

Review

A Paradox: The Roles of Inositolphosphoglycans in mediating insulin sensitivity and Hyperandrogenism in the Polycystic Ovary Syndrome

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Current evidence suggests that insulin resistance contributes to the pathophysiologic features of the polycystic ovary syndrome (PCOS). The observation that insulin resistance is a central feature of PCOS leads to studies of the insulin signaling system in this disorder. This article reviews the possible role of putative insulin mediators, inositolphosphoglycans (IPG) mediators, in PCOS. We will focus on how IPGs mediate (a) hyperandrogenism and (b) insulin sensitivity in this disorder.

INSULIN RESISTANCE AND THE PATHOGENESIS OF PCOS

PCOS affects approximately 6-10% of women of reproductive age, and is characterized by chronic anovulation and hyperandrogenism. Insulin resistance, with its compensatory hyperinsulinemia, is a virtually

universal feature of PCOS¹⁻³. There is a form of insulin resistance that is intrinsic to the disorder and is present even in lean women with PCOS³. In addition, women with PCOS have an added component of insulin resistance from adiposity. About 50% to 80% of women with PCOS are obese, and obesity itself induces insulin resistance and hyperinsulinemia⁴.

Insulin resistance, along with its concomitant hyperinsulinemia, is critical in the pathogenesis of hyperandrogenism in PCOS. *In vitro* evidence shows that in isolated thecal cells from PCOS women, insulin stimulates ovarian testosterone production⁵. *In vivo* evidence also supports the role of hyperinsulinemia in the elevated circulating levels of testosterone in PCOS. Suppression of insulin release by diazoxide decreases both serum total and free testosterone levels in women with PCOS. In addition, when insulin-sensitizing agents such as metformin⁶⁻⁸, troglitazone^{9,10}, and more recently, rosiglitazone^{11,12}, are administered to women with PCOS, serum testosterone concentrations decrease together with serum insulin concentrations. Besides playing a role in the anovulatory infertility of PCOS, insulin resistance in PCOS is associated with an increased risk of hypertension^{13,14}, dyslipidemia¹⁵⁻¹⁸, type 2 diabetes¹⁹⁻²³, metabolic syndrome²⁴, and cardiovascular disease²⁵⁻²⁹.

Key words: Inositolphosphoglycans, D-chiro-inositol, Myo-inositol, Polycystic ovary syndrome, Insulin resistance

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The above observations suggest that the hyperinsulinemia of PCOS plays an important role in the hyperandrogenism of the disorder. However, these observations also suggest a contradictory tenet to the insulin resistance hypothesis in PCOS: while PCOS women are "insulin resistant" in terms of glucose disposal, their ovarian tissues remain sensitive to insulin's stimulation in terms of testosterone biosynthesis. In an attempt to provide a unifying hypothesis for these phenomena, we propose that ovarian androgen biosynthesis and glucose homeostasis in PCOS may be mediated via a signaling system for insulin that is separate from the classical tyrosine phosphate signaling mechanism.

THE INOSITOLPHOSPHOGLYCAN SIGNALING SYSTEM

Some actions of insulin may involve low molecular weight inositolphosphoglycan (IPG) mediators, also known as putative insulin mediators or second messengers³⁰⁻³². When insulin associates with its receptor, mediators of this class are generated by hydrolysis of glycosylphosphatidylinositol lipids and/or proteinated species located at the outer leaflet of the cell membrane. Released mediators are then internalized and alter the activities of purified enzymes^{33,34} and the metabolism of intact cells^{32,35} in an insulin mimetic fashion. Two different IPG mediators have been extracted from rat liver³⁶. One of these is the D-*chiro*-inositolphosphoglycan mediator (DCI-IPG) which activates pyruvate dehydrogenase (PDH) phosphatase. The other IPG mediator is the *myo*-inositolphosphoglycan mediator (MYO-IPG) which inhibits cyclic AMP-dependent protein kinase (PKA). These IPG mediators may mediate the alternate insulin signaling mechanism.

IPGS AND OVARIAN ANDROGEN BIOSYNTHESIS IN PCOS

Over a decade ago, experiments from our laboratory examined whether IPGs mediate insulin's steroidogenic actions in human placental tissue. In studies using placental cytotrophoblasts isolated from human placentae, purified liver extracts containing either the MYO-IPG or the DCI-IPG mimicked insulin's inhibitory action on aromatase³⁷ and stimulatory action on 3 β -hydroxysteroid dehydrogenase³⁸ in a

concentration dependent fashion³⁹. In this system, it was also shown that MYO-IPG may be more potent than DCI-IPG³⁹. Preincubation of the cytotrophoblasts with a polyclonal antibody that blocked IPGs completely abolished insulin's ability to either inhibit aromatase or stimulate 3 β -hydroxysteroid dehydrogenase activity. These studies suggested that the IPG system may be responsible for transducing insulin's regulation of human placental steroidogenesis.

Similarly, Romero et al. demonstrated that IPG mediators mimicked insulin's stimulation of progesterone production by swine granulosa cells⁴⁰. In addition, insulin's action on progesterone synthesis was abolished when the swine cells were preincubated with an antibody that blocked the IPG mediators⁴⁰. These observations suggested that IPGs mediate insulin's steroidogenic actions in swine granulosa cells.

In order to assess specifically in PCOS the mediation of insulin stimulated steroidogenic action by IPGs, our laboratory and collaborators in Caracas, Venezuela, obtained ovarian tissue from PCOS women undergoing gynecologic surgery⁴¹. Thecal cells, the cells responsible for ovarian testosterone biosynthesis, were isolated and cultured. Upon exposure to insulin, testosterone biosynthesis increased 10- to 12- fold in these primary thecal cultures. Experiments were also performed using a putative *chiro*-IPG mediator, named "INS-2" (provided by Insmad Pharmaceuticals). INS-2 is a synthetic pseudo-disaccharide of pinitol (3-O-methyl D-*chiro*-inositol) and galactosamine that is believed to be closely related to the pH 2.0 DCI-IPG putative insulin mediator described by Larner and colleagues^{41,43}. Exposure to the INS-2 mediator led to thecal testosterone biosynthesis in a dose-dependent fashion. In addition, the INS-2 mediator in micromolar concentrations stimulated thecal testosterone biosynthesis to a degree at least equal to and sometimes greater than that of insulin in maximal concentrations. These observations suggested that the synthetic INS-2 mediator could mimic insulin's action in stimulating thecal testosterone biosynthesis.

To confirm that insulin stimulated testosterone biosynthesis via the IPG signal transduction system, PCOS thecal cells were preincubated with the polyclonal anti-inositol antibody A23939. Preincubation with A23939 completely inhibited insulin's ability to stimulate testosterone biosynthesis. These results were confirmed by using a second anti-inositolglycan anti-

body, aIPG, that had been shown to block the action of inositolglycan mediators⁴⁴. Preincubation with aIPG similarly abolished the ability of insulin to stimulate thecal testosterone biosynthesis. The inhibitory effect of the anti-inositol antibodies was specific for insulin, since preincubation with these blocking antibodies did not affect stimulation of testosterone biosynthesis by hCG.

The results of these studies demonstrated that a synthetic DCI-IPG (INS-2) mimicked insulin's stimulation of thecal testosterone biosynthesis in a dose-responsive manner and to a degree at least equal to that of insulin. These studies also suggested that IPG mediators may serve as the signal transduction system for insulin's stimulation of human thecal testosterone biosynthesis. It is important to note, however, that these studies were not designed to identify which of the many IPGs was primarily responsible for mediating insulin's action on steroidogenesis, but as reported previously, the MYO-IPG was more potent in human placental steroidogenesis than DCI-IPG.

IMPROVEMENT OF INSULIN SENSITIVITY IN PCOS BY D-CHIRO-INOSITOL

Besides IPGs' role in transducing androgen production in PCOS, available data suggest that the IPG system also mediates glucose metabolism. Among the different species of IPG mediators that have been identified, several lines of evidence support the hypothesis that a deficiency specifically of DCI-IPG may result in insulin resistance. Although several studies have linked insulin resistance (as determined by the hyperinsulinemic-euglycemic clamp technique) with decreased urinary excretion of total DCI⁴⁵⁻⁴⁸, large urinary losses of DCI have been shown in both type 1 and type 2 diabetes using a more sensitive gas chromatography/mass-spectroscopy technology⁴⁹. This urinary loss of DCI was out of proportion to that expected in diabetic glycosuria. Furthermore, significantly decreased muscle DCI-IPG bioactivity and decreased total DCI content have been noted in needle biopsies⁴⁸ and autopsy specimens⁵⁰ from type 2 diabetic subjects compared with controls. In a study of rats, intragastric administration of DCI significantly decreased hyperglycemia in low-dose streptozocin rats and improved glucose tolerance in normal rats⁵¹. Administered intravenously to monkeys with varying degrees of insulin resistance, DCI accelerated glucose

disposal and increased the rate of decrease of plasma insulin⁵¹. These observations suggested that a deficiency in DCI-IPG may lead to insulin resistance, and that exogenous administration of DCI may enhance insulin sensitivity and improve insulin action in insulin-resistant individuals.

To determine if DCI-IPG is deficient specifically in women with PCOS, a preliminary study was conducted to compare the total DCI content in urine and blood of women with PCOS to that of age- and weight-matched normal women, and to determine if this difference may be accompanied by (a) decreased release of DCI-IPG (as determined by bioactivity) in blood of women with PCOS during an oral glucose challenge and (b) decreased whole-body insulin sensitivity⁵².

As reported in preliminary form, urinary DCI clearance was 5-fold higher in PCOS as compared with normal women ($p=0.006$). Urinary DCI clearance was also correlated inversely with insulin sensitivity as defined by the Bergman minimal model ($p=0.0003$). Furthermore, DCI-IPG release was impaired in response to insulin release in women with PCOS upon oral glucose challenge, as shown by a 3-fold $AUC_{DCI-IPG}/AUC_{Insulin}$ decrease in PCOS ($p<0.0001$)⁵².

These results confirmed a functional deficiency of DCI in women with PCOS, and suggested that DCI deficiency may be related to increased urinary DCI clearance and may contribute to insulin resistance of the syndrome. These data also corroborated a previous study showing that urinary DCI excretion was increased in insulin resistant states⁴⁹. However, these data did not rule out the existence of either an intra-tissue deficiency of DCI, or a defect in incorporating DCI into the DCI-IPG mediator.

If a functional deficiency of this specific IPG mediator, DCI-IPG, is responsible for insulin resistance in PCOS, then administration of DCI might replenish DCI-IPG stores and improve insulin sensitivity. To test this hypothesis, 44 obese women ($BMI >28 \text{ kg/m}^2$) with PCOS were given DCI 1200 mg orally or placebo once daily for six to eight weeks⁵³. Fasting serum steroids were measured and oral glucose tolerance tests were performed before and after the treatment period. Serum progesterone was obtained weekly to monitor for ovulation.

In the women given DCI ($n=22$), the mean $AUC_{Insulin}$ ($\pm SD$) curve after oral glucose challenge decreased

from 13417 ± 2467 to 5158 ± 1431 $\mu\text{U}/\text{ml}/\text{min}$ ($P=0.007$)⁵³. In women who demonstrated impaired glucose tolerance at baseline, $\text{AUC}_{\text{glucose}}$ during the OGTT decreased from 13983 ± 3625 to 10945 ± 2410 $\text{mg}/\text{dl}/\text{min}$ ($P=0.03$), resulting in normal glucose tolerance curves in the six women given DCI, but no change in the women given placebo. Diastolic ($P<0.001$) and systolic ($P=0.05$) blood pressures decreased by 4 mmHg, and plasma triglyceride concentrations decreased from 184 ± 88 to 110 ± 61 mg/dl ($P=0.002$ compared to the placebo group). These parameters did not change substantially in the placebo group. Moreover, ovulation occurred in 19 of the 22 women (86%) who received DCI, but only in 6 of the 22 women (27%) in the placebo group ($P<0.001$). Serum free testosterone in all women treated with DCI decreased by 53% from 1.1 ± 0.8 to 0.5 ± 0.5 ng/dl and did not change substantially in the placebo group ($p=0.006$ for the comparison).

The above study showed that administration of DCI enhanced insulin action in PCOS, thereby improving ovulation and decreasing serum androgen concentrations. DCI administration in PCOS also ameliorated components of the metabolic syndrome. These results support the idea that the insulin resistance of PCOS is, at least in part, related to a functional deficiency in DCI, and presumably DCI-IPG.

To further test the hypothesis that DCI deficiency contributes to the insulin resistance of PCOS independent of adiposity, a study similar to the above investigation was conducted in lean women with PCOS to determine if DCI administration was beneficial in this subset⁵⁴. In this study, 600 mg DCI or placebo once daily was administered for six to eight weeks. As in the previous study, DCI administration improved insulin sensitivity, ovulation and serum androgen levels in these lean women with PCOS. These findings confirmed that lean women with PCOS also suffered from insulin resistance and a functional deficiency in DCI may contribute to their insulin resistance.

Since DCI deficiency seems to contribute to insulin resistance in PCOS, we wondered whether some of the beneficial effects of insulin sensitizing drugs in this disorder may be mediated by improvements in DCI-IPG release. We utilized stored serum samples from a study where we administered metformin or placebo for 4 to 8 weeks, and quantified DCI-IPG release from these stored samples⁵⁵. Nineteen obese

($\text{BMI} \geq 27.5 \text{ kg}/\text{m}^2$) women with PCOS who took metformin ($n=10$) or placebo ($n=9$) were investigated. Insulin and DCI-IPG release during an oral glucose tolerance test were assessed. Treatment with metformin decreased the $\text{AUC}_{\text{insulin}}$ ($\pm\text{SE}$) compared to placebo during oral glucose challenge (-3574 ± 962 vs. $+1367 \pm 1021$ $\text{mIU}/\text{min} \times \text{ml}$, $p=0.003$). In addition, after metformin therapy, the bioactive DCI-IPG per unit of insulin ($\text{AUC}_{\text{DCI-IPG}}/\text{AUC}_{\text{insulin}}$) increased by 160%. In comparison, this value decreased by 29% after placebo ($p=0.002$). This study suggested the possibility that metformin improved insulin sensitivity in PCOS in part by improving insulin-stimulated release of DCI-IPG mediators.

In summary, these findings support the idea that DCI deficiency contributes to insulin resistance in PCOS. They further suggest that DCI deficiency in PCOS is unrelated to adiposity, since it appears to be present in both obese and lean women with PCOS. In addition, exogenous DCI administration in lean and obese women with PCOS can overcome DCI deficiency and alleviate insulin resistance in the disorder. Finally, some of the improvement in insulin sensitivity mediated by metformin may be related to an improvement in insulin-stimulated release of DCI-IPG mediators.

A UNIFYING HYPOTHESIS: THE ROLE OF IPGS IN BOTH GLUCOSE HOMEOSTASIS AND HYPERANDROGENEMIA IN PCOS

The above findings present us with an apparent paradox. If IPG mediators are responsible in part for mediating insulin sensitivity and also transducing ovarian androgen production, then one might expect that a deficiency of IPG mediators in PCOS would logically lead to insulin resistance and a low level of circulating androgens. DCI administration would therefore increase circulating androgens in women with PCOS. Yet this is not the case since DCI administration to women with PCOS improves insulin sensitivity, improves ovulation and lowers circulating androgens^{53,54}. How does the IPG transduction system explain both the insulin resistance and the hyperandrogenism of this disorder?

In this regard, it is important to emphasize that while studies have shown that DCI-IPG deficiency in PCOS may contribute to the insulin resistance of the

syndrome, studies in steroidogenesis have not identified which of the many IPGs are responsible for transducing insulin’s signal *in vivo* for testosterone biosynthesis. In human placental cytotrophoblasts *in vitro*, MYO containing IPG seems to be more potent than DCI-IPG in mediating steroidogenesis³⁹. We postulate that MYO-IPG, but not DCI-IPG, may be the primary mediator for testosterone biosynthesis in PCOS, although this theory awaits validation by further studies. In contrast, evidence suggests that DCI-IPG mediates insulin sensitivity in PCOS.

Figures 1 A-C depict our hypothesis. Levels of DCI-IPG and MYO-IPG are normal in the absence of PCOS (Figure 1A). In PCOS, a functional deficiency in DCI-IPG exists as supported by available data, and we hypothesize that there may be an increased level of MYO-IPG due to compensatory hyperinsulinemia resulting from insulin resistance (Figure 1B). Administration of DCI restores normal levels of DCI-IPG, and may improve insulin sensitivity. Decreased circulating insulin then results in decreased MYO-IPG release, reducing ovarian testosterone biosynthesis (Figure 1C). To date, we have preliminary data supporting a functional deficiency of DCI in PCOS⁵². Additional studies are underway to assess whether there is

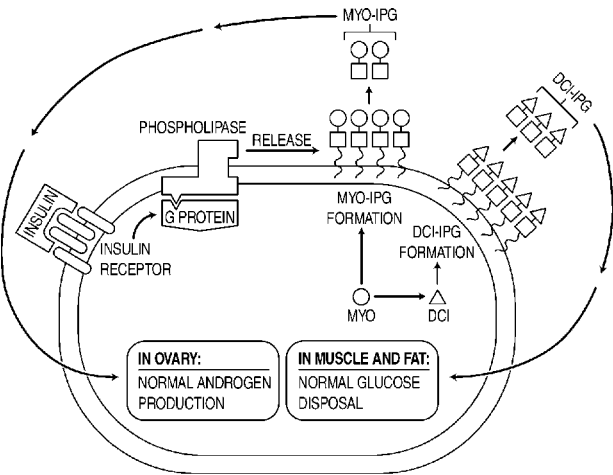


Figure 1A. Hypothesis – normal women.

Tissue IPG system	Endocrine/Metabolic Consequences
Normal DCI-IPG	→ Normal insulin sensitivity
Normal MYO-IPG	→ Either no release (insulin did not attain threshold) or Normal/minimal MYO-IPG release and constitutive ovarian androgen production

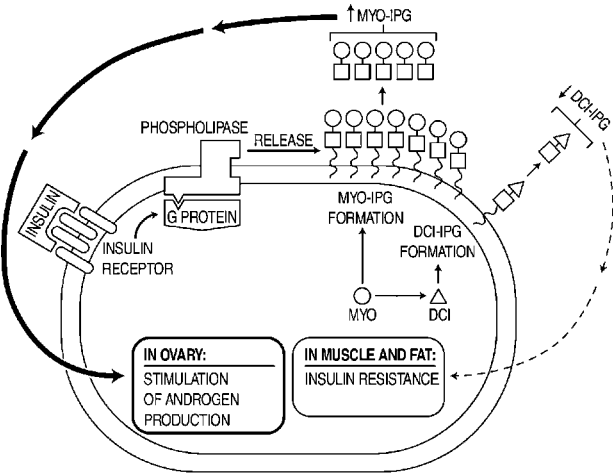


Figure 1B. Hypothesis – women with PCOS.

Tissue IPG system	Endocrine/Metabolic Consequences
Decreased DCI-IPG	→ INSULIN RESISTANCE
	↓
	Hyperinsulinemia
	↓
	Increased MYO-IPG release
	↓
	Stimulated ovarian androgen production (HYPERANDROGENIM)

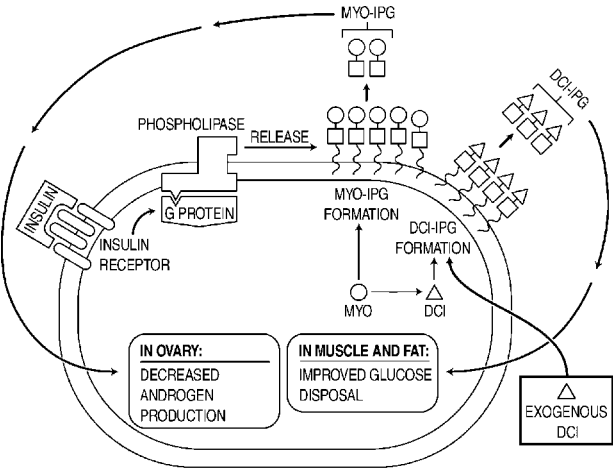


Figure 1C. Hypothesis – DCI administration in PCOS.

Tissue IPG system	Endocrine/Metabolic Consequences
Administration of DCI-IPG	→ IMPROVED INSULIN SENSITIVITY
	↓
	Reduced circulating insulin
	↓
	Decreased MYO-IPG release
	↓
	DECREASED OVARIAN ANDROGEN PRODUCTION

an excess of MYO-IPG in this disorder.

CONCLUSION AND FUTURE DIRECTIONS

IPGs play a role in both ovarian testosterone biosynthesis and insulin resistance in PCOS. Deficiency of DCI-IPG may be in part responsible for the insulin resistance in PCOS. DCI administration ameliorates insulin resistance, hyperandrogenemia and improves ovulation in this disorder. We postulate that MYO-IPG may be the primary IPG responsible for ovarian testosterone biosynthesis, but this awaits validation by future studies. Additional studies should also investigate the ratio of DCI-IPG to MYO-IPG in ovarian tissues in PCOS women. Therapeutic moieties to decrease MYO-IPG or increase DCI-IPG may be a possible research avenue to correct the hyperandrogenism and insulin resistance of this disorder.

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