Puberty and polycystic ovary syndrome
Selma Feldman Witchel *
Children's Hospital of Pittsburgh, University of Pittsburgh, 3705 Fifth Avenue, Pittsburgh, PA 15213, USA

Abstract
Polycystic ovary syndrome (PCOS) is a heterogeneous familial disorder characterized by chronic anovulation and hyperandrogenism. This multi-system, polygenic, multi-factorial disorder is associated with an increased risk for metabolic abnormalities such as type 2 diabetes mellitus. Signs and symptoms of PCOS often emerge during the peri-pubertal years with premature pubarche (PP) being the earliest manifestation for some girls. Insulin resistance and hyperinsulinemia are important pathophysiologic features that are common to both PP and PCOS. Future investigations are needed to uncover the relevant genetic and hormonal factors and identify effective interventions.

© 2006 Elsevier Ireland Ltd. All rights reserved.
Keywords: Polycystic ovary syndrome; Premature pubarche; Premature adrenarche; Insulin resistance

1. Introduction
Polycystic ovary syndrome (PCOS) is a heterogeneous familial disorder characterized by chronic anovulation and hyperandrogenism which affects approximately 5% of reproductive aged women (Ehrmann, 2005; Dunaf and Thomas, 2001; Aziz et al., 2004; Knochenhauer et al., 1998). The hyperandrogenism is due to excessive ovarian and/or adrenal androgen secretion. Cutaneous manifestations associated with the hyperandrogenism include hirsutism, acne, and male-pattern baldness. The chronic anovulation is associated with oligo/amenorrhea and infertility. Polycystic ovaries may be apparent on ultrasound, but are not a requisite diagnostic feature. Ovaries from women with PCOS are characterized by multiple small follicles and the theca cell compartment may be hypertrophied. Signs and symptoms of PCOS often emerge during the peri-pubertal years. For some girls, premature pubarche (PP), defined as the development of pubic hair prior to 8 years of age, is the earliest manifestation of this chronic disorder (Ibáñez et al., 1993, 2000).

Whereas medical students and patients consider PCOS to be a specific entity, physicians and scientists struggle to reach a consensus definition of this multifaceted disorder. In 2003, a conference sponsored by the American Society for Reproductive Medicine (ASRM) and the European Society of Human Reproduction and Embryology (ESHRE) revised the diagnostic criteria for PCOS to include two of three findings: (1) oligo- or anovulation; (2) clinical and/or biochemical signs of hyperandrogenism; and (3) polycystic ovaries along with exclusion of other disorders (ASRM/ESHRE, 2004).

2. Multi-system disorder
In addition to the hirsutism, irregular menses, and infertility, women with PCOS manifest a number of metabolic abnormalities including hyperinsulinemia, insulin resistance, and dyslipidemia. These metabolic features are components of the metabolic syndrome also known as "syndrome X". Women with PCOS have an increased risk to develop impaired glucose tolerance (IGT), gestational diabetes mellitus (GDM), type 2 diabetes mellitus (T2DM), obesity, and endometrial disease (Legro et al., 1999; Dokras et al., 2005). Since insulin resistance and metabolic syndrome are associated with an increased risk for cardiovascular disease, it is presumed that women with PCOS also have an increased risk for coronary artery disease. A recent study found the prevalence of the metabolic syndrome to be higher among adolescent girls with PCOS compared to the general adolescent population (Coviello et al., 2006). The demonstration of dyslipidemias, insulin resistance, and hyperinsulinemia among girls with PP and adolescent girls with hyperandrogenism (HA)/PCOS provides additional support that PP and PCOS are related disorders. This "thread" of insulin resistance/hyperinsulinemia appears to be an important pathophysiologic feature (Lewy et al., 2001). Clinical improvement in non-obese women with PCOS treated with insulin sensitizers, e.g. metformin and rosiglitazone, provides additional evidence supporting the role of insulin resistance in the pathophysiology...
androstenedione. DHEA can be converted to DHEAS through identical enzymes, the adrenal cortex synthesizes DHEA and can be converted to testosterone through enzymes capable of 17α-hydroxylase activity and then to DHEA through the actions of a steroid sulfotransferase enzyme, SULT2A1, which is expressed in the zona reticularis (Suzuki et al., 2000).

Theca cells obtained from women with PCOS have an inherent tendency to synthesize and secrete excessive amounts of androgens (Gilling-Smith et al., 1994). This phenotype persists despite many passages in cell culture indicating that it is an inherent property of PCOS theca cells (Nelson et al., 1999). A number of strategies, i.e. cell culture, transient transfection, suppressive subtractive hybridization, and cDNA micro-array analyses, are being used to discern the differences between PCOS and normal theca cells (Strauss et al., 2002; Wood et al., 2004). These studies suggest that increased CYP17, CYP11A1, and HSD3B2 expression contribute to the excessive androgen biosynthesis (Wickenheisser et al., 2004, 2005). In addition to increased transactivation of the CYP17 promoter, increased steady-state accumulation of CYP17 mRNA due to slower degradation has been detected in PCOS theca cells (Wickenheisser et al., 2005).

Many women with PCOS exhibit LH hypersecretion with increased LH/FSH ratio, LH pulse amplitude, LH pulse frequency, and LH responses to GnRH stimulation. It has been suggested that primary LH hypersecretion is one potential cause of PCOS (Yen et al., 1970; Taylor et al., 1997; Waldstreicher et al., 1988). LH hypersecretion could reflect a primary intrinsic abnormality of the GnRH pulse generator perhaps related to prenatal programming (Abbott et al., 2005). Corroborating data for this hypothesis include the masculinization of LH secretory patterns among women with classical congenital adrenal hyperplasia (CAH) and in animals exposed to prenatal androgen treatment (Barnes et al., 1994; Foster et al., 2002; Abbott et al., 2005). Another possibility is that LH hypersecretion is the consequence of chronic anovulation and/or the abnormal insulin, androgen, estrogen, and progesterone concentrations (Wildt et al., 1981; Berga et al., 1993; Kazer et al., 1987; Arroyo et al., 1997; Dunaif et al., 1996; Ehrmann et al., 1997; Sir-Petermann et al., 1993; Venturoli et al., 1987). Hence, both prenatal programming and post-pubertal hyperandrogenemia can be invoked as potential mechanisms for LH hypersecretion in women with classical CAH (Einars et al., 1983; Lobo and Carmina, 1994; Barnes et al., 1994; Ghizzi et al., 1996; Eagleson et al., 2000; Patel et al., 2004). Given the familial nature of PCOS, it is conceivable that prenatal programming could occur in the offspring of mothers with PCOS.

No matter what the inciting event might be, the GnRH pulse generator in some women with PCOS is more resistant to negative feedback inhibition by estradiol and progesterone leading to persistently increased GnRH pulsatility (Daniels and Berga, 1997; Pastor et al., 1998). This relative insensitivity to progesterone can be attenuated by anti-androgens (Eagleson et al., 2000). Results of a study investigating gonadotropin pulsatility among adolescent girls with hyperandrogenism, suggested that both ethnicity and the duration of androgen exposure may influence the sensitivity of the GnRH pulse generator to physiological estradiol and progesterone concentrations (Chhabra et al., 2005). Other factors modulating LH secretion in women with PCOS include body mass index and estradiol and insulin concentrations (Patel et al., 2004; Pasquali et al., 2000; Arroyo et al., 2000).
in later life (Huopio et al., 2000). Hence, genetic variants in the sulfonylurea receptor (SUR1) can progress to diabetes. The observation that primary hyperinsulinism due to mutations in the insulin signal transduction and insulin resistance in specific tissues secretion downregulates insulin receptors leading to decreased and diabetes. Yet, an alternative is that primary excessive insulin secretion culminates inmary insulin resistance, (Rothman et al., 1995).

For PCOS and T2DM, traditional dogma has assumed that insulin resistance is the primary defect. And, in the face of primary insulin resistance, β cells secrete increasing amounts of insulin to maintain euglycemia culminating in β cell exhaustion and diabetes. Yet, an alternative is that primary excessive insulin secretion downregulates insulin receptors leading to decreased insulin signal transduction and insulin resistance in specific tissues. Circumstantial evidence for the latter possibility includes the observation that primary hyperinsulinism due to mutations in the sulfonylurea receptor (SUR1) can progress to diabetes in later life (Huopio et al., 2003). Hence, genetic variants in genes coding for proteins involved in β cell glucose-stimulated insulin secretion may produce small functional effects on the β cell KATP channel current; these variants may be associated with predisposition to hyperinsulinism in youth and/or increase the risk to develop diabetes in later life (Ashcroft, 2005).

Irrespective of the molecular mechanism(s) responsible for the insulin resistance and hyperinsulinemia and despite its presence, the mitogenic actions of insulin are generally preserved. Insulin also inhibits hepatic synthesis of sex hormone binding globulin resulting in increased bioavailable testosterone. The elevated insulin concentration acts synergistically with LH and/or ACTH, respectively, to provoke excessive ovarian and/or adrenal androgen biosynthesis (Barbieri et al., 1986). In this sense, the ovary becomes a target of the metabolic derangements.

5. Premature pubarche and PCOS

Development of pubic or axillary hair prior to age 8 years in girls and age 9 years in boys is considered to be premature pubarche (PP). The differential diagnosis of PP includes premature adrenal pubertal maturation (premature adrenarche), CAH, Cushing’s syndrome, androgen secreting tumors, and hyperprolactinemia. Children with PP due to premature adrenarche (PA) typically have androgen concentrations commensurate to the stage of pubic hair development. In general, skeletal maturation is not advanced or only slightly advanced. Growth velocity is normal to slightly accelerated. The diagnosis of PA is based on clinical parameters and exclusion of other disorders associated with PA and hyperandrogenism. Girls with PP due to PA share several features with women with PCOS. In addition to increased androgen concentrations for chronologic age, they have hyperinsulinemia, insulin resistance, dyslipidemia, and decreased sex hormone binding globulin concentrations (Ibáñez et al., 1997). IGF-1 concentrations may be elevated in children with PP (Silfen et al., 2002). When compared to their mothers and population control women, girls with premature pubarche experience menarche earlier than expected (Ibáñez et al., 1992).

There appear to be two groups of adolescent girls with PCOS. In one group, premature pubarche precedes PCOS. In the second group, symptoms typical of PCOS develop during or after adolescence. Prior to gonadarche with resurgence of the GnRH pulse generator, adrenal androgen secretion accounts for most of the circulating androgens. Upon gonadarche with increased gonadotropin secretion, ovarian androgen secretion increases. Hence, girls in whom PP precedes PCOS presumably have a component of adrenal hyperandrogenism that persists and may be exacerbated by the onset of gonadarche. Among girls who develop PCOS during the adolescent years, the relative proportions of ovarian vs. adrenal contributions to the androgen excess are likely to be more variable. Among Spanish girls, the frequency of anovulatory cycles among adolescent girls with a history of PP did not increase when compared to the control subjects until three or more years after menarche (Ibáñez et al., 1999a). In this population, laboratory findings associated with PCOS such as higher basal serum LH concentrations, higher free androgen indices, hyperinsulinemia, and lower SHBG concentrations were associated with anovulation after PP. Although the precise molecular mechanism(s) and sequence of events remain uncertain, persistent hyperandrogenism and altered gonadotropin secretion appear to evolve into a self-perpetuating vicious cycle associated with disruption of synchrony in LH-androgen secretion culminating in chronic anovulation (Veldhuis et al., 2001).

6. Genetic aspects of PCOS

Much evidence indicates that genetic factors influence the predisposition to PCOS, and perhaps, PP. For example, approximately 35% of mother and 40% of sisters of women with PCOS also have PCOS (Kahsar-Miller et al., 2001). Family members, of women with PCOS, often have T2DM, impaired glucose tolerance, insulin resistance, and/or hyperinsulinemia (Diamanti-Kandarakis et al., 2004; Norman et al., 1996; Yildiz et al., 2003). When family pedigrees are examined, the inheri-
classical CAH due to homozygotic carriers, ascertained through family members with androgen excess (Knochenhauer et al., 1997). Yet, it has become apparent regarding PCOS phenotype. Increased LH stimulation of ovarian steroidogenesis, at the time of gonadarche, appears to influence onset of symptoms of PCOS.

Fig. 2. Pathophysiology of PCOS. PCOS is a complex genetic disorder influenced by environmental factors. Food choices, exercise frequency and intensity, and exposure to endocrine disruptors are potential environmental factors. Body composition and distribution of adipose tissue may influence expression of the PCOS phenotype. Increased LH stimulation of ovarian steroidogenesis, at the time of gonadarche, appears to influence onset of symptoms of PCOS.

Despite the many impediments, some progress has been made to identify genetic markers, i.e. 21-hydroxylase (CYP21) gene, associated with PP and PCOS. A paradox has become apparent regarding CYP21 mutations. Typically, obligate heterozygotic carriers, ascertained through family members with classical CAH due to CYP21 mutations, manifest few signs of androgen excess (Knochenhauer et al., 1997). Yet, it has become apparent that some heterozygotic female carriers present with clinical features of PP or PCOS (Witchel et al., 1997; Witchel and Aston, 2000). Several series, consisting largely of Caucasian female subjects, have found that 20-30% of girls and women with hyperandrogenism are heterozygotic carriers of CYP21 mutations (Escobar-Morreale et al., 1999; Blanché et al., 1997; Knorr et al., 1986; Lajic et al., 2002). Among Greek children with PP, 36% were heterozygotic carriers of CYP21 mutations (Dacou-Voutetakis and Dracopoulou, 1999). Although the specific factors which differ between obligate and manifesting heterozygotes remain to be determined, one potential explanation includes differences in the extent of insulin resistance and/or hyperinsulinemia.

Nevertheless, the functional significance of heterozygosity for CYP21 mutations seems to differ between populations. The frequency of CYP21 mutations was comparable among girls with PP (25%) and controls (23%) in Barcelona (Potau et al., 2002). Comparing children and adolescent girls from western Pennsylvania to adult women with PCOS from Alabama, the frequency of CYP21 mutations was higher among children with PP and adolescents with PCOS compared to adult women with PCOS (Witchel et al., 2005). Given that the source of androgens may differ in PP (primarily adrenal) compared to PCOS (ovarian and adrenal) and may differ by ethnic origin, it is not surprising that the frequency of heterozygosity for CYP21 mutations varies between populations. Thus, heterozygosity for CYP21 mutations may be one of several risk factors for PP and/or PCOS that play a greater role in the pathophysiology of these disorders in some populations, i.e. American and Greek children, than in others, i.e. adult women with PCOS.

A polymorphism in another gene, PPARγ2, appears to influence outcome for girls with premature pubarche (Witchel et al., 2001). The PPARs are orphan nuclear receptors involved in adipocyte differentiation, glucose homeostasis, and inflammation (Kersten et al., 2000). A common genetic variant of the PPARγ2 gene, P12A, is a mild loss of function mutation which, in large population studies, appears to be protective against T2DM (Lohmueller et al., 2003; Altshuler et al., 2000). Effects of this variant are influenced by context and environmental factors (Luan et al., 2001). In our study, we found that the P12A variant of PPARγ2 is more common in obese children with premature pubarche is associated with an increased risk for obesity during adolescence independent of risk due to pre-existing obesity. Curiously, the lean mutation carriers tended to remain lean. Hence, the P12A variant may be a genetic marker indicating risk for obesity persisting into adulthood (Witchel et al., 2001a). How PPARγ2 translates nutritional and metabolic signals into changes in gene expression appears to be influenced by gene-environmental interactions (Luan et al., 2001).

It has been suggested that common genetic variants contribute to the risk for PCOS, T2DM, hypertension, and coronary artery disease. Consistent with this hypothesis, we found that children with PP and adolescent girls with incipient PCOS tended to have more genetic variants in the candidate genes studied than did the healthy control women (Witchel et al., 2001b). A genetic variant in the aromatase gene has been associated with PP in Spanish girls and with PCOS in young British women, respectively (Petry et al., 2005).

7. Low birth weight

Accumulating evidence suggests that low birth weight is associated with increased risks for coronary artery disease and T2DM in adulthood (Hales and Barker, 2001). Among Spanish
8. Summary

PCOS is a common heterogeneous polygenic multi-factorial disorder associated with increased risks for abnormalities of carbohydrate metabolism and infertility. PP due to PA pre-disorder associated with increased risks for abnormalities of glucose metabolism resulting in mild diabetes in adulthood (Hattersley et al., 1998). In a different population, intra-uterine growth retardation was associated with hyperinsulinemia and not hyperandrogenism (Jaquet et al., 2000). More recent data implicate the transition from low birth weight to greater post-natal catch-up growth with disease risk (Bhargava et al., 2004; Forsen et al., 2000). Even in normal children, there appears to be a relationship between birth weight, post-natal growth rate, and onset of adrenarche in that highest DHEAS concentrations were found among the lowest birth weight children with the highest rate of post-natal weight gain (Ong et al., 2004).

In addition to environmental factors associated with intra-uterine growth retardation, birth weight and risk for T2DM may be influenced by genetic factors. This is illustrated by comparison of mothers and infants carrying mutations in the glucokinase (GCK) gene. Mutations in the GCK gene are associated with MODY2, an autosomal dominant monogenic form of diabetes mellitus. When mothers and infants are concordant for presence or absence of mutations, infants have normal and comparable birth weights. When only the mother carries the mutation, the infant is large as would be anticipated for the infant of a diabetic mother. However, when only the infant carries the mutation, the infant has a lower birth weight presumably due to lower fetal insulin concentrations as well as an increased risk to develop mild diabetes in adulthood (Hattersley et al., 1998).

<table>
<thead>
<tr>
<th>Potential factors influencing development of PCOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic factors</td>
</tr>
<tr>
<td>Hypoandrogenism</td>
</tr>
<tr>
<td>Low birth weight and post-natal catch-up growth</td>
</tr>
<tr>
<td>Excessive peri-pubertal weight gain</td>
</tr>
<tr>
<td>Insulin resistance</td>
</tr>
<tr>
<td>Hypersensitivity</td>
</tr>
<tr>
<td>Sedentary lifestyle</td>
</tr>
<tr>
<td>Body composition/flat distribution</td>
</tr>
</tbody>
</table>

Fig. 3. Outcome of PP. PP is associated primarily with excessive adrenal androgen secretion. With gonadarche, ovarian androgen, estrogen, and progesterone secretion increase. Post-pubertal weight gain appears to be one factor associated with progression from PP to PCOS.

Acknowledgements

Supported in part by grants from the National Institutes of Health R29-HD334808 (SFW) and 5M01-RR-00084 (GCRC), Pharmacia Educational Research Fund (SFW), and the American Heart Association (SFW).

References
Dernaik, A., Bird, R.S., Dunaif, A., 2006. Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance. J. Clin. Endocrinol. Metab. 91, 492-497.
are not at increased risk for hyperandrogenism. J. Clin. Endocrinol. Metab. 85, 470–485.


