Predictive factors of polycystic ovary syndrome in girls with precocious pubarche

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Abstract

Objective: The aim of this study is to clarify, in girls with premature pubarche (PP), the influence of premature androgenization on the prevalence of polycystic ovary syndrome (PCOS).

Design and patients: Ninety-nine PP girls, 63 who developed PCOS and 36 who did not develop PCOS, were retrospectively included. Clinical, anthropometric, and metabolic parameters were evaluated at time of diagnosis of PP and after 10 years from menarche to find predictive factors of PCOS.

Results: Young females with PP showed a PCOS prevalence of 64% and showed higher prevalence of familial history of diabetes (p= 0.004) and lower prevalence of underweight (p= 0.025) than PP-NO-PCOS. In addition, girls with PP-PCOS showed higher BMI (p<0.001), waist circumference (p<0.001), total testosterone (p= 0.026), visceral adiposity index (VAI) (p= 0.013), total cholesterol (p<0.001), LDL-cholesterol (p<0.001), non-HDL-cholesterol (p<0.001) and lower age of menarche (p=0.015), ISI-Matsuda (p<0.001), DIo (p= 0.002), HDL-cholesterol (p= 0.026) than PP-NO-PCOS. Multivariate analysis showed that WC (p=0.049), ISI-Matsuda (p<0.001), oral disposition index (DIo) (p<0.001), VAI (p<0.001), total testosterone (p<0.001) and LDL-cholesterol (p<0.001) are independent predictive factors for PCOS in girls with PP.

Conclusions: Our study established a strong association between multiple risk factors and development of PCOS in PP girls. These risk factors are predominantly related to the regulation of glucose, lipid, and androgen metabolism. Among these factors, WC, ISI-Matsuda, DIo, VAI, total testosterone and LDL-cholesterol predict PCOS.
Introduction

Girls with precocious pubarche (PP) (<8 years) tend to develop early and rapidly progressive puberty leading to early menarche (<8 years), to an adult height below target level, and to features of polycystic ovary syndrome (PCOS) (1,2). Insulin resistance with total, visceral, and hepatic adiposity is thought to be a major driver of such early maturation (3,4). Non-classical congenital adrenal hyperplasia or heterozygous carriers of 21-hydroxilase deficiency can sometimes show PP (5).

PCOS is characterized by a combination of signs and symptoms of androgen excess (hirsutism and/or hyperandrogenaemia) and ovarian dysfunction (oligo-ovulation) and/or polycystic ovarian morphology (PCOM)(6). The most severe clinical manifestation is the classic PCOS phenotype, which presents with both hyperandrogenism and oligo-ovulation, irrespectively of the presence of PCOM (6). A less severe phenotype is ovulatory PCOS (characterized by the co-presence of hyperandrogenism and PCOM), while the non-hyperandrogenic phenotype, consisting of oligo-ovulation and PCOM, is the least severe phenotype (6). In this light, PCOS is a heterogeneous disorder in terms of its link with insulin resistance and metabolic dysfunction. This association is much stronger in women with the classic PCOS phenotype than in those with ovulatory PCOS (7). Interestingly, women with the non-hyperandrogenic phenotype (8) rarely show insulin resistance (7) or metabolic dysfunction, although they might have mildly decreased levels of sex hormone-binding globulin (8). The reported prevalence of PCOS in women of reproductive age ranges from 6% to 20% depending on the different diagnostic criteria applied (9-13).

The age at menarche in women with PCOS may be earlier than in the general population on the basis of various conditions, such as genetic variants, androgen levels and low birth weight (2,14,15). Both PP and PCOS are characterized by an excess of androgen levels showing an adverse metabolic phenotype, including hyperinsulinism, dyslipidaemia, and augmented risk for metabolic syndrome and diabetes mellitus in adulthood (16).
The aim of the current study was to identify metabolic factors in girls with PP that are associated with the development of PCOS and to establish predictive factors for PCOS in this population.

Materials and Methods

This retrospective cohort study was carried out in patients with PP who developed or did not develop PCOS, attending the Unit of Endocrinology, University of Palermo (Italy), from January 2010 to December 2020. The study was approved by the Ethical Committee of Policlinico Paolo Giaccone, University of Palermo and carried out in accordance with the Declaration of Helsinki for experiments that involved humans. At the time of observation the parents, regularly informed of the aim of the study, signed an informed consent for scientific use of their data.

Population study

Ninety-nine Caucasian naive-treatment girls with PP (pubic hair <8 years) and exclusion of congenital adrenal hyperplasia who developed or did not develop PCOS (PP-PCOS and PP-NO-PCOS) followed up from childhood to adulthood in our dedicated Outpatients Clinic were retrospectively enrolled. Girls were evaluated at time of diagnosis of PP and after 10 years from menarche to evaluate the presence of PCOS. The diagnosis of PCOS was based on the diagnostic criteria of the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) Rotterdam Consensus (17) including the presence of at least two of the three following characteristics: clinical or biochemical hyperandrogenism, oligomenorrhea and/or PCO morphology in ultrasound. Applying the diagnostic criteria of the Rotterdam Consensus (17), 64/99 (64.6%) girls with history of PP developed PCOS. Exclusion criteria were the following: congenital adrenal hyperplasia or Cushing’s syndrome.

The following relevant data were obtained from our databases: family history of diabetes, age of menarche, weight, BMI, waist circumference (WC) and Ferriman–Gallwey (FG) score.
At time of diagnosis of PP, girls were tested for FSH, LH, 17-estradiol (E2), 17OH-Pg, basal prolactin, total and free testosterone, DHEA-S and ∆4androstenedione, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, triglycerides, glutamic–pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT). On the same day an oral glucose tolerance test (OGTT) (75 g glucose) measuring glycaemia and insulinaemia was performed at 0, 30, 60, 90 and 120 min. The Matsuda index of insulin sensitivity (ISI-Matsuda) [(10000/glucose (mg/dl) x insulin (mU/ml) x glucose mean x insulin mean] (18), the oral disposition index (DIo) [(ΔInsulin0–30/ΔGlucose0–30) x (1/fasting insulin)] (19) and the area under the curve for insulin (AUC2h insulinaemia) and glucose (AUC2h glycaemia) were calculated.

The classes of obesity were defined according to BMI values. Girls were defined underweight if having a BMI below the 3rd percentile, overweight as having a BMI between 90 and 95th percentile, and obese for a BMI above the 95th percentile (20).

At the time of re-evaluation, after 10 years of menarche, women were trained to track a menstrual cycle calendar. The mean duration of menstrual cycles (days) of the last 6 months was obtained for each woman. All patients were tested for FSH, LH, 17-estradiol (E2), 17OH-Pg, basal prolactin, total and free testosterone, DHEA-S, ∆4androstenedione, and SHBG, during the follicular phase (from the third to the fifth day of the spontaneous menstrual cycle or 7 days after withdrawal of bleeding, administering a single 100-mg im dose of medroxyprogesterone acetate in cases of secondary amenorrhea). On the same day, we also tested for total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, triglycerides, glutamic–pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT) and performed an OGTT (75 g glucose) measuring glycaemia and insulinaemia at 0, 30, 60,90 and 120 min. The serum progesterone level was determined between days 20 and 24 of the menstrual cycle and chronic oligoanovulation was established if two consecutive cycles were anovulatory (Pg level <3 ng/mL [International System:< 9.54 nmol/L]). Biochemical hyperandrogenism was diagnosed when androgen levels were as follows: total testosterone >2.84 nmol/L, DHEA-S >12.14 mmol/L, ∆4androstenedione >10.72 nmol/L (calculated on the basis of the 95th percentile upper limits of basal serum androgen concentrations.
in 144 healthy normal Sicilian eumenorrheal women without hirsutism and with no family history of PCOS [used as a control group in a previous study]) (21). Transvaginal ovarian ultrasound scanning was performed between days 5 and 10 after the beginning of the last period using a 7.5-MHz vaginal probe transducer (General Electric LOGIQ 400MD). Ovaries were classified as polycystic if 12 or more follicles measuring 2 to 8 mm in diameter were present in each ovary, and/or there was an increase in ovarian volume (>10 mL) (11).

Metabolic syndrome was diagnosed according to the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) III definition (22) and diagnosis of DM according to the American Diabetes Association criteria (23).

**Assay methods**

The samples were collected in the morning between 8 and 10 am after an overnight fast of at least 8 h and stored at -80°C until processed. All hormones were measured in our centralized laboratory by chemiluminescence assays (Immulite, Diagnostic Products). These included FSH (mUI/mL), LH (mUI/mL), 17-β-E2 (pg/mL), PRL (ng/mL), total testosterone (ng/mL), DHEA-S (µg/dL) and insulin (mUI/L; the intra- and interassay coefficient of variance were ≤4% and ≤3.6%, respectively). Mass spectometry assays were used for Δ4androstenedione (ng/mL) and 17OHPg (ng/mL).

Blood glucose levels, total cholesterol, HDL and triglycerides were measured in our laboratory using standard assays. HDL cholesterol levels were calculated with Friedewald’s formula. The conversion factors for the International System were the following: glucose (mg/dL vs. mmol/L: 0.0555), insulin (mUI/L vs. pmol/L: 6.945), total cholesterol (mg/dL vs. mmol/L: 0.0259), total testosterone (ng/mL vs. nmol/L: 3.467), DHEA-S (µg/dL vs. µmol/L: 0.0272), Δ4androstenedione (ng/mL vs. nmol/L: 3.492), 17-β-E2 (pg/mL vs. pmol/L: 3.671), 17OHPg (ng/mL vs. nmol/L: 3.026), PRL (ng/mL vs. µg/L: 1), FSH (mUI/mL vs. IU/L: 1) and LH (mUI/mL vs. IU/L: 1).
Visceral adiposity index (VAI) was calculated as described \((24, 25)\) using the following sex-specific equation, where TG is triglyceride levels expressed in mmol/L and HDL is HDL cholesterol levels expressed in mmol/L:

Females: \(\text{VAI} = \left(\frac{\text{WC/36.58} + (1.89 \times \text{BMI})}{0.81}\right) \times \left(\frac{\text{TG}}{1.52}\right) \times \left(\frac{1}{\text{HDL}}\right)\).

### Statistical analysis

SPSS version 17 and MedCalc version 11.3 were used for data analysis. Baseline characteristics were presented as mean \(\pm\) SD for continuous variables; rates and proportions were calculated for categorical data. Normality of distribution for quantitative data was assessed by the Shapiro-Wilk test. The differences between PP-PCOS and PP-NO-PCOS were detected by Student's t test for continuous variables and by the chi-square test for categorical variables. Crude odds ratios (OR) and their 95% CI for the association of PCOS with potential risk factors in PP were calculated by univariate analysis at the time of diagnosis of PP. Adjusted OR were calculated by stepwise logistic regression analysis to identify factors independently associated with the development of PCOS. Only factors significantly associated with PCOS by univariate analysis were included in the logistic regression analysis. The receiver operating characteristic (ROC) analysis was performed to investigate the diagnostic ability of significantly associated risk factors to predict PCOS development. The ROC curve is plotted as sensitivity versus 1-specificity. The area under the ROC curve (AUC) was estimated to measure the overall performance of the predictive factors for PCOS. A \(p\) value of \(<0.05\) was considered statistically significant.

### Results

Out of 99 girls with PP, 63 (64.6%) developed PCOS (PP-PCOS), while 36 did not develop PCOS (PP-NO-PCOS). Girls with PP-PCOS showed higher prevalence of familial history of diabetes \((p=0.003)\) and lower prevalence of underweight \((p=0.001)\) than PP-NO-PCOS. In addition, PP-PCOS showed higher BMI.
(p<0.001), WC (p<0.001), total testosterone (p=0.026), VAI (p=0.013), total cholesterol (p<0.001), LDL-cholesterol (p<0.001), non-HDL-cholesterol (p<0.001) and lower age of menarche (p=0.015), ISI-Matsuda (p<0.001), Dio (p=0.002), HDL-cholesterol (p=0.026) compared with PP-NO-PCOS (Table 1). After 10 years from menarche girls with history of PP who developed PCOS had higher WC (p<0.001), LH (p=0.001), LH/FSH ratio (p=0.040), VAI (p=0.001), Dio (p=0.031), total (p=0.017), LDL (p=0.008) and non-HDL cholesterol (p=0.005) and lower frequency of underweight (p = 0.001), lower 17-β-E2 (p=0.006), progesterone (p=0.018) and ISI-Matsuda (p=0.001) values than those who did not develop PCOS (Table 2).

A multiple logistic regression model was fitted by using the above-mentioned risk factors as potential predictors for PCOS (Table 3). Our model demonstrates that ISI-Matsuda (p<0.001), oral disposition index (Dio) (p<0.001), VAI (p<0.001), total testosterone (p<0.001) and LDL-cholesterol (p<0.001) at time of diagnosis of PP were statistically significant factors for predicting the development for PCOS (Figure 1). A ROC curve was constructed, and a prediction model was established with a moderately robust power (AUC = 0.73) to predict PCOS in PP women. A p value <0.005 was statistically significant.

Discussion

This retrospective cohort study of PP girls confirms the interesting relationship between PCOS and PP and the higher prevalence of PCOS in girls with PP compared to that in the general population (26). We established a significant association with a number of metabolic factors after diagnosis of PP, with the development of PCOS, including WC, age of menarche, ISI-Matsuda, Dio, VAI, total testosterone, total, LDL, non-HDL and HDL-cholesterol and familial history of diabetes. In the multivariate analysis, WC, ISI-Matsuda, Dio, VAI, total and LDL-cholesterol were found to be significant predictive factors for PCOS. In addition, our model performs moderately well in predicting incidence of PCOS.

The relationship between PP and PCOS has been widely investigated. The first hypothesis of a relationship between PP and PCOS was reported by Ibanez et al, who found that 15 out of 35 girls with PP developed PCOS. This suggests that the development of PCOS in girls with PP may be related to metabolic factors, such as WC, age of menarche, ISI-Matsuda, Dio, VAI, total testosterone, total, LDL, non-HDL and HDL-cholesterol, and familial history of diabetes. Therefore, it is important to consider metabolic factors when assessing the risk of PCOS in girls with PP.
PCOS (27). The coexistence of PCOS and PP is compatible with the concept that the ovarian and adrenal
dysfunction of PCOS represents an inborn dysregulation of steroidogenesis (28). Besides the pubarchal
androgen levels, other factors such as low birth weight and genotype have been suggested to be associated
with the development of PCOS in girls with PP (2,29). Although, some studies reported that low birth
weight was associated with a greater hyperinsulinism leading to PP and PCOS (2,29,30), other studies did
not confirm these data (31).

Interestingly, some genetic variants and the presence of high BMI values were also reported to strongly
influence menarche age in women with PCOS (29,30). Girls with PCOS who were overweight in the pubertal
window had an earlier menarche age, whereas those who were thin tended to have a later menarche age
(29-31).

In another set of studies, Ibañez et al. (32,33) reported that, as in PCOS, PP girls were excessively insulin-
resistant from mid-childhood through puberty. This was associated with central adiposity and dyslipidemia
but not obesity (34). In agreement with these authors, other studies documented that PP patients were
insulin-resistant and obese and reported a correlation between insulin resistance and androgen levels (35).
Early metformin treatment in low birth weight girls with PP was associated with a delay in the menarche
age of about 1 year compared to girls who were not treated with metformin (36). In addition, early
treatment was more effective in reducing hirsutism, androgen excess, oligomenorrhea and PCOS than late
treatment (37). Early adrenarche was therefore correlated with high risk of adipose tissue dysfunction as a
consequence of androgen excess (38).

A Parisian collaborative group identified Caucasian PP patients with no hormone abnormality who were
postpubertal (31) and showed that these girls did not differ from controls in hirsutism score, acne,
prevalence of menstrual irregularity, BMI, or glucose and insulin during an OGTT. The investigators
concluded that only 15-20% of their PP population developed hyperandrogenism after menarche. Their
study did not clarify whether the subgroup of girls with exaggerated adrenarche was also at increased risk
of developing PCOS.
In line with these studies, our study shows that girls with PP who develop PCOS are more insulin-resistant than PP who do not develop PCOS. In the current study the insulin resistance degree was evaluated by ISI-Matsuda, and the insulin secretion by Dio. ISI-Matsuda is a useful tool to evaluate the insulin resistance degree of the whole body. It derives from OGTT and more closely correlates with the M-value of the euglycaemic hyperinsulinemic clamp, which represents the gold standard of insulin sensitivity measurement (18). Lower ISI-Matsuda values in patients with PP-PCOS clearly show an insulin resistance condition in these patients, compared to PP-NO-PCOS. Interestingly, girls with PP-NO-PCOS showed higher values than PCOS-NO-PP, suggesting that the coexistence of PP and PCOS may have an additive adverse effect on insulin sensitivity. Dio is an interesting tool that expresses the ability of β-cells to adequately compensate insulin resistance through increased insulin secretion and is a predicting factor of diabetes development in adults (19). Girls with PP-PCOS showed lower Dio values PP-NO-PCOS, further supporting the hypothesis of an additive effect of premature androgenization and the development of PCOS tends to worsen insulin sensitivity and secretion.

An interesting finding in this study is the evaluation of cardiometabolic risk by VAI. VAI is an index of altered visceral fat function and distribution. It has been shown that VAI is a marker of visceral adiposity, insulin resistance, impaired adipocytokine production and inflammatory product secretion, which better correlates with the computed tomographic scan (38-41) in women with PCOS. Although, VAI is not validated in pediatric population, many studies report its usefulness in predicting metabolic syndrome in children (42) and its association with insulin resistance, adipokines and subclinical inflammation (43). Girls with PP-PCOS showed higher VAI compared to both PP-NO-PCOS both at time of diagnosis of PP and at the diagnosis of PCOS. These data suggest that the coexistence of PP and PCOS is responsible for an increased cardiometabolic risk. This hypothesis is confirmed by the knowledge that increasing androgen levels in females, as documented in PCOS women, results in abdominal adiposity, adipose tissue dysfunction, insulin resistance and metabolic disturbances (44). On this concept there has been created the theory of the
“metabolic valley of death”, as a window of circulating androgen excess associated with adverse metabolic manifestations in both sexes.

There remains to be further explored the physiological role of androgens in regulating multiple aspects of insulin signalling in females which becomes evident early in utero and possibly determines PP. Indeed PP may represent the most pronounced example, where clinical signs of androgen action (pubic/axillary hair, acne, greasy hair and accelerated stature growth) appear early in girls and are associated with an increase in adrenal androgen precursors, such as dehydroepiandrosterone and its dehydroepiandrosterone sulfate (44). These girls are often more overweight than control groups of the same age and show an adverse metabolic phenotype, including hyperinsulinism, dyslipidemia, and augmented risk of metabolic disturbances in adulthood.

Although the exact mechanisms leading to development of PCOS in patients with PP are not clearly and definitely established, on the basis of our data we hypothesize that both early endogenous hyperandrogenism together with a higher metabolic dysfunction may lead to enhanced hyperinsulinemia contributing to PCOS development.

Using multivariate analysis, we identified six risk factors – WC, ISI-Matsuda, Dlo, VAI, total testosterone and LDL-cholesterol – for development of PCOS. A risk prediction model was constructed using these 6 risk factors. Our risk prediction model was moderately robust in predicting PCOS with an AUC of 0.73. Interestingly, our study is the first to identify the above-mentioned parameters as predictive factors for PCOS in girls with PP. However, these findings need to be further validated in larger studies.

The limitations of the study are the small sample of patients with PP and the retrospective design of the study. Our study is the first to report a high percentage of PCOS in girls with PP, compared to other studies conducted on smaller population, which may be due to a longer period of observation.

In conclusion, in this study we identified a number of metabolic factors in PP that are associated with the development of PCOS. These risk factors represent some of the key metabolic abnormalities of PCOS. Our
prediction model proposes that in PP, WC, ISI-Matsuda, Dlo, VAI, total testosterone and LDL-cholesterol are significant predictive factors for PCOS. Taken together, these findings support the metabolic pathogenesis of PCOS and suggest easy tools to identify girls who may develop PCOS in order to establish an early therapeutic approach.

Declaration of interest: Authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported

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Legend to Figure 1. A Forest Plot showing odds ratio values and 95% confidence intervals for: PP and PCOS, adjusted by age of menarche, total, non-HDL and LDL-cholesterol, familial history of diabetes and underweight. The x-axis represents the odds ratio (circles, square and triangles) and 95% confidence intervals (whiskers). The dashed vertical line indicates an OR value of 1.
Table 1. Clinical, anthropometric, and biochemical characteristics of girls with premature pubarche developing or not PCOS at baseline

|                                             | Premature pubarche who develop PCOS (N=63) | Premature pubarche who do not develop PCOS (N=36) | p       |
|---------------------------------------------|--------------------------------------------|--------------------------------------------------|---------|
| BMI (Kg/m²)                                 | Mean ± SD 18.3 ± 2.09                      | Mean ± SD 15.3 ± 1.48                            | <0.001  |
| Waist Circumference (cm)                    | Mean ± SD 68.6 ± 3.81                      | Mean ± SD 64.2 ± 3.92                            | <0.001  |
| FG score                                    | Mean ± SD 14.5 ± 5.38                      | Mean ± SD 15.8 ± 5.99                            | 0.574   |
| Birth weight (g)                            | Mean ± SD 2930 ± 642.1                     | Mean ± SD 3150 ± 173.2                           | 0.244   |
| Subjects (%)                                | Mean ± SD                                  | Mean ± SD                                      |         |
| Familial history of diabetes mellitus       |                                             |                                                 |         |
| Underweight                                 | 2 (3.2%)                                   | 6 (16.7%)                                       | 0.025   |
| Normal weight                               | 60 (96.8%)                                 | 30 (83.3%)                                      | 0.025   |
| Overweight                                  | 1 (1.6%)                                   | 0                                               | 0.636   |
| Hormonal profile                            | Mean ± SD                                  | Mean ± SD                                      |         |
| FSH (IU/l)                                  | Mean ± SD 0.66 ± 0.28                      | Mean ± SD 0.77 ± 0.23                           | 0.901   |
| LH (IU/l)                                   | Mean ± SD 0.67 ± 0.28                      | Mean ± SD 0.58 ± 0.17                           | 0.126   |
| LH/FSH ratio                                | Mean ± SD 0.87 ± 0.35                      | Mean ± SD 0.81 ± 0.34                           | 0.574   |
| 17-β-E2 (pmol/L)                            | Mean ± SD 20.1 ± 12.8                      | Mean ± SD 20.1 ± 8.66                           | 0.996   |
| 17OHPg (nmol/L)                              | Mean ± SD 0.64 ± 0.31                      | Mean ± SD 0.74 ± 0.32                           | 0.417   |
| Prolactin (µg/L)                             | Mean ± SD 11.1 ± 3.84                      | Mean ± SD 11.2 ± 3.19                           | 0.989   |
| Total testosterone (nmol/L)                 | Mean ± SD 0.28 ± 0.31                      | Mean ± SD 0.11 ± 0.04                           | 0.026   |
| DHEA-S (µmol/L)                              | Mean ± SD 2.16 ± 0.91                      | Mean ± SD 2.12 ± 0.87                           | 0.857   |
| Δ4androstenedione (nmol/L)                  | Mean ± SD 4.32 ± 1.02                      | Mean ± SD 4.42 ± 1.13                           | 0.722   |
| Metabolic profile                           | Mean ± SD                                  | Mean ± SD                                      |         |
| ISI Matsuda                                  | Mean ± SD 2.70 ± 1.13                      | Mean ± SD 4.48 ± 1.75                           | <0.001  |
| AUC₂h Insulin (pmol · L⁻¹ · 120min)         | Mean ± SD 8639.9 ± 604.8                   | Mean ± SD 7799.9 ± 624.9                        | 0.880   |
| AUC₂h Glucose (mmol · L⁻¹ · 120min)         | Mean ± SD 747.7 ± 190.2                    | Mean ± SD 735.5 ± 120.1                         | 0.959   |
| Oral Disposition Index (Dlo)                | Mean ± SD 2.14 ± 0.92                      | Mean ± SD 3.34 ± 1.76                           | 0.002   |
| VAI                                         | Mean ± SD 1.91 ± 0.53                      | Mean ± SD 1.48 ± 0.43                           | 0.013   |
| Total cholesterol (mmol/l)                  | Mean ± SD 3.31 ± 0.44                      | Mean ± SD 2.68 ± 0.67                           | <0.001  |
| HDL cholesterol (mmol/l)                    | Mean ± SD 1.10 ± 0.13                      | Mean ± SD 1.18 ± 0.13                           | 0.026   |
| Calculated LDL cholesterol (mmol/l)         | Mean ± SD 1.97 ± 0.45                      | Mean ± SD 1.28 ± 0.62                           | <0.001  |
| Non-HDL cholesterol (mmol/l)                | Mean ± SD 2.21 ± 0.44                      | Mean ± SD 1.49 ± 0.63                           | <0.001  |
| Triglycerides (mmol/l)                      | Mean ± SD 1.14 ± 0.29                      | Mean ± SD 1.07 ± 0.23                           | 0.256   |
| GOT (U/l)                                   | Mean ± SD 15.4 ± 2.96                      | Mean ± SD 23.8 ± 12.6                           | 0.216   |
| GPT (U/l)                                   | Mean ± SD 20.4 ± 12.4                      | Mean ± SD 28.8 ± 16.6                           | 0.392   |

Abbreviations: 17-β-E2, 17βestradiol; 17OHPg, 17OHProgesterone; VAI, Visceral Adiposity Index; AUC₂h Insulin, area under curve 2 hours of insulin; AUC₂h glucose, area under curve 2 hours of glucose.
## Table 2. Clinical, anthropometric, and biochemical characteristics of women with history of premature pubarche at time of diagnosis of PCOS

|                      | **Premature pubarche with PCOS (N=63)** | **Premature pubarche without PCOS (N=36)** | p   |
|----------------------|----------------------------------------|--------------------------------------------|-----|
| Age at evaluation (years) | 21.2 ± 4.48                          | 22.4 ± 3.42                               | 0.509 |
| BMI (Kg/m²)           | 32.6 ± 7.99                           | 30.2 ± 7.36                               | 0.127 |
| Waist Circumference (cm) | 104.6 ± 16.1                         | 98.1 ± 12.5                               | <0.001 |
| FG score              | 16.6 ± 6.42                           | 18.1 ± 6.45                               | 0.574 |
| **Subjects (%)**      |                                       |                                            |     |
| **Classes of obesity** |                                       |                                            |     |
| Underweight           | 2 (3.1%)                              | 8 (22.8%)                                  | 0.001 |
| Normal weight         | 27 (42.1%)                            | 14 (40%)                                   | 0.840 |
| Overweight            | 15 (23.4%)                            | 6 (17.1%)                                  | 0.465 |
| Obese class I         | 7 (10.9%)                             | 3 (8.5%)                                   | 0.705 |
| Obese class II        | 5 (7.8%)                              | 2 (5.8%)                                   | 0.712 |
| Obese class III       | 8 (12.5%)                             | 2 (5.8%)                                   | 0.293 |
| Obesity               | 20 (31.7%)                            | 7 (19.4%)                                  | 0.188 |
| **Metabolic syndrome*** |                                        |                                            | 0.154 |
| Impaired fasting glucose (IFG) | 1 (1.5%)                          | 0                                           | 0.468 |
| Impaired glucose tolerance (IGT) | 0                                 | 0                                           | /    |
| IFG + IGT             | 0                                      | 0                                           | /    |
| Diabetes mellitus     | 0                                      | 0                                           | /    |

**Hormonal profile**

|                      | **Mean ± SD** | **Mean ± SD** | p |
|----------------------|--------------|--------------|---|
| FSH (IU/l)           | 5.66 ± 1.74  | 5.13 ± 2.48  | 0.538 |
| LH (IU/l)            | 7.77 ± 2.73  | 4.54 ± 2.02  | 0.001 |
| LH/FSH ratio         | 1.47 ± 0.61  | 1.01 ± 0.56  | 0.040 |
| 17-β-E2 (pmol/L)     | 151.9 ± 97.3 | 325.9 ± 203.7 | 0.006 |
| LHOPg (pmol/L)       | 5.71 ± 3.05  | 3.87 ± 1.91  | 0.066 |
| Prolactin (µg/L)     | 18.1 ± 6.99  | 15.6 ± 6.51  | 0.279 |
| Pg (nmol/L)          | 11.1 ± 10.4  | 23.8 ± 11.7  | 0.018 |
| DHEA-S (µmol/L)      | 10.5 ± 4.92  | 9.35 ± 6.75  | 0.801 |
| Δ4androstenedione (nmol/L) | 13.4 ± 5.37 | 12.1 ± 6.56 | 0.707 |

**Metabolic profile**

|                      | **Mean ± SD** | **Mean ± SD** | p |
|----------------------|--------------|--------------|---|
| Homa2-IR             | 3.75 ± 2.41  | 3.33 ± 1.13  | 0.868 |
| ISI Matsuda          | 2.78 ± 1.14  | 5.51 ± 1.64  | 0.001 |
| AUC_{2h Insulin} (pmol · L⁻¹ · 120min) | 8639.9 ± 604.8 | 7799.9 ± 624.9 | 0.729 |
| AUC_{2hglucose} (mmol · L⁻¹ · 120min) | 747.7 ± 190.2 | 735.5 ± 120.1 | 0.961 |
| Oral Disposition Index (Dlo) | 2.08 ± 1.09 | 1.56 ± 1.18 | 0.031 |
| VAI                  | 2.65 ± 1.41  | 1.38 ± 0.42  | 0.001 |
| Total cholesterol (mmol/l) | 5.11 ± 0.71 | 3.27 ± 0.38 | 0.017 |
| HDL cholesterol (mmol/l) | 1.25 ± 0.31 | 1.29 ± 0.15 | 0.789 |
| Calculated LDL cholesterol (mmol/l) | 3.55 ± 0.82 | 1.78 ± 0.38 | 0.008 |
| Non-HDL cholesterol (mmol/l) | 3.84 ± 0.86 | 1.98 ± 0.39 | 0.005 |
| Triglycerides (mmol/l) | 1.27 ± 0.74 | 0.98 ± 0.19 | 0.104 |
| GOT (U/l)            | 15.4 ± 2.96  | 23.8 ± 12.6  | 0.216 |
| GPT (U/l)            | 20.4 ± 12.4  | 28.8 ± 16.6  | 0.392 |

**WHO classification; (2010); ** *According to Adult Treatment Panel (ATP) III criteria

Abbreviations: 17-β-E2, 17βestradiol; LHOPg, 17OHprogesterone; VAI, Visceral Adiposity Index; AUC_{2h Insulin}, area under curve 2 hours of insulin; AUC_{2h glucose}, area under curve 2 hours of glucose.
Table 3. Risk factors associated with PCOS in girls with premature pubarche (PP) at diagnosis

| Variable                   | PP-PCOS (N°=63) | PP-NO-PCOS (N°=36) | Crude OR (95% CI) |
|----------------------------|-----------------|-------------------|------------------|
| **Age of menarche**        |                 |                   |                  |
| 11.1-13.11 years           | 14 (38.9%)      | 22 (61.1%)        | 4.37 (1.61-11.8) |
| 8.6-11 years               | 8 (12.7%)       | 55 (87.3%)        | 1                |
| **Total testosterone**     |                 |                   |                  |
| ≤ 0.13 mmol/L              | 34 (94.4%)      | 2 (0.6%)          | 14.8 (3.08-70.9) |
| > 0.13 mmol/L              | 23 (36.5%)      | 40 (63.5%)        | 1                |
| **ISI-Matsuda**            |                 |                   |                  |
| > 4.3                      | 23 (63.9%)      | 13 (36.1%)        | 12.6 (4.21-38.1) |
| ≤ 4.3                      | 9 (14.3%)       | 54 (85.7%)        | 1                |
| **Dio**                    |                 |                   |                  |
| > 4                        | 25 (69.4%)      | 11 (30.6%)        | 29.8 (3.62-245.6) |
| ≤ 4                        | 6 (9.5%)        | 57 (90.5%)        | 1                |
| **VAI**                    |                 |                   |                  |
| ≤ 1.75                     | 25 (69.4%)      | 11 (30.6%)        | 4.74 (1.86-12.1) |
| > 1.75                     | 21 (33.3%)      | 42 (66.7%)        | 1                |
| **Total cholesterol**      |                 |                   |                  |
| ≤ 2.88 mmol/L              | 27 (75%)        | 9 (25%)           | 8.18 (2.9-23.1)  |
| > 2.88 mmol/L              | 11 (17.5%)      | 52 (82.5%)        | 1                |
| **LDL-cholesterol**        |                 |                   |                  |
| ≤ 1.66 mmol/L              | 31 (86.1%)      | 5 (13.9%)         | 12.3 (3.84-39.3) |
| > 1.66 mmol/L              | 23 (36.5%)      | 40 (63.5%)        | 1                |
| **Non-HDL cholesterol**    |                 |                   |                  |
| ≤ 1.86 mmol/L              | 25 (69.4%)      | 11 (30.6%)        | 5.72 (1.67-19.5) |
| > 1.86 mmol/L              | 21 (33.3%)      | 42 (66.7%)        | 1                |
| **Familial history of diabetes** |     |                   |                  |
| No                         | 30 (83.3%)      | 6 (16.7%)         | 2.5 (0.9-6.94)   |
| Yes                        | 42 (66.7%)      | 21 (33.3%)        | 1                |
| **Underweight**            |                 |                   |                  |
| No                         | 61 (96.8%)      | 30 (83.3%)        | 1                |
| Yes                        | 2 (3.2%)        | 6 (16.7%)         | 0.16 (0.03-0.86) |
254x190mm (307 x 307 DPI)