Lipoprotein Subclass Patterns in Women with Polycystic Ovary Syndrome (PCOS) Compared with Equally Insulin-Resistant Women without PCOS

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Objectives: Women with polycystic ovary syndrome (PCOS) are more insulin resistant and display an atherogenic lipid profile compared with normal women of similar body mass index (BMI). Insulin resistance (IR) at least partially underlies the dyslipidemia of PCOS, but it is unclear whether PCOS status per se confers additional risk.

Research Design and Methods: Using a case-control design, we compared plasma lipids and lipoprotein subclasses (using polyacrylamide gel tube electrophoresis) in 70 women with PCOS (National Institutes of Health criteria) and 70 normal women pair matched for age, BMI, and IR (homeostasis model assessment-IR, quantitative insulin sensitivity check index, and the Avignon Index). Subjects were identified as having a (less atherogenic) type A pattern consisting predominantly of large low-density lipoprotein (LDL) subfractions or a (more atherogenic) non-A pattern consisting predominantly of small-dense LDL subfractions.

Results: Total, high-density lipoprotein, or LDL cholesterol, or triacylglycerol did not differ between the groups, but very low-density lipoprotein levels \( (P < 0.05) \) were greater in women with PCOS, whereas a non-A LDL profile was seen in 12.9% compared with 2.9% of controls \( (P < 0.05, \chi^2) \). Multiple regression analysis revealed homeostasis model assessment-IR and waist circumference to be independent predictors of very low-density lipoprotein together explaining 40.2% of the overall variance. Logistic regression revealed PCOS status to be the only independent determinant of a non-A LDL pattern (odds ratio 5.48 (95% confidence interval 1.082–27.77; \( P < 0.05 \)).

Conclusions: Compared with women matched for BMI and IR, women with PCOS have potentially important differences in lipid profile with greater very low-density lipoprotein levels and increased rates of a more atherogenic non-A LDL pattern. (J Clin Endocrinol Metab 95: 3933–3939, 2010)
low concentration of large particles) correlate with progression of atherosclerosis and earlier and more severe cardiovascular disease (11–13).

Women with PCOS are usually insulin resistant (14–17), and it is likely that insulin resistance (IR) at least partially underlies these abnormalities of lipid metabolism (18, 19). It is not known whether PCOS, through hyperandrogenemia or other mechanisms, confers an additional deleterious effect on plasma lipids. This information is important both to further understand the metabolic effects of PCOS and also to improve long-term evaluation of cardiovascular risk.

The aim of this study was, using a case-control design, to compare plasma lipid profiles and lipoprotein sub-classes in women with PCOS with equally insulin-resistant women without PCOS. IR was determined using the homeostasis model assessment (HOMA) and quantitative insulin sensitivity check index (QUICKI) models and also using the Avignon index of insulin sensitivity (20), which has previously been demonstrated to be the surrogate measure of IR that correlates most closely with data derived from the hyperinsulinemic euglycemic clamp in PCOS (21). Lipoprotein subclasses were evaluated using the Quantimetrix Lipoprint System, which measures LDL and HDL particle size by comparing particle electrophoretic mobility with the electrophoretic mobility of particles with known sizes (22).

Subjects and Methods

Experimental subjects

Two hundred seven premenopausal women with (n = 103) and without (n = 104) PCOS were recruited for the study. Women with PCOS were recruited from endocrinology outpatient clinics (Adelaide and Meath Hospital, Tallaght, Dublin, Ireland). Normal women were recruited by local advertisement. PCOS was defined according to the National Institutes of Health (NIH) criteria as chronic oligomenorrhea (less than nine cycles per year) and hyperandrogenism (clinical and/or biochemical); °, controls were studied in the follicular phase of their menstrual cycle.

FIG. 1. Study design. °, Defined according to the NIH criteria (1990); oligomenorrhea (less than nine cycles per year) and hyperandrogenism (clinical and/or biochemical); °, controls were studied in the follicular phase of their menstrual cycle.

Their written signed consent to the study, which was approved by the Research Ethics Committee of the Adelaide and Meath Hospital and St. James’ Hospital (Dublin, Ireland).

Study design

This was a case-control study (Fig. 1). Cases were matched with controls for age (± 3 yr); BMI (± 3 kg/m²) and insulin resistance (HOMA-IR ± 1). This resulted in 70 matched pairs. All subjects were studied after a 12-h fast and having avoided excessive exercise and alcohol for the previous 24 h.

Measurement of body composition, nutritional status, and IR

All subjects underwent estimation of body composition using auxiological methods and bioimpedance analysis. Height (measured with a Harpenden stadiometer) and weight were measured in a hospital gown. Waist circumference (WC) and hip circumference were measured with a nondistensible flexible tape measure at the waist and hip. Percent body fat (%BF) was estimated using the Bodystat 1500 system (Bodystat Ltd., Isle of Man, UK). A health and lifestyle questionnaire, which included details on smoking history and alcohol consumption that could potentially impact on lipid parameters, was completed by each participant. Physical activity was assessed by a validated questionnaire (24). Subjects completed a 3-d food diary and this was analyzed using Master Wisp (Tinuviel Software, UK) and exported to SPSS (Chicago, IL) for statistical analysis.

All subjects underwent a standard 75-g oral glucose tolerance test. Glucose and insulin levels in the fasting state and postglucose load were used to calculate HOMA-IR, QUICKI, and the Avignon index of insulin sensitivity (SiM) (21).

Laboratory methods

Glucose was measured by an enzymatic (hexokinase) method on the Roche P Module (Roche, Stockholm, Sweden); insulin was measured by electrochemiluminescence immunoassay on the Roche E Module; and nonesterified fatty acids (NEFAs) were measured by a kit using the Randox Colorimetric Method (Randox, Antrim UK) and analyzed on a Hitachi modular analyzer (Tokyo, Japan) [coefficients of variation (CVs) <5% for all]. Plasma fatty acids were analyzed by gas chromatography on a Shimadzu GC 2010 (Shimadzu, Kyoto, Japan) using a fused sil-
ica capillary column Omegawax 250 (30 m × 0.25 mm inner diameter, 0.25 μm film) (Supelco, Bellefonte, PA).

SHBG, DHEAS, estradiol, TSH, free T₄, prolactin, and cortisol were measured by standard chemiluminescence immunoassays (CVs <5% for all). Total testosterone was measured by electrochemiluminescence immunoassay on the Roche E Module. Free testosterone was calculated from total testosterone and SHBG using the law of mass action (25, 26). Free androgen index (FAI) was calculated by the formula, FAI = 100 × (total testosterone/SHBG). Androstenedione was measured by RIA (CV <5%).

Measurement of plasma lipids and lipoprotein subclasses

Total cholesterol, triacylglycerol (TAG), and HDL cholesterol (HDL-C) were measured using standard laboratory techniques (CV <5%). LDL cholesterol (LDL-C) was calculated using the Friedewald equation. The reciprocal relationship between HDL-C and TAG was expressed as the atherogenic index of plasma (27), which is defined as log[TAG/HDL-C] and is inversely correlated with LDL particle size (28). Atherogenic index of plasma values increase with increasing cardiovascular risk (29). Apolipoprotein B (apo B) was measured by standard nephelometry on a BN II nephelometer (Dade Behring, Deerfield, IL). Blood samples for LDL and HDL subclass analysis were collected in EDTA-containing tubes. These were centrifuged at 3000 rpm for 10 min at 4°C, and plasma was stored at −80°C until the end of the study. LDL and HDL subfractions were separated using the Quantimetrix Lipoprint system (Redondo Beach, CA) (22). High-resolution 3% polyacrylamide gel tubes were used for electrophoresis. Twenty-five microliters of sample were mixed with 200 μL of Lipoprint loading gel, which contained Sudan Black B dye to stain the lipoproteins. This was placed on the upper part of 3% polyacrylamide gel. After 30 min of photopolymerization at room temperature, samples were electrophoresed for 60 min with 3 mA for each gel tube. The electrophoresis was followed by letting the tubes in the dark for 1 h before performing densitometry. Densitometry was performed at 610 nm. Raw data from the densitometer were imported into a Microsoft excel spreadsheet and using a computerized method that was developed for the Quantimetrix Lipoprint system using NIH image program version 1.62 (Bethesda, MD), and subfractions were identified and quantified. The Lipoprint system (Quantimetrix) was used as quality control. Each chamber had two quality controls. Very low-density lipoprotein (VLDL), seven LDL, and 10 HDL subclasses were quantified and further classified as large, intermediate, and small subfractions. Using a weighted scoring system developed by the manufacturers, LDL scores were also calculated. On the basis of LDL migration rates on the scan, LDL phenotypes A (predominantly large, buoyant LDL) or non-A (predominantly small, dense LDL) were assigned to scores of less than 5.5 for phenotype A and greater than 5.5 for non-A.

Statistical analysis

Statistical analysis was performed using SPSS for Windows (version 11.5). Data are presented as mean ± SD. Skewed variables were logarithmically transformed. Statistical analysis of clinical characteristics was made using Student’s paired t test, and correlations were performed using Pearson’s correlation coefficient with a P < 0.05 considered significant. Multiple linear regression analysis was used to explore the determinants of VLDL in PCOS and logistic regression was used to explore determinants of a non-A LDL pattern.

Results

Baseline characteristics

Women with PCOS and normal women were closely matched for anthropometric characteristics, IR, and nutritional and lifestyle factors (physical activity and smoking), with the exception of waist to hip ratio, which was significantly greater in women with PCOS (Table 1). As expected, total and free testosterone, androstenedione, DHEAS, and FAI were greater in women with PCOS.

Plasma lipids and lipoprotein subclasses

There were no differences between groups in total cholesterol, HDL-C, LDL-C, TAG, NEFA, or apo B (Table 2). VLDL was significantly greater in women with PCOS (P < 0.05). There was an increase of borderline significance (P = 0.05) in the atherogenic index of plasma in the PCOS cohort. There was a trend toward a greater percentage of small dense LDL in women with PCOS and a significantly greater number of women with PCOS had a non-A LDL pattern compared with controls (x², P = 0.05). There were no differences in HDL subclasses expressed as absolute values or percentages between the groups.

Determinants of VLDL

Percent VLDL correlated significantly (P < 0.01) with markers of body composition [BMI (r = 0.506), WC (r = 0.539)], %BF (r = 0.492) and IR (HOMA-IR (r = 0.572) and SI (r = −0.522)]. Percent VLDL levels also correlated (P < 0.01) with androgens [free testosterone (r = 0.277) and FAI (r = 0.316)]. Multiple regression analysis revealed HOMA-IR and WC to be independent predictors of percent VLDL together explaining 40.2% of the overall variance.

Determinants of a non-A LDL pattern

Direct logistic regression was performed to assess which independent variables predicted a non-A LDL phenotype. The model contained four independent variables (BMI, C-reactive protein, HOMA-IR, and PCOS status). Markers of body composition, inflammation, and IR were included in the model because these variables are known to be associated with LDL particle size. The full model containing all predictors was statistically significant [x² (4, n = 116) = 11.38, P < 0.05]. The model as a whole explained between 9.3% (Cox and Snell R²) and 20.5% (Nagelkerke R²) of the variance in LDL phenotype. Only PCOS status made a unique statistically significant con-
trolling for all other factors in the model.

likely to have a non-A LDL pattern than controls, con-

crating that subjects with PCOS were 5.48 times more

* P

SD. ns, not significant; BP, blood pressure.

a P value derived from Student’s paired t test.

| TABLE 1. Anthropometric, biochemical, and nutritional characteristics in women with PCOS and normal women |
|-----------------------------|-----------------------------|-------------|
|                            | PCOS                        | Normal      | P value a  |
| Age (yr)                   | 30.0 ± 5.3                  | 30.0 ± 5.5  | ns         |
| BMI (kg/m²)                | 31.5 ± 6.9                  | 31.6 ± 5.9  | ns         |
| WC (cm)                    | 99.1 ± 15.0                 | 96.5 ± 12.8 | ns         |
| Hip circumference (cm)     | 112.9 ± 12.7                | 114.7 ± 15.1| ns         |
| Waist to hip ratio         | 0.87 ± 0.06                 | 0.84 ± 0.07 | <0.001     |
| Systolic BP (mm Hg)        | 122 ± 1.3                   | 124 ± 1.4   | ns         |
| Diastolic BP (mm Hg)       | 83 ± 9                      | 83 ± 10     | ns         |
| %BF                        | 39 ± 8                      | 40 ± 7      | ns         |
| Fasting glucose (mmol/liter)| 4.7 ± 0.5                   | 4.7 ± 0.4   | ns         |
| Fasting insulin (mU/liter) | 12.0 ± 6.6                  | 11.5 ± 7.4  | ns         |
| 2-h postprandial glucose (mmol/liter) | 4.6 ± 1.2 | 4.6 ± 1.1 | ns         |
| 2-h postprandial insulin (mU/liter) | 32.0 ± 32.7 | 33.1 ± 39.0 | ns         |
| HDMA-IR                    | 2.56 ± 1.49                 | 2.42 ± 1.64 | ns         |
| QUICKI                      | 0.16 ± 0.06                 | 0.15 ± 0.07 | ns         |
| SiM                         | 5.8 ± 8.4                   | 6.2 ± 7.1   | ns         |
| Energy (KJ/d)              | 1743 ± 458                  | 1756 ± 466  | ns         |
| Saturated fat (g/d)        | 25 ± 10                     | 26 ± 12     | ns         |
| Carbohydrate (g/d)         | 205 ± 68                    | 209 ± 56    | ns         |
| Saturated fatty acid (%)   | 24 ± 11                     | 26 ± 10     | ns         |
| Monounsaturated fatty acid (%) | 25 ± 4     | 26 ± 4     | ns         |
| Polyunsaturated fatty acid (%) | 44 ± 5     | 43 ± 6     | ns         |
| Smokers (%)                | 18.6                        | 25.7        | <0.05      |
| Baecke physical activity score | 7.4 ± 0.9 | 7.7 ± 1.0 | ns         |
| Total testosterone (nmol/liter) | 2.7 ± 1.2 | 1.7 ± 8   | <0.0001    |
| Free testosterone (nmol/liter) | 0.053 ± 0.038 | 0.028 ± 0.016 | <0.0001  |
| Androstenedione (nmol/liter) | 16 ± 6   | 11 ± 4     | <0.0001    |
| DHEAS (μmol/liter)         | 6.3 ± 3.0                   | 5.2 ± 2.4   | <0.0001    |
| FAS                         | 14.7 ± 39                   | 4.7 ± 3.2   | <0.0001    |
| SHBG (nmol/liter)          | 37.0 ± 17.8                 | 48.6 ± 23.3 | <0.0001    |

Mean ± SD. ns, not significant; BP, blood pressure.

a P value derived from Student’s paired t test.

tribution to the model, recording an odds ratio of 5.48
(95% confidence interval 1.082–27.77, P < 0.05), indi-
cating that subjects with PCOS were 5.48 times more
likely to have a non-A LDL pattern than controls, con-
trolling for all other factors in the model.

**Discussion**

The original findings of this study are that compared with
equally overweight and insulin-resistant women with sim-
ilar nutritional and lifestyle factors, women with PCOS

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have increased VLDL levels and are more likely to have an atherogenic non-A LDL particle size pattern. Although the presence of PCOS was the single most important determinant of the likelihood of having a non-A LDL pattern, we did not detect an independent contribution of hyperandrogenemia to this abnormality.

Hypertriacylglycerolemia, reduced HDL, and reduced LDL particle size are the characteristic lipoprotein abnormalities associated with both PCOS and IR (30–32). Through a combination of increased VLDL production and reduced clearance by lipoprotein lipase, IR causes hypertriacylglycerolemia. Hypertriacylglycerolemia in turn, through the reverse cholesterol transport pathway, leads to low HDL-C and increased small dense LDL (33). The findings of the current study demonstrate a further small but significant increase in VLDL-cholesterol and also a greater atherogenic index of plasma in women with PCOS compared with equally insulin-resistant women. This alteration in the well-documented reciprocal relationship between triglyceride and HDL-C suggests that additional variables in PCOS contribute to a reduction in HDL-C. This is potentially important because even small reductions in HDL-C are independently associated with increased risk of cardiovascular events (34, 35). We also demonstrated a 12.9% prevalence of an atherogenic non-A LDL profile in women with PCOS compared with 2.9% of women without PCOS, and indeed a diagnosis of PCOS was the only independent determinant of this pattern. This is important because an increase in small dense LDL is associated with a 3- to 7-fold increase in coronary heart disease, which is independent of LDL cholesterol concentration (9, 36).

The mechanisms through which PCOS exerts an effect on lipoprotein metabolism independent of insulin resistance are not known, but hyperandrogenemia and increased central adiposity are the most likely causal agents. Although we demonstrated correlations between androgens and VLDL and small dense LDL levels, androgens were not independent predictors of any of the variables studied. However, plasma androgen levels do not always correlate closely with clinical hyperandrogenemia, and it is thought that other factors such as tissue sensitivity to androgens are involved. Similarly, it is possible that the relationship between hyperandrogenemia and lipid metabolism might not be entirely reflected in plasma androgen levels. Studies involving the use of antiandrogens might help clarify this.

Central adiposity is independently associated with larger VLDL and small dense LDL (37, 38). In this study, waist to hip ratio, a marker of central adiposity, was greater in women with PCOS despite no difference in BMI, %BF, or IR. Why women with PCOS have greater central adiposity than BMI matched controls is not known but may be an effect of androgen excess early in life. Prepubertal daughters of women with PCOS have higher DHEAS concentrations than BMI-matched controls (39), raising the possibility that priming of adipocytes by androgens in early life predisposes to the dyslipidemia associated with PCOS. Conversely, it is also possible that hyperandrogenemia is a consequence of more metabolically active adipocytes. Microarray studies have demonstrated differential gene expression in omental fat from women with PCOS compared with normal women including genes involved in insulin resistance signaling pathway, lipid metabolism, oxidative stress processes, immune function, and adipocyte differentiation (40). A recent proteomic study confirmed that changes at the mRNA level translated to differences in protein expression, with overexpression of several proteins involved in glucose and lipid metabolism, inflammation, and oxidative stress processes in omental fat of obese women with PCOS compared with controls (41). It is possible therefore that the adverse effects of central obesity in PCOS extend beyond simply increasing IR.

A potential limitation of this study is that insulin sensitivity was not determined using the euglycemic-hyperinsulinemic clamp method. To obtain an adequate number of matched pairs to determine relatively small differences in lipids and lipoprotein subclasses, it was necessary to estimate insulin sensitivity in more than 200 women, and we did not consider it feasible to carry out clamp studies in this sample size. Our groups, however, were very closely matched for IR estimated by three different methods, one of which takes into account postchallenge glucose and insulin levels as well as those measured in the fasting state. Furthermore, SiM has recently been shown to be the marker of insulin sensitivity that most closely correlates with clamp-derived data in PCOS (21).

There is also a potential for bias in our patient selection process. Women with PCOS were recruited from endocrinology clinics, whereas our control subjects were recruited from local advertisement. However, we performed extensive assessments of various lifestyle factors that could account for differences in lipid parameters. Nutritional status was assessed by a 3-d food diary and plasma fatty acid analysis. Physical activity was assessed by using a validated questionnaire (24). There were no differences in any of these variables.

Another potential limitation is the lack of correction for multiple comparisons. The study was designed to investigate what were expected to be relatively small changes between two very closely matched groups. Use of correction for multiple comparisons would have required study of a much greater number of subjects and might not have
allowed delineation of relatively subtle differences. Although it is, of course, possible that the significant differences observed could have occurred by chance, it is very reassuring that a consistent and physiologically plausible pattern was seen across all measured variables, i.e., increased VLDL, increased atherogenic index of plasma, and an increased fraction of smaller LDL particles in PCOS. The likelihood that the results are real and not just due to statistical chance are further strengthened by the observed significant correlations between androgens and VLDL and the outcome of logistic regression analysis demonstrating PCOS status to be the major determinant of a non-A LDL pattern.

In conclusion, compared with women matched for BMI and IR, women with PCOS demonstrated potentially important differences in lipid profile with greater VLDL and increased rates of a more atherogenic non-A LDL pattern. The mechanisms underlying these effects require further investigation.

Acknowledgments

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