human reproduction   **ORIGINAL ARTICLE** *Reproductive endocrinology*

Milder forms of atherogenic dyslipidemia in ovulatory versus anovulatory polycystic ovary syndrome phenotype

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BACKGROUND: Dyslipidemia is common in women with polycystic ovary syndrome (PCOS) but its prevalence in different PCOS phenotypes is still largely unknown.

METHODS: We measured plasma lipids and lipoproteins in 35 anovulatory PCOS (age: 25 ± 6 years, BMI: 28 ± 6 kg/m^2), 15 ovulatory PCOS (age: 30 ± 6 years, BMI: 25 ± 3 kg/m^2) and 27 healthy women (controls) age- and BMI-matched with ovulatory PCOS. PCOS was diagnosed by the presence of clinical or biologic hyperandrogenism associated with chronic anovulation and/or polycystic ovaries at ultrasound. In women with normal menses chronic anovulation was indicated by low serum progesterone levels (<9.54 nmol/l) during midluteal phase (days 21–24) in two consecutive menstrual cycles.

RESULTS: Total cholesterol, triglycerides and low-density lipoprotein (LDL)-cholesterol levels increased and high-density lipoprotein (HDL)-cholesterol decreased from controls to ovulatory and then to anovulatory PCOS (all $P < 0.05$). Levels of lipoprotein(a) (Lp(a)) and small, dense LDL increased ($P < 0.0001$ for both) and LDL size reduced ($P < 0.005$) between groups. Insulin resistance (by HOMA) showed a positive correlation with triglycerides and small, dense LDL and an inverse correlation with HDL-cholesterol and LDL size ($P < 0.05$ for all) in both PCOS phenotypes. No significant correlations were found with testosterone levels. At multivariate analysis, insulin resistance was independently associated with HDL-cholesterol and small, dense LDL in both PCOS phenotypes and with triglyceride concentrations in ovulatory PCOS only.

CONCLUSIONS: Women with ovulatory PCOS showed milder forms of atherogenic dyslipidemia than anovulatory PCOS and this seemed to be related to the extent of insulin resistance. Future prospective studies are needed to assess the relative contribution of such alterations on cardiovascular risk.

Key words: polycystic ovary syndrome / lipids / lipoproteins / cardiovascular risk

Introduction

Polycystic ovary syndrome (PCOS) probably constitutes the most frequently encountered endocrinopathy in women, affecting up to 10% of women of reproductive age (Carmina and Lobo, 1999; Lobo and Carmina, 2000). Although PCOS is known to be associated with reproductive morbidity and increased risk for endometrial cancer, diagnosis is especially important because the presence of PCOS significantly increases the cardiovascular risk, a finding which has been consistently reported across several geographic areas and ethnic groups (Rizzo *et al.*, 2009). Women with PCOS are more likely than normally cycling women to have insulin resistance, central adiposity and hypertension (Guzick, 2004). In addition, several markers of clinical and subclinical atherosclerosis are altered in women with PCOS (Talbott *et al.*, 2000; Christian *et al.*, 2003; Orio *et al.*, 2004; Carmina *et al.*, 2006).

Dyslipidemia may represent the most common metabolic abnormality in PCOS, with a prevalence of up to 70% according to the National Cholesterol Education Program criteria (Legro et al., 2001). Dyslipidemia usually includes low high-density lipoprotein (HDL)-cholesterol, elevated triglyceride concentrations and less often increased low-density lipoproteins (LDL) and total cholesterol levels (Cussons et al., 2006). Evidences is accumulating that, beyond plasma lipids, different lipoprotein alterations significantly increase cardiovascular risk, including elevated lipoprotein(a) (Lp(a)) levels, increased concentrations of small, dense LDL and increased LDL particle number, that can be measured indirectly as apolipoprotein B (apoB) levels (National Cholesterol Education Program, 2002). However, available data are not fully consistent on the prevalence of the different forms of atherogenic dyslipidemia in women with PCOS, which may be related to genetic and environmental factors as well as the heterogeneity of PCOS phenotypes.

New diagnostic criteria have enlarged the spectrum of PCOS phenotypes, now including ovulatory hyperandrogenic and anovulatory normoandrogenic women (Rotterdam, 2004; Azziz et al., 2006). Such subgroups usually show a milder form of the syndrome and may differ from classic PCOS (i.e. anovulatory hyperandrogenic patients) by their gonadotrophin and steroid hormone secretion pattern, severity of insulin resistance as well as body weight and cardiovascular risk (Carmina, 2006; Barber et al., 2007). Yet, the exact prevalence of atherogenic forms of dyslipidemia in women with ovulatory versus anovulatory PCOS is unknown thus limiting the utility of information we have on their cardiovascular risk. Therefore, we studied 50 Mediterranean women with both anovulatory and ovulatory PCOS, as well as 27 healthy women as controls, in order to assess the differences in plasma lipids and lipoproteins between groups.

Materials and Methods

Patients and control subjects

We have included in the present study 50 women of reproductive age (age: 18–40 years) who were consecutively diagnosed with PCOS at the Endocrine Unit of the Department of Internal Medicine and Emerging Diseases (University of Palermo, Italy). They were all referred because of androgen excess (hirsutism or increased circulating androgens) and serum levels of testosterone, dehydroepiandrosterone sulfate, 17-hydroxyprogesterone, progesterone and pelvic sonography were evaluated at the moment of their referral to our Endocrine Unit. Serum androgens and 17-hydroxyprogesterone were measured during follicular phase (days 5–8) although serum progesterone was determined in day 21–24 of menstrual cycle. Patients with increased 17-hydroxyprogesterone ($>$9.1 nmol/l) were excluded from the study. Biochemical hyperandrogenism was defined as serum testosterone $>$2.08 nmol/l and/or serum dehydroepiandrosterone sulfate $>$7800 nmol/l. These values of hyperandrogenism have been previously calculated in our population with the same assays (Carmina, 1998).

Anovulation was defined as serum progesterone $<$9.54 nmol/l. In patients with normal menses, at least two consecutive menstrual cycles were studied and finding of low levels of serum progesterone ($<$9.54 nmol/l) during midluteal phase (days 21–24) in both cycles indicated the presence of chronic anovulation (Fisher, 2004). Thus, the study included both anovulatory ($n = 35$) and ovulatory ($n = 15$) PCOS. In all patients transvaginal sonography was performed using a Hitachi H21 instrument by an experienced sonographer (e.g.) on day 5 of menstrual cycle. Ovarian sonographies had to report ovarian size (by measurement of the main three ovarian diameters in both ovaries) and presence, size and number of ovarian microcysts. Finding of increased ovarian size and/or of at least 12 follicular cysts measuring 2–9 mm were considered indicative of the presence of polycystic ovaries (Balen et al., 2003). According to this definition, all studied patients had polycystic ovaries. The diagnosis of PCOS was based on the presence of clinical or biologic hyperandrogenism associated with chronic anovulation and/or polycystic ovaries at ultrasound (Azziz et al., 2006).

The project design included a medical examination and biochemical analyses. The adopted procedures were in agreement with the Helsinki Declaration of 1975 (6th revision, 2008) and the study was approved by the local Ethics Council. All subjects gave their informed consent to participate in the study. At admission all subjects underwent a medical examination and also answered a questionnaire on personal and medical items, including age, past medical history and use of medications. Exclusion criteria included the presence of type-2 diabetes, renal or hepatic diseases able to modify plasma lipids and lipoproteins, including levels of triglyceride-rich lipoproteins and small, dense LDL, as well as the use of hypolipidemic drugs. The control group consisted of 27 healthy women with the same exclusion criteria described above, which were age- and BMI-matched with the group of ovulatory PCOS. They were recruited from family members of hospital co-workers. Controls were women with regular menses, normal ovulatory menses (progesterone during midluteal phase $>$22.3 nmol/l) (Carmina and Lobo, 2004) and normal androgen and 17-hydroxyprogesterone levels.

Height and weight were recorded and BMI was calculated as kg/m^2. Waist circumference was measured according to the American Heart Association/National Heart, Lung and Blood Institute guidelines (Grundy et al., 2005). We located top of right iliac crest and we placed a measuring tape in a horizontal plane around abdomen at level of iliac crest. Before reading tape measure, we ensured that tape was snug, not compressing the skin and parallel to the floor. Measurement was made at the end of a normal expiration. Among the cardiovascular risk factors, hypertension (systolic or diastolic blood pressure, respectively, $\geq$140 or $\geq$90 mmHg or previous pharmacological therapy with antihypertensive drugs), diabetes (fasting glucose plasma concentrations higher than 7.0 mmol/l or previous pharmacological therapy with antidiabetic drugs or insulin), smoking habit and family history of cardiovascular diseases (in first degree male relatives younger than 55 years of age and in first degree female relatives younger than 65 years of age) (National Cholesterol Education Program, 2002) were also considered.

Laboratory analyses

A blood sample was collected from each subject after 14 h overnight fast in sodium-EDTA tubes. Plasma total cholesterol and triglycerides were measured on a Roche Modular System using commercial reagents (Roche Diagnostics, Rotkreuz, Switzerland) with a coefficient of variation of 2.3 and 2.4%, respectively. HDL-cholesterol, Lp(a) and apoB levels were measured on a Roche Integra 800 analyzer using commercial assays (Roche Diagnostics) with a coefficient of variation of 4.1, 2.3 and 1.2%, respectively. LDL-cholesterol was calculated according to the Friedewald formula (Friedewald et al., 1972). Insulin resistance was determined by the homeostasis model assessment (HOMA) using the formula: [fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5] (Matthews et al., 1985). Serum hormone levels were quantified by well-established methods which had been validated previously in our laboratory. All steroids were measured by specific radioimmunoassays after extraction using previously described methods (Carmina et al., 1992) with intra-assay and inter-assay coefficients of variation not exceeding 6 and 15%, respectively.

LDL size and subclasses were assessed by non-denaturing polyacrylamide gradient gel electrophoresis of whole plasma in Switzerland in the laboratory of K.B. at 10–14°C in 2–16% polyacrylamide gradient gels. Gels were subjected to electrophoresis for 24 h at 125 V in tris borate buffer (pH 8.3) as described elsewhere (Krauss and Burke, 1982). Gels were fixed and stained for lipids in a solution containing oil red O in 60% ethanol at 55°C. Gels were placed on a light source and photographed using a Luminescent Image Analyzer, LAS-3000 of Fujifilm, detection using white transmitted light source. Migration distance for each absorbance peak was determined and the molecular diameter corresponding to each peak was calculated from a calibration curve generated from the migration distance of size standards of known diameter, which includes carboxylated latex beads (Duke Scientific, Palo Alto, CA, USA), thyroglobulin and apoferritin (HMW Std, Pharmacia, Piscataway, NJ, USA) having molecular diameter of 380, 170 and 122 Å, respectively, and lipoprotein calibrators of previously determined particle size. LDL subclass distribution as percent of total LDL was calculated as previously described (Krauss and Burke, 1982). Total small, dense LDL particles were considered those obtained by the sum of individual LDL-III + LDL-IV subclasses (e.g. LDL-IIIA + LDL-IIIB + LDL-IVA + LDL-IVB).

In order to assess if plasma lipids or lipoproteins were abnormal in women with ovulatory and anovulatory PCOS, the following cut-offs were considered (National Cholesterol Education Program, 2002; Grundy *et al.*, 2005): high triglycerides if >1.7 mmol/l, low HDL-cholesterol if <1.29 mmol/l, high LDL-cholesterol if >4.1 mmol/l, elevated Lp(a) if >30 mg/dl, elevated apoB if >100 mg/dl. Higher levels of small, dense LDL were considered as those greater than mean +2SD of the values of controls, as previously described (Berneis *et al.*, 2007).

Statistical analysis

Statistical analyses were performed using Statview 5.0 (SAS Institute, USA). Since Lp(a) concentrations were not normally distributed, a log transformation was necessary to obtain a normal distribution. Univariate analyses were performed using the Mann–Whitney *U* non-parametric test for the numeric variables whereas the differences in the prevalence for the nominal variables were analyzed by the Fisher's exact test. ANOVA was performed to assess the differences in clinical and biochemical parameters between groups, after adjustment for age and BMI. Correlation analyses were performed using the Spearman rank correlation method. Multivariate analysis (by multiple regression) was performed in order to determine the effects of clinical and laboratory parameters on plasma lipids and lipoproteins in ovulatory and anovulatory PCOS. As shown in Table V, we built different models of multivariate analysis for

ovulatory and anovulatory PCOS with the following dependent variables: HDL-cholesterol, triglycerides, Lp(a), apoB and small, dense LDL. The independent variables were represented in each model by age, BMI, waist circumference, HOMA and testosterone.

Results

As shown in Table I, ovulatory PCOS had similar age and BMI than controls, although anovulatory PCOS were younger and had a larger waist circumference (*P* < 0.01 for both). A clear trend towards higher testosterone and insulin concentrations and HOMA values from controls to ovulatory and to anovulatory PCOS (*P* < 0.0001 for all) was noted. No significant differences were found between the three groups with respect to hypertension, diabetes, smoking habits and family history of cardiovascular diseases (data not shown). Total-cholesterol, triglyceride and LDL-cholesterol concentrations were higher and HDL-cholesterol levels were lower in controls versus ovulatory versus anovulatory PCOS (all *P* < 0.05, see Table II).

Similarly, concentrations of Lp(a), as log-transformed, and small, dense LDL were higher in anovulatory as compared with ovulatory PCOS (*P* < 0.0001 for both) although no changes were found in apoB levels. A clear trend towards alterations in LDL subclass distribution was noted between groups too (see Fig. 1): larger particles decreased and smaller, more dense LDL increased from controls to ovulatory and to anovulatory PCOS. As depicted in Table III, women with ovulatory PCOS displayed milder forms of atherogenic dyslipidemias in relation to those with the anovulatory phenotype. By correlation analysis we found that testosterone levels did not correlate with any plasma lipid or lipoprotein parameter in either PCOS phenotypes (Table IV); by contrast, HOMA indices correlated positively with triglyceride concentrations and small dense LDL and inversely with HDL-cholesterol and LDL size in both PCOS phenotypes.

We also performed multivariate analysis (multiple regression) in order to determine potential independent predictors of plasma lipids and lipoproteins in ovulatory and anovulatory PCOS (see Table V). We found that in both groups insulin resistance was independently associated with HDL-cholesterol (*P* = 0.0385 in ovulatory and *P* = 0.0210 in anovulatory PCOS) and small, dense LDL levels (*P* = 0.0468 in ovulatory and *P* = 0.0335 in anovulatory PCOS) as well as with triglyceride concentrations in the group of ovulatory

Table I Clinical and laboratory characteristics in all subjects

	Controls (*n* = 27)	Ov-PCOS (*n* = 15)	Anov-PCOS (*n* = 35)	ANOVA *P
Age (years)	30 ± 8	30 ± 6	25 ± 6[a,b]	0.0053
Body-mass index (kg/m²)	25 ± 4	25 ± 3	28 ± 6	0.0264
Waist (cm)	80 ± 6	85 ± 7	94 ± 14[a]	0.0044
Total testosterone (nmol/l)	1.0 ± 0.4	2.1 ± 0.8	3.0 ± 1.2	<0.0001
Insulin (pmol/l)	45 ± 13	65 ± 25[a]	109 ± 44[a,b]	<0.0001
HOMA	1.1 ± 0.3	2.0 ± 0.8[a]	3.3 ± 1.4[a,b]	<0.0001

Results are shown as mean ± SD.
*Age and BMI adjusted.
[a]*P* < 0.05 versus Controls; [b]*P* < 0.05 versus Ov-PCOS.
HOMA, homeostasis model assessment.

**Table II** Plasma lipids and lipoproteins in all subjects

	Controls (n = 27)	Ov-PCOS (n = 15)	Anov-PCOS (n = 35)	ANOVA *P
Total cholesterol (mmol/l)	4.3 ± 1.2	4.6 ± 1.0	4.9 ± 0.8[a]	0.0440
Triglycerides (mmol/l)	0.6 ± 0.4	1.0 ± 0.5[a]	1.0 ± 0.4[a]	0.0014
HDL-cholesterol (mmol/l)	1.5 ± 0.7	1.4 ± 0.3	1.2 ± 0.3[a,b]	0.0209
LDL-cholesterol (mmol/l)	2.7 ± 1.6	2.6 ± 1.2	3.5 ± 1.0[a,b]	0.0282
Lp(a) (mg/dl)	6 ± 6	21 ± 37	40 ± 38[a,b]	0.0106
Log Lp(a)	0.60 ± 0.39	0.86 ± 0.62	1.39 ± 0.50[a,b]	<0.0001
apoB (mg/dl)	81 ± 44	84 ± 20	86 ± 25	ns
LDL size (Å)	290 ± 19	278 ± 12[a]	276 ± 10[a]	0.0040
Total small, dense LDL (%)	31 ± 6	37 ± 7[a]	42 ± 8[a,b]	<0.0001

Results are shown as mean ± SD.
*Age and BMI adjusted.
[a]$P < 0.05$ versus Controls; [b]$P < 0.05$ versus Ov-PCOS.
HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); apoB, apolipoprotein B.
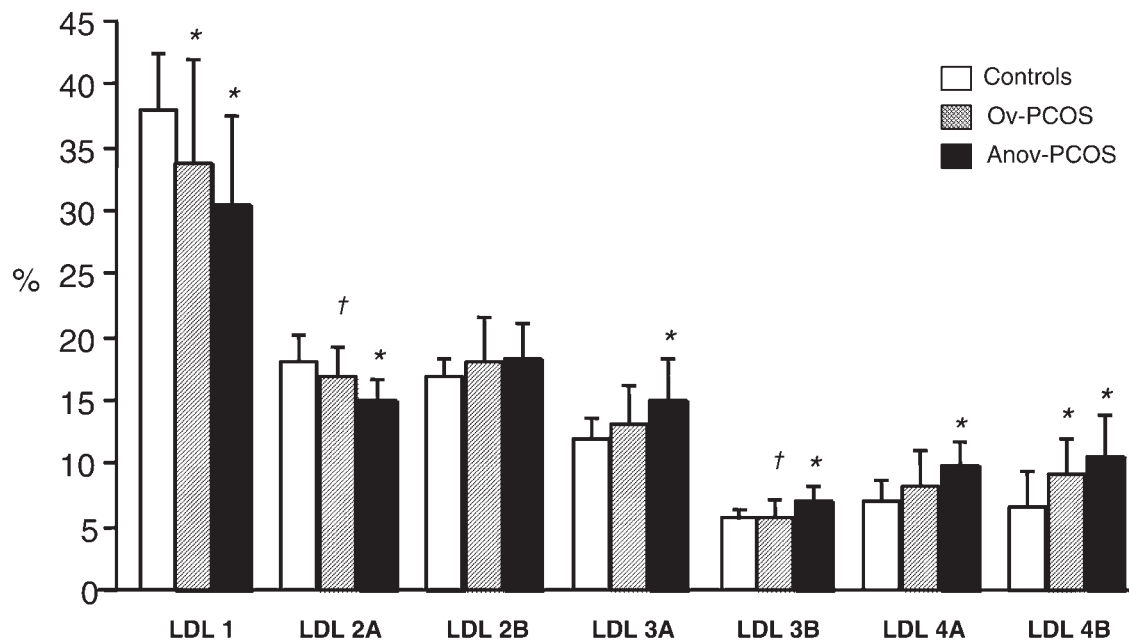
Figure 1 LDL subclasses in all subjects (mean + SD).
*$P < 0.05$ versus controls; †$P < 0.05$ versus Anov-PCOS.

PCOS only ($P = 0.0433$). Further, waist circumference was independently associated with levels of small, dense LDL in the group of anovulatory PCOS ($P = 0.489$).

Discussion

Cardiovascular diseases represent the major cause of death in both genders, but women have hormonal protection before menopause thus delaying the onset of cardiovascular diseases by 10–15 years in comparison to men (National Cholesterol Education Program, 2002); however, young women may show increased cardiovascular risk when affected by PCOS (Lobo and Carmina, 2000). The explanation for such increased risk is still not clear; hyperandrogenism, as isolated androgen excess, has not been recognized so far as a risk factor for cardiovascular disease (Legro, 2003) and prospective studies performed on pre- and post-menopausal women failed to show a clear association between hyperandrogenism and future cardiovascular events (Cussons et al., 2006). By contrast, other metabolic conditions, including insulin resistance and dyslipidemia, seems to play a major role on cardiovascular risk in PCOS (Rizzo et al., 2009). Yet, to what extent dyslipidemia may contribute to this increased risk is still largely unknown.

Table III Prevalence of plasma lipid and lipoprotein alterations in ovulatory and anovulatory PCOS

	Ov-PCOS (n = 15)	P =	Anov-PCOS (n = 35)
High triglycerides (>1.7 mmol/l), n (%)	1 (7%)	ns	3 (9%)
High LDL-cholesterol (>4.1 mmol/l), n (%)	1 (7%)	ns	8 (23%)
Low HDL-cholesterol (<1.29 mmol/l), n (%)	5 (33%)	0.0299	24 (69%)
Elevated Lp(a) (>30 mg/dl), n (%)	3 (20%)	0.0566	19 (54%)
Elevated apoB (>100 mg/dl), n (%)	1 (7%)	ns	11 (31%)
High small, dense LDL (LDL-III and -IV), n (%)	3 (20%)	0.0302	20 (57%)

HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); apoB, apolipoprotein B.

Lipid alterations are common in women with PCOS (Legro et al., 2001; Cussons et al., 2006) and we have previously reported in our Mediterranean PCOS population that triglyceride and LDL-cholesterol concentrations are usually in the normal range, although significantly increased compared with controls, although HDL-cholesterol levels are decreased (Essah et al., 2008). Evidence is accumulating that, beyond plasma lipids, alterations in lipoprotein patterns significantly increase cardiovascular risk (Rizzo and Berneis, 2007; Wierzbicki, 2008). We have previously shown that Mediterranean women with PCOS display increased concentrations of Lp(a) and small, dense LDL, with no alterations in apoB levels (Berneis et al., 2007, 2009). However, this observation is not consistent with findings by other authors, particularly in terms of LDL size (Legro et al., 1999), apoB (Demirel et al., 2007) and Lp(a) (Sahin et al., 2007) concentrations. This may be due to genetic and environmental factors and may reflect the heterogeneity of PCOS phenotypes.

According to new diagnostic criteria (Rotterdam, 2004; Azziz et al., 2006) PCOS may indeed represent a wide spectrum of abnormalities, including women with milder forms of the syndrome who generally present less severe clinical and hormonal abnormalities (Carmina,

Table IV Spearman correlations between lipid parameters and testosterone, insulin and insulin resistance (by HOMA) in ovulatory and anovulatory PCOS

	Ov-PCOS			Anov-PCOS		
	Insulin	HOMA	Testosterone	Insulin	HOMA	Testosterone
Total cholesterol	0.001	0.030	0.264	−0.088	−0.066	0.207
Triglycerides	0.343	0.427[a]	0.056	0.351[a]	0.392[a]	0.204
HDL-cholesterol	−0.481[a]	−0.465[a]	−0.133	−0.381[a]	−0.338[a]	−0.158
LDL-cholesterol	−0.062	−0.030	0.252	0.163	0.119	0.221
Log Lp(a)	0.084	0.042	−0.140	0.168	0.162	−0.023
apoB	0.036	0.070	−0.103	0.091	0.125	−0.047
LDL size	−0.302	−0.419[a]	−0.134	−0.525[b]	−0.492[b]	−0.210
Total small, dense LDL	0.427[a]	0.461[a]	0.115	0.548[b]	0.518[b]	0.078

HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); apoB, apolipoprotein B; HOMA, homeostasis model assessment.
[a]P < 0.05; [b]P < 0.005.

Table V Multivariate analysis (by multiple regression) determining independent predictors of plasma lipids and lipoproteins in ovulatory and anovulatory PCOS

	Ov-PCOS					Anov-PCOS				
	HDL-cholesterol	TG	Lp(a)	apoB	Small, dense LDL	HDL-cholesterol	TG	Lp(a)	apoB	Small, dense LDL
Age	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
BMI	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Waist circumference	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.0489
HOMA	0.0385	ns	ns	ns	0.0468	0.0210	0.0433	ns	ns	0.0335
Testosterone	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

TG: triglycerides; apoB: apolipoprotein B.

2006; Barber *et al.*, 2007). In particular, patients with the ovulatory form of PCOS are generally leaner and have lower insulin concentrations and higher insulin sensitivity than patients with the classic anovulatory hyperandrogenic phenotype (Carmina, 2006; Barber *et al.*, 2007). In the present study we have confirmed that ovulatory PCOS patients are less obese and display lower testosterone and insulin concentrations than women with the classic anovulatory phenotype. It should be noted, however, that there are several issues concerning the measurement of female testosterone (Rosner *et al.*, 2007), and it may influence the reported results.

We also found that women with ovulatory PCOS have lower total-cholesterol, triglyceride and LDL-cholesterol concentrations and higher HDL-cholesterol levels as compared with anovulatory PCOS; further, they showed increased Lp(a) concentrations and reduced levels of small, dense LDL. The elevation in triglyceride levels may represent the main contributor for the production of small, dense LDL: these particles are usually formed by the action of hepatic lipase from lipoprotein precursors enriched in triglycerides (Rizzo and Berneis, 2007). Therefore, although the Friedewald's formula is appropriate for the calculation of LDL-cholesterol concentrations when triglycerides are below 4.5 mmol/l, its use in the presence of abnormal lipoproteins may be problematic. Overall, women with ovulatory PCOS display a milder form of atherogenic dyslipidemia as compared with the anovulatory phenotype. Correlation analysis revealed that such alterations may reflect the extent of insulin resistance. Of interest, no significant correlations were found between testosterone levels and plasma lipids and lipoproteins in either PCOS phenotype, suggesting a limited role of testosterone in modulating atherogenic dyslipidemia. This is consistent with previous findings in PCOS populations (Pirwany *et al.*, 2001).

It should be noted that in the present study we did not match ovulatory and anovulatory PCOS for age, BMI or waist circumference. Although the difference in BMI could contribute to differences in insulin resistance, we preferred to not match these two subgroups for such parameters in order to include women with their usual and distinct phenotypes. In fact, ovulatory and anovulatory PCOS generally exhibit significant differences in BMI and waist circumference. Matching ovulatory with anovulatory PCOS for BMI and waist circumference would exclude the majority of women with ovulatory PCOS, including only those with elevated BMI and waist circumference (i.e. mostly overweight and obese subjects). This may lead to a bias, including only ovulatory PCOS women with unusual phenotype. Yet, we adjusted all laboratory data for age and BMI in order to remove the effects of these potential confounding factors.

We also performed multivariate analyses in order to determine potential independent predictors of plasma lipids and lipoproteins in ovulatory and anovulatory PCOS. We found that insulin resistance was independently associated with HDL-cholesterol and small, dense LDL levels in both groups of PCOS as well as with triglyceride concentrations in the group of ovulatory PCOS only. Further, waist circumference was independently associated with levels of small, dense LDL in the group of anovulatory PCOS. These findings suggest a limited role of obesity, specifically abdominal obesity, in determining dyslipidemia in PCOS, although insulin resistance seems to have a greater role.

At present we do not know whether therapeutic modulation of such pro-atherogenic lipid alterations reduces cardiovascular risk.

Management of atherogenic dyslipidemia in PCOS is in debate; weight reduction and increasing physical activity should constitute first-line measures. Lipid-lowering drugs, including statins, nicotinic acid and fibrates as well as insulin-sensitizing medications or the combination pioglitazone + metformin may be an option for patients with severe dyslipidemia (Rizzo *et al.*, 2008). It remains to be tested in future prospective studies whether hypolipidemic and/or insulin-sensitizing agents may reduce the cardiovascular risk in women with PCOS by decreasing atherogenic dyslipidemia.

In conclusion, this is the first report focusing on alterations in plasma lipids and lipoproteins in ovulatory versus anovulatory PCOS phenotype. We found milder forms of atherogenic dyslipidemia in ovulatory than anovulatory PCOS and these alterations were linked to the degree of insulin resistance. Thus, measurement of atherogenic lipoproteins such as Lp(a) and small, dense LDL in women with different PCOS phenotypes may potentially help to assess cardiovascular risk and adapt the treatment goals.

Authors' roles

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