Polycystic ovary syndrome, a pathway to type 2 diabetes

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Numerous studies have established a relation between insulin resistance (IR) and polycystic ovary syndrome (PCOS), a highly prevalent (5% to 10%) endocrine metabolic dysfunction in premenopausal women. Even though this syndrome was described in 1935 by Stein and Leventhal, it remains a matter of great controversy and interest due to its heterogeneity and complex physiopathology, which has opened a debate as to whether it is a single or a multiple disorder. Currently there are two definitions; one was proposed by the National Institute of Health Consensus Conference (1990), which defined PCOS as the presence of hyperandrogenism and oligo-ovulation in the absence of other specific causes of ovarian, adrenal, or hypophyseal origin, and the other was proposed by the Rotterdam ESHRE/ASRM Consensus Conference (2003), which defined PCOS as an ovarian dysfunction characterized by hyperandrogenism and polycystic ovarian morphology. Most women with PCOS (60% to 80%) exhibit peripheral IR, which affects predominantly muscle and adipose tissue, and a compensatory hyperinsulinemia that can be manifested independent of obesity. IR plus pancreatic β-cell dysfunction constitute a frequent comorbidity in these patients and play a major role in the long-term metabolic consequences of the syndrome, among which type 2 diabetes and cardiovascular disease should be highlighted. More than 40% of these patients develop glucose intolerance and 16% develop type 2 diabetes at the end of the fourth decade of life. Moreover, it has been demonstrated that coronary disease is more frequent in women with PCOS and that the risk of developing a myocardial infarction is seven times higher than in normal women. These important findings recently led Reaven to incorporate PCOS into the syndrome X [1], or Reaven's plurimetabolic syndrome, with its other components (type 2 diabetes, arterial hypertension, dyslipidemia, etc.); as a consequence, PCOS, which was initially described as a distinctly reproductive disorder, is currently defined as a mainly endocrine metabolic disorder. Therefore, according to these arguments, it is evident that PCOS should be considered a public health problem that affects a woman beyond her reproductive function but nevertheless leads her to consult a physician at a young age, thus offering us the valuable opportunity for the early detection of associated metabolic abnormalities. Due to the high prevalence of affected women, several years ago it was postulated that this syndrome might have a genetic basis. This has been evaluated in different populations through phenotypic and family aggregation studies. Most of this research has been conducted in relation to hyperandrogenism or ovarian morphology as assessed by ultrasound, and a high frequency of PCOS in mothers and sisters of these patients has been established. A scarce (limited) number of studies has evaluated the metabolic compromise in other family members of women with PCOS; one suggested that first-degree relatives of patients with PCOS exhibit high insulin levels and another suggested that β-cell dysfunction in families of women with PCOS is a heritable trait. According to my experience and that of my colleagues, a first study on family aggregation, based on personal interviews with healthy women, women with PCOS, and their parents, we established that the probability that a family member (brothers, sisters, parents, and grandparents) of a woman with PCOS would develop type 2 diabetes is significantly higher than in a family member of a control woman. To confirm this finding, we designed a second study in which we evaluated glucose tolerance, IR, and frequency of type 2 diabetes in 120 parents of healthy women and in 200 parents of women with PCOS. These data [2] suggest that parents of women with PCOS exhibit IR and type 2 diabetes more frequently than do those of healthy women, thus constituting a high-risk group but an ideal cohort to detect and prevent the development of type 2 diabetes. This apparently simple concept is of utmost importance because it means that patients with PCOS, due to their genetic charge, have a higher probability of developing a metabolic pathology and, hence, constitute a high-risk population. In addition, PCOS

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may be considered a marker of a family pathology because it identifies metabolic risks; as a consequence, the timely diagnosis of PCOS may allow installation of therapeutic and preventive measures, not only in the affected cases but also in the family group. Another aspect of this work, which deserves to be highlighted, is that fathers of women with PCOS exhibited metabolic alterations more frequently than did mothers. Some previous studies had suggested that the heritability of PCOS could be mainly paternal, based on the proportion of affected mothers and sisters of women with PCOS that, although high, is not absolute. Until now, it has not been possible to exclude an X-linked or autosomal dominant heritability, although the form of heritability is probably more complex, mainly due to the heterogeneity of the syndrome and the absence of the male phenotype. If PCOS has a genetic basis, then a man and a woman should carry susceptibility gene(s). Some studies have suggested that the "male PCOS phenotype" could be carried by a man with premature temporary male balding. Likewise, it has been estimated that at least two genetic alterations must coexist for the syndrome to be expressed: one of them, which is related to androgen synthesis, is referred to as the "PCOS gene," and the other is associated with the insulin signalling pathway.

In the complex physiopathology of PCOS, three types of mutually inter-related alterations stand out: a neuroendocrine dysfunction characterized by lactate dehydrogenase Luteinizing hormone (LH) hypersecretion, a metabolic disorder characterized by IR and hyperinsulinemia, and a dysfunction in ovarian and adrenal steroidogenesis. The latter is strictly necessary for the syndrome to occur and consists of hyperactivity of the enzyme CYP17 (cytochrome P450), which has 17-hydroxylase and 17-20 lyase activity and catalyzes the conversion of progesterone to 17αOH progesterone and of the latter to androstenedione (Δ4A). It has been suggested that this is a dysfunction exclusive to PCOS and may be a primary or secondary event to LH and/or insulin excess. The increase in LH and insulin in PCOS leads to an overexpression of the enzyme, resulting in higher production of intraovaric androgens and chronic anovulation through the inhibition of follicular development.

During the past 20 y, I have developed a line of research with the objective of studying the physiopathologic and etiopathogenic mechanisms of PCOS. The collaboration of other investigators, the financial support of research grants from national and international agencies, and the development and mounting of different research techniques have allowed me to accomplish these objectives. Our results have been published in various peer-review journals, the resulting publications have received international acknowledgement, and our laboratory currently serves as a national reference centre for the study of this pathology. This has resulted in a line of research that constitutes a continuum of different aspects of this syndrome, which are complementary to one another.

From 1987 and 1989, during a visiting professor scholarship funded by the Alexander von Humboldt Foundation (Germany) and under the mentorship of Professor Ludwig Wildt, head of the Unit of Reproductive Medicine and Gynecologic Endocrinology of the University of Chile, I began to study the neuroendocrine component of PCOS. We developed a simple sampling system that allowed the continuous withdrawal of blood through a heparinized catheter [3], which facilitated the study of ultradian and circadian secretion rhythms of various hormones and metabolites. While I was working on these studies, the gonadotrophic effect of insulin on the ovary was demonstrated; subsequently, a growing body of evidence emerged about the relation between IR and PCOS that constituted a true revolution in the approach to this syndrome. These studies gave origin to several Ph.D. theses in medicine and to a large number of publications.

Upon my return from Germany, in cooperation with some researchers from the Unit of Diabetes, Department of Medicine, San Juan de Dios Hospital—Gloria López, M.D., Pilar Durruty, M.Sc., and Virginia Riesco, M.Sc.—I set up my own laboratory at the Faculty of Medicine of the University of Chile (Santiago, Chile). With Marcelo Calvillán, M.Sc., Teresita Castillo, M.Sc., and Manuel Maliqueo, M.Sc., my principal colleague, we mounted and validated several biological assays and methods for the evaluation of tissue insulin sensitivity.

Years later (from 1995 to 1997), we began studies to evaluate the association between ovarian hyperandrogenism and IR in Chilean women, which demonstrated that a large percentage of women with PCOS (80%) have IR. As part of this study, we observed that administration of metformin, an insulin sensitizer and one of the drugs employed to study the mechanisms involved in IR of these patients, showed a favorable effect on lipid profile, body weight, insulin sensitivity, and reproductive alterations. This rendered us pioneers in the use of metformin as an important therapeutic tool in the management of IR in patients with PCOS in Latin America.

Always with the purpose of inquiring more into the physiopathology and origin of PCOS, in the third step of this line of research (1997 to 2000), we evaluated neuroendocrine and metabolic aspects of the syndrome by using the pregnancy/postpartum period as a study model. During the pregnancy/postpartum period, the gonadal axis presents an inhibition and reactivation phase comparable to what occurs during prepuberty and puberty but in a shorter period. We were able to establish that ultradian and circadian LH secretions were altered in women who had PCOS during lactational amenorrhea and that this phenomenon could not be explained by the dysfunction of a specific neurotransmitter. Instead, we assumed that it was probably due to a reprogramming of the Gonadotrophin-releasing hormone (GnRH) pulse generator that results from excessive exposure to androgens. There were two very important observations that supported this hypothesis: 1) during lactational amenorrhea, lactating women with PCOS exhibited enlarged ovaries associated with higher androstenedione con-

centrations compared with normal lactating women and 2) pregnant women with PCOS exhibited significantly higher androstenedione levels compared with normal pregnant women, which led us to speculate that, during pregnancy, ovaries of women with PCOS remained steroidogenically active [4]. Subsequently, we were able to show a significant increase in androgen concentrations during this period by evaluating peripheral serum androgen concentrations in normal women and those with PCOS during pregnancy. This led us to propose that these androgen concentrations served as a potential source of prenatal androgen excess for the fetus, without leading to fetal virilization [5]. Other observations that suggested a possible "fetal reprogramming" were that about 30% of our patients who had PCOS had the antecedent of having been small for gestational age and that a significant percentage of the babies born to our mothers who had PCOS exhibited the same condition [6]. To establish the effect of intrauterine life as an environmental factor in the origin of PCOS, we continued with further neuroendocrine and metabolic studies in children born to these women at different stages of sexual development. Because there is no an experimental model to address these issues in humans, it was necessary to turn to animal models.

Currently we are collaborating with studies of an experimental animal model of prenatal androgenization led by Sergio Recabarren, Ph.D., from the University of Concepción [7], which may provide us with some clues with respect to the effect of prenatal exposure to androgens in the development of PCOS. In addition and in the context of two M.Sc. theses, one in physiology and the other in clinical nutrition, we introduced the measurement of leptin. This led to three additional publications with high international acceptance [8].

More recently (2000 to 2005), we commenced a study of genetic markers of PCOS, focusing particularly on polymorphisms in genes related to insulin signaling, such as those that encode substrates 1 and 2 of the insulin receptor and that link PCOS to type 2 diabetes. Moreover, we examined other polymorphisms associated with obesity (Trp64Arg of β-3-adrenergic receptor) [9] and with hyperandrogenism (polymorphism T→C, −34 bp, of gene CYP17 promoter). To perform these studies, different molecular biology techniques were developed in collaboration with Francisco Pérez-Bravo, Ph,D., from the Institute of Nutrition and Food Technology of the University of Chile (INTA). In this process, the evaluation of a large number of families of normal women and those with PCOS led to family studies of the syndrome, which have also received good international acceptance and allowed us to initiate the study of the male phenotype of PCOS [10]. In the first project, we examined the association between the Gly972Arg genotype of Insulin Receptor Substrate 1 (IRS-1) and PCOS in a case-control and case-parent study. The most relevant results of this study showed that the frequency of the heterozygote form of the codon 972 variant was higher in patients with PCOS than in healthy women. In women with PCOS, this polymorphism interacted with obesity to influence IR; in healthy women, this polymorphism appeared to be associated with a decrease in insulin secretion. During a journal club meeting, while discussing the paper by J. Mann [11] entitled "Gene nutrient interactions in type 2 diabetes a clinical perspective," we questioned whether, through some nutrient, we could make the insulin secretion deficit more evident in a simple way. This led us to a new bibliographic search, where we came upon a publication by J. J. Ägren [12] who used oral fat-loading tests to study the postprandial responses of individual fatty acids in subjects who were homozygous for the threonin-54 or alanine encoding allele (Ala54). In our case, however, different carbohydrates were needed. Based on the concept of the glycemic index, we first proposed using breakfasts with high and low glycemic indexes but, after a discussion of the protocol with another investigator, Erik Díaz, Ph.D., from INTA, we realized how complex it was to obtain standardized breakfasts because the glycemic load of the breakfasts would depend not only on the blend of foodstuffs and dietary fiber content but also on the cooking time and other physicochemical variables that are difficult to control for the breakfasts to be perfectly comparable. We were quite disappointed but decided to employ a single high glycemic carbohydrate such as glucose and a single low glycemic carbohydrate such as fructose, followed by continuous blood sampling for several hours to assess the dynamics of secretion of plasma glucose and insulin. For this purpose, we applied our old sampling device that was initially designed to study the gonadotrophin pulsatile pattern in the early stages of our line of research.

The results indicated that PCOS could have a genetic predisposition that may manifest itself even before menarche and that environmental factors during prenatal or postnatal life could lead to clinical and biochemical expressions of the syndrome in adult life. It is unlikely that PCOS could be explained on the basis of only one genetic disorder. Identification of genetic markers may allow, in the first instance, identification and prevention of the development of type 2 diabetes and its complications in women with PCOS and their family members and, in a second instance, to establish some dietary recommendations for carriers of a specific polymorphism.

Due to the high prevalence of type 2 diabetes in women with this syndrome and their relatives, PCOS should be considered a marker of a family pathology, a pathway to type 2 diabetes, and a public health problem.

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References

[1] Reaven GM. The metabolic syndrome: requiescat in pace. Clin Chem 2005;51:931–8.

[2] Sir-Petermann T, Angel B, Maliqueo M, Carvajal F, Santos JL, Pérez-Bravo F. Prevalence of type ii diabetes mellitus and insulin resistance in parents of women with polycystic ovary syndrome. Diabetologia 2002;45:959–64.

[3] Sir-Petermann T, Recabarren S, Bittl A, Jäger W, Wild L. A simple device for serial blood collection in human subjects and animals. Exp Clin Endocrinol Diabetes 1995;103:398–401.

[4] Sir Petermann T, Devoto L, Maliqueo M, Peirano P, Recabarren SE, Wildt L. Resumption of ovarian function during lactational amenorrhoea in breastfeeding women with polycystic ovary syndrome: endocrine aspect. Hum Reprod 2001;16:1603–10.

[5] Sir-Petermann T, Maliqueo M, Angel B, Lara HE, Pérez-Bravo F, Recabarren SE. Maternal serum androgens in pregnant women with polycystic ovary syndrome: possible implications in prenatal androgenization. Hum Reprod 2002;17:2573–9.

[6] Sir-Petermann T, Hitchsfeld C, Maliqueo M, Codner E, Echiburú B, Gacitúa R, et al. Birth weight in offspring of PCOS mothers. Hum Reprod 2005; in press.

[7] Recabarren SE, Padmanabhan V, Codner E, Lobos A, Durán C, Vidal M, et al. Postnatal developmental consequences of altered insulin sensitivity in female sheep treated prenatally with testosterone. Am J Physiol 2005; in press.

[8] Sir-Petermann T, Maliqueo M, Palomino A, et al. Episodic leptin release is independent of luteinizing hormone secretion. Hum Reprod 1999;14:2695–9.

[9] Pérez-Bravo F, Echiburú B, Maliqueo M, Santos JL, Sir-Petermann T. Trp64Arg polymorphism of β-3-adrenergic receptor in Chilean women with polycystic ovary syndrome. Clin Endocrinol 2005;62:126–31.

[10] Sir Petermann T, Cartes A, Maliqueo M, Gutiérrez C, Toloza H, Echiburú B, Recabarren SE. Patterns of hormonal response to the gonadotrophin releasing hormone agonist leuprolide in brothers of women with polycystic ovary syndrome: a pilot study. Hum Reprod 2004;19:2742–7.

[11] Mann J. Gene nutrient interactions in type 2 diabetes—a clinical perspective. Exp Clin Endocrinol Diabetes 2002;110:2–5.

[12] Agren JJ, Vidgren HM, Valve RS, Laakso M, Uusitupa MI. Postprandial responses of individual fatty acids in subjects homozygous for the threonine- or alanine-encoding allele in codon 54 of the intestinal fatty acid binding protein 2 gene. Am J Clin Nutr 2001;73:31–5.