Atherogenic forms of dyslipidaemia in women with polycystic ovary syndrome

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Disclosures
None.

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SUMMARY

Objective: Dyslipidaemia is very common in patients with polycystic ovary syndrome (PCOS) but, beyond plasma lipids, atherogenic lipoprotein (Lp) and apolipoprotein (apo) alterations are still ill defined. **Design:** We measured concentrations of apoB, Lp(a) and small, dense low-density lipoprotein (LDL) in 42 patients with PCOS [age: 28 ± 7 years, body mass index (BMI): 27 ± 5 kg/m^2] vs. 37 age- and BMI-matched healthy controls. **Methods:** Elevated Lp(a) levels considered were those > 30 mg/dl while elevated apoB concentrations were those > 100 g/l. **Results:** Polycystic ovary syndrome showed increased triglycerides levels (p = 0.0011) and lower high-density lipoprotein (HDL)-cholesterol concentrations (p = 0.0131) while total- and LDL cholesterol were similar. PCOS also showed smaller LDL size (p = 0.0005), higher levels of total small, dense LDL (p < 0.0001), higher concentrations of Lp(a), as considered as absolute values (p = 0.0143) and log-transformed (p = 0.0014), while no differences were found in apoB levels. Elevated Lp(a) concentrations were found in 24% of PCOS, while elevated apoB levels were relatively uncommon (14%). Spearman correlation analysis revealed that Lp(a) concentrations were weakly correlated only with HDL-cholesterol levels (r = −0.378, p = 0.0431). In addition, 36% of patients with PCOS with normal plasma lipid profile showed elevated levels of Lp(a), apoB or small, dense LDL. **Conclusions:** Atherogenic Lp abnormalities may be found in one-third of women with PCOS who have a normal lipid pattern. Future prospective studies are needed to test to which extent such atherogenic forms of dyslipidaemia may contribute to the increased cardiovascular risk in young women with PCOS.

What's known
Women with PCOS have increased cardiovascular risk. Dyslipidaemia is very common and usually includes low HDL-cholesterol levels and elevated triglyceride concentrations. Yet, the exact prevalence of atherogenic dyslipidaemia in women with PCOS is still unknown and this limits the utility of information we have in this category of young patients at higher cardiovascular risk.

What's new
We investigated the presence of atherogenic dyslipidaemia in Mediterranean women with PCOS. PCOS had increased concentrations of Lp(a) and of small, dense LDL. As these alterations were partially linked to insulin resistance, it may be possible in the future to individualise specific interventions if more than the traditional lipids are taken into account. This may potentially help assess cardiovascular risk and adapt treatment goals thereafter.

Introduction

Polycystic ovary syndrome (PCOS) probably constitutes the most frequently encountered endocrinopathy in women, affecting up to 10% of women in reproductive age (1,2). 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 and this finding has been consistently reported across several geographical areas and ethnic groups (1,2). Women with PCOS are more likely than normally cycling women to have insulin resistance, central adiposity and hypertension (3). In addition, several markers of clinical and subclinical atherosclerosis have been found to be altered in women with PCOS (4–8).

Dyslipidaemia is also very common in women with PCOS and usually includes low HDL-cholesterol levels and elevated triglyceride concentrations (1,2). In recent years, evidence has suggested that, beyond plasma lipids, different lipoprotein (Lp) and apolipoprotein (apo) alterations significantly increase cardiovascular risk (9). These alterations include elevated Lp(a) levels as well as increased concentrations of apoB; yet, the exact prevalence of these forms of atherogenic dyslipidaemia in women with PCOS is still unknown and this limits the utility of information we have in this category of young patients at higher cardiovascular risk.

Therefore, we included in this study 42 Mediterranean patients with PCOS and 37 healthy female subjects matched for age and body mass index (BMI) as controls, to assess: (i) whether levels of Lp(a) or apoB are altered in our PCOS population in relation

to controls; (ii) whether their levels are related to plasma lipids or other atherogenic Lp including small dense low-density lipoproteins (LDL) and (iii) whether patients with PCOS with normal plasma lipid profile may show 'hidden' atherogenic lipid abnormalities [e.g. elevated levels of Lp(a), apoB or small, dense LDL].

Materials and methods

Patients and control subjects

Forty-two women of reproductive age with PCOS, all referred because of androgen excess to our Endocrine Unit of the Department of Clinical Medicine and Emerging Diseases (University of Palermo) were included in this study. The diagnosis of PCOS was based on the presence of clinical or biological hyperandrogenism associated with chronic anovulation and/or polycystic ovaries at the ultrasound (10). Anovulation was defined as serum progesterone < 3 ng/ml (< 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 (< 3 ng/ml) in both cycles indicated the presence of chronic anovulation. Therefore, the study included both anovulatory ($n = 31$) and ovulatory ($n = 11$) PCOS patients (7,11). The presence of polycystic ovaries was determined by intravaginal sonography. Findings of increased ovarian size (12) and/or of at least 12 follicular cysts measuring 2–9 mm were considered indicating the presence of polycystic ovaries (13). All studied patients had polycystic ovaries.

The project design included a medical examination and biochemical analyses. The adopted procedures were in agreement with the Helsinki Declaration of 1975 as revised in 1983 and the study was approved by the local Ethic 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 renal or hepatic diseases capable of modifying plasma Lp and the use of hypolipidaemic drugs. None of patients had type 2 diabetes or was taking medications from at least 3 months before entering the study.

As controls, we selected a group of 37 healthy women, matched for age and BMI with the same exclusion criteria described above. They were recruited from family members of hospital co-workers. Controls were women with regular menses, normal fertility and normal hormonal levels. Height and weight were recorded and BMI was calculated as kg/m^2. Waist circumference was assessed in both controls and women with PCOS. Among the cardiovascular risk factors, hypertension (systolic or diastolic blood pressure respectively ≥ 140 or ≥ 90 mmHg or previous pharmacological therapy with antihypertensive drugs), diabetes (fasting glucose plasma concentrations higher than 126 mg/dl or previous pharmacological therapy with antidiabetic drugs or insulin) and smoking habit were also considered.

Laboratory analyses

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 analyser 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. Insulin and insulin resistance were determined by different methods including fasting insulin, the homeostasis model assessment (HOMA) and the quantitative insulin sensitivity check index (QUICKI). Yet, it has to be considered that we did not perform oral glucose tolerance test. The estimate of insulin resistance by HOMA score was calculated using the formula: fasting serum insulin (μU/ml) $\times$ fasting plasma glucose (mmol/l)/22.5 (14). The QUICKI is derived by calculating the inverse of the sum of logarithmically expressed values of fasting insulin and glucose (15).

Low-density lipoprotein size and subclasses were assessed by non-denaturing polyacrylamide gradient gel electrophoresis of whole plasma in Switzerland 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 (16). 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 (Tokyo, Japan), 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), thyroglobulin and apoferritin (HMW Std, Pharmacia, Piscataway, NJ) having molecular diameter of 380, 170 and 122 Å, respectively, and Lp calibrators of previously determined particle size. LDL subclass distribution as percent of total LDL was calculated as previously

described (16). 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).

To assess if plasma lipids or Lp were abnormal in women with PCOS, we considered the following cut-offs according the most recent international guidelines (9,17): high triglycerides if > 1.7 mmol/l, low HDL-cholesterol if < 1.1 mmol/l, high LDL-cholesterol if > 4.1 mmol/l, elevated Lp(a) if > 30 mg/dl, elevated apoB if > 100 g/l. Higher levels of small, dense LDL were considered as those greater than mean + 2 SD of the values of controls.

Statistical analysis

Statistical analyses were performed using Statview 5.0 (SAS Institute, Cary, NC, USA). As Lp(a) concentrations were not normally distributed, a log-transformation was necessary to obtain a normal distribution. Univariate analyses were performed using Student's unpaired t-test for the continuous variables, while the differences in the prevalences for the nominal variables were analysed by chi-square test. Correlation analyses were performed using the Spearman-rank correlation method.

Results

Patients and controls have similar BMI, according to the inclusion criteria. However, waist circumference was larger in women with PCOS than in those in control (91 ± 13 vs. 80 ± 6 cm, p < 0.01). As shown in Table 1, patients with PCOS showed increased levels of triglycerides (p = 0.0011) and lower levels of HDL cholesterol (p = 0.0131) while total and LDL cholesterol did not differ significantly in relation to controls. In addition, women with PCOS showed increased values of insulin and insulin resistance, as assessed by HOMA and QUICKI (all p < 0.0001). The prevalence of elevated triglycerides or LDL cholesterol in PCOS was low (9% and 14% respectively) and not significantly different from that found in controls. Low HDL-cholesterol concentrations were found in 33% of women with PCOS vs. 14% of controls (p = 0.0460). Consistent with our previous report (18), we also found that women with PCOS showed a smaller LDL size (p = 0.0005) (data not shown) because of a reduction in largest particles (LDL-I and -IIA) with a concomitant increase in medium- and smaller-sized subspecies (LDL-IIB, -IIIA, -IIIB and -IVA) (Figure 1); therefore, levels of total small, dense particles were strongly increased in PCOS compared to controls (40 ± 8% vs. 31 ± 6%, p < 0.0001).

As shown in Table 2, women with PCOS had significantly higher concentrations of Lp(a), as considered as absolute values (p = 0.0143) and log-transformed (p = 0.0014); by contrast, no difference was found in apoB levels. Elevated Lp(a) levels were found in about 1/4 of women with PCOS (24%), while elevated apoB concentrations were

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Table 1 Clinical and laboratory characteristics in all subjects

	With PCOS (n = 42)	p = (t-test/χ^2 test)	Controls (n = 37)
Age (years)	28 ± 7	ns	31 ± 2
Body mass index (kg/m^2)	27 ± 5	ns	26 ± 4
Hypertension (%)	2	ns	0
Diabetes (%)	0	ns	0
Smoking (%)	16	ns	19
Family history of cardiovascular diseases (%)	7	ns	11
Total cholesterol (mmol/l)	4.7 ± 0.9	ns	4.3 ± 1.2
Triglycerides (mmol/l)	1.0 ± 0.5	0.0011	0.6 ± 0.4
HDL cholesterol (mmol/l)	1.2 ± 0.3	0.0131	1.5 ± 0.7
LDL cholesterol (mmol/l)	3.1 ± 1.1	ns	2.7 ± 1.6
High triglycerides (> 1.7 mmol/l), (%)	9	ns	3
Low HDL cholesterol (< 1.1 mmol/l), (%)	33	0.0460	14
High LDL cholesterol (> 4.1 mmol/l), (%)	14	ns	8
Insulin (μU/ml)	13 ± 5	< 0.0001	7 ± 2
HOMA	2.8 ± 1.1	< 0.0001	1.1 ± 0.3
QUICKI	0.33 ± 0.02	< 0.0001	0.37 ± 0.01

Results are shown as mean ± SD. PCOS, polycystic ovary syndrome; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; QUICKI, quantitative insulin sensitivity check index.
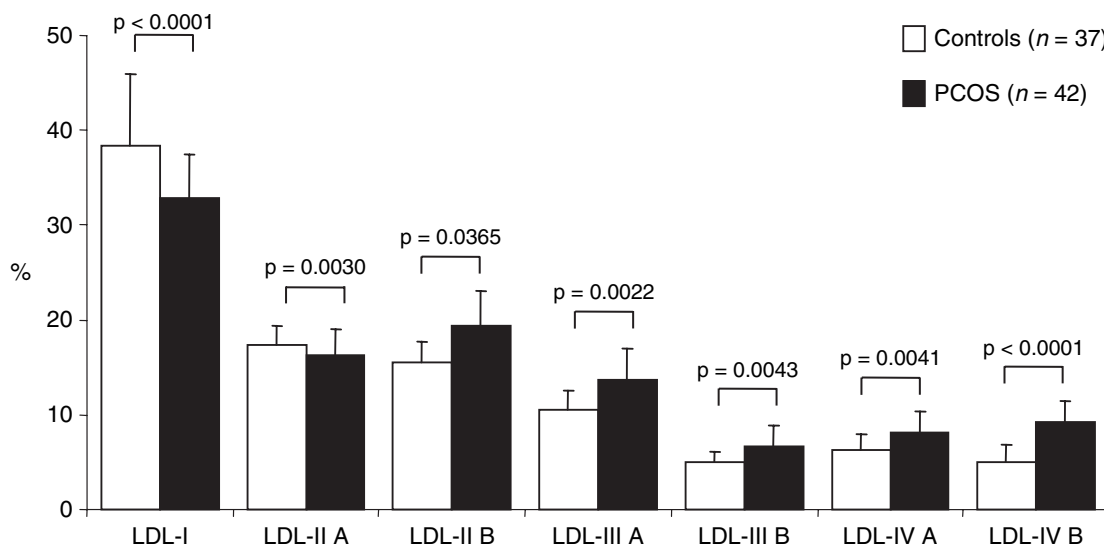
Figure 1 Low-density lipoprotein (LDL) subclasses in women with polycystic ovary syndrome (PCOS) and controls

Table 2 Lipoprotein (a) and apoB levels in all subjects

	With PCOS (n = 42)	p = (t-test/χ² test)	Controls (n = 37)
Lp(a) (mg/dl)	24 ± 26	0.0143	5.2 ± 5.1
Log Lp(a)	1.12 ± 0.53	0.0014	0.57 ± 0.35
Elevated Lp(a) (> 30 mg/dl), (%)	24	< 0.0001	0
Apolipoprotein B (g/l)	79 ± 24	ns	81 ± 44
Elevated apoB (> 100 g/l), (%)	14	ns	15

Results are shown as mean ± SD. Lp, lipoprotein; apo, apolipoprotein; PCOS, polycystic ovary syndrome.

relatively uncommon (14%). Spearman correlation analysis (Table 3) was performed to assess the potential correlations between Lp(a) and age, BMI, plasma lipids, LDL size and subclasses, insulin and insulin resistance. We found that Lp(a) levels were weakly correlated only with HDL-cholesterol levels (r = −0.378, p = 0.0431). In addition, we found that 25 patients with PCOS had a normal plasma lipid profile, but further analysis revealed in nine of them (36%) the presence of 'hidden' pro-atherogenic lipid abnormalities (e.g. elevated levels of Lp(a) or small, dense LDL).

Discussion

Cardiovascular diseases represent the major cause of death in both genders, but women have hormonal protection before menopause and the onset of cardiovascular diseases is usually delayed by 10–15 years in comparison with that in men (9,19); however, young women may show an increased cardiovascular risk when affected by PCOS (2). The explanation for such increased risk is still not clear; hyperandrogenism, as isolated androgen excess, has not been recognised so far as a risk factor for cardiovascular disease (20) and prospective studies performed on pre- and postmenopausal women failed to show a clear association between hyperandrogenism and future cardiovascular events (21). In contrast, other metabolic conditions including insulin resistance and dyslipidaemia seems to play a major role on cardiovascular risk in PCOS (22). Yet, to which extent dyslipidaemia may contribute to this increased risk is still unknown.

Lipid alterations are common in women with PCOS (1,2,7) but are strongly influenced by environmental factors including diet and physical activity (23). As we have previously reported in our PCOS population (7,18,23), triglyceride levels are usually in the normal range, although significantly increased

Table 3 Spearman correlations between Lp(a) levels (as log-transformed) and age, BMI and biochemical parameters in women with PCOS

	Spearman correlation analysis	
	r	p-value
Age (years)	−0.110	ns
BMI	0.098	ns
Total cholesterol (mg/dl)	−0.033	ns
Triglycerides (mg/dl)	0.196	ns
HDL cholesterol (mg/dl)	−0.378	0.0431
LDL cholesterol (mg/dl)	0.205	ns
Apolipoprotein B (g/l)	0.116	ns
LDL size (Å)	−0.023	ns
LDL-I (%)	0.063	ns
LDL-II A (%)	−0.018	ns
LDL-II B (%)	−0.266	ns
LDL-III A (%)	−0.213	ns
LDL-III B (%)	−0.130	ns
LDL-IV A (%)	0.117	ns
LDL-IV B (%)	0.166	ns
Insulin (μU/ml)	−0.318	ns
HOMA	−0.339	ns
QUICKI	0.376	ns

HOMA, homeostasis model assessment; QUICKI, quantitative insulin sensitivity check index; PCOS, polycystic ovary syndrome; Lp, lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index.

compared to controls, and it cannot therefore surprise that the prevalence of elevated triglycerides in the present study was low (9%). High LDL-cholesterol levels were uncommon too (14%), while low HDL-cholesterol concentrations were found in about one-third of subjects (33%). Beyond plasma lipids, in recent years, evidence has suggested that different Lp and apo alterations significantly increase cardiovascular risk (9,24). Notably, elevated levels of small, dense LDL represented the most common lipid alteration (44%) in our Mediterranean PCOS population.

To assess if other 'hidden' pro-atherogenic lipid abnormalities are present in women with PCOS, we also measured levels of apoB and Lp(a). ApoB is the chief structural component of LDL and its assessment represents a more accurate measurement of the relative number of LDL particles than LDL-cholesterol measurement (9). ApoB is a true indicator of the number of atherogenic particles: in a multivariate model for the estimation of change in coronary artery percent stenosis, the best fit included percent changes in apo B levels (25). Yet, we did not find any difference between women with PCOS and

controls in apoB levels and its elevated concentrations were uncommon too (14%). These findings are consistent with those recently found in a PCOS population from the Netherlands (26), but differ from those found in non-obese Turkish adolescent with PCOS where apoB levels were slightly increased compared to controls (27). Genetic and environmental components may determine differences in lipid patterns (23) and, based on available data, measurement of apoB should not be recommended in women with PCOS.

Lipoprotein(a) is a specific heterogeneous class of Lp particles consisting of an apo(a) molecule attached to an apoB-100 and a lipid-rich LDL-like core. Lp(a) is metabolically distinct from LDL and its levels are genetically determined and remain relatively stable over an individual's lifetime (28). Elevated Lp(a) is an established independent risk factor for cardiovascular events associated with an increased risk for myocardial infarction, stroke and coronary artery disease (28–30). We found that women with PCOS had significantly higher concentrations of Lp(a), as considered as absolute values and log-transformed; in addition, elevated Lp(a) levels were found in about one-fourth of subjects (24%). These findings are somewhat consistent with previous reports (31,32). Although issues related to measurement of Lp(a) in clinical practice have not been fully resolved (9), we have used the cutoff commonly accepted for defining its high levels (33). Notably, it has been recently shown in large-scale prospective data that Lp(a) concentrations are strong predictors of future coronary heart disease (34). Interestingly, such association between Lp(a) and coronary heart disease is independent of insulin resistance (35). Yet, it has to be considered that we did not assess apo(a) isoform size that may represent a cardiovascular risk factor independently of Lp(a) levels (36).

Increased Lp(a) together with higher small, dense LDL may potentially increase cardiovascular risk with a synergistic effect. Interestingly, we further found in this study that these two Lp variables were not significantly correlated and that Lp(a) did not correlate with insulin or insulin resistance, suggesting that dyslipidaemia in PCOS may arise by different metabolic pathways and may be independent on insulin resistance. To what extent such different atherogenic forms of dyslipidaemia contribute to the increased cardiovascular risk in PCOS is at present unknown and future prospective studies are needed. Yet, our study demonstrates that more than one-third of patients with PCOS with normal plasma lipid profile have 'hidden' pro-atherogenic lipid abnormalities [including elevated levels of

Lp(a) or small, dense LDL]. Notably, it has been recently reported that Lp(a) and LDL size are together related to the severity of coronary artery disease (37) and this may be at least in part due to their combined effects on haemostasis (38). Moon et al. (37) also found a strong correlation between LDL size and Lp(a) in patients with coronary stenosis and this may be linked to the role of Lp(a) as a carrier of pro-inflammatory oxidative products (39,40) that ultimately led to increased levels of oxidised LDL in coronary arteries (41).

At present it is unknown whether the therapeutic modulation of such pro-atherogenic lipid alterations may significantly reduce cardiovascular risk. Management of atherogenic dyslipidaemia in PCOS remains on debate: weight reduction and increased physical activity should constitute first-line therapy while lipid-lowering drugs, including statins, nicotinic acid and fibrates, should remain an option only for patients with severe dyslipidaemia (42). Insulin-sensitising medications are also effective agents and combined therapy remains an option; in particular, the combination pioglitazone + metformin may be beneficial (42). Notably, statins are almost ineffective on Lp(a) concentrations, while nicotinic acid significantly reduce them (43) as well as levels of small, dense LDL (44). No conclusive data are available on metformin, which showed contrasting effects in modulating levels of Lp(a) in women with PCOS (45,46). It remains therefore to be tested by future prospective studies whether hypolipidaemic and/or insulin-sensitising agents may be able to reduce cardiovascular risk in women with PCOS by decreasing atherogenic dyslipidaemia in the short term (e.g. reducing carotid intima media thickness) and long term (e.g. reducing cardiovascular morbidity and mortality).

In conclusion, we investigated in this study the presence of atherogenic dyslipidaemia in Mediterranean women with PCOS. We found that our PCOS patients have increased concentrations of Lp(a) while apoB concentrations are not altered. As these alterations seem to be only in part linked to insulin resistance, we cannot exclude that in the future it might be possible to individualise specific interventions if more than the traditional lipids are taken into account. Particularly, measurement of Lp(a) in women with PCOS may potentially help assess cardiovascular risk and adapt the treatment goals thereafter.

Acknowledgements

Author contributions

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