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Insulin Resistance in PCOS

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Polycystic ovary syndrome (PCOS) is the commonest endocrinopathy affecting women of reproductive age, manifested with a variety of clinical signs, none of which is pathognomonic. The association of insulin resistance and reproductive abnormalities with clinical hyperandrogenism in a woman was first demonstrated by Achard and Thiers in the "diabetes of bearded woman." The link of PCOS with insulin resistance was subsequently established by clinical studies characterizing the profound insulin resistance in obese and lean PCOS patients. Insulin resistance, hyperinsulinemia, and beta-cell dysfunction are very common in PCOS, but are not required for the diagnosis. The numerous in vivo and in vitro data supporting the central role of insulin resistance in the pathogenesis of PCOS found a broad clinical application in the management of the syndrome, where the regulation of cycle abnormalities and the facilitation of pregnancy in obese PCOS patients was assisted by co-administration of agents such as the well-known insulin sensitizers. The documentation of the presence of insulin resistance contributed substantially to unravel several metabolic components present in the syndrome. Today our knowledge about PCOS appears to have broader health implications and to have profoundly altered our view of the gravity of this condition.

Key Words: Insulin resistance; PCOS; DM type 2.

Introduction

Polycystic ovary syndrome (PCOS), a complex and heterogeneous disorder, is widely accepted as the commonest cause of anovulatory infertility. It is the most common endocrine disorder of women in reproductive age and affects 6–6.7% in this population *(1–3)*. The syndrome is characterized by clinical and/or biochemical signs of excess androgen secretion and chronic anovulation. About 50–70% of all women with PCOS have some degree of insulin resistance, and this hormone insensitivity probably contributes to the hyperandrogenism of PCOS *(4)*. The definition of this enigmatic disorder still remains an unsolved problem and has been an issue of great and continuous debate among experts.

In 1990, the National Institutes of Health (NIH) sponsored a conference on PCOS where they put forward the diagnostic criteria of chronic anovulation and hyperandrogenemia, after exclusion of related disorders *(4)*.

Recently, in 2003, an experts meeting in Rotterdam, sponsored by European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), suggested that the definition of PCOS should include two of the following three criteria: (i) oligo- and/or anovulation, (ii) clinical and/or biochemical signs of hyperandrogenism, (iii) polycystic ovaries on ultrasonography, and exclusion of other hyperandrogenic disorders *(5,6)*.

Further guidelines may be necessary in order to include all possible clinical phenotypes of this syndrome, as it is evident by the preponderance of combinations of diagnostic criteria used among different groups of the specialists *(7)*. Among many hormonal and metabolic aberrations that have been associated with the syndrome, insulin resistance appears to play a leading role in the pathogenesis of PCOS, linked with increased prevalence of impaired glucose tolerance (IGT) and type 2 diabetes mellitus (DM2) *(8,9)*. In non-obese PCOS women, IGT was found in 10.3% and DM2 in 1.5% *(10)*. In the Mediterranean region, a study by Gambineri et al. showed that in women of reproductive age with PCOS (range 14–37 yr), the prevalence of IGT and of DM2 was 15.7% and 2.5%, respectively. Most interestingly, as in other insulin resistant states, PCOS appears to be associated with increased cardiovascular risk factors, dyslipidemias, endothelial dysfunction, and defective fibrinolysis *(11,12)*.

Definition of Insulin Resistance

Insulin has a broad range of metabolic and mitogenic actions in many tissues *(13)*. It is important to specify the biological action of insulin being measured as well as the tissue being considered, because its action is regulated not only by changes in its concentration but also through changes in the sensitivity of target tissues to hormone action *(13)*. Insulin resistance has been defined as a state (of a cell, tissue,

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or organism) in which a greater than normal amount of insulin is required to elicit the appropriate response *(14)*. Increased insulin secretion by β-cells is the normal response and compensatory hyperinsulinemia follows. As long as hyperinsulinemia overcomes insulin resistance, glucose levels remain normal; if β-cells compensatory response declines, relative or absolute insulin insufficiency develops, with metabolic consequences, i.e., IGT and DM2.

The WHO describes insulin resistance as a glucose uptake below the lowest quartile under hyperinsulinemic euglycemic conditions for the background population *(15)*. Reaven originally identified 25% of the general population as insulin resistant *(16)*. The European Group for the Study of Insulin Resistance took a more restricted view, defining insulin resistance as the sensitivity index (SI) of the lowest 10% of a nonobese, nondiabetic, normotensive Caucasian population *(17)*. The concept of insulin resistance is relatively easy to understand, but identifying precisely the individual with insulin resistance is a more complicated task; therefore, establishing limits for normal degrees of insulin sensitivity is arbitrary.

Great variability among populations has been shown and is influenced by age, ethnicity, and obesity; therefore, caution should be exercised when applying values from one population to another. Furthermore, although the pathogenetic mechanisms of insulin resistance remain under intensive investigation, the intracellular pathways of insulin action are only just being uncovered *(18–20)*.

Because not all women with PCOS are insulin resistant, assessment of insulin sensitivity quantitatively may allow identification of patients at higher risk for metabolic sequelae, including the development of diabetes and may also allow the selection of patients most likely to respond to treatment with insulin-sensitizing drugs.

Mechanisms of Insulin Resistance in PCOS

PCOS is characterized by hyperandrogenemia and chronic anovulation *(21–23)*, and is associated with a number of cardiovascular risk factors like insulin resistance, glucose intolerance, dyslipidemia, coagulopathy, endothelial dysfunction, and inflammation *(4–6,24,25)*.

Dunaif, in very elegant studies, has shown that insulin resistance is a unique and common finding in women with PCOS, independent of obesity, and has demonstrated that the OGTT in lean and obese women with PCOS had an abnormal hyperinsulinimic response compared with matched controls and hirsute women. Studies with the euglycemic clamp technique indicate that insulin resistance is present in both obese and nonobese women with PCOS compared to age- and weight-matched normal women *(26)*.

It has been also shown by other investigators *(27)* in lean PCOS patients compared with lean controls, that reduced insulin sensitivity is a feature of the syndrome. Furthermore, a twofold reduction in insulin sensitivity in obese PCOS has been observed, suggesting the additive role of obesity to insulin resistance in PCOS *(26,27)*.

Contradictory data also exist in the literature. Reports using an intravenous glucose tolerance test or a hyperinsulinemic euglycemic clamp found normal insulin action in normal weight PCOS patients. Ovesen et al. found in a case-control study normal basal and insulin-stimulated glucose utilization in lean PCOS patients with marked hyperandrogenemia *(28)*.

The heterogeneity of studied populations is one of the major factors that have enhanced the discrepancies between the results from different research groups. The variation depends on diagnostic criteria, methods of assessment of insulin resistance *(29)*, ethnic background, and the presence of family history of DM2 *(30)*.

The mechanisms of the impaired glucose utilization in PCOS continue to be largely unknown, despite extensive study. However, it appears that it is a result of a combined abnormality between defective insulin response of insulin-sensitive tissues to insulin action and inappropriate β-cell response to the increased demands.

β-Cell Dysfunction

β-Cell function has been shown to be significantly reduced in PCOS *(31)* during a frequently sampled intravenous glucose tolerance test. O'Meara et al. *(32)* analyzed β-cell function in PCOS and seven weight-matched controls using the hyperinsulinemic euglycemic clamp. Although glucose concentrations in both groups were within the normal range, PCOS patients had higher basal and 24-h insulin concentrations. The increased insulin concentrations reflected both a reduced clearance and an increased secretion of insulin in PCOS. By contrast, their incremental insulin secretory response to meals was markedly reduced. This reduction in postprandial response resulted from a reduction in the amplitude rather than from a reduction in the frequency of pulses. These secretory patterns resemble those of DM2 *(32)*. Furthermore, it has been found that obese adolescents with PCOS and IGT display impaired first-phase insulin secretion, decreased glucose disposition index, and increased hepatic glucose production *(33)*. Both obese and nonobese PCOS women may exhibit β-cell dysfunction and insulin resistance, but it has been shown that only obese PCOS women have increased glucose production *(30)*.

This effect of obesity and PCOS on hepatic glucose production is an important factor in the pathogenesis of glucose intolerance as is also seen in DM2, where genetically impaired insulin action is enhanced by insulin resistance, induced by environmental factors. In another study the insulin disposition index was significantly decreased by PCOS and obesity suggesting that obesity and PCOS exert a synergistic negative effect *(34)*.

Colilla et al. *(35)* examined insulin sensitivity and insulin secretion in families of PCOS patients and disclosed a heritable component to β-cell dysfunction in families of

women with PCOS. The authors concluded that heritability of β-cell dysfunction is likely to be a significant factor in the predisposition to diabetes in PCOS. Weight loss results in significantly improved insulin resistance, but the β-cell defect remains *(36)*, suggesting that it may be the primary abnormality in PCOS *(37)*.

Molecular Defects in Insulin Action

The molecular mechanisms underlying insulin resistance are not yet completely understood, but seem to be different than other insulin-resistant states, such as DM2, and are considered to be a unique and intrinsic features of PCOS *(38,39)*. The defect in insulin action in PCOS women appears to be selective, affecting metabolic but not mitogenic actions including glucose metabolism but not cell growth *(38)*. Under physiological conditions, the binding of insulin to the cell surface receptors gives rise to a long cascade of molecular alterations that lead to signal transduction and result in initiation of its actions in target tissues *(18)*. Insulin action is mediated through a protein tyrosine kinase receptor. The β-subunit of the insulin receptor contains a tyrosine kinase, whose activity is enhanced via autophosphorylation of the tyrosine residues and inhibited by serine phosphorylation. The activated tyrosine kinase phosphorylates substrates inside the cell, in order to initiate signal transduction. Among these substrates are IRS-1 and IRS-2 *(20)*. In muscle tissue, IRS-1 acts as a major docking protein that is phosphorylated in regions which contain specific amino acid sequence motifs that act as recognition sites for proteins containing SH2 domains. In the liver, IRS-2 is the primary docking protein. Phosphorylation of the tyrosine residues of IRS-1 leads to the activation of PI3-kinase, which is composed of an 85-kDa regulatory subunit and a 110-kDa catalytic subunit, resulting in the stimulation of glucose transport and activation of glycogen synthase.

To evaluate the postbinding defect in insulin action in PCOS, Book, Dunaif, and colleagues *(40)* examined the metabolic and mitogenic actions of insulin and IGF-I in cultured skin fibroblasts from PCOS and control women. They concluded that there is a selective defect in insulin action in PCOS fibroblasts that affects metabolic, but not the mitogenic signalling pathways *(40)*.

This defective signaling in insulin action was present in only half of the PCOS women examined, while in the remaining 50% of PCOS women there was no such defect, suggesting the possibility of an impairment of the signal transduction in other steps of intracellular pathways such as phosphorylation of IRS-1 or activation of PI3-kinase. Dunaif continued her research and studied an insulin-sensitive tissue, skeletal muscle, where biopsies in the quadriceps muscles were performed in PCOS and control women. The data from this experiment confirmed the presence of a postbinding defect in insulin action as indicated by the significantly decreased activity of the PI3-kinase in association with insulin-mediated glucose disposal *(41)*.

Furthermore, additional experiments by the same investigator as well as others revealed different defects of insulin intracellular signal transduction. GLUT-4 glucose transporters (found in insulin-sensitive tissues, muscle and adipocytes) have been shown to be decreased in adipocytes of PCOS women, independent of obesity *(42)*.

Recently, several substances produced by adipose tissue (adipocytokines) have been shown to come into play in the multiple pathophysiological defects of PCOS. An overexpression of the *resistin* gene in adipocytes may be a local determinant factor in the pathogenesis of PCOS. Resistin is a protein hormone thought to modulate glucose tolerance and insulin action. Seow et al. *(43)* compared serum resistin levels in PCOS women and in lean, healthy, age-matched non-PCOS women and showed that serum resistin levels were similar in PCOS patients and controls. However, resistin mRNA levels were twofold higher in adipocytes from PCOS patients. Panidis et al. *(44)* analyzed resistin levels, which were found to be significantly increased in anovulatory women with PCOS (BMI >25 kg/m^2). These findings suggest that resistin is unlikely to be a major determining factor of PCOS-associated insulin resistance. Adiponectin is regarded as a possible link between adiposity and insulin resistance in PCOS. Recent reports have shown that serum adiponectin levels were reduced in obese PCOS women compared to lean PCOS women and healthy controls *(45,46)*.

Insulin Resistance and Ovary Function

Several studies have demonstrated a positive correlation between fasting insulin and androgen levels in women with PCOS *(47)*. The primary defect remains unresolved as it is unclear whether hyperandrogenism results from the hyperinsulinemia or vice versa. Both insulin and IGF-1 stimulate the synthesis of androgens in ovarian tissue as was first shown in vitro experiments from polycystic ovarian tissue *(48)*.

During the past two decades a large number of in vitro studies have shown that in the ovaries of PCOS women excess insulin is capable of stimulating steroidogenesis and excessive androgen production from the theca cell system *(49)*.

Nestler et al. showed, in very well designed studies, that insulin produced a greater increase in androgen production by theca cells isolated from women with PCOS than in cells obtained from subjects without PCOS, and that this effect is mediated specifically through the insulin receptor rather than through the IGF-1 receptor *(50)*. It is possible that hyperinsulinemia as a result of insulin resistance, potentiates LH effect on ovarian theca cells to cause androgen excess, because the theca cells from PCOS women are intrinsically programmed to overproduce androgens *(51)*. The excess in local ovarian androgen production induced by excess circulating insulin may also cause premature follicular atresia and possibly contribute to anovulation *(50,52)*.

There are two other important actions of insulin, which contribute to hyperandrogenism in PCOS. The inhibition

of SHBG synthesis in liver by excess insulin may further increase the delivery of free androgens to target tissues. This relationship is so strong that SHBG concentrations have been proposed as good markers for insulin resistance *(53)*. In vivo, numerous studies have subsequently shown that both acute and chronic hyperinsulinaemia can stimulate testosterone production and that suppression of insulin levels can conversely decrease blood androgen concentrations *(54)*.

On the other hand, decreasing hyperandrogenemia by bilateral oophorectomy *(55)* or the administration of a GnRH agonist *(56)* has not demonstrated improvement in insulin resistance in PCOS.

Diamanti-Kandarakis et al. *(57)* have also reported that antiandrogen therapy did not alter insulin sensitivity assessed by euglycemic clamp, in lean and obese PCOS women.

It is possible, however, that androgens may contribute to some extent to the associated insulin resistance of PCOS, as some investigators have found that insulin resistance was partially reversed during androgen suppression *(58)* or with antiandrogen treatment *(59)*. It should be made clear, however, that not all patients with hyperinsulinemia also have hyperandrogenemia. Conn et al. *(60)* have shown that although 82% of women with DM2 had polycystic ovaries on ultrasound, only 52% had clinical evidence of hyperandrogenism and/or menstrual disturbance, suggesting that hyperinsulinemia alone is not sufficient for the expression of the syndrome. In some studies has been claimed that women with the PCOS hyperandrogenism and chronic anovulation appear to be more insulin resistant than women hyperandrogenism alone *(61)*.

References

1. Diamanti-Kandarakis, E., Koulie, C. R., Bergiele, A. T., et al. (1999). *J. Clin. Endocrinol. Metab.* **84,** 4006–4011.
2. Asuncion, M., Calvo, R. M., San Millan, J. L., Sancho, J., Avila, S., and Escobar-Morreale, H. F. (2000). *J. Clin. Endocrinol. Metab.* **85,** 2434–2438.
3. Azziz, R., Woods, K. S., Reyna, R., et al. (2004). *J. Clin. Endocrinol. Metab.* **8,** 2745–2749.
4. Zawdaki, J. K. and Dunaif, A. (1992). In: *Polycystic ovary syndrome*. Dunaif, A., Givens, J. R., Haseltine, F., and Merriam, G. R. (eds.). Blackwell Scientific: Boston, pp. 377–384.
5. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. (2004). *Fertil. Steril.* **81,** 19–25.
6. Rotterdam ESHRE-ASRM Sponsored PCOS consensus workshop group. (2004). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Hum. Reprod.* **19,** 41–47.
7. Cussons, A. J., Stuckey, B. G., Walsh, J. P., Burke, V., and Norman, R. J. (2005). *Clin. Endocrinol. (Oxf.)* **62(3),** 289–295.
8. Legro, R. S., Kunselmann, A. R., Dodson, W. C., and Dunaif, A. (1999). *J. Clin. Endocrinol. Metab.* **84,** 165–169.
9. Ehrmann, D. A., Barnes, R. B., Rosenfield, R. L., Cavaghan, M. K., and Imperial, J. (1999). *Diabetes Care* **22,** 141–146.
10. Gambineri, A., Pelusi, C., Manicardi, E., et al. (2004). *Diabetes* **53(9),** 2353–2358.
11. Legro, R. S., Kunselman, A. R., and Dunaif, A. (2001). *Am. J. Med.* **111(8),** 607–613.
12. Diamanti-Kandarakis, E., Spina, G., Kouli, C., and Migdalis, I. (2001). *J. Clin. Endocrinol. Metab.* **86,** 4666–4673.
13. Kahn, C. R. (1985). *Ann. Rev. Med.* **36,** 429–451.
14. Mantzoros, C. S. and Flier, J. S. (1995). *Adv. Endocrinol. Metab.* **6,** 193–232.
15. World Health Organization (WHO) Expert Committee on Diabetes Mellitus Second Report. (1980). Technical Report Series 646. World Health Organization, Geneva, Switzerland.
16. Reaven, G. M. (1988). *Diabetes* **37,** 1595–1607.
17. Balkau, B., Charles, M. A., Drivsholm, T., et al. (2002). *Diabetes Metab.* **28,** 364–376.
18. Rosen, E. D. and Spiegelman, B. M. (1999). *Curr. Opin. Endocrin. Diab.* **6,** 170–176.
19. White, M. F. and Kahn, C. R. (1994). *J. Biol. Chem.* **269,** 1–4.
20. White, M. F. (1998). *Mol. Cell Biochem.* **182,** 3–11.
21. Velazquez, M. E., Bellabarba, G. A., Mendoza, S., and Sanchez, L. (2000). *Fertil. Steril.* **74(6),** 1159–1163.
22. Goodarzi, M. O., Erickson, S., Port, S. C., Jennrich, R. I., and Korenman, S. G. (2003). *Metabolism* **52(6),** 713–719.
23. Talbott, E. O., Zborowski, J. V., Boudreaux, M. Y., McHugh-Pemu, K. P., Sutton-Tyrrell, K., and Guzick, D. S. (2004). *J. Clin. Endocrinol. Metab.* **89,** 6061–6067.
24. Diamanti-Kandarakis, E., Piperi, C., Kalofoutis, A., and Creatsas, G. (2005). *Clin. Endocrinol. (Oxf.)* **62(1),** 37–43.
25. Diamanti-Kandarakis, E., Palioniko, G., Alexandraki, K., Bergiele, A., Koutsouba, T., and Bartzis, M. (2004). *Eur. J. Endocrinol.* **150**, 795–800.
26. Dunaif, A., Segal, K. R., Futterweit, W., and Dobrjansky, A. (1989). *Diabetes* **38,** 1165–1174.
27. Morales, A. J., Laughlin, G. A., Butzow, T., Maheshwari, H., Baumann, G., and Yen, S. S. (1996). *J. Clin. Endocrinol. Metab.* **81,** 2854–2864.
28. Ovesen, P., Moller, J., Ingerslev, H. J., et al. (1993). *J. Clin. Endocrinol. Metab.* **77,** 1636–1640.
29. Diamanti-Kandarakis, E., Kouli, C., Alexandraki, K., and Spina, G. (2004). *J. Clin. Endocrinol. Metab.* **89(3),** 1273–1276.
30. Dunaif, A. and Finegood, D. T. (1996). *J. Clin. Endocrinol. Metab.* **81,** 942–947.
31. Ehrmann, D. A., Sturis, J., Byrne, M. M., Karrison, T., Rosenfield, R. L., and Polonsky, K. S. (1995). *J. Clin. Invest.* **96,** 520–527.
32. O'Meara, M., Blackman, J. D., Ehrmann, D. A., et al. (1993). *J. Clin. Endocrinol. Metab.* **76,** 1241–1247.
33. Arslanian, S. A., Lewy, V. D., and Danadian, K. (2001). *J. Clin. Endocrinol. Metab.* **86,** 66–71.
34. Dunaif, A. (1997). *Endocr. Rev.* **18,** 774–800.
35. Colilla, S., Cox, N. J., and Ehrmann, D. A. (2001). *J. Clin. Endocrinol. Metab.* **86,** 2027–2031.
36. Holte, J., Bergh, T., Berne, C., and Lithell, H. (1994). *Clin. Endocrinol. (Oxf.)* **41(4),** 463–471.
37. Holte, J., Bergh, T., Berne, C., Wide, L., and Lithell, H. (1995). *J. Clin. Endocrinol. Metab.* **80,** 2586–2593.
38. Sozen, I. and Arici, A. (2000). *Obstet. Gynecol. Surv.* **55(5),** 321–328.
39. Book, C. B. and Dunaif, A. (1999). *J. Clin. Endocrinol. Metab.* **84(9),** 3110–3116.
40. Dunaif, A., Xia, J., Book, C.-B., Schenker, E., and Tang, Z. (1995). *J. Clin. Invest.* **96,** 801–810.
41. Dunaif, A., Wu, X., Lee, A., and Diamanti-Kandarakis, E. (2001). *Am. J. Physiol. Endocrinol. Metab.* **281(2),** E392–399.
42. Rosenbaum, D., Haber, R. S., and Dunaif, A. (1993). *Am. J. Physiol.* **264(2 Pt 1),** E197–202.
43. Seow, K. M., Juan, C. C., Wu, L. Y., et al. (2004). *Hum. Reprod.* **19,** 48–53.
44. Panidis, D., Koliakos, G., Kourtis, A., Farmakiotis, D., Mouslech, T., and Rousso, D. (2004). *Fertil. Steril.* **81(2),** 361–366.
45. Panidis, D., Kourtis, A., Farmakiotis, D., Mouslech, T., Rousso, D., and Koliakos, G. (2003). *Hum. Reprod.* **18,** 1790–1796.

46. Orio, F., Palomba, S., Cascella, T., et al. (2003). *J. Clin. Endocrinol. Metab.* **88,** 2619–2623.
47. Burghen, G. A., Givens, J. R., and Kitabchi, A. E. (1980). *J. Clin. Endocrinol. Metab.* **50,** 113–116.
48. Barbieri, R. L., Smith, S., and Ryan, K. J. (1988). *Fertil. Steril.* **50(2),** 197–212.
49. Sam, S. and Dunaif, A. (2003). *Trends Endocrinol. Metab.* **14(8),** 365–370.
50. Nestler, J. E., Jakubowicz, D. J., de Vargas, A. F., Brik, C., Quintero, N., and Medina, F. (1998). *J. Clin. Endocrinol. Metab.* **83,** 2001–2005.
51. Gilling-Smith, C., Willis, D. S., Beard, R. W., and Franks, S. (1994). *J. Clin. Endocrinol. Metab.* **79,** 1158–1165.
52. Poretsky, L., Cataldo, N. A., Rosenwaks, Z., and Giudice, L. C. (1999). *Endocr. Rev.* **20,** 535–582.
53. Lindstedt, G., Lundberg, P. A., Lapidus, L., Lundgren, H., Bengtsson, C., and Bjorntorp, P. (1991). *Diabetes* **40,** 123–128.
54. Gambineri, A., Pelusi, C., Vicennati, V., Pagotto, U., and Pasquali, R. (2002). *Int. J. Obesity* **26,** 883–896.
55. Nagamani, M., Van Dinh, T., and Kelver, M. E. (1986). *Am. J. Obst. Gynec.* **154,** 384–389.
56. Geffner, M. E., Kaplan, S. A., Bersch, N., Golde, D. W., Landaw, E. M., and Chang, R. J. (1986). *Fertil. Steril.* **45,** 327–333.
57. Diamanti-Kandarakis, E., Mitrakou, A., Hennes, M. M., et al. (1995). *Metabolism* **44,** 525–531.
58. Elkind-Hirsch, K. E., Valdes, C. T., and Malinak, L. R. (1993). *Fertil. Steril.* **60,** 634–641.
59. Moghetti, P., Tosi, F., Castello, R., et al. (1996). *J. Clin. Endocrinol. Metab.* **81,** 952–960.
60. Conn, J. J., Jacobs, H. S., and Conway, G. S. (2000). *Clin. Endocr.* **52,** 81–86.
61. Dunaif, A., Graf, M., Mandeli, J., Laumas, V., and Dobrjansky, A. (1987). *J. Clin. Endocrinol. Metab.* **65,** 499–507.