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Neuroendocrine Dysfunction in PCOS: A Critique of Recent Reviews

Suhail A. R. Doi, PhD, FRCP

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Reprint Requests:
Dr. Suhail A.R. Doi
Department of Medicine
Kuwait University
P.O. Box 24923 Safat
13110 Kuwait
Fax +44 709 2377990
Email: sardoi@gmx.net

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Blank et al[1,2] have written comprehensively on neuroendocrine dysfunction in polycystic ovary syndrome (PCOS) in the recent literature, but have made several assertions which I find difficult to tie in with the current knowledge base. In this critique, I therefore focus on the three areas of disagreement below and argue the case for a more satisfactory explanation of these three issues based on the evidence available to us today and studies from our group.

The Relationship Between Luteinizing Hormone (LH) and Hyperandrogenism

Blank et al[2] state that "...the concomitant disruption of neuroendocrine function would serve to further exacerbate hyperandrogenaemia..." and this is also depicted in their schema.[1,2] Therefore, in their reviews they tend to imply that LH excess worsens hyperandrogenism through ovarian stimulation of androgenesis. This is unlikely to be valid despite the fact that a major neuroendocrine component of PCOS involves alterations in LH secretion, and primary LH excess has long been considered the cause of the excess ovarian androgen secretion in PCOS. This concept simply arose because of the known stimulatory effect of LH on theca cell function and the elevation of serum LH levels at baseline and in response to gonadotrophin releasing hormone (GnRH) in classic PCOS. Such a primary role of LH excess is arguable, however. Theca cells from polycystic ovaries secrete abnormal amounts of steroids in culture, both before and after LH stimulation.[3]

Furthermore, when patients with PCOS are given a fixed dose of the LH analogue human chorionic gonadotropin, they have 17-hydroxyprogesterone and androstenedione hyper-responses[4,5] that continue to be manifest despite correction of their baseline LH abnormality by 1 month of GnRH agonist treatment.[6] This observation even led to the use of GnRH as a specific test of the combined function of the ovarian follicular compartments.

Patients with classic PCOS (hyperandrogenism with elevated LH or ultrasonographic abnormality or both) will characteristically respond to such a GnRH agonist challenge test with hyper-responsiveness of plasma 17-hydroxyprogesterone and, to a lesser extent, androstenedione.[7,8] This pattern

of steroid secretion suggests that there is generalized dysregulation of ovarian androgen secretion, which is particularly prominent at the level of 17-hydroxylase and 17, 20-lyase activities. This is in contrast to normal women who, when given a single dose of a potent GnRH agonist like nafarelin or leuprolide, have a premature LH and follicle stimulating hormone surge that is of preovulatory magnitude[7,8] and in whom plasma estradiol and estrone rise 3-fold within 24 hours. This process in normal women is fairly efficient and there is relatively little elevation in the blood level of estradiol precursors.

The former type of ovarian dysfunction (the excess rise in estrogen previously mentioned) typical of PCOS has been found in more than half of hyperandrogenic patients, many of whom lack the classic criteria for the diagnosis of PCOS. This was demonstrated in a study[9] where an elevated plasma 17-hydroxyprogesterone response to GnRH agonist was found in 58% of hyperandrogenic patients. Interestingly, 96% of such responders had an abnormal dexamethasone androgen-suppression test, suggesting that it too was equally specific for PCOS.

It can be argued that these data, however, do not resolve the question of whether the abnormal theca cell response is simply a consequence of residual thecal cell hyperplasia from antecedent LH stimulation,[10,11] but data have begun to accumulate that are against such a primary role for LH excess in this type of ovarian dysfunction. To begin with, such ovarian syndromes (ovarian hyperandrogenism) have the same frequency with or without the classic PCOS feature of LH excess[9] even though it could be argued that these patients may secrete an LH molecule with enhanced bioactivity,[12,13] or that serum gonadotropin levels inversely correlate with the adiposity of patients with PCOS,[14,15] and as such the hyperinsulinemia of obesity amplifies the effect of normal gonadotropin levels. Perhaps the most important factor weighing against a primary role of LH excess as a cause of hyperandrogenism is the desensitization process.

The downregulation response in normal women (called homologous desensitization) is because normal ovarian thecal cells respond to LH in a biphasic manner, increasing their steroid output in response to LH levels only to a point (at LH levels in the range normal for the early to midfollicular phase of the menstrual cycle), and ceasing to respond when LH levels exceed this. The process of desensitization may in part involve downregulation of the number of LH receptor sites on thecal cells, and may be partly due to downregulation of thecal androgen biosynthesis by the local paracrine factors produced by the granulosa cells in response to LH.[16] In PCOS, there is escape from such desensitization and implies that the ovarian dysfunction must be independent of LH excess.

Normal thecal cells are very sensitive to the downregulating effect of LH levels within the physiologic range.[3,17] Maximal stimulation of 17-hydroxyprogesterone and androstenedione in culture normally occurs at LH concentrations approximating the upper portion of the normal range for follicular phase serum LH levels, and a further increase in LH dosage leads to no further rise. Nevertheless, the possibility exists that the disproportionate 17-hydroxyprogesterone response to stimulation by gonadotropins in patients with PCOS (and functional ovarian hyperandrogenism) might be explained by their LH levels on the LH-steroid dose-response curve being at a higher point where there could be incipient downregulation of 17,20-lyase at LH levels stimulatory to 17-hydroxylation. To refute this possibility, workers have shown that LH-steroid dose-response relationships during GnRH agonist tests[18] are similarly abnormal in patients with or without elevated serum LH levels. Following GnRH agonist administration, the responses of estradiol fall along the normal LH-steroid dose-response slope, but those of estradiol precursors do not. The apparent slope of the LH-steroid dose-response relationship is markedly abnormal for 17-hydroxyprogesterone, above but parallel to normal for androstenedione, and slightly increased for testosterone.

These data suggest that although 17,20-lyase efficiency is increased, it is increased less than that of 17-hydroxylation. These results also suggest that patients with functional ovarian hyperandrogenism, regardless of whether they have classic or nonclassic PCOS, have an LH-17-hydroxyprogesterone dose-response curve that is shifted upward and to the left. Since the steroid responses do not fall along the normal LH-steroid dose-response curve, the defect in steroidogenesis must therefore be the result of escape from normal downregulation of thecal cell secretion rather than over-stimulation by LH. If we accept these results, then the fundamental defect underlying the androgen excess of PCOS is ovarian hyper-responsiveness to gonadotropin action because of escape from downregulation and not a primary result of excess LH per se.

In vitro studies using cultured individual theca cells from polycystic ovaries also seem to be consistent with this conclusion and have again shown that the LH-steroid dose-response curves are displaced upward and leftward.[3] Using a system of propagating human theca cells in long-term culture, other workers have also shown that enhanced production of progesterone, 17-α-hydroxyprogesterone, and testosterone is a persistent biochemical phenotype of PCOS theca cells.[19] As these theca cell cultures can be maintained through multiple population doublings, the increased steroidogenic activity of PCOS theca cells compared with normal theca cells has been thought to be unlikely to reflect the influence of *in vivo* hormonal stimulation (i.e., increased LH levels associated with PCOS).

Taken together, all these observations suggest that dysregulation of androgen biosynthesis is an intrinsic property of PCOS theca cells. Nevertheless, it can still be argued that the increased transcription of steroidogenic

enzyme genes could still be the result of a stable metabolic imprinting of the theca cells imposed *in vivo* and retained *in vitro*,[19,20] rather than genetic imprinting. Also, PCOS theca cells may have increased sensitivity to some component of the culture medium that stimulates the expression of steroidogenic enzymes.[19] Studies of decreased *in vivo* steroidogenesis after pharmacologic therapy suggest that the latter mechanism may be important in the genesis of the hyperandrogenism, but this does not necessarily exclude a genetic factor that results in hypersensitive theca cells. For example, increased synthesis of testosterone and its precursors *in vivo* after human chorionic gonadotropin is alleviated by metformin therapy[21] but insulin is unlikely to be directly involved as both normal and PCOS theca cells respond equivalently to insulin in terms of steroid production.[19] In, conclusion, the evidence suggests that LH is not a major player in the hyperandrogenism of PCOS, and excess LH may be a consequence of the metabolic alterations in PCOS.

Why is There LH Excess in the First Place?

Blank et al[2] go on in their review to propose that neuroendocrine abnormalities are partly related to androgen-dependent decreases in GnRH pulse generator sensitivity to the negative feedback actions of ovarian steroids. Our group had already proposed this in 2005,[22] and hence their claim is not valid. Second, we have taken this concept much further than is depicted by Blank et al in their reviews.[1,2] We demonstrate that rather than testosterone, there is a significant association of elevated androstenedione levels with neuroendocrine dysfunction in PCOS.[22] This conclusion was reached via inferential studies as follows. Since it is known that the absence of physiologic levels of estradiol allows rises in gonadotrophins in both men[23] and women,[24] this suggests that it is estradiol rather than androgens that modulate GnRH feedback. However, experiments in the Rhesus monkey have shown that the suppression of pulsatile GnRH release is relatively constant in the face of widely varying levels of estradiol in the peripheral circulation,[25] suggesting that modulation of the hypothalamic GnRH "pulse generator" by estradiol in the physiologic range is subject to another factor. This factor must be ovarian since total blockade of the electrical activity of this pulse generator has been demonstrated in ovariectomized monkeys by physiological levels of exogenous estradiol.[25] This raises the possibility that estradiol feedback is at the hypothalamus but is modulated by an ovarian factor that is normally present during the menstrual cycle and keeps this neuronal system uncoupled from excessive inhibition by estrogen. We then demonstrate by mathematical modeling[22] that androstenedione might be the factor that enhances uncoupling of hypothalamic GnRH secretion from the effect of estradiol, thus leading to accelerated activity of this pulse generator. This uncoupling hypothesis is not new and was actually first suggested as a mechanism for excess LH in PCOS by Yen[26] back in 1980. What had not been recognized then was the putative role of androgens in the pathogenesis of this effect.

Of the various factors identified in our study,[22] we only were able to associate androstenedione with hypothalamic uncoupling since insulin resistance was the only other (non-estradiol) factor identified by our model as being a predictor of LH status but was not associated with high LH. In retrospect, other studies[27] also support such a role for androgens, since it has been shown that serum testosterone is the baseline variable that differs between LH groups in PCOS, and controlling for testosterone removes the differences in LH responsiveness between groups. As testosterone and androstenedione are linearly related in PCOS,[22] this could very well have been an effect of androstenedione rather than testosterone. In our study, almost none of the women with high LH had a normal androstenedione level, suggesting that androstenedione might be the factor that uncouples GnRH from inhibition by estradiol. Indeed the data previously reported by the same group[28] supports our finding, since they demonstrate that blockade of androgen action for 5 weeks restores the sensitivity of the GnRH pulse generator to feedback inhibition by estradiol and progesterone. Further support of this is lent to by studies where anti-androgens like flutamide do reduce LH levels in women with PCOS but not in normal women. Indeed, flutamide administration to hyperandrogenic women is followed by a decrease in LH pulse amplitude associated with an apparent decline in mean LH concentrations.[29-31] Androstenedione concentrations significantly decrease during flutamide administration, while sex-hormone-binding globulin and estradiol are not affected.[31] On the other hand, reduction in estradiol alone via treatment with aromatase inhibitors does not alter LH significantly and in fact increases LH pulse amplitude and frequency slightly in association with increased androgens.[32] Furthermore, in patients with androgen secreting ovarian neoplasms, LH is high but returns to normal after tumor removal.[33] Other studies have also documented that decreases in circulating androgens precede the fall in LH and ovulation following both ovarian wedge resection[34] and ovarian cauterization.[35]

However, in addition to a high androstenedione, a high normal estradiol seems to be a prerequisite for neuroendocrine dysfunction. Such estradiol levels alone, however (in the absence of high androstenedione), were very rarely associated with high LH.[22] This suggests that both hypothalamic uncoupling and an excess estradiol state are required to lead to neuroendocrine dysfunction. The mechanism of this effect might be related to the fact that estradiol is also thought to sensitize the pituitary to GnRH, providing part of the basis for the LH surge[36] and, at least in humans, there is also evidence for reduced production of GnRH at the time of the LH surge, placing even greater importance on such estradiol-mediated gonadotrope sensitivity to GnRH.[37] Estrogen has also been shown to increase the fraction of gonadotropes that respond to GnRH,[38] such that small GnRH pulses (normally insufficient to stimulate LH secretion) may result in discernible LH

pulses. Hypothalamic uncoupling may therefore enhance this positive feedback mechanism at the pituitary level in PCOS, and indeed this has been shown in other studies to correlate with the basal levels of both estrone and 17-β-estradiol.[39] Studies of normal women suggest a threshold for the estradiol effect at a minimum serum estradiol concentration sustained at 734 pmol/L (200 pg/mL) for at least 50 hours.[40] In PCOS, hypothalamic uncoupling may reduce this threshold and result in pituitary sensitization after chronic exposure to just high-normal estradiol levels as demonstrated recently by us.[22]

What is the Role of Obesity and Insulin Resistance?

Blank et al[2] imply in their review that hyperinsulinemia may bring about important changes in hypothalamic function indirectly by increasing androgen levels. On the contrary, one of the androgens, androstenedione, has clearly been shown to be negatively associated with body mass index (BMI) (and presumably insulin levels) and this decrease occurs in both PCOS and non-PCOS related obesity,[41-43] while testosterone levels and the free androgen index remain constant throughout the entire range of BMI in controls.[41] The view put forward by Blank et al[2] is based mainly on evidence from studies of insulin lowering[44-50] and may not necessarily be a correct interpretation of the relationship between insulin and neuroendocrine dysfunction. Studies that acutely increased insulin have shown that there are no significant correlations between either basal, peak, or area under the curve-insulin response during the oral glucose tolerance test and intravenous glucose tolerance test with basal testosterone or androstenedione concentrations or between insulin and androgen levels measured at 30-minute intervals throughout the oral glucose tolerance test.[51] Also, clinical studies of insulin infusion for several hours to normal and hyperandrogenic women fail to increase testosterone levels.[52] It is possible, therefore, that the effect on androgens demonstrated in the literature is merely associated with insulin lowering and in fact may result from changes in insulin-like growth factor-1 induced paracrine regulation that influences LH-induced androgen secretion.[53,54] Indeed, we were only able to demonstrate either no overall change in testosterone with obesity or a clear decline in androstenedione,[22] except for an increase in free androgen index because of insulin mediated decreases in sex hormone-binding globulin.

It is also well known that obese PCOS patients, who are also expected to be hyperinsulinemic, demonstrate reduced values for LH compared with leaner PCOS patients who tend to have higher follicular phase values than normal women.[14,55] Nevertheless, LH pulse frequency is increased in women with PCOS in comparison with normal women across the spectrum of BMI with no difference between lean and obese PCOS patients.[14,55] The evidence on the interaction between LH and obesity in PCOS is conflicting with some studies, suggesting that a higher BMI is associated with a lower basal LH,[56-59] while others do not.[60,61] We could demonstrate no difference when all PCOS women were analyzed but when only oligomenorrhoic women were included in the analysis; then a clear interaction between LH and BMI emerged.[22] The varying results in the literature may be a consequence of the varying definitions of PCOS utilized and suggests that in the absence of obesity, the mere presence of regular cycles in lean PCOS may preclude the occurrence of neuroendocrine dysfunction. In the presence of irregular cycles, mainly lean women have neuroendocrine dysfunction, suggesting that factors associated with obesity exert an effect on endogenous GnRH action at the central level in PCOS women,[14] and in contrast to the conclusions reached by Blank et al,[2] our results suggest that a fall in androstenedione and increase in insulin[22,62] might be the factors that preclude the occurrence of neuroendocrine dysfunction.

We have also documented that PCOS women with neuroendocrine dysfunction are anovulatory.[63] We also found that in women without neuroendocrine dysfunction, insulin resistance was associated with anovulation.[63] Other studies[64-66] confirm our findings that insulin resistance is associated with anovulation. Indeed, studies that use ovarian morphology to classify women with PCOS have found that only anovulatory hyperandrogenic women with polycystic ovarian morphology are insulin resistant.[65-67] Women with regular ovulatory menses and hyperandrogenism[64] or with PCOS detected by ovarian ultrasound[65,66] are not insulin resistant. We have shown in a recent study[22] that alterations in steroid hormones lead to neuroendocrine dysfunction and anovulation, and in another study[63] that alterations in progesterone/estradiol ratio leads to anovulation. Insulin resistance seems associated with both an altered central response to the steroid hormones leading to absence of neuroendocrine dysfunction in PCOS women (despite high levels of both androstenedione and estradiol), as well as altered progesterone/estradiol ratio leading to anovulation. Indeed, those women without excess LH are more obese and more insulin resistant.[22] Since multivariate analysis retains insulin sensitivity over BMI, this suggests a direct role for insulin resistance in decreasing LH, even in the presence of increased androstenedione. Since LH secretion and gonadotrophin responses to GnRH have not been shown to be directly influenced by insulin administration,[68] again this effect of insulin resistance, while associated with excess insulin, seems also to be mediated indirectly (we do not know how) and not directly through an increase in insulin levels. That the overall effect demonstrated in our studies is an interaction between the effects of steroid hormones and insulin resistance is further suggested by the fact that in other studies the LH/follicle stimulating hormone ratio does not change with differences in insulin status alone.[69]

Conclusion

Ultimately, it is getting clearer that the LH dysregulation associated with PCOS is not primary but secondary to the peripheral events within the ovary. However, in contrast to what has been published recently, the mechanism of neuroendocrine dysfunction resulting in an elevated LH in

PCOS may be an uncoupling of hypothalamic estradiol inhibition by elevated ovarian androstenedione. This abnormal secretion of ovarian androstenedione seems to be an intrinsic property of PCOS theca/granulosa cells. At a particular threshold (determined by estradiol levels), this uncoupling is associated with an estradiol-related sensitization of pituitary LH release and hence an increase in LH secretion. Finally, a case has been made to support the view that in some hyperandrogenic women with PCOS, obesity leads to decreased androstenedione synthesis or the development of insulin resistance, both of which seem to decrease LH secretion independently of each other.

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Author Affiliation

Suhail A. R. Doi, PhD, FRCP
Division of Endocrinology
Mubarak Al-Kabeer Hospital and
Kuwait University, Kuwait