Reproductive and metabolic features during puberty in sons of women with polycystic ovary syndrome

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Abstract

Context: Intratuerine life may be implicated in the origin of polycystic ovary syndrome (PCOS) modifying the endocrine and metabolic functions of children born to PCOS mothers independently of the genetic inheritance and gender. The aim of the present study was to evaluate the reproductive and metabolic functions in sons of women with PCOS during puberty.

Methods: Sixty-nine PCOS sons (PCOSs) and 84 control sons of 7–18 years old matched by the Tanner stage score were studied. A complete physical examination was conducted including anthropometric measurements (weight, height, waist, hip and body mass index). An oral glucose tolerance test was performed and circulating concentrations of luteinizing hormone, follicle-stimulating hormone (FSH), sex hormone-binding globulin, testosterone, androstenedione (A4), 17α-hydroxyprogesterone (17-OHP) and AMH were determined in the fasting sample.

Results: Waist-to-hip ratio, FSH and androstenedione levels were significantly higher in the PCOSs group compared to control boys during the Tanner stage II–III. In Tanner stages II–III and IV–V, PCOSs showed significantly higher total cholesterol and LDL levels. Propensity score analysis showed that higher LDL levels were attributable to the PCOSs condition and not to other metabolic factors. AMH levels were comparable during all stages. The rest of the parameters were comparable between both groups.

Conclusions: Sons of women with PCOS show increased total cholesterol and LDL levels during puberty, which may represent latent insulin resistance. Thus, this is a group that should be followed and studied looking for further features of insulin resistance and cardiovascular risk markers. Reproductive markers, on the other hand, are very similar to controls.

Introduction

Polycystic ovary syndrome (PCOS) is a common familial endocrine-metabolic disorder that affects women of reproductive age, characterized by irregular menses, chronic anovulation, infertility and hyperandrogenism (1, 2). A genetic cause of the syndrome was suggested many years ago (3), and phenotypic and family aggregation studies have demonstrated that a significant number of female relatives of PCOS patients are affected...
with the condition (4, 5, 6, 7, 8). Moreover, it has been proposed that intrauterine life, as an environmental factor, is implicated in the origin of PCOS (8, 9, 10, 11) and may modify the endocrine and metabolic functions of a child born to a PCOS mother independently of the genetic inheritance and gender (12, 13, 14). Therefore, it is possible to suggest that both daughters and sons born to PCOS mothers could be affected showing reproductive and metabolic abnormalities similar to those observed in women with PCOS.

We have studied daughters of women with PCOS during different stages of development finding several features that may represent early metabolic and reproductive markers of the syndrome and that may be modified by interventions that improve the adverse pregnancy environment present in PCOS women (13, 15).

On the other hand, the reproductive and metabolic features of male members of PCOS families have been less documented in the literature. Some of the phenotypes proposed include abnormalities in hair distribution, such as increased hair growth and balding or premature male balding (16). Other studies have described abnormalities in plasma luteinizing hormone (LH) levels (17) and dehydroepiandrosterone sulphate concentrations in male members of PCOS families (18). Our group reported that brothers of PCOS women show increased 17-hydroxyprogesterone levels in response to leuprolide acetate resembling those described in women with PCOS (19). In a further study, we reported that basal concentrations of gonadotropins, sex steroids and inhibin B were comparable between PCOS sons (PCOSs) and control sons (Cs) during early infancy, childhood and adulthood. We also obtained similar results in stimulated gonadotropin and sex steroid concentrations. However, AMH serum concentrations were higher in PCOSs compared with Cs during early infancy and childhood with no difference during adulthood indicating a possible early dysfunction of Sertoli cells. Nevertheless, the sperm-count analysis was similar between both groups (14). Regarding metabolic features, we observed increased weight from an early age and some indicators of insulin resistance during adulthood (12).

Puberty is a transition period between childhood and adulthood accompanied by profound changes in the body composition, insulin sensitivity and sex steroid production, and then it is a critical period in the setting of metabolic and reproductive features observed during adulthood (20).

Therefore, the aim of the present study was to evaluate the reproductive and metabolic functions in sons of women with PCOS during puberty in order to analyse the transition from childhood to adulthood in this setting.

**Materials and methods**

We included 69 PCOSs and 84 Cs between 7 and 18 years of age. The study was designed to analyse PCOSs and controls transversally. Boys were sub-grouped into three categories according to their Tanner stage score for testis development: Tanner I, Tanner II–III and Tanner IV–V. The boys included in the study were not taking any medication. All boys were born from singleton pregnancies. Some of these boys participated in our previous studies during infancy and childhood (12, 14).

Mothers with PCOS were recruited from patients attending the Unit of Endocrinology and Reproductive Medicine, University of Chile, Santiago, Chile. The diagnosis of PCOS was made according to the National Institutes of Health Consensus Criteria which was the criteria used at the time of their diagnosis during reproductive age before pregnancy (21). Mothers with PCOS exhibited chronic oligomenorrhea or amenorrhea and hirsutism during reproductive age. In addition, PCOS women showed the characteristic ovarian morphology of polycystic ovaries in ultrasound, based on the criteria described by Adams and coworkers (22). These inclusion criteria for PCOS mothers were similar to those previously reported (15). As control mothers, we selected women who had a history of regular 28- to 32-day menstrual cycles, absence of hirsutism and other manifestations of hyperandrogenism and no history of infertility or pregnancy complications.

**Study protocol**

The boys were admitted with their mothers to our Clinical Research Centre. We performed a complete physical examination on each boy, including anthropometric measurements (weight, height, waist, hip, body mass index (BMI) and BMI standard deviation (s.d.) score (SDS) calculated using the Growth Analyser Program and the US BMI-for-age growth charts) (23). These growth curves have been shown to be applicable to contemporary Chilean population (24). Obesity was defined as a body weight >95th percentile. Testis volume was determined by a single endocrinologist (N C) using the Prader orchidometer.

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In both groups of boys, an oral glucose tolerance test (1.75 g/kg, up to a maximum of 75 g glucose in 250 mL water) after a 12-h overnight fast was performed. Blood samples (5 mL) were drawn before 30, 60 and 120 min after the glucose load. Serum glucose and insulin were determined in each sample. Glucose tolerance was evaluated using the American Diabetes Association criteria (25). The homeostasis model assessment for insulin resistance (HOMA-IR) and the whole-body insulin sensitivity composite index (ISI) were calculated as previously described (26, 27). In addition, circulating concentrations of LH, follicle-stimulating hormone (FSH), sex hormone-binding globulin (SHBG), testosterone, androstenedione (A4), 17α-hydroxyprogesterone (17-OHP), AMH and lipid profile were determined in the fasting sample. Basal serum SHBG and testosterone were used to calculate the FAI as the ratio of serum testosterone/SHBG × 100.

The protocol was approved by the institutional review board of the University of Chile. All parents signed informed consents, and boys gave their assent before entering the study.

## Assays

Serum AMH was assayed by enzyme immunoassay (Immunotech-Beckman Coulter, Marseille, France). Analytical sensitivity was 2.1 pmol/L and intra- and interassay coefficients of variation were 5.3% and 8.7%, respectively. Serum glucose and lipid profile were determined by the glucose oxidase method (Biosystem). The intraassay coefficient of variation of this method was <1.2% and interassay was <2.7%. LDL cholesterol was calculated according to Friedewald formula.

Serum insulin was assayed by IRMA (Diasource, Nivelles, Belgium since 2010) with a sensitivity of 1.0 µIU/mL and intra- and interassay coefficients of variation of 3 and 7%, respectively. Serum LH, FSH and SHBG were determined by IRMA izotop (Institute of Isotops Co. Ltd. (Izotop), Budapest, Hungary). Assay sensitivities were 0.05 IU/mL, 0.021 IU/mL and 0.22 nmol/L, respectively. Intra- and interassay coefficients of variation were 1.0% and 3.1% for LH, 2.5% and 2.7% for FSH and 4.9% and 3.8% for SHBG, respectively.

Serum testosterone, androstenedione and 17-OHP were assayed by RIA (Diagnostic Systems Laboratories and Diasource) and the limits of detection were 0.1, 0.1 and 0.03 ng/mL. The intra- and interassay coefficients of variation were 3.5 and 5.5%, 3.8% and 7.5% and 9.4 and 18%, respectively.

## Statistical evaluation

The present study was designed to analyse PCOS boys and controls transversally. The number of subjects was calculated based on our previous studies showing significant differences in LDL and insulin levels in adult PCOSs (12). Data are expressed as median and interquartile range. Data distribution was assessed by the Kolmogorov–Smirnov test showing a not normal distribution. Differences between study groups were assessed with the Mann–Whitney test. Correlations were assessed using the Spearman test adjusted for BMI and age. Statistical analysis was performed with Graph Pad Prism 6.0 package and the SPSS package version 22. A P value of less than 0.05 was considered statistically significant. A propensity score analysis, using the variables age, Tanner stage, waist-to-hip ratio (WHR), z score BMI, testosterone, androstenedione, ISI score, HOMA and trygliceride levels, was used to establish if the differences observed in LDL levels were attributable only to the PCOSs condition.

## Results

Table 1 shows the clinical and anthropometric characteristics of both groups classified in three sub-groups according to the Tanner stage. WHR was significantly higher in the PCOSs group during the Tanner stage II–III. There were no other anthropometric differences between control and PCOSs in none of the stages studied. Testicular volumes were similar between both groups within the different Tanner stage sub-groups.

Table 2 shows the hormonal and metabolic data in both groups classified according to the Tanner stage. During Tanner stages II–III, PCOSs showed higher FSH and androstenedione levels compared to control boys. AMH levels were comparable during all stages.

During Tanner stages II–III and IV–V, PCOSs showed significantly higher total cholesterol and LDL levels.

Propensity score analysis using the variables age, Tanner stage, WHR, z score BMI, testosterone, androstenedione, ISI score, HOMA and triglyceride levels established that there is a significant difference in LDL levels (73.03 s.d.: 4.21 vs 88.4 s.d.: 4.89; P=0.022) between PCOSs and Cs that is attributable to the PCOSs condition.

Table 3 shows the correlations found between the different parameters in the whole group and according to the Tanner stage adjusted by BMI and age, showing a positive correlation among waist, LDL and total cholesterol with surrogates of insulin resistance.
### Table 1 General anthropometric characteristics of PCOS sons (PCOSs) and control sons (Cs) at the different Tanner stages analysed.

<table>
<thead>
<tr>
<th>Tanner</th>
<th>Cs</th>
<th>PCOSs</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>31</td>
<td>20</td>
<td>0.823</td>
</tr>
<tr>
<td>II–III</td>
<td>31</td>
<td>26</td>
<td>0.446</td>
</tr>
<tr>
<td>IV–V</td>
<td>33</td>
<td>20</td>
<td>0.538</td>
</tr>
</tbody>
</table>

- **n**: number of participants
- **Age (years)**: median and interquartile range
- **Weight (kg)**: median and interquartile range
- **Height (m)**: median and interquartile range
- **BMI (kg/m²)**: median and interquartile range
- **BMI SDS**: median and interquartile range
- **Waist (cm)**: median and interquartile range
- **Hip (cm)**: median and interquartile range
- **WHR**: median and interquartile range
- **Right testes volume (mL)**: median and interquartile range
- **Left testes volume (mL)**: median and interquartile range

Data are median and interquartile range.

### Table 2 Hormonal and metabolic parameters of PCOS sons (PCOSs) and control sons (Cs) at the different Tanner stages analysed.

<table>
<thead>
<tr>
<th>Tanner</th>
<th>Cs</th>
<th>PCOSs</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>31</td>
<td>20</td>
<td>0.999</td>
</tr>
<tr>
<td>II–III</td>
<td>33</td>
<td>20</td>
<td>0.953</td>
</tr>
<tr>
<td>IV–V</td>
<td>20</td>
<td>0.285</td>
<td></td>
</tr>
</tbody>
</table>

- **n**: number of participants
- **SHBG (nmol/L)**: median and interquartile range
- **LH (IU/L)**: median and interquartile range
- **FSH (IU/L)**: median and interquartile range
- **Testosterone (ng/mL)**: median and interquartile range
- **Androstenedione (ng/mL)**: median and interquartile range
- **17 OH progesterone (ng/mL)**: median and interquartile range
- **FAI**: median and interquartile range
- **AMH (mM)**: median and interquartile range
- **Glucose basal (mg/dL)**: median and interquartile range
- **Glucose 30 min (mg/dL)**: median and interquartile range
- **Glucose 60 min (mg/dL)**: median and interquartile range
- **Glucose 120 min (mg/dL)**: median and interquartile range
- **Insulin basal (µIU/mL)**: median and interquartile range
- **Insulin 30 min (µIU/mL)**: median and interquartile range
- **Insulin 60 min (µIU/mL)**: median and interquartile range
- **Insulin 120 min (µIU/mL)**: median and interquartile range
- **HOMA-IR**: median and interquartile range
- **ISI composite**: median and interquartile range
- **Tryglycerides (mg/dL)**: median and interquartile range
- **Total cholesterol (mg/dL)**: median and interquartile range
- **HDL cholesterol (mg/dL)**: median and interquartile range
- **LDL cholesterol (mg/dL)**: median and interquartile range

Data are median and interquartile range.
Table 3  Spearman correlations adjusted by age and BMI in the whole groups.

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole group</td>
<td></td>
<td></td>
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<tr>
<td>Waist</td>
<td></td>
<td></td>
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<tr>
<td>Basal insulin</td>
<td>0.275</td>
<td>0.01</td>
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<tr>
<td>30 min insulin</td>
<td>0.215</td>
<td>0.01</td>
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<tr>
<td>HOMA-IR</td>
<td>0.267</td>
<td>0.03</td>
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<tr>
<td>HDL</td>
<td>−0.210</td>
<td>0.02</td>
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<tr>
<td>Tanner II–III</td>
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<td></td>
</tr>
<tr>
<td>Waist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal insulin</td>
<td>0.342</td>
<td>0.02</td>
</tr>
<tr>
<td>30 min insulin</td>
<td>0.341</td>
<td>0.02</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.317</td>
<td>0.03</td>
</tr>
<tr>
<td>HDL</td>
<td>−0.308</td>
<td>0.04</td>
</tr>
<tr>
<td>Total cholesterol</td>
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<td></td>
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<tr>
<td>120 min glucose</td>
<td>0.330</td>
<td>0.02</td>
</tr>
<tr>
<td>30 min insulin</td>
<td>0.335</td>
<td>0.02</td>
</tr>
<tr>
<td>60 min insulin</td>
<td>0.348</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal glucose</td>
<td>0.331</td>
<td>0.02</td>
</tr>
<tr>
<td>30 min insulin</td>
<td>0.344</td>
<td>0.02</td>
</tr>
<tr>
<td>60 min insulin</td>
<td>0.357</td>
<td>0.01</td>
</tr>
<tr>
<td>Composite</td>
<td>−0.394</td>
<td>0.01</td>
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<tr>
<td>Tanner IV–V</td>
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<td></td>
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<tr>
<td>Waist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal insulin</td>
<td>0.398</td>
<td>0.02</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.408</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Discussion

In the present study, we analysed the pubertal period of sons born to PCOS women, a stage of profound changes in the body composition, insulin sensitivity and sex steroid production. We are, thus, completing our previous observations in PCOSs during infancy, childhood and adulthood.

We found an increased WHR along with higher cholesterol and LDL levels during Tanner stages II–III. PCOSs also showed higher FSH and androstenedione levels during this stage. The cholesterol alterations were also observed during the Tanner stage IV–V but with no anthropometric differences.

Despite being comparable in age, BMI and Tanner stage, PCOSs seem to develop early derangements in cholesterol levels. We have previously reported this observation in children and adult PCOSs (12). Nevertheless, in that case, PCOSs BMI was also significantly higher and the differences disappeared when controlled by BMI. In the present study, BMIs were comparable and propensity score analysis showed that this difference was attributable to the PCOs condition. Thus, cholesterol alterations in this group seem to represent an intrinsic independent feature probably associated with an incipient state of insulin resistance. Although insulin and glucose levels were comparable during all Tanner stages, during Tanner stages II–III, LDL was positively correlated with basal glucose, 30, 60 and average insulin levels and negatively correlated with ISI composite. Thus, indirectly LDL levels might be reflecting insulin resistance at this stage.

The increase in WHR and a trend to higher waist circumference (WC) in Tanner II–III boys may also represent a latent insulin resistance that is expressed later on during adulthood. In a larger study, waist was associated with insulin resistance during adolescence. In this regard, associations between adiposity measures, such as WC, and insulin resistance have been shown to be stronger in children during puberty compared with those who had completed pubertal development (28). In this regard, in the current study, WC was correlated with different surrogates of insulin resistance during Tanner stages II–V. Thus, this might be a good marker for metabolic disruption in pubertal boys.

Daughters of women with PCOS show increased insulin and triglyceride levels from an early stage (15). On the other hand, boys show comparable insulin and triglyceride levels but significantly higher LDL and total cholesterol levels. This feature is still present during adulthood but is accompanied by increased insulin levels (12). Thus, we can suggest that there is a sexual dimorphism regarding the expression and onset of insulin resistance in the offspring of PCOS women, with an early appearance of hypertriglyceridemia and hyperinsulinemia in girls and an early increased LDL and late hyperinsulinaemia in boys.

This sexual dimorphism has also been found in normal Chilean girls, showing higher triglyceride levels in adolescent girls compared to boys (29). Thus, the expression of insulin resistance in daughters and sons of PCOS women seems to be determined in part by these naturally occurring gender differences.

Later, during adult life, this insulin resistance is expressed as increased insulin levels and higher BMI in PCOS women and adult PCOSs as we have previously reported (12).

Opposite to what we found in younger PCOSs, in whom we observed increased AMH levels, pubertal PCOSs show normal AMH levels, same as the adult PCOSs group in our previous report (14). Thus, it seems like, despite having a period of possible delayed maturation during infancy and childhood, Sertoli cells seem to have an appropriate function during puberty.
Starting puberty, we observed increased FSH levels and higher androstenedione levels that then normalize during the next stage of puberty. Higher androstenedione may come from the testis or the adrenal gland. A possible explanation is that there is an increased secretion of androgens from the adrenal gland as has been suggested in PCOS women (30, 31), and then as the testis takes over androgen secretion, this difference disappears. Higher FSH levels are harder to interpret and may reflect a slightly earlier maturation of the gonadal axis.

In conclusion, sons of women with PCOS show increased total cholesterol and LDL levels during puberty, which may represent latent insulin resistance that is later on translated into higher insulin levels and a higher BMI. Thus, this is a group that should be followed and studied looking for further features of insulin resistance and cardiovascular risk markers. Reproductive markers, on the other hand, are very similar to controls so there is no important evidence pointing at any significant reproductive difference in this group.

Declaration of interest
N C, B E, M M. H. L, P. R, S R and T. S-P declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Authors contribution statement
Nicolas Crisosto: patient recruitment and evaluation, data analysis, manuscript preparation; Bárbara Echiburú: data analysis, hormonal determinations; Manuel Maliqueo: data analysis, hormonal determinations, Pedro Rojas: data analysis; Sergio Recabarren: data analysis; Teresa Sir-Petermann: general coordination of the project, patient recruitment and evaluation, manuscript preparation.

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