterone; <sup>‡</sup>free testosterone; <sup>§</sup>fasting plasma glucose; <sup>||</sup>homeostatic model assesment-pancreatic  $\beta$  cell function; <sup>|||</sup>homeostatic model assesment-insulin resistance; <sup>°</sup>quantitative insulin sensitivity check index; <sup>^</sup>Total cholesterol; <sup>#</sup>high density lipoprotein cholesterol; <sup>°</sup>low density lipoprotein cholesterol; <sup>□</sup>very low density lipoprotein cholesterol; <sup>^</sup>triglycerides; <sup>B</sup>small dense low density lipoprotein; <sup>A</sup>carotid intima media thicknesses; \*statistically significant.

(44.1% vs 9.5%, respectively,  $p=0.006$ , Table 4). Neither the demographic (BMI, WHR, age) nor the hormone parameters differed between cases with or without NAFLD ( $p>0.05$ ). When patients with or without insulin resistance were compared, NAFLD was diagnosed in 45% of the insulin resistant cases whereas this rate was 22% in patients without insulin resistance ( $p=0.087$ ) (Table 5). Regardless of having PCOS, cases with NAFLD were found to have significantly higher levels of VLDL and TG when compared with patients without NAFLD (Table 2). In addition, CIMT was significantly higher in cases with NAFLD (Table 2). The predictive value of the parameters on the risk for subsequent NAFLD development was examined by multivariable analysis using the variables that might be associated with NAFLD. The final model in logistic regression analysis of the statistically significant continous variables showed that ALT and VLDL levels are predictive for NAFLD [VLDL OR(95%CI):1.14(1.011-1.298),  $p=0.033$ ; ALT OR(95%CI): 1.06(1.006-1.122),  $p=0.029$ ].

## Discussion

In this study, insulin resistance was found in 68% of young, normal weight PCOS cases, which is consistent with previous data (65-70% prevalence) [16]. BMI and WHR were higher in insulin resistant PCOS cases. Insulin resistance is the determinant factor in accumulation of triglycerides in the liver and oxidative stress plays role

**Table 4.** Liver function tests, NAFLD and metabolic syndrome in PCOS and control groups

Parameters	Control group n=21	PCOS group n=34	p
ALT <sup>†</sup> (U/L)			
Median (min-max)	12(5-19)	15(8-83)	0.110
GGT <sup>‡</sup> (U/L)			
Median (min-max)	9.4(6.5-18)	12(6-28)	0.610
NAFLD <sup>§</sup> n(%)	2(9.5)	15(44.1)	0.007*
Metabolic syndrome n(%)	0(0)	2(5.8)	-

<sup>†</sup>alanin aminotransferaz; <sup>‡</sup>gamma-glutamyl transferase; <sup>§</sup>nonalcoholic fatty liver disease (NAFLD); \*statistically significant.

**Table 5.** Liver Disease in cases with and without insulin resistance

Liver ultrasonography	NAFLD <sup>†</sup> (+) n=17	NAFLD <sup>†</sup> (-) n=38	%
IR <sup>†</sup> (-)	8	27	%23 (8/35)
IR <sup>†</sup> (+)	9	11	%45 (9/20)

<sup>†</sup>Insulin resistance, <sup>†</sup>Nonalcoholic fatty liver disease.

in inflammation, hepatic cell destruction, necrosis and fibrosis. Therefore, increased risk of liver disease in especially young patients with PCOS, needs to be clarified. In the general population, prevalence of NAFLD ranges from 3% to 24% [17]. In women with PCOS, increased prevalence (15-55%) of NAFLD based on abnormal aminotransferase levels and/or ultrasonographic evidence of hepatic steatosis, were shown by a few studies [3,18,19]. Our results were parallel to the literature documenting 44% NAFLD in young normal weight PCOS patients depending upon ultrasonographic diagnosis. Even if the histologic diagnosis is superior, it is not an applicable procedure for all patients. Therefore, ultrasonography is a practical tool for early detection of NAFLD. Diagnosis and intervention at this stage, may slow, or even stop progression to NASH. A previous study by Gambarin-Gelwans and colleagues [19] found a 40% prevalence of NAFLD in lean (BMI<25) PCOS cases, similar to ours. In our study BMI and insulin resistance parameters did not differ between patients with or without NAFLD, but patients with insulin resistance had two-fold more NAFLD when compared with cases without insulin resistance. In addition to insulin resistance, abnormal lipid profile was found to be associated with NAFLD in PCOS [20]. The finding of significantly higher levels of TG and VLDL in PCOS patients with NAFLD strengthens this aspect. The regression analysis performed in our study showed that ALT and VLDL levels were best predictors for NAFLD.

In PCOS patients, other than well documented dyslipidemia, increased levels of proatherogenic lipid alterations such as small dense lipoprotein particles have been

reported [6,21]. One third of women with PCOS who have a normal lipid pattern, may have atherogenic lipoprotein abnormalities [21]. Confirming these previous data, we found higher levels of sd-LDL in PCOS patients. Moreover, in the insulin resistant PCOS, significantly lower levels of HDL-C and higher levels of TG, VLDL and sd-LDL were found. The correlation between insulin resistance parameters and sd-LDL is in accordance with the report of Rizzo et al [22]. Additionally, positive correlation between TG and VLDL levels and sd-LDL in our study, supports the previously documented association with plasma TG concentrations [6]. Insulin resistance in accordance with hypertriglyceridemia seems to be the crucial factor for increased cardiovascular risk in PCOS.

Recently, a meta-analysis was conducted to determine whether CIMT is higher in women with PCOS compared to women without PCOS [23]. The results of the analysis showed that the mean difference in CIMT among women with PCOS compared with controls was 0.072 mm (95% CI: 0.040-0.105,  $p < 0.0001$ ) [23]. Regarding these results, the authors suggest that women with PCOS are at a greater risk for premature atherosclerosis [23]. In our study, significantly higher CIMT was found in women with PCOS when compared to controls. Neither the demographic features nor the hormone or lipid levels were found to be correlated with CIMT. However, total testosterone was positively correlated with CIMT. Similarly, Coksuer et al [24] also showed a positive correlation of CIMT with testosterone levels in obese young women with PCOS. Larger studies with PCOS population are required to draw strong conclusions, but until then androgen excess seems to be the determining factor for increased CIMT in women with PCOS [25]. The lack of association between insulin resistance parameters and CIMT in our study supports the previous suggestion that insulin resistance per se may not be adequate for endothelial dysfunction in young, lean patients with PCOS [26]. Older age, duration of insulin resistance and obesity may be confounding factors for endothelial deterioration in PCOS.

In conclusion, screening for insulin resistance and dyslipidemia should be the first step in young PCOS patients. When detected, insulin resistance and hypertriglyceridemia in PCOS should be treated to prevent long term consequences of the disease. Screening for NAFLD may be valuable for early detection and prevention of liver disease in insulin resistant patients. And these patients should be under strict control as they age. Larger population based long term observational studies are required to better understand if treatment of comorbidities associated with PCOS decrease the later cardiovascular risk for these patients.

### **Acknowledgements**

The authors wish to thank all patients for their participation in this study, and all personnel at the obstetrics and gynecology department for their enthusiastic contribution. This study has no financial support. G.S.C. collected

the data, and performed the analysis and interpretation of data. M.K. and E.D.O. contributed to the acquisition of data. Manuscript preparation was performed by G.S.C., M.K and R.P. D.K. is the radiologist performing the carotid intima-media thickness measurements and abdominal ultrasound in all cases. S.D performed the biochemical analyses of the serum samples. Drafting the article or revising it critically for important intellectual content was performed by all authors. All authors have approved the final version of the manuscript.

### **Conflict of Interest**

There are no conflicts of interest among the authors.

### **References**

- [1] Ehrmann DA. Polycystic ovary syndrome. *N Engl J Med* 2005; 352(12):1223-36.
- [2] Neuschwander-Tetri BA. Fatty liver and the metabolic syndrome. *Curr Opin Gastroenterol* 2007; 23(2):193-8.
- [3] Cerda C, Pérez-Ayuso RM, Riquelme A, Soza A, Villaseca P, et al. Nonalcoholic fatty liver disease in women with polycystic ovary syndrome. *J Hepatol* 2007; 47(3):412-7.
- [4] McCullough AJ. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin Liver Dis* 2004; 8(3):521-33.
- [5] Conway GS, Agrawal R, Betteridge DJ, Jacobs HS. Risk factors for coronary artery disease in lean and obese women with the polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 1992; 37(2):119-25.
- [6] Pirwany IR, Fleming R, Greer IA, Packard CJ, Sattar N. Lipids and lipoprotein subfractions in women with PCOS: relationship to metabolic and endocrine parameters. *Clin Endocrinol (Oxf)* 2001; 54(4):447-53.
- [7] Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, et al. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA* 1988; 260(13):1917-21.
- [8] Talbott EO, Guzik DS, Sutton-Tyrrell K, McHugh-Pemu KP, Zborowski JV, et al. Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. *Arterioscler Thromb Vasc Biol* 2000; 20(11):2414-21.
- [9] The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004; 82(suppl 3):1193-7.
- [10] Ferriman D, Gallway JD. Clinical assessment of body hair growth in women. *J Clin Endocrinol Metab* 1961; 21:1440-7.
- [11] Grundy SM, Cleeman JJ, Daniels SR, Donato KA, Eckel RH, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005; 112(17):2735-52.
- [12] Onwubuya EI, Anisiuba BC, Osuji CU, Ahaneke JE. Changes in lipids and lipoprotein indices in relation to the severity of hypertension in newly diagnosed hypertensive Nigerians. *ISRN Cardiol* 2012; 2012:972341.
- [13] Hirano T, Ito Y, Saegusa H, Yoshino G. A novel and simple method for quantification of small, dense LDL. *J Lipid Res* 2003; 44(11):2193-201.
- [14] Neuschwander-Tetri BA1, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; 37(5):1202-19.
- [15] Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* 1986; 74(6):1399-406.

- [16] DeUgarte CM, Bartolucci AA, Azziz R. Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. *Fertil Steril* 2005; 83(5):1454-60.
- [17] Clark JM. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; 40 Suppl 1:5-10.
- [18] Setji TL, Holland ND, Sanders LL, Pereira KC, Diehl AM, et al. Nonalcoholic steatohepatitis and nonalcoholic Fatty liver disease in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006; 91(5):1741-7.
- [19] Gambarin-Gelwan M, Kinkhabwala SV, Schiano TD, Bodian C, Yeh HC, et al. Prevalence of nonalcoholic fatty liver disease in women with polycystic ovary syndrome. *Clin Gastroenterol Hepatol* 2007; 5(4):496-501.
- [20] Vassilatou E, Lafoyianni S, Vryonidou A, Ioannidis D, Kosma L, et al. Increased androgen bioavailability is associated with non-alcoholic fatty liver disease in women with polycystic ovary syndrome. *Hum Reprod* 2010; 25(1):212-20.
- [21] Berneis K, Rizzo M, Hersberger M, Rini GB, Di Fede G, et al. Atherogenic forms of dyslipidaemia in women with polycystic ovary syndrome. *Int J Clin Pract* 2009; 63(1):56-62.
- [22] Rizzo M, Berneis K, Hersberger M, Pepe I, Di Fede G, et al. Milder forms of atherogenic dyslipidemia in ovulatory versus anovulatory polycystic ovary syndrome phenotype. *Hum Reprod* 2009; 24(9):2286-92.
- [23] Meyer ML, Malek AM, Wild RA, Korytkowski MT, Talbott EO. Carotid artery intima-media thickness in polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod Update* 2012; 18(2):112-26.
- [24] Coksuer H, Koplay M, Oghan F, Haliloglu B, Keskin N. Evaluation of carotid wall thickness and vertebro-basilar system insufficiency in patients with obese polycystic ovary syndrome. *J Obstet Gynaecol Res* 2011; 37(8):997-1003.
- [25] Luque-Ramírez M, Mendieta-Azcona C, Alvarez-Blasco F, Escobar-Morreale HF. Androgen excess is associated with the increased carotid intima-media thickness observed in young women with polycystic ovary syndrome. *Hum Reprod* 2007; 22(12):3197-203.
- [26] Arikan S, Akay H, Bahceci M, Tuzcu A, Gokalp D. The evaluation of endothelial function with flow-mediated dilatation and carotid intima media thickness in young nonobese polycystic ovary syndrome patients; existence of insulin resistance alone may not represent an adequate condition for deterioration of endothelial function. *Fertil Steril* 2009; 91(2):450-5.