REVIEW

Developmental origin of polycystic ovary syndrome – a hypothesis

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Abstract

Polycystic ovary syndrome (PCOS) is a common but complex endocrine disorder and is a major cause of anovulation and consequent subfertility. It is also associated with a metabolic disturbance, characterized by hyperinsulinaemia and insulin resistance that carries an increased risk of type 2 diabetes in later life. Despite its prevalence little is known about its aetiology, but there is increasing evidence for an important genetic involvement. On the basis of experimental observations in the prenatally androgenized sheep and rhesus monkey, and supported by data from human studies, we propose that the clinical and biochemical features of PCOS can arise as a consequence of genetically determined hypersecretion of androgens by

the ovary during, or very likely long before, puberty. The resulting hyperandrogenism results in ‘programming’ of the hypothalamic–pituitary unit to favour excess LH secretion, and encourages preferential abdominal adiposity that predisposes to insulin resistance. The severity of hyperinsulinaemia and insulin resistance (which has a profound influence on the phenotype of PCOS) is further influenced by both genetic factors (such as polymorphism in the insulin gene regulatory region) and environmental factors, notably obesity. This hypothesis therefore suggests a unifying, ‘linear’ model to explain the aetiology of the heterogeneous phenotype.

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Introduction

Polycystic ovary syndrome (PCOS) is the most common, yet complex, endocrine disorder affecting women in their reproductive years. Its complexity stems from the syndrome’s typical heterogeneity (Table 1) and its unknown aetiology. There is increasing evidence to support a major genetic basis for PCOS, since the syndrome is strongly familial (Franks *et al.* 1997, Legro *et al.* 1998a). It is clear, however, that more than one gene (and probably several) contribute to the heterogeneous phenotype (Franks *et al.* 1997, Urbanek *et al.* 1999) and the clinical and biochemical presentation is undoubtedly influenced by additional environmental factors, such as diet and exercise (Huber–Buchholz *et al.* 1999). Given the complex interactions of such variables on the PCOS phenotype, a single developmental origin for the heterogeneous PCOS characteristics (Table 1) might seem unlikely. Nevertheless, results from recent experiments using animal models, together with supporting clinical evidence, lead us to propose that the development of PCOS is a linear process with an origin before adolescence (the contemporary clinical perception of age of onset of PCOS). Super-

imposed on this developmental process are interacting genetic and environmental factors that may alter phenotypic expression of PCOS during adult life, particularly the susceptibility to anovulation (White *et al.* 1995, Franks *et al.* 1997, Chang *et al.* 2000).

In utero androgen excess programmes for PCOS in non-human primates and sheep

Adult, female rhesus monkeys exposed, *in utero*, to levels of testosterone equivalent to those found in fetal males show many clinical and biochemical features of PCOS. They particularly exhibit hypersecretion of luteinizing hormone (LH), abnormal insulin secretion or action and, in obese (hyperinsulinaemic) individuals, hyperandrogenic anovulation (Abbott *et al.* 1998, Eisner *et al.* 2000). These observations have been verified in recent studies of sheep, in which exposure of the pregnant ewe to large doses of testosterone causes increased LH secretion and abnormal ovarian cycles in female offspring (Padmanabhan *et al.* 1998, Robinson *et al.* 1999). Interestingly, prenatally androgenized females in both species develop enlarged ovaries with multiple, medium-sized antral follicles.

Table 1 Important disorders of reproduction, metabolism and general health that are manifest in women with polycystic ovaries: their combination and degree of expression are highly variable between individuals, including first-degree relatives

Reproductive disorders

Polycystic ovaries
Hyperandrogenism (hirsutism, acne, androgenic alopecia)
Anovulation (amenorrhoea, oligomenorrhoea)
Hypersecretion of LH
Increased risk of early miscarriage

Metabolic disorders

Hyperinsulinaemia and insulin resistance
Impaired pancreatic β -cell insulin secretion and type 2 diabetes
Obesity (including preferential abdominal adiposity)
Hyperlipidaemia

Disorders of general health

Increased cardiovascular disease risk factors
Endometrial cancer

How, then, do these studies further our understanding of the aetiology of PCOS in women? They certainly suggest that abnormal LH and androgen secretion, menstrual cyclicity and insulin secretion and action represent exposure of the female fetus to very high levels of androgen. A similar phenomenon in PCOS seems unlikely since any maternal source of excess androgen production is unlikely to affect the human female fetus. Even pregnant women with extremely high circulating levels of testosterone (due, for example, to an ovarian thecoma) are unlikely to have a virilized female child (McLamrock & Adashi 1992). Together, high circulating concentrations of sex hormone-binding globulin and efficient placental metabolism of androgens provide an effective buffer against excess maternal androgen reaching the fetal circulation. In other words, it is difficult to imagine that hyperandrogenism is commonly passed across the placenta from a mother with PCOS to a previously unaffected daughter, unless other circumstances exist that compromise placental function, such as placental aromatase deficiency, stress or inadequate diet.

It is more likely that a hyperandrogenic fetal ovary (Barbieri *et al.* 1986, Beck-Peccoz *et al.* 1991), hyperandrogenic adrenal cortex (Barnes *et al.* 1994), or both, are sources of excess prenatal androgen production. Barnes *et al.* (1994) noted that patients with adrenal hyperandrogenism, due to 21-hydroxylase deficiency, also showed evidence of polycystic ovaries and excess ovarian androgen production. Adrenal androgens may be used as a substrate for ovarian androgen production. Both the fetal and adult ovary are able to convert steroid precursors, including adrenal dehydroepiandrosterone sulphate, to potent androgens (and thence to oestrogens) (Payne & Jaffe 1974, Bonser *et al.* 2000).

Androgens produced during differentiation are potent gene transcription factors and induce other critical transcription factors (such as *c-fos*) that interact with their own receptors in many fetal tissues, permanently enhancing

gene expression (including increased serine phosphorylation of the cAMP response element) (Auger *et al.* 2001). It is therefore feasible that fetal androgen excess in human females simultaneously reprogrammes multiple organ systems that will later manifest the heterogeneous phenotype of PCOS (Table 1). Virilization of female genitalia, as a potential phenotypic consequence of fetal androgen excess, does not occur in women with PCOS. Such virilization, however, does not reliably accompany fetal androgen excess in either humans (Barnes *et al.* 1994) or rhesus monkeys (Herman *et al.* 2000), illustrating the subtle, but permanent, effects androgen reprogramming can exert on female physiology.

Evidence for a primary ovarian disorder affecting androgen production in PCOS

Irrespective of the mode of clinical presentation or degree of menstrual cycle dysfunction, excess production of androgen is the most consistent biochemical feature of both women with PCOS (Franks 1991, Legro *et al.* 1998b) and prenatally androgenized female rhesus monkeys (Abbott *et al.* 1998). Even women or female monkeys with typical ovarian morphology and normal menstrual cyclicity have biochemical evidence of hyperandrogenism. Although the adrenal may contribute to excess testosterone circulating in women with PCOS (Azziz *et al.* 1998) and in prenatally androgenized female rhesus monkeys, the major source of excess androgens is the ovary (Ehrmann *et al.* 1995, Gilling-Smith *et al.* 1997, Eisner *et al.* 2002).

Both *in vivo* and *in vitro* studies of theca cell function show an intrinsic abnormality of ovarian steroidogenesis. The ovarian androgen response is exaggerated in women with PCOS following stimulation by exogenous human chorionic gonadotrophin (hCG) (Gilling-Smith *et al.* 1997) or by endogenous gonadotrophin (after treatment with exogenous gonadotrophin-releasing-hormone (GnRH) analogue) (Ehrmann *et al.* 1995, White *et al.* 1995). A pronounced ovarian androgen response is also evoked by exogenous hCG in adult female rhesus monkeys, androgenized prenatally (Eisner *et al.* 2002). In women with PCOS, increased thecal steroid production in response to hCG remains evident after long-term LH suppression by a GnRH analogue (Gilling-Smith *et al.* 1997).

Cultured human theca cells from polycystic ovaries produce 20 times more androstenedione than similar cells from normal ovaries (Gilling-Smith *et al.* 1994). Recently, these observations have been confirmed in long-term cultures of human theca cells, in which increased mRNA expression for many steroidogenic enzymes was evident (Wickenheisser *et al.* 2000). These findings reflect the increase in progesterone and 17-hydroxyprogesterone accumulation (as well as androstenedione) observed in primary theca cell cultures (Gilling-Smith *et al.* 1994) and suggest a global enhancement of steroidogenesis.

Figure 1 Diagrammatic representation of our hypothesis for the developmental origin of PCOS. During gestation, placental hCG, fetal pituitary LH and genes regulating folliculogenesis and steroidogenesis individually, or in concert, result in fetal ovarian hyperandrogenaemia leading to prenatal (and potentially prepubertal) exposure of the developing female to excess androgen. Post-pubertally, the early exposure to androgen excess (i) diminishes steroid hormone negative feedback on pituitary LH producing abnormal LH secretion and (ii) predisposes to preferential accumulation of abdominal (central) adiposity that exaggerates insulin resistance (the latter are enhanced by genes regulating adipocyte differentiation, and insulin secretion and action). The resultant hyperinsulinaemia synergistically interacts with LH hypersecretion to augment ovarian steroidogenesis and to induce premature arrest of follicle development and anovulation.

These findings in cultured human theca cells prompted consideration of genes encoding steroidogenic enzymes as candidate loci in the aetiology of PCOS. One polymorphism – a pentanucleotide repeat – was identified in the promoter region of *CYP11a*. Evidence has been found for association and linkage of variants at the *CYP11a* locus with hyperandrogenism in women with PCOS (Gharani *et al.* 1997). While it is unlikely to be the exclusive cause of PCOS, variation at this locus may contribute to excess androgen production, supporting the view that there is a genetically determined abnormality of ovarian function. It is possible that abnormal theca cell function is the consequence of abnormal ovarian follicular development. As yet, there is little evidence for a genetic basis for impaired follicular development in PCOS since the recent findings implicating the follistatin gene in the aetiology of the syndrome (Urbanek *et al.* 1999) remain unconfirmed (Urbanek *et al.* 2000b). Nevertheless, such studies reinforce the possibility that abnormal ovarian folliculogenesis may indeed be the key ovarian abnormality.

Abnormal LH secretion is secondary to ovarian dysfunction

In anovulatory PCOS women, the predominant reason for high serum LH concentrations (representing

increased LH pulse amplitude and – in some studies – pulse frequency) is abnormal negative feedback that would otherwise be provided by cyclical changes in gonadal steroids. If, for example, LH levels are monitored regularly over a period of several weeks, tonically high serum LH concentrations may fall into the normal range if a spontaneous ovulatory cycle occurs (Franks 1989, Taylor *et al.* 1997). Nevertheless, LH secretion remains higher than normal (although significantly lower than in anovulatory subjects) in women with polycystic ovaries and regular cycles, but who have symptoms and signs of hyperandrogenism (Franks 1989, 1991). This also is a feature of prenatally androgenized rhesus monkeys and ewes, suggesting that *in utero* exposure to androgen may permanently diminish hormonal negative feedback on the hypothalamic–pituitary axis, thereby stimulating androgen hypersecretion. Indirect evidence for such *in utero* programming in humans is provided by elevated serum LH levels in women with hyperandrogenaemia from classical 21-hydroxylase deficiency, an adrenal cause of hyperandrogenism (Ehrmann *et al.* 1995). The mechanism for this LH hypersecretion is not entirely clear, but recent data suggest that it involves impaired negative feedback on LH secretion mediated by either oestradiol or progesterone in women with PCOS (Eagleson *et al.*

2000), prenatally androgenized female rhesus monkeys (Steiner *et al.* 1976) and prenatally androgenized ewes (Robinson *et al.* 1999).

The origin of insulin resistance and its relationship to the mechanism of anovulation

Anovulatory women with PCOS are relatively hyperinsulinaemic and more insulin resistant than weight-matched control subjects (Dunaif 1997). Causes of the metabolic abnormalities in PCOS remain uncertain, but include an intrinsic abnormality of post-receptor insulin signalling (e.g. excess serine phosphorylation) and abnormal insulin secretion (Holte *et al.* 1995, Dunaif 1997, Eisner *et al.* 2002). Female rhesus monkeys exposed to androgen excess *in utero* also exhibit specific impairments of insulin secretion or insulin action depending on whether the androgen excess occurred during early or late gestation respectively (Eisner *et al.* 2000). The key question is whether these features represent a primary defect in the insulin-signalling pathway (or in the β cell) or whether they reflect the abnormal androgen environment. In support of the former, three recent reports have implicated polymorphisms in women with PCOS for genes involved with insulin secretion and with the insulin receptor (Waterworth *et al.* 1997, Urbanek *et al.* 2000a, Tucci *et al.* 2001). On the other hand, Holte *et al.* (1995) observed that weight reduction in obese women with PCOS significantly improved insulin sensitivity, noting also that the post-diet insulin sensitivity index, after reduction of abdominal adiposity, was normalized compared with weight-matched control subjects. This finding supports the hypothesis that body fat distribution is a major determinant of insulin insensitivity in PCOS. Our hypothesis is that the endocrine environment (in particular hyperandrogenaemia) during development (and especially during prenatal life and puberty) has a profound effect on body fat distribution, with a proclivity to abdominal adiposity, thus predisposing to insulin resistance. This hypothesis is supported by data from prenatally androgenized rhesus monkeys that selectively deposit fat intra-abdominally and exhibit insulin resistance. Of course, other factors may affect insulin secretion and sensitivity, including the age of the female fetus when exposed to androgen excess (Eisner *et al.* 2000). Thus, evidence for abnormal insulin receptor phosphorylation or impaired β -cell function does not refute the possibility that androgen-dependent body fat distribution is a cause of insulin resistance in PCOS.

Finally, we suggest that hyperinsulinaemia contributes to the mechanism of anovulation in PCOS in women, prenatally androgenized female rhesus monkeys and ewes. Ovarian steroidogenesis is spared from the effects of insulin resistance and therefore is responsive to the high circulating concentrations of insulin. Insulin synergistically interacts with LH to augment steroidogenesis and to

induce premature arrest of follicle development (Willis *et al.* 1998).

Prenatal androgen excess provides the developmental origin for PCOS

We therefore conclude that although PCOS is a complex, heterogeneous disorder, most – if not all – of the clinical and biochemical features can be explained on the basis of a developmental disorder of ovarian androgen production (Fig. 1). This fetal (and/or prepubertal; Ibanez *et al.* 1999) androgen excess ‘programmes’ the hypothalamic–pituitary control of LH, enhances visceral fat distribution (thus predisposing to insulin resistance and anovulation) and causes the clinical manifestations of hyperandrogenism in adulthood. Other secondary genetic and environmental (particularly dietary) factors may interact with this underlying linear process to modify the final phenotype and produce the heterogeneous nature of the syndrome that afflicts so many women (Fig. 1). Such a defined, developmental aetiology for PCOS holds great promise for targeted clinical interventions that not only eliminate expression of the adult phenotype, but also improve the constellation of metabolic derangements associated with this disorder.

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