INSULIN PARADOX AND POLYCYSTIC OVARIAN SYNDROME: IMPLICATIONS ON MECHANISM AND PATHOGENESIS

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Abstract

Polycystic ovarian syndrome is a part of the most rampant metabolic syndrome experienced by women in their reproductive age. PCOS is characterized by hyperandrogenemia, oligo/anovulation and polycystic ovaries. The individuals are at high risk of long term health effects; cardio-vascular disorders, type 2 diabetes mellitus, hypertension and reduced fertility. The etiology of this disorder is not well understood. Over the years it has been noticed that PCOS can originate as a metabolic sequelae of insulin resistance and glucose metabolic anomalies. According to the insulin hypothesis, defects in insulin signaling either at the ligand –receptor level or the post receptor binding, triggers the metabolic abnormalities culminating in the pathogenesis of PCOS. Altered insulin signalling leads to insulin resistance and hyperinsulinemia effecting (1) increased gonadotropin secretion of LH hormone, (2) abnormal ovarian androgen secretion (3) reduced hepatic production of SHBG and (4) follicular atresia in ovaries. The factors inducing errors in insulin signalling can be endogenous or exogenous. Lifestyle patterns like lack of exercise, sleep and proper diet can lead to insulin resistance and initiate PCOS. Even though concrete information on the molecular mechanism of insulin action are lacking, there are substantial evidences to support the central role of “insulin hypothesis” in the pathogenesis of PCOS.

Introduction

Polycystic ovarian syndrome is the most common endocrine disorder among women of reproductive age. According to statistics by National Women’s Health Information Centre (NWHIC) about 5-10% of women of child bearing age (20-40) have PCOS. The disorder was first reported by Stein and Leventhal in 1936 in a small population of overweight women presenting symptoms of hyperandrogenemia and bilateral ovaries. The manifestation of the disorder is extremely heterogeneous and variable with following features
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- Oligo/anovulation (manifested as irregular periods i.e., oligo/amenorrhoea)
- Hyperandrogenism (high male hormones) and its physiological evidences such as hirsutism (excess body hair in ‘male pattern’ areas such as the chin and upper lip), acne or hyperseborrhea.
- Characteristic polycystic ovaries (enlarged with peripherally distributed multiple cysts) confirmed by ultrasound examination.

The complex nature of the syndrome presents constrains in diagnosis and research in the related areas. Polycystic ovarian syndrome is a polygenic trait with multiple gene and environmental factors contributing to the development and expression. Familial clustering, linkage and association studies showed candidate genes for PCOS. Genetic studies suggest PCOS as an autosomal dominant trait with low penetrance. Foetal reprogramming under prenatal androgen excess in uterus is hypothesized to be one of the reasons leading to PCOS. But the data generated is insufficient to shed light on the pathophysiology of the disorder. Hence, even after 40 years of study and research the etiology of the disorder is still a mystery.

It is only in recent times that the havoc and peril associated with PCOS has come to the fore-front. The prevalence of PCOS has significantly increased from 8% to nearly 20% globally within the past two decades. The Indian scenario is also not different; the number of women diagnosed with PCOS has doubled in the last ten years. 40-60 % of obese adolescent women in Kerala are manifesting some features of PCOS. The incidence of the disorder is irrespective of ethnicity, affluence and education. PCOS can be considered as a forerunner to other lifestyle diseases as diabetes, obesity, cardiovascular problems and reduced fertility. So more than being a gynaecological allegory PCOS has other strong health implications too. The above mentioned factors have changed the face of PCOS from an ovarian disease to a metabolic disorder embedded in factors like sedentary lifestyle and diet. Thus PCOS shows many hallmarks of the metabolic syndrome.

Many theories have been proposed towards the origin and pathogenesis of PCOS as in androgen excess hypothesis, insulin hypothesis and the genetic theory of autosomal inheritance. Many candidate genes from metabolic pathways like steroid hormone metabolism and synthesis, gonadotropin action, insulin signalling, and energy consumption are shown to have
positive association with PCOS susceptibility. But none of them were strong enough to correlate with the origin of PCOS. Strikingly, these pathways are interdependent in control and execution of the pertained roles. The protagonist in the arena is to be recognized to develop future diagnostic and management strategies.

In this review we are going to discuss the molecular networking prominent in the pathogenesis of PCOS. Being a multifactorial endocrinopathy, numerous pathways are involved in PCOS etiology. Over the past 20 years, the role of insulin signalling in the metabolic and reproductive disturbances of PCOS is renowned. The alterations in insulin signalling pathway can play as a molecular hub controlling various other key networks such as steroid signaling and gonadotropin signaling culminating in PCOS. Interestingly lifestyle and environmental modifiers of the above mentioned molecular hub can also direct towards PCOS in future. In this review we will focus on the pathophysiological role of insulin signalling in PCOS, different parameters disturbing insulin signalling and the concomitant health risks.

**Insulin signalling: A brief overview**

Insulin is a polypeptide hormone secreted by pancreatic β cells, with various metabolic and physiological functions. In addition to its dominant role in glucose homeostasis, insulin induces protein synthesis, lipid metabolism, cell growth and differentiation. The major target tissues of insulin action are liver, muscle and adipose tissue. Insulin impacts its feat through insulin receptor which belongs to a family of tyrosine kinase receptors INSR, IGF-1, epidermal growth factor, fibroblast growth factor and platelet derived growth factor. Insulin signalling action starts with binding of insulin to extracellular α subunits of insulin receptor (INSR) which causes a conformational change leading to activation of intrinsic tyrosine kinase and autophosphorylation of its β subunits. Activated INSR phosphorylates a number of downstream substrates like the insulin receptor substrate family (IRS 1–4), Gab-1, Cbl, APS and Shc isoforms, and signal regulatory protein (SIRP) family members which actively bind to INSR. Phosphorylated IRS proteins act as docking sites for several intracellular proteins with SH2 domains including phosphatidylinositol 3-Kinase (PI3K), which mediate different metabolic and mitogenic actions of insulin.

PI3K activation is a crucial step for GLUT4 translocation to the cell surface leading to glucose transport, glycogen synthesis and protein synthesis. Downstream signaling proteins of PI3K pathway are involved in
the activation of Akt (Protein kinase B) and PKC isoforms λ and ζ. Once activated, AKT enters the cytoplasm where it leads to the phosphorylation and inactivation of glycogen synthase kinase 3 (GSK3). A major substrate of GSK3 is glycogen synthase, the enzyme catalyzing the final step in glycogen synthesis. Phosphorylation of glycogen synthase by GSK3 inhibits glycogen synthesis; therefore the inactivation of GSK3 by AKT promotes glucose storage as glycogen. Another pathway leading to GLUT4 translocation involves the insulin receptor-mediated phosphorylation of the scaffolding protein CAP (c-Cbl Associated Protein) and formation of the CAP:Cbl:CrkII complex. This complex, through its interaction with flotillin, localizes to lipid rafts facilitating GLUT4 translocation. The mitogenic action (cell growth and differentiation) is mediated through binding of phosphorylated IRS1/2 or Shc with Grb-2/SOS complex leading to p21Ras and Raf-1 activation of mitogen-activated protein kinase pathway (MAPK). Inactivation of GSK3 by AKT upon receipt of an insulin signal, leads to the dephosphorylation of eIF2B thereby promoting protein synthesis and the storage of amino acid.1 AKT promotes protein synthesis also by activating mammalian target of rapamycin (mTOR). Insulin promotes the uptake of fatty acids and the synthesis of lipids, whilst inhibiting lipolysis through decreasing cellular concentrations of cAMP by activating a cAMP specific phosphodiesterase in adipocytes.

Insulin hypothesis of PCOS

Hyperinsulinaemia and peripheral insulin resistance are major metabolic dysfunctions intrinsic and unique to women with PCOS (irrespective of the BMI). About 60-80% of PCO women have insulin resistance. Insulin resistance can lead to severe consequences as Type 2 Diabetes Mellitus (T2DM). PCOS women have higher risk of developing T2DM than their normal counterparts.2

Insulin resistance is a condition where the target tissues of insulin are unable to take up glucose under normal level of insulin sensitization. But the body maintain the normal glucose level by a feedback control mechanism, by stimulating β cells to secrete excess insulin. The elevated level of insulin i.e., hyperinsulinaemia in body causes adverse effects in target tissues. The overload on β cells to produce surplus insulin, subsequently lead to its functional incompetence. Insulin level falls and blood glucose level rises leading to a phase called ‘impaired glucose tolerance’ paving the way to Type 2 diabetes mellitus.

There is an “insulin paradox” correlating the simultaneous association of
hyperinsulinaemia and peripheral insulin resistance. The insulin defects in PCOS can be of two major causes, the first one at the receptor level and the latter at the post receptor insulin signalling. According to ‘insulin paradox’ a cross reaction of increased insulin pulse resulting in suppression of its homologous receptors IGF-1 and IGF-2 is tenable in PCOS. There can be a hybrid receptor comprising half INSR dimerized with half IGF-1 receptor. Tissue specific variations in insulin sensitivity also explain to the hypothesis. The targeted resistant organ may be physiologically restrained to hyper respond under all conditions.

Another face of the insulin hypothesis of hyperglycemia induced insulin resistance in PCOS pathogenesis is the post receptor insulin signalling due to constitutively excess serine phosphorylation of insulin receptors and its downstream proteins, IRSs. The phosphorylation alters IRSs interaction with insulin receptors i.e., a decreased insulin stimulation in IRS-1 activation in turn reducing GLUT4 expression. The condition reworks insulin action on different tissues resulting in metabolic abnormalities. The serine phosphorylation is performed by extrinsic factors which can interact with insulin downstream targets and then blocking the signal propagation. The candidate molecules inducing serine phosphorylation are protein kinase C3 (PKC) and TNF α. Most importantly, TNFα need not be extrinsic always. Hyperglycemic condition can initiate reactive oxygen species induced oxidative stress in PCOS women which in turn activates pro inflammatory cytokine; nuclear factor κB (NFκB) thereby promoting the transcription of TNFα. TNFα consecutively increases the serine phosphorylation of insulin receptor substrate, leading to inhibition of downstream insulin signaling and glucose uptake. The identity of serine kinase carrying out the phosphorylation is unknown till now.

According to insulin hypothesis a unique defect in insulin action leading to hyperinsulinaemia potentiate excess androgen secretion (augmentation of LH stimulation enhancing ovarian androgen production) and anovulation. Precisely, the insulin hypothesis proposes that surplus insulin in females alter hypothalamo–gonadal-pituitary axis, ending up in PCOS characteristic features; hyperandrogenemia, follicular atrophy, oligo/anovulation and development of multiple cystic follicles in the ovaries. The raised insulin has adverse effects on adrenal gland, muscle, ovary, adipocytes, pituitary gland and liver that are not normally effects of insulin. The cumulative effects are parts of the ‘metabolic syndrome’, including high blood pressure,
increased visceral adiposity and altered lipid profile. The subjects are predisposed to increase risk of cardio-vascular diseases, obesity, stress and diabetes.

**Insulin action exacerbate GnRH signaling pathway to effect the ovarian functions**

Gonadotropin releasing hormone (GnRH) is a trophic peptide hormone synthesized in hypothalamus. It is responsible for the release of Leutinizing Hormone (LH) and Follicle stimulating hormone (FSH) from the anterior pituitary. In females LH stimulates the ovarian production of sex steroid hormones; testosterone, estrogen and progesterone and FSH is involved in the maturation of ovarian follicles. The secretion of GnRH as well as the release of LH and FSH is occurring in a pulsatile fashion. The set of neurons secreting GnRH in a synchronized manner with unique pulse frequency and amplitude are referred to as GnRH pulse generator. In human the frequency is one pulse per 60 minutes. A variation in the GnRH pulse frequency is linked to alteration in the normal LH/FSH ratio. Rapid pulse frequency of GnRH favours LH and low frequency weigh towards FSH synthesis. PCOS women are characterized by high amount of LH whereas FSH level remains normal and the normal LH/FSH ratio will be increased from 1:1 to 2:1 or 3:1. The excess LH concentration stimulates the ovarian androgen synthesis resulting in hyperandrogenemia, hirsuitism and acne.

The role of insulin on the regulation of GnRH secretion in PCOS subjects is still an intriguing question. The hypothalamic neurons secreting GnRH express insulin receptor, IGF-1 and IGF-2. The activation of IGF-1 enhances the GnRH gene expression and secretion. This mechanism underlies the fact that the metabolic derangements in insulin can affect the GnRH pulse generator. Even though studies on isolated rat pituitary cells had proven the stimulatory effect of insulin on gonadotropin releasing hormone, there is no human counterpart study. But human studies had shown that reducing insulin could ameliorate aberrant GnRH signalling.

The altered LH/FSH ratio also leads to chronic anovulation playing an apparent central role in PCOS pathogenesis. The high insulin stimulates the basal level of LH concentration which subjects the ovarian follicles to premature luteinisation. High insulin causes the developing follicle to respond to LH sensitization at an early development stage of approximately 4 mm rather than 9.5 mm in normal conditions. Further follicular growth becomes stalled.
after LH stimulation thereby resulting in follicular atresia leaving follicles immature to ovulate. Interestingly PCOS granulosa cells are shown to express higher number of LH receptors in comparison with normal cells. The concept of insulin mediated follicular premature luteinisation is further supported by reports showing an increase in LH induced progesterone secretion from smaller (<9mm) follicles retrieved from PCOS granulosa cells. But it is still not clear how pervasive will be the effect of LH surge on granulosa cell viability. Follicular atrophy is the primary cause of the oligo/anovulation, which is manifested as oligo /a monorrhoea. The stunted follicles which are functionally incompetent later turn out to be fluid filled follicles denoted as ‘cysts’ in PCOS.

Insulin action augments hyperandrogenemia to alter obesity and circadian rhythm in women with PCOS

The classical association between androgen excess in women and diabetes was first described by two French physicians Emile Achard and Joseph Thiers in 1921 as ‘diabete des femmes a barbe’ (diabetes of the bearded lady). The major concept of PCOS pathogenesis centres around insulin mediated steroidogenesis. In PCOS women with insulin resistance the surplus insulin stimulates a bifunctional enzyme cytochrome P450 17-α (has both 17alpha-hydroxylase and 17, 20-lyase activity) in ovaries and adrenal glands to produce excess androgens. It clearly explains that the male hormones in PCOS women are secreted from both ovary and adrenal gland. In ovarian theca cells, P450c17α through 17alpha-hydroxylase activity catalyzes the conversion of progesterone to 17 hydroxyprogesterone which is further via 17, 20 lyase activity converted to androstenedione. Androstenedione is later converted to testosterone by enzyme 17 β reductase. The increased P450c17α activity thus enhances the testosterone production in PCOS women. The primary mechanism underlying insulin regulation on steroidogenesis is indistinct. Substantial evidences suggest that insulin, IGF-1 and other intra ovarian factors can stimulate gonadotropin mediated steroidogenesis by elevating LH receptor messenger RNA levels, and also enhance CYP11A and CYP17 mRNA accumulation. Meanwhile LH independently also can stimulates P450c17α. It may also be due to a joint stimulation of LH and insulin to augment androgen production. Both LH and insulin have distinct signalling mechanisms. Where LH acts through G protein-linked receptor-mediated cAMP and PKA-dependent signaling, insulin kicks off a complex cascade of tyrosine phosphorylation-dependent reactions to alter gene transcription. Therefore their synergistic
enhancement of theca-cell steroidogenesis likely entails important interactions between these pathways. Precisely it is not justifiable to neglect the possibility of some common genes regulated collectively by LH-insulin venture to boost androgen synthesis. Studies on porcine theca cells demonstrated that the bihormonal effects in concert could enhance StAR and CYP17 gene expression profoundly escalating thecal cell steroidogenesis. But very less information is available on the actual molecular mechanism behind this.

Additionally insulin increases the testosterone bioavailability in PCOS women by reducing hepatic production of sex hormone binding globulin (SHBG). Circulating SHBG binds to testosterone in the blood stream thereby checking male hormone concentration. High testosterone concentration increases abdominal obesity changing the visceral mass of women. The adipose tissue in obese PCOS women is thought to be the source of TNF-α, thereby exacerbating the chaos. Visceral obesity lead to further worsening of the metabolic manifestation of PCOS as it has strong correlation with development of obstructive sleep apnea (OSA). It has also been shown that in obese men OSA, insulin resistance and obesity are allied partners in crime. A single night partial sleep deprivation is enough to contribute to hyperglycemia and insulin resistance in healthy subjects. Even though research evidences in this context are currently inadequate, the potential circadian control on the metabolic and reproductive activities cannot be overruled. These findings are strong implication towards the impact of changing lifestyle in the present era like shift working and fast food habits in making PCOS and related metabolic anomalies prevalent among the younger generation.

On the basis of the above discussed points, PCOS can be considered as an acquired disorder and thus women at the premenopausal phase can better manage the syndrome by lifestyle modifications. Its long term health implications like CVD, endometrial cancer, hypertension, obesity and fertility will affect the quality of life. Future priorities in relation to PCOS involve improvement of physical symptoms through enhancement of psycho-social situations of women.
Conclusion

Polycystic ovarian syndrome is a multiorgan endocrine failure among adolescent women, presently acknowledged as a socio-economic burden to afflicted people. In this review insulin signalling was interpreted as the dominant factor to explain the ‘theory of chaos’ in PCOS. We could discuss the chain of metabolic fates in PCOS pathogenesis initiated by aberrant insulin signalling. The extrinsic as well as intrinsic factors contributing to glucose metabolism abnormalities lead to insulin resistance thereby triggering malfunctions in hypothalamo-pituitary–ovarian axis (Figure 1). The above factor constitutes the
cornerstone in PCOS etiology. The manifestations of PCOS are normally conceived as effects rather than causes of this syndrome. But we strongly suggest a reverse psychology approach, why we should not consider these small disturbances in the starting itself as the prelude of a much precarious one.

**Figure 1.** Pictorial representation of the “insulin hypothesis” in PCOS pathogenesis. Elevated level of insulin secretion by pancreas changes the hepatic, hypothalamic and ovarian functions leading to development of hyperandrogenism and anovulation.

References:


