# HETEROGENOUS FORMS OF DYSLIPIDEMIAIN WOMEN WITH POLYCYSTIC OVARY SYNDROME

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*[Forme eterogenee di dislipidemia in donne con sindrome dell'ovaio policistico]*

## SUMMARY

Forms of dyslipidemia are very common in individuals affected by polycystic ovary syndrome (PCOS), but in addition to plasmatic lipids, it is necessary to evaluate the alterations in the atherogenic lipoproteins (Lp) and apolipoproteins (apo).

In our study we measured the concentrations of apoB, Lp(a) and low density lipoproteins (LDL) in 42 patients with PCOS (age: 28 +/- 7 years, body mass index: 27 +/- 5 kg/m_) and 37 healthy women (of the same age and body mass index).

**Methods**: values of Lp(a) >30 mg/dl were considered high, whereas for apoB, values >100 g/l were considered high.

**Results**: the patients with PCOS showed an increase in triglycerides (p=0.0011) and low levels of high density lipopro-teins (HDL) (p=0.0131), but the total cholesterol and the LDLs were not significantly different to those of the control group. High levels of Lp(a) were found in 24% of the individuals with PCOS, and a smaller number showed high levels of apoB (14%).

This analysis shows that the concentrations of Lp(a) are only correlated to the HDL levels (r =0.378, p= 0.0431). 36% of the patients with PCOS with normal levels of plasmatic lipids show high levels of Lp(a) and apoB, and small and dense LDLs.

**Conclusions**: alterations in the plasmatic lipids are pre-sent in 1/3 of the women affected by PCOS. More research is necessary to better understand the mechanisms responsible to reduce the risk of cardiovascular problems in young women with polycystic ovary syndrome.

**Key words:** Polycystic ovary syndrome, body mass index, atherogenic lipoproteins

## RIASSUNTO

*Le forme di dislipidemia sono molto comuni nei soggetti affetti da sindrome dell'ovaio policistico(PCOS) ma, oltre ai lipidi plasmatici, è necessario valutare le alterazioni delle lipo - proteine aterogene (Lp) e le apolipoproteine (apo).*

*Nel nostro studio, abbiamo misurato le concentrazioni di apoB, Lp(a) e delle lipoproteine a bassa densità (LDL) in 42 pazienti con PCOS (età: 28 ± 7 anni, body mass index: 27 ± 5 kg/m2 ) e 37 donne sane (di pari età e body mass index).*

***Metodi***: *Sono stati considerati elevati i valori di Lp(a) >30 mg/dl, mentre per apoB, valori >100 g/l.*

***Risultati***: *le pazienti con PCOS mostravano un aumento dei trigliceridi (p=0.0011) e bassi valori di lipoporteine ad alta densità (HDL) (p=0.0131), mentre il colesterolo totale e le LDL non differivano in modo significativo rispetto ai controlli.*

*Elevati valori di Lp(a) furono trovati nel 24% dei sogget - ti con PCOS, mentre una minoranza presentava elevati livelli di apoB (14%). Tale analisi rivela che le concentrazioni di Lp(a) sono correlate solo con i livelli di HDL (r =0.378, p= 0.0431). Il 36% delle pazienti con PCOS con normali valori dei lipidi plasmatici presentano elevati livelli di Lp(a), apoB e LDL piccole e dense.*

***Conclusioni***: *le alterazioni dei lipidi plasmatici sono presenti in 1/3 delle donne affette da PCOS. Sono necessari ulteriori studi per conoscere meglio i meccanismi responsabili al fine di ridurre il rischio cardiovascolare in giovani donne con sindrome dell'ovaio policistico.*

*Parole chiave: Sindrome dell'ovaio policistico, indice di massa corporea, lipoproteine aterogene*

## Introduction

Polycystic ovary syndrome (PCOS) is the most frequent form of endocrinopathy found in women; in fact more than 10% of those of reproductive age are affected. Despite it being well-known that PCOS is linked to reproductive pathologies and an increa-sed risk of endometrial cancer, diagnosis of PCOS is of great interest for the cardiovascular risks entailed, a consistent datum especially in some geographical areas and ethnic groups[1, 2].

In women with PCOS, as opposed to women with normal cycles, insulin resistance, android obe-sity and hypertension can be found[3]. Severe indices of arteriosclerosis, clinical and subclinical, have been found in women with PCOS[4-8]; dyslipidemia is very common and is characterised by low chole-sterol values and hypertriglyceridemia[1, 2].

Over the last few years, several studies have suggested how, among the plasma lipids, different lipoproteins (Lp) and abnormal apoliproproteins increase the risk of cardiovascular pathology.

These alterations include high levels of Lp(a), as well as high levels of apoB; at the moment the exact incidence of these forms of dyslipidemia in women with PCOS is not yet known and this limits the usefulness of the information.

In our study we included 42 patients of Caucasian origin, from the Mediterranean, affected by PCOS and 37 healthy women chosen by age and body mass index with the aim of evaluating:

a) whether the levels of Lp(a) or apoB changed in subjects with PCOS compared to control;

b) whether these levels could be correlated with the levels of lipids circulating in the plasma or with the presence of arteriogenic lipoproteins such as small dense low density lipoproteins (LDL);

c) whether the patients with PCOS with normal levels of lipids in the plasma might show the presence of abnormal arteriogenic lipids (e.g. high levels of Lp(a), apoB or small dense LDL).

## Methods and materials

42 women of reproductive age affected by PCOS were enlisted at the Department of Clinical Medicine and Emerging Pathologies, Clinical of Internal Medicine, University of Palermo.

Diagnosis of PCOS was based on clinical and biological evidence of hyperandrogenism associated with chronic anovulation and/or ultrasound demonstration of a polycystic ovary[10].

Anovulation was defined as serum levels of progesterone lower than 3 ng/ml (< 9.54 nmol/L). In patients with regular cycles, at least two consecutive cycles were studied and the ascertainment of low serum levels of progesterone (<3ng/ml) in both cycles were indicative of anovulation.

Patients with anovulatory[31] and ovulatory[11] PCOS were thus identified[7, 11].

The presence of ovarian polycystosis was investigated through suprapubic and transvaginal ecographic investigation. The criteria for indicating the presence of polycystic ovaries was based on the confirmation of enlarged ovaries and/or the presence of at least 12 follicular cysts with a diameter of 2-9 mm[13]. All the patients in the study showed these characteristics.

The study included a medical check-up and biochemical surveys.

The procedures adopted were in accordance with the Helsinki Declaration of 1975 as revised in 1983 and the study was approved by the local ethics council.

All subjects gave their informed consent to participate in the study.

The patients, after undergoing a medical examination, were asked to fill in a questionnaire on personal and medical items, including age, past medical history and any use of medications. Exclusion criteria included renal or hepatic diseases capable of modifying the plasma concentration of lipoproteins or the use of hypolipemizing medication. No patient had type 2 diabetes mellitus or was taking medication from at least three months before the study. In the control group there were 37 healthy women, matched for age and body weight using the same exclusion criteria described above.

Control subjects were women with regular menstrual cycles and normal hormonal values. The following were recorded: weight, height, BMI and waist circumference for both groups. Hypertension (systolic or diastolic, respectively, 140mmHg or 90 mmHg also in treatment with hypertensive medication), diabetes (fasting plasma glucose concentration greater than 126mg/dl) and smoking were among the cardiovascular risk factors taken into consideration.

## Laboratory tests

The cholesterol and triglyceride plasma concentrations were measured on a Roche (Roche Diagnostics, Rotkreuz, Switzerland) commercial modular system using reagents with a variation coefficient of 2.3% - 2.4% respectively. The levels of HDL cholesterol, Lp(a) and apoB were measured with a Roche Integra 800 analyzer, using commercial tests (Roche Diagnostic), with a variation of 4.1%, 2.3% and 1.2% respectively. The LDL values were calculated using the Friedeward formula. Insulinemia and any insulin resistance were calculated by different methods, including fasting insulinemia, the HOMA (homeostasis model assessment) method, and QUICKI (quantitative insulin sensitivity check index).

The estimate for insulin resistance through the "HOMA score" method was calculated using the following formula: serum levels of fasting insulin (μU/ml) x plasma levels of fasting glucose (mmol/l)/22.5 (14). The QUICKI method was derived from the inverse calculation of the sum of the logarithmic values of fasting insulin and glucose (15). Low density lipoproteins and subclasses were evaluated by electrophoresis of the plasma at 10-14°C with polyacrylamide gel.

Initially the gels underwent electrophoresis for 24 hours at 124 V in a tris borate buffer (pH 8.3) (16). They were then fixed and coloured in a solution of red oil in 60% ethanol at 55°C; they were subsequently exposed to a light source and photographed using a Fujifilm LAS 3000 Luminescent Image Analyzer. The migration distance for each absorbance peak and the molecular diameter corresponding to each peak were determined; everything was calculated using a calibration curve generated by the distance migration of standard particles with known diameter, which included carboxylated latex beads (Duke Scientific, Palo Alto, CA), thyroglobulin and apoferritin (HMW Std, Pharmavia, Piscataway, N), with molecular diameters of 380, 170 e 122 Å, respectively. Lp calibration was also carried out after the particle size had been determined.

LDL percentage subclass distribution of total LDL, was calculated as previously described. The total of small dense LDL was obtained by the sum of the single values of LDL III and LDL IV subclasses (LDL-IIIA+ LDL-IIIB + LDL-IVA + LDL-IVB).

|  | PCOS(n=42) | Controls(n=37) | p= |
|---|---|---|---|
| TC (mmol/l) | 4.7 ± 0.9 | 4.3 ± 1.2 | ns |
| TG (mmol/l) | 1.0 ± 0.5 | 0.6 ± 0.4 | 0.001 |
| HDL (mmol/l) | 1.2 ± 0.3 | 1.5 ± 0.7 | 0.013 |
| LDL (mmol/l) | 3.1 ± 1.1 | 2.7 ± 1.6 | ns |
| TG (>1.7 mmol/l), (%) | 9 | 3 | ns |
| HDL (<1.1mmol/l)(%) | 33 | 14 | 0.046 |
| LDL (>4.1 mmol/l)(%) | 14 | 8 | ns |
| Insulina (μU/ml) | 13 ± 5 | 7 ± 2 | <0.0001 |
| HOMA | 2.8 ± 1.1 | 1.1 ± 0.3 | <0.0001 |
| QUICKI | 0.33 ± 0.02 | 0.37± 0.01 | <0.0001 |

|  | PCOS(n=2) | Controls (n=37) | p= |
|---|---|---|---|
| Età (anni) | 28 ± 7 | 31 ± 2 | ns |
| BMI (kg/m2) | 27 ± 5 | 26 ± 4 | ns |
| Hypertension (%) | 2 | 0 | ns |
| Diabetes (%) | 0 | 0 | ns |
| Smoke (%) | 16 | 19 | ns |
| GCV(%) | 7 | 11 | ns |

**Table 1:** caratteristiche cliniche e dati laboratoristici delle pazienti affette da PCOS e del gruppo controllo.

To evaluate whether the plasma lipids or Lp were abnormal in women with PCOS, we considered the following cutoffs in accordance with the more recent international guidelines[9,17]: high triglycerides if > 1.7 mmol/l, low cholesterol if < 4.1 mmol/l, high Lp (a) values if > 30 mg/dl, high apoB if > 100 g/l. Higher levels by 2DS of small dense LDL were found in the patients with PCOS compared to the controls.

**Results**

The patients with PCOS and the control group had a similar BMI, in compliance with the inclusion criteria. Nevertheless, the abdomen circumference was greater in women with PCOS compared to the healthy women (91+/- 13 v. 80+/- 6 cm, p<0.01). As shown in table 2, patients with PCOS showed an increase in triglycerides and low total levels of cholesterol, whilst total cholesterol and LDL did not differ significantly compared to the controls.

Women with PCOS also showed an increase in insulin values and insulin resistance, as shown by HOMA and QUICKI (all p=0.0001). The prevalence of high levels of triglycerides and LDL cholesterol in women with PCOS was low (9% and 14% respectively) and there were no significant differences compared to the values found in the controls. Low levels of HDL cholesterol were found in 33% of women with PCOS, against 14% of the controls (p=0.0460). We also found that the women with PCOS showed small size (p=0.0005) LDL (data not shown) because of a reduction in larger particles with a concomitant increase of the medium and small subspecies (LDL-IIB, -IIIA, -IIIB e –IVA); thus, the total levels of dense small LDL were strongly increased in the women with PCOS compared to the controls (40± 8% vs. 31 ± 6%, p< 0.0001).

|  | PCOS (n=42) | Controlli (n=37) | p= |
|---|---|---|---|
| Lp(a) (mg/dl) | 24 ± 26 | 5.2 ± 5.1 | 0.0143 |
| Log Lp(a) | 1.12 ± 0.53 | 0.57 ± 0.35 | 0.0014 |
| LP(a) (> 30 mg/dl) (%) | 24 | 0 | < 0.0001 |
| ApoB (g/l) | 79 ± 24 | 81 ± 44 | ns |
| apoB (<100 g/l) (%) | 14 | 15 | ns |

**Table 2:** Levels of di Lipoprotein(a) and of ApoB

The women with PCOS also had high concentrations of Lp(a), taken both as absolute values (p=0.0143) and after logarithmic transformation (p=0.0014); conversely no difference was found for the apoB levels. High levels of Lp(a) were found in _ of the women with PCOS (24%), whilst high levels of apoB were relatively scarce (14%).

We also found a normal lipids profile in 25 patients with PCOS, but further analysis showed the presence of "hidden" altered pro-arteriogenic lipoproteins in 9 subjects (e.g. high levels of Lp(a) or dense small LDL).

## Discussion

Cardiovascular disease is the major cause of death in the world for both sexes, but women are hormonally protected before the menopause and the onset of cardiovascular disease is normally delayed by 10-15 years compared to men[9,19]; nevertheless, young women may show an increased cardiovascular risk when affected by PCOS[2]. The reason for the increase in the risk is not yet clear; hyperandrogenism has not yet been recognised as a risk factor for cardiovascular disease and studies on pre- and post-menopausal women do not show a clear association between hyperandrogenism and the risk of future cardiovascular events[21].

Conversely, altered metabolic conditions like insulin resistance and dyslipidemia seem to have an important role on the risk of cardiovascular pathology in women with PCOS[22], but it is still unknown to what degree dyslipidemia may contribute to this risk increase. Lipid alterations are common in women with PCOS[1,2,7], but are heavily influenced by other factors like diet and physical exercise[23]. As we have shown in our population of women with PCOS[7,18,23], the triglyceride levels are within the norm, although significantly increased compared to the controls. Also high levels of LDL cholesterol were not common (14%), whereas low concentrations of HDL cholesterol were found in a third of the subjects (33%).

In the last few years several studies have suggested that, as well as plasma lipids, different alterations of Lp and apoB significantly increase the cardiovascular risk[9,24]. High LDL levels represent the most common lipid alteration (44%) in women with PCOS. In order to evaluate if there are other lipids promoting arteriosclerosis in women with PCOS, we also evaluated the Lp(a) and apoB concentrations.

ApoB is the main structural component of LDL and its evaluation represents a more accurate measurement of the relative number of LDL particles compared to the more common measurement of LDL cholesterol[9]. ApoB is a true indicator of the number of particles promoting arteriosclerosis. We found no difference in apoB levels between women with PCOS and the controls, and their high concentration was not common (14%).

These results are consistent with those found recently in a study of subjects with PCOS in Holland[26], but differ from those found in Turkish adolescents, not obese, with PCOS where the apoB levels were significantly increased compared to the controls. Probably, genetic and environmental factors may determine different lipid patterns[23] and, on the basis of data not available, the determination of apoB levels is not indicated in women with PCOS.

Lipoprotein(a) is a heterogeneous class of lipoproteins made up of an apo(a) molecule linked to a apoB-100 and a lipid. Lp(a) is metabolically distinct from LDL and its levels are determined genetically, with its concentration remaining stable throughout the life of a subject[28]. High Lp(a) levels represent an independent risk factor for cardiovascular events, linked to an increased risk of myocardium infarction, stroke and coronary heart disease [28-30]. In our study we have shown greater concentrations of Lp(a) in women with PCOS, both in absolute values and after logarithmic transformation; furthermore, high levels of Lp(a) were found in about a quarter of the subjects (24%).

Even if the questions relating to the measurement of Lp(a) cannot be carried out in clinical practice, we have used the maximum commonly accepted limit to define those levels[33]. It has also been recently demonstrated in large scale prospective studies that the concentrations of Lp(a) are strong predictors of cardiac disease[34]. It is interesting to note that said association of Lp(a) and coronary disease is independent of the insulin resistance factor.

The simultaneous increase of Lp(a) and LDL could increase the cardiovascular risk with synergic effect. We also found in this study that these two lipoprotein variables are not significantly correlated and that Lp(a) is not correlated with levels of insulin or insulin resistance, suggesting that dyslipidemia can stem from different metabolic mechanisms. At the moment it is not known to what degree these different forms of dyslipidemia may contribute to increasing this cardiovascular risk in PCOS and future prospective studies are needed.

Our study also demonstrates that more than a third of the patients with PCOS with a normal lipid profile have altered lipids that can cause arteriosclerosis (high levels of Lp(a) or LDL are included). It was recently shown that Lp(a) and LDL are correlated with severe pictures of coronary heart disease[37] and this can be at least in part because of their combined effects on haemostasis[38]. Moon et al.[37] have found a strong correlation between LDL and Lp(a) in patients with stenosis of the coronary arteries and this can be linked to the role of Lp(a) as a vehicle for pro-inflammatory products[39, 40], which in the last analysis led to an increase of oxidized LDL levels in the coronary arteries[41].

At the moment it is not known whether therapeutic modulation of these lipids can significantly reduce the cardiovascular risk.

Management of dyslipidemia in PCOS is still under debate, weight reduction and greater physical activity are the first level of therapy, whilst medication that reduces the cholesterol levels, including statins, nicotinic acid and fibrates, are only used in patients with severe dyslipidemia[42]. Oral hypoglycemizing drugs are also effective agents and combined therapy remains an option; in particular the combination of pioglitazone + metformin could be beneficial[42]. It is well known that statins are rather ineffective on Lp(a) concentrations, whereas nicotinic acid markedly reduces both Lp(a) an LDL levels[44]. No data on metformin is available, which demonstrates the contrasting effects on modulation of Lp(a) levels in women with PCOS[45,46].

We must thus wait for the results from prospective studies to know whether hypolipemizing and/or insulin sensitizing drugs are capable of reducing cardiovascular risk in women with PCOS; these, in the short term, act by reducing the dyslipidemia promoting arteriosclerosis (reduction of carotid myointimal thickness) and, in the long term (reduction of cardiovascular morbidity and mortality).

In conclusion, this study was conducted to underline the presence of artherogenic dyslipidemia in women with PCOS in the Mediterranean basin. We found that our patients with PCOS show increased concentrations of Lp(a), whereas the concentrations of apoB are unaltered. These changes seem in part to be linked to insulin resistance, but it cannot be excluded that other responsible mechanisms may be identified in the near future. In particular, the Lp(a) measurement in women with PCOS could facilitate the evaluation of cardiovascular risk and adapt the treatment on the basis of objectives to be reached.

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