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Review

Inositol in Polycystic Ovary Syndrome: Restoring Fertility through a Pathophysiology-Based Approach

Antonio Simone Laganà [1,3,*] Simone Garzon,[2,3] Jvan Casarin,[1] Massimo Franchi,[2] and Fabio Ghezzi[1]

Myo-inositol (MI) and D-chiro-inositol (DCI) are insulin second messengers, and MI is involved in follicular gonadotropin pathways which orchestrate ovulation. The tissue-specific MI/DCI ratio is modulated by insulin through aromatase and is altered in insulin resistance (IR), with reduced epimerization of MI to DCI in insulin-sensitive tissues. In ovaries, the MI/DCI ratio is 100:1, but is dramatically reduced by insulin-stimulated epimerase in hyperinsulinemic women with polycystic ovary syndrome (PCOS). Inositols have proved to be effective in PCOS, improving metabolic and hormonal state, and restoring spontaneous ovulation. In assisted reproductive technology, inositol improved ovarian stimulation parameters, although data concerning fertility outcomes are conflicting. Given their functions, inositols are an attractive treatment option for PCOS, although well-designed studies on spontaneous and non-spontaneous fertility are needed.

Highlights

PCOS is a heterogeneous, multifaceted, and complex disorder associated with metabolic and hormonal impairments, ovarian dysfunction, menstrual irregularity, and infertility.

PCOS results from a vicious circle of androgen excess favoring abdominal adipose tissue deposition and visceral adiposity by inducing insulin resistance and compensatory hyperinsulinism which further facilitates androgen secretion by the ovaries and adrenal glands.

Oral supplementation with MI, DCI, or their combination can improve metabolic patterns and ovarian function in PCOS patients.

An MI:DCI ratio of 40:1 is considered an appropriate strategy to improve fertility outcomes, whereas an excess of DCI may have a detrimental effect on oocyte development.

Introduction

Polycystic ovary (see Glossary) syndrome (PCOS) is a heterogeneous, multifaceted and complex disorder associated with metabolic and hormonal impairments, ovarian dysfunction, menstrual irregularity, and **infertility** (Box 1) [1].

Although the Rotterdam criteria have been widely accepted, it has recently become clear that dysmetabolic features of IR are a further clinical element that needs to be taken into account [2]. In the past decade, substantial *in vitro* and *in vivo* evidence has supported the pivotal role of IR and compensatory hyperinsulinemia in the pathogenesis of PCOS (which is present in ~80% of obese women with PCOS, and in 30–40% of lean women) [1,3] (Box 2).

Nevertheless, although the role of IR and related hyperinsulinemia is widely accepted, some women who exhibit extreme obesity and IR do not develop PCOS [4]. Therefore, a prerequisite for developing PCOS may be the concomitant abnormal secretion of **androgens**, and a primary defect that favors androgen excess is thought to be essential for PCOS development in response to **insulin** or other triggering factors [5] (Box 3).

Whether IR or abnormal androgens secretion are the primary causes of PCOS is a subject of major debate, and constant efforts are being made to understand the complex pathogenic network underling the syndrome [6–8]. Regardless of the initiating factor of PCOS, treatment of IR and hyperinsulinemia could return the metabolic and hormonal state to homeostasis, and thereby alleviate ovarian dysfunction, anovulation, and finally infertility.

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Box 1. Polycystic Ovary Syndrome

Women with PCOS are characterized by hyperandrogenemia, hyperinsulinemia, and hypothalamic–pituitary–ovarian axis dysfunction [62]. First described by Drs Stein and Leventhal in 1935 [63], PCOS is one of the most common causes of infertility in industrialized countries (prevalence 6–25%) [8,64]. As recommended by an National Institutes of Health Expert Panel on PCOS, this syndrome should be diagnosed according to the Rotterdam criteria [65] when at least two of the following features are present: (i) clinical or biochemical hyperandrogenism, (ii) oligo-anovulation, and (iii) polycystic ovaries [66]. Four different phenotypes of PCOS have been identified (type A, hyperandrogenism, chronic anovulation, and polycystic ovaries; type B, hyperandrogenism and chronic anovulation; type C, hyperandrogenism and polycystic ovaries; and type D, chronic anovulation and polycystic ovaries) [6].

A range of reproductive, endocrine, and metabolic traits are associated with PCOS, and these include anovulation, infertility, hyperandrogenism, obesity, dyslipidemia, IR, hyperinsulinism, and an increased risk of T2DM and cardiovascular disease [3,67]. IR is a major risk factor for the development of T2DM, and 30–50% of obese PCOS women develop either **impaired glucose tolerance** (IGT) or T2DM by the age of 30 [68–70]. The prevalence of **metabolic syndrome** in women with PCOS is 2–4-fold higher than in the general population, with a prevalence of metabolic syndrome in PCOS women between the ages of 30 and 40 years of >50% [71].

Women with PCOS develop a higher prevalence of cardiovascular risk factors at an earlier age than the general population [67,72]. Indeed, PCOS women often have classic cardiometabolic risk markers that are characteristics of the syndrome: obesity, reduced nitric oxide (NO) synthesis, enhanced synthesis of vasoconstricting agents, dyslipidemia, hypertension, hyperandrogenemia, and finally impaired glucose metabolism, IGT, and T2DM [67,73].

The process of atherosclerosis is characterized by early endothelial dysfunction and chronic inflammation, and both coexist in young women with PCOS who have increased serum markers of inflammation (high-sensitivity C-reactive protein) and endothelial activation (endothelin-1, soluble intercellular adhesion molecule-1, soluble vascular cell adhesion molecule-1), even without the abovementioned cardiovascular risk factors [74]. This pre-atherosclerotic vascular impairment could be related to the increased serum level of **advanced glycation end-products** (AGEs) that are reported to be an early distinct finding in women with PCOS, regardless of obesity or serum glucose levels [73]. In addition, oxidative stress may also play a key role in the development of this syndrome [38,75].

Given the possible pathogenic role of IR in the endocrine, reproductive, and metabolic disturbances of PCOS, several pharmacological and non-pharmacological approaches have been proposed to counteract the hyperinsulinemic IR [6]. For example, an improvement in insulin sensitivity through diet-induced and exercise-induced weight loss has been shown to reduce circulating androgens and improve fertility [6,9–11]. Similarly, insulin-sensitizing drugs such as metformin and thiazolidinediones have been studied, and have proved to be beneficial for the treatment of infertility in women with PCOS [6]. Nevertheless, although effective, insulin-sensitizing drugs can cause side effects such as nausea and diarrhea (in the case of metformin) and increased body weight (in the case of pioglitazone) that may reduce patient compliance and limit the use of these drugs [11]; in addition, troglitazone was withdrawn from the market in 2000 owing to serious idiosyncratic hepatotoxicity. Furthermore, although metformin use during pregnancy has not been associated with increased incidence of fetal abnormalities [12], other insulin-sensitizing drugs carry a teratogenic risk and should not be prescribed to women desiring pregnancy [13].

In this scenario, the two stereoisomers of inositol, MI and DCI, may have a key therapeutic role in PCOS owing to their action as modulators of insulin sensitivity. MI and DCI could be useful as a treatment particularly in women desiring pregnancy and in patients with contraindications to other insulin-sensitizing drugs: considering these points, in this article we address the role of both MI and DCI as potential drugs for PCOS-related infertility.

Inositols: Key Molecules for Oocyte Development

In addition to insulin-sensitizing and insulin-response modulatory effects, inositols act as gonadotropin second messengers in the ovary. Given the connection between ovulatory

Glossary

Advanced glycation end-products (AGEs): heterogeneous highly reactive products of non-enzymatic glycation or glycol oxidation of the amino groups of proteins, nucleic acids and aminolipids.
Androgens: steroid hormones synthesized in the testis, ovary, and adrenal gland. The most important are testosterone, dihydrotestosterone, dehydroepiandrosterone, dehydroepiandrosterone sulfate, androstenedione, and androstenediol.
Anti-Müllerian hormone (AMH): a glycoprotein hormone produced by follicle granulosa cells of the preantral and small antral follicles which regulates their sensitivity to FSH and consequent recruitment in the ovarian cycle.
Assisted reproductive technology (ART): techniques that, for the purpose of reproduction, include the *in vitro* handling of both male and female gametes or of embryos.
Follicle-stimulating hormone (FSH): heterodimeric glycoprotein that supports and regulates the growth and development of ovarian follicles and stimulates estrogen production by granulosa cells.
Gonadotropin-releasing hormone (GnRH): a peptide hormone produced by GnRH neurons in the hypothalamus.
Heterotrimeric G protein complex (G protein): heterotrimeric complexes made up of α, β, and γ subunits that are involved in signaling across the cell membrane when activated by G protein-coupled receptors. There are many classes of Gα subunits: Gαs (cAMP pathway; adenylyl cyclase activation), Gαi (G adenylyl cyclase inhibition), Gαq (activates PLP).
Hyperandrogenism: an endocrine disorder characterized by clinical manifestation of androgens in females (hirsutism, acne after adolescence, alopecia, and deepening of voice) and/or high blood concentration of androgens.
Impaired glucose tolerance (IGT): a prediabetic condition characterized by hyperglycemia and insulin resistance.
***In vitro* fertilization (IVF):** a technique that involves

Box 2. Insulin Resistance and Hyperinsulinemia in the Pathogenesis of PCOS

Both lean and obese women with PCOS manifest IR, with a prevalence ranging from 44% to 70%. IR is associated with compensatory hyperinsulinemia due to the increased amount of insulin required for metabolic action [2,6]. Insulin sensitivity measured by the clamp method showed an intrinsic reduction of 27% in PCOS patients, independently of body mass index (BMI). Moreover, BMI independently exacerbates IR in women suffering from PCOS, and has a greater impact on IR in women with PCOS than in controls [69]. In PCOS the prevalence of IGT ranges from 23% to 35%, while the prevalence of T2DM ranges from 4% to 10%, with their respective rates being threefold and 7.5–10-fold higher than in healthy control women of similar age [70].

In vitro studies demonstrated that decreased insulin sensitivity is the most consistent defect of adipocytes of PCOS women, with decreased insulin-stimulated glucose transport and insulin responsiveness. These effects might be mediated by post-receptor events, such as a decrease in the abundance of glucose transporter type 4 (GLUT4) in adipocytes of subcutaneous tissue and/or decreased insulin receptor β subunit abundance in visceral adipose tissue [6]. Furthermore, women with PCOS exhibit defective β cell function, although it is unclear whether β cell dysfunction is secondary to IR as a result of progressive β cell exhaustion, or is a primary defect [76].

Accumulating evidence suggests that IR and hyperinsulinemia play an important pathogenic role in hyperandrogenism and anovulation of women affected by PCOS [7,8,32,68,77]. Hyperinsulinemia may alter physiologic gonadotropin secretory dynamics, increasing LH levels that promote ovarian androgen production acting synergistically with insulin [6,8]. In addition, hyperinsulinemia was associated with an increase of GnRH, altered LH to FSH ratio, and disturbances in the production and action of LH, FSH, insulin-like growth factor-1 (IGF-1) and AMH. These elements can result in follicular dysfunction and anovulation [2]. Moreover, hyperinsulinemia and androgen overproduction could inhibit hepatic SHBG production in women with PCOS, resulting in a markedly increased bioavailability of circulating free testosterone [7,8].

The IR state in PCOS was associated with elevated serum AGEs, even in normoglycemic women. AGEs, that are involved in oxidative stress and cardiovascular risk of PCOS women, are able to interfere with insulin signaling pathways and are implicated in insulin resistance mechanisms in different tissues. Furthermore, AGEs can interfere with insulin signaling as well as with LH and FSH signaling in humanized granulosa cells, suggesting that imbalance of these molecules is detrimental to granulosa cell metabolism, potentially leading to ovulation failure [78,79].

dysfunction, hyperinsulinemic IR, and **hyperandrogenism**, inositols have been studied to assess their effects on PCOS symptoms and signs, including the possibility of improving fertility and reproductive outcomes [14]. Both MI and DCI are fundamental biologically active molecules, act as insulin second messengers, and mediate different actions of insulin in humans [1,15] (Box 4 and Figure 1).

In ovaries, MI is one of the second messengers of **follicle-stimulating hormone** (FSH) and **luteinizing hormone** (LH), whose pathways are highly complex and nonlinear. The dominant FSH pathway is cAMP/protein kinase A (PKA)-mediated and leads to steroidogenesis, where aromatase induction is considered to be a primarily effect. cAMP is modulated primarily by Gs protein through activation of adenylyl cyclase, and the latter is activated by Gq protein [16]. Conversely, MI pathway compared to the cAMP/PKA pathway may require a higher concentration of the hormone and a higher density of the receptor that activates Gq protein. Thereafter PLP-C, activated by Gq protein, modulates LH/FSH activity through the MI pathway, releasing inositol trisphosphate (IP3) and diacylglycerol. IP3 interacts with its receptors and controls intracellular Ca^{2+} release [16].

This dual signaling mechanism has an impact on ovulation. On the one hand, FSH receptor stimulation in the early follicular phase activates the cAMP pathway that at high concentration maintains oocyte in prophase 1 and stimulates the proliferation and growth of granulosa cells. On the other hand, modulation of FSH receptor concentration in the dominant follicle and the ovulatory LH surge activate the MI pathway. The change of cAMP and increased calcium concentration promote the resumption of meiosis and release the mature oocyte [16]. Indeed,

extracorporeal fertilization of gametes by coincubation of oocytes with sperm *in vitro*.
Infertility: disease defined as failure of a couple to achieve clinical pregnancy after 12 months of regular, unprotected sexual intercourse.
Inositolphosphoglycan (IPG): second messengers released by heterotrimeric G protein-regulated hydrolysis (phospholipase-mediated) of membrane phosphatidylinositols.
Insulin: a dimeric peptide hormone (51 amino acids) composed of an A chain and a B chain linked by disulfide bonds. It is produced by β cells of pancreatic islets.
Insulin resistance: reduced cellular response to insulin.
Intracytoplasmic sperm injection (ICSI): a technique that involves extracorporeal fertilization of gametes in which a single spermatozoon is injected into the oocyte cytoplasm.
Luteinizing hormone (LH): a heterodimeric glycoprotein that triggers ovulation and induces subsequent development of the corpus luteum. In follicle thecal cells LH induces androgen production as a precursor to estrogen produced by granulosa cells.
Metabolic syndrome: a disorder of energy utilization and storage defined by the presence of at least three of the five following medical conditions: high blood pressure, high blood glucose, abdominal obesity, high serum triglycerides, and low high-density lipoprotein (HDL) levels.
Ovarian hyperstimulation syndrome: a pathologic condition characterized by an exaggerated systemic response to ovarian stimulation.
Phosphatidylinositols (PIs): a family of lipids of the phosphatidylglyceride class that can be phosphorylated to the phosphoinositides PI phosphate (PIP), PI bisphosphate (PIP2), and PI trisphosphate (PIP3) that play important roles in cell signaling and trafficking.
Phosphatidylinositol 3-kinase (PI-3-K): heterodimeric enzymes that phosphorylate PIs and are regulated by G protein-coupled receptors and tyrosine kinase receptors.
Phospholipase (PLP): a family of enzymes that hydrolyze

Box 3. Hyperandrogenism and Insulin Resistance: The Chicken or the Egg?

Although the contributions of IR and hyperinsulinemia to PCOS are increasingly recognized, androgens play a central role in the pathogenesis of PCOS. Clinical or biochemical hyperandrogenism is a key diagnostic trait for PCOS, and findings from clinical and animal model studies have provided substantial evidence to support a driving role for androgens acting via the androgen receptor in the development of PCOS [80]. High circulating androgen levels in combination with disrupted gonadotropin secretion may lead to unregulated follicle growth, with an absence of dominant follicle formation and subsequent anovulation [2,7]. In PCOS women, high androgen levels are also associated with hypothalamic reduced sensitivity to the inhibitory action of progesterone [3,80]. *In vivo* studies demonstrate altered androgenic programming in the pathways leading to aberrant LH surge generation in PCOS, and this supports the rationale for using androgen antagonists to improve ovulation rates in PCOS patients [80].

The origins of IR in PCOS may be related to androgens. Androgens can impair insulin action, promote IR, and predispose to pancreatic β cell failure [75]. Nontargeted and targeted studies suggest that the genomic, transcriptomic, and proteomic profiles of visceral adipose tissue from women with PCOS are very different from those of healthy women, and resemble those of men, indicating that androgen excess contributes to their adipose tissue dysfunction that leads to IR and associated hyperinsulinemia [5,80–82]. In this view, clinical characteristics of PCOS could evolve from genetically and/or epigenetically determined hypersecretion of androgens, which may further start during the fetal period as a consequence of excess androgen exposure, either during this vulnerable period or at puberty [83].

Genetic predisposition is also like to underlie both the primary steroidogenic abnormality and triggering factors such as IR, and this may explain the frequent hereditability of PCOS (>70% concordance in monozygotic twins) [5]. Genetic/epigenetic mechanisms have been proposed to contribute to the pathogenesis of IR and hyperinsulinemia [68,84,85], and both male and female first-degree relatives of PCOS subjects have reproductive and metabolic abnormalities, with glucose-stimulated hyperinsulinemia developing at an early age and persisting through puberty [85].

On that basis, it is hypothesized that PCOS results from a vicious circle of androgen excess favoring abdominal and visceral adipose tissue deposition, that induces IR and compensatory hyperinsulinemia, further facilitating androgen secretion by the ovaries and adrenal glands. This cyclical pathogenetic interaction between IR, hyperinsulinemia, and hyperandrogenism, in combination with hypothalamic–pituitary dysfunction, leads to further ovarian dysfunction that can result in anovulation and infertility [5]. Therefore, different points of this vicious cycle could be considered as possible therapeutic targets to recover ovarian function, ovulation, and overall fertility [5,6].

phospholipids. Phospholipase C hydrolyzes phospholipids such as PIP2 before the phosphate moiety, releasing diacylglycerol and inositol trisphosphate (IP3).

Polycystic ovaries: at least one ovary with 12 or more follicles measuring 2–9 mm in diameter at ultrasound evaluation (Rotterdam criteria).

Sex hormone-binding globulin (SHBG): a glycoprotein that binds hydrophobic sex steroid hormones, with highest affinity for androgens.

Type 2 diabetes mellitus (T2DM): a chronic disease characterized by reduced population and insulin secretory function of β cells and/or by a reduced response of peripheral tissue to insulin (insulin resistance).

binding of IP3 to its receptor 1 (IP3-R1) seems to be necessary for oocyte maturation, especially in the final stages of development that are tightly calcium-dependent [17]. MI derivatives appear to promote meiotic progression of oocytes into fertilization-competent eggs in the mouse model, whereas depletion of MI intracellular stores within the ovary may alter the physiological processes previously described [18]. In mice with a selective mutation of Gq protein there was no increase of inositol second messengers following LH administration, accompanied by impaired ovulation and fertility [19]. Moreover, MI derivatives seem to participate in cytoskeleton regulation, and are necessary to accelerate oviduct transport of oocytes [20]. Finally, literature data indicate that MI signaling may adjust the level of **anti-Müllerian hormone** (AMH) production induced by FSH in granulosa cells. AMH, decreasing oocyte sensitivity to FSH, participates in regulating follicle maturation [20] (Figure 2).

Role of Abnormal Epimerase Activity in PCOS

Each organ can regulate the intracellular balance of inositol levels and has a tissue-specific intracellular MI:DCI ratio that modulates metabolic processes [21]. The tissue-specific MI:DCI ratio depends on a process of epimerization, and the intracellular epimerase enzyme is regulated by insulin [22]. IR and **type 2 diabetes mellitus** (T2DM) have been associated with reduced availability and level of DCI, supporting its role as an insulin second messenger and insulin-sensitizing agent (Box 4). An explanation of this imbalance comes from studies on animal models: in particular, in insulin-sensitive tissues (muscle, liver, and fat) MI conversion to DCI was reduced from about 20–30% in control rats to <5% in T2DM rats, and this was associated with reduced epimerase activity [23,24]. Based on these data, deficiency in DCI,

Box 4. Inositol Functions and Their Roles as Insulin Second Messengers

Inositol is considered to be part of the vitamin B complex, although in both prokaryotic and eukaryotic cells inositol can be synthesized from glucose [16]. However, in mammals it is primarily obtained from dietary sources as inositol-6-phosphate [51]. Inositol uptake is regulated via Na^+/MI cotransporters and H^+/MI cotransporters localized on the cell membrane of most cells. Inositols can be present within cells in a free form or as components of cell-membrane phospholipids, playing both a structural and functional role [16]. Among phospholipids, MI and DCI are present as **phosphatidylinositols** (PIs). Phosphatidylinositol phosphate (PIP) and its phosphorylated derivative phosphatidylinositol bisphosphate (PIP2) represent the bulk of these lipids [86]. PIP2 may be the starting point of a different pathway with different effects through the differential actions of **phospholipases** (PLPs), phosphatases, and **phosphatidylinositol 3-kinase** (PI-3-K) [86]. MI and DCI, following hydrolysis of PIs by PLPs, are converted to inositolphosphoglycan (IPG) and function as second messengers (MI-IPG and DCI-IPG) [1,15].

Both MI and DCI function as insulin second messengers and mediate different actions of insulin in humans [1,15]. MI is involved primarily in cellular glucose uptake, and is high in tissues with high glucose utilization and consumption, such as brain and heart [1,28]. MI also inhibits adenylyl cyclase, thus reducing the release of free fatty acids from adipose tissues [1]. Conversely, DCI levels are high in tissues which store glycogen, such as liver, muscle, and fat, and low in tissues with high glucose utilization, such as brain and heart [28]. The roles of DCI as an insulin second messenger and insulin-sensitizing agent are supported by evidence that IR is associated with reduced availability of DCI [87], and with increased urinary clearance of DCI in both PCOS and non-PCOS women with IR [88–90]. Moreover, several studies have reported reduced DCI-IPG release in the blood of diabetic subjects during a glucose tolerance test [91], and lack of DCI-IPG release in women with PCOS during an insulin clamp [89]. Along with these findings, dietary supplementation with DCI reduces IR in diabetic rats or monkeys affected by hyperglycemia, and studies on a mouse model suggested that the DCI may be more effective than MI in partially restoring insulin sensitivity and glycogen synthesis [92].

The cellular roles of MI and DCI in insulin-regulated glucose metabolic pathways are described in detail in Figure 1 in the main text.

with a consequent increased ratio of MI to DCI in insulin-sensitive tissues, is suggested to be caused by defective epimerization of MI to DCI [25].

In contrast to other tissues, ovarian theca and granulosa cells in PCOS women do not develop IR and have been reported to be exquisitely sensitive to insulin. Therefore, within ovaries of patients with PCOS and hyperinsulinemia related to IR, epimerization of MI to DCI is enhanced, producing MI deficiency that would impair FSH signaling [26].

Heimark *et al*. [22] studied well-characterized theca cells from normal cycling women with normal insulin sensitivity, and theca cells from PCOS women with hyperinsulinemic IR, and evaluated the intracellular ratio of MI to DCI and the activity of epimerase. They reported that the ratio of MI to DCI in the theca cells from the PCOS women was lower than in healthy women. In addition, thecal epimerase activity was increased in cells obtained from PCOS women compared to theca cells from healthy women. These results are consistent with those reported by Unfer *et al*. [21] who measured MI and DCI levels in the follicular fluid of a small sample of PCOS patients, who manifested hyperinsulinemic IR, and in a small sample of healthy women. They reported that the follicular ratio of MI to DCI was 100:1 in healthy women, compared to only 0.2:1 in patients with PCOS, and this imbalance was explained by a dramatic reduction in follicular MI and increased DCI in the PCOS women.

In full agreement with these data, it was suggested that epimerization of MI to DCI is enhanced in patients with PCOS and hyperinsulinemia, which in turn yields MI deficiency in the ovaries, thereby impairing FSH signaling, leading to reduced oocyte quality, deficient oocyte maturation, and anovulation, as well as an increased risk of **ovarian hyperstimulation syndrome** [27]. The highly insulin-sensitive thecal cells from PCOS patients are exposed to hyperinsulinemia, which results in higher insulin-stimulated epimerase activity and thus increased conversion of MI to DCI [21,22,27].

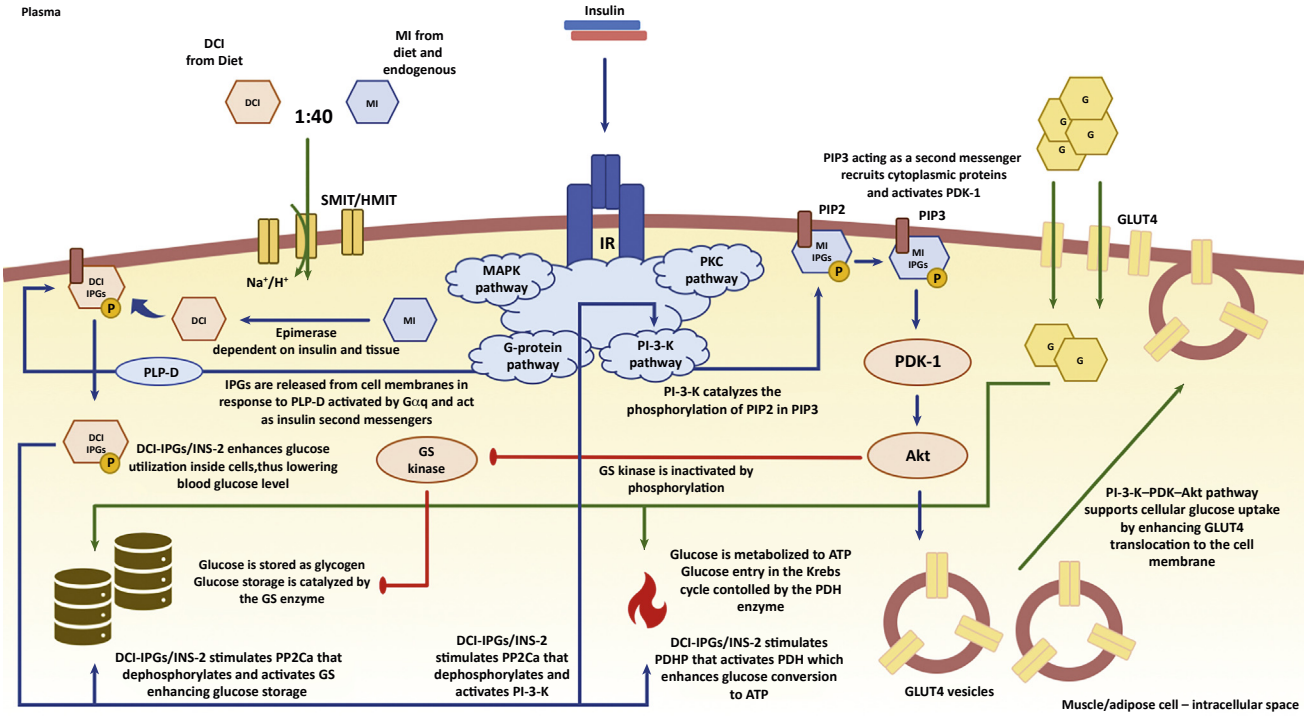

Figure 1. Roles of Myo-Inositol (MI) and D-Chiro-Inositol (DCI) in Cellular Insulin-Regulated Glucose Metabolic Pathways. The three main signal transduction pathways of insulin are the phosphatidylinositol (PI) 3-kinase (PI-3-K) pathway, the mitogen-activated protein kinase (MAPK) pathway, and the protein kinase C (PKC) pathway. PI-3-K catalyzes the phosphorylation of PI bisphosphate (PIP2) to PI trisphosphate (PIP3), that acts as a second messenger recruiting cytoplasmic proteins and mediating the activation of PI-dependent kinase-1 (PDK-1), which in turn phosphorylates and activates Akt kinase. This cascade induces glucose transporter type 4 (GLUT4) translocation to the cell membrane, and inactivation of glycogen synthase (GS) kinase (GSK) by phosphorylation leads to improvement of GS activity. A fourth alternative insulin pathway involves the activation of the **heterotrimeric G protein complex** (G protein). Gq protein activates phospholipase D (PLP-D), which hydrolyzes the cell-membrane PIs to produce inositol phosphoglycans (IPGs). One such IPG is a DCI-IPG called insulin second messenger, INS-2, that acts as an insulin sensitizer. DCI-IPG/INS-2 binds to phosphatase 2Cα (PP2Ca) protein, which dephosphorylates and activates GS and PI-3-K, stimulating GS and glucose uptake in insulin-sensitive tissues. Furthermore, DCI-IPG improves glycolysis by activating the enzyme pyruvate dehydrogenase phosphatase (PDHP) that activates pyruvate dehydrogenase (PDH), thus supporting ATP production by stimulating the oxidative metabolism of glucose via the Krebs cycle. Abbreviations: G, glucose; Gαq: α subunit of heterotrimeric Gq protein; HMIT, H^+/myo-inositol cotransporter; IR, insulin receptor; P, phosphate; PDH: pyruvate dehydrogenase; PDK-1, phosphatidylinositol-dependent kinase-1; PKC, protein kinase C; SMIT, Na^+/myo-inositol cotransporter.

This hypothesis shed light on the importance of the ratio of MI to DCI in restoring normal ovary functionality [28,29]. Indeed, a correlation between MI concentration in the follicular fluid and high oocyte quality was found, and several studies have reported that MI improves oocyte quality [30,31]. Furthermore, increased intrafollicular DCI could be converted to DCI **inositol-phosphoglycan** (IPG) that could then act locally in the ovary to increase thecal androgen production [32].

Inositols as a Treatment for Women with PCOS

As reported in recent reviews [28,33], it is currently accepted that oral administration of MI alone, DCI alone, or the combination of MI and DCI can alleviate much of the metabolic dysregulation that is typical of PCOS.

DCI administration was tested since the initial report by Nestler *et al*. [34]. Several studies [35,36] investigated the effects of oral DCI administration in women with PCOS with a daily

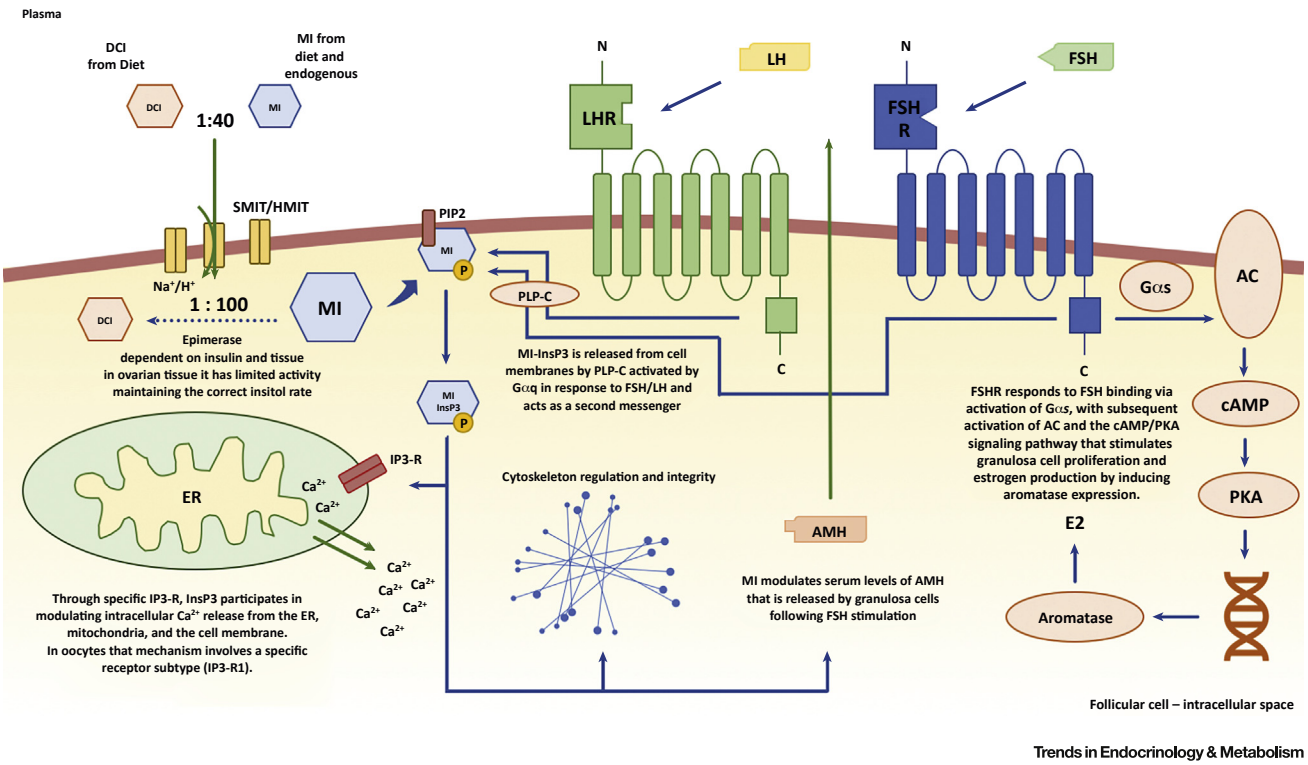

Figure 2. Roles of Inositol as Second Messenger in Follicle-Stimulating Hormone (FSH)/Luteinizing Hormone (LH) Signaling Pathways within the Ovary. The multiple effects of FSH and LH stimulation on granulosa and thecal cell proliferation and maturation suggest that the signaling pathways activated by FSH receptor (FSHR) and LH receptor (LHR) are highly complex and nonlinear. FSH induces cAMP/protein kinase A (PKA)-mediated events leading to granulosa cell proliferation and steroidogenesis, and aromatase induction is considered to be a primary effect. A higher density of FSHR and the LH surge induces Ca^{2+} pathways by releasing Ca^{2+} from intracellular stores or via influx of extracellular Ca^{2+} through plasma-membrane channels. This pathway is mediated by one or more members of the phospholipase C (PLP-C) family of enzymes that induce hydrolysis of phosphatidylinositol bisphosphate (PIP2) to inositol trisphosphate (IP3) and diacylglycerol. Myo-inositol (MI) mediates LH/FSH activity via IP3, that participates in modulating intracellular Ca^{2+} release, and in oocytes this involves a specific receptor subtype (IP3-R1) that seems to play a pivotal role in oocyte maturation, promoting meiotic progression during the final stages of oogenesis when oocyte sensitivity to calcium fluctuations reaches the maximal value. Furthermore, MI derivatives participate in cytoskeletal regulation and modulate anti-Müllerian hormone (AMH) serum levels. AMH, released after FSH stimulation, decreases oocyte sensitivity to FSH and participates in regulating follicle maturation. Abbreviations: AC, adenylyl cyclase; DCI, D-chiro-inositol; E2, estradiol; ER, endoplasmic reticulum; Gαs, α subunit of heterotrimeric Gs protein; Gαq, α subunit of heterotrimeric Gq protein; HMIT: H^+/myo-inositol cotransporter; IPG, inositolphosphoglycan; P, phosphate; SMIT, Na^+/myo-inositol cotransporter.

dosage ranging from 500 to 1200 mg, over a period from 8 to 24 weeks. Significant decreases in the waist-to-hip ratio, systolic and diastolic blood pressure, and plasma total cholesterol and triglyceride concentrations were reported. Furthermore, in DCI group the composite whole-body insulin-sensitivity index increased by 84%, with reduced glucose and insulin plasma levels during the oral glucose-tolerance test. Moreover, DCI treatment was associated with a decrease in the serum free testosterone and dehydroepiandrosterone sulfate concentrations, and an increase in serum **sex hormone-binding globulin** (SHBG) concentration. Finally, ovulation and menstrual cycle regularity were restored in 60–86% of women treated with DCI, and the LH response to **gonadotropin-releasing hormone** (GnRH) bolus was significantly normalized after treatment. Recent evidence suggests that treatment with oral DCI 1 g daily decreases the production of reactive oxygen species (ROS) in ovary [37] which are known to play a detrimental role in PCOS [38]. As far as oxidative stress is concerned, DCI was further reported to reduce ROS levels even in endothelial cells improving vessel endothelium function, as previously reported for metformin [39,40]. In

addition, the combined treatment with DCI plus metformin was reported to restore homeostasis at the level of thyroid-stimulating hormone in infertile PCOS patients affected by subclinical thyroid dysfunction, a condition potentially associated with unfavorable reproductive outcomes [41].

MI administration was also studied and gave similar results to DCI for metabolic and hormonal function [42–46]. The available studies investigated the effects of oral MI administration in women with PCOS, with a daily dosage ranging from 2000 to 4000 mg for 12–24 weeks. Among the most important findings, several authors found decreased plasma triglycerides, systolic and diastolic blood pressure, and insulin plasma concentration after oral administration of glucose, with improved insulin sensitivity. Moreover, decreased serum concentrations of total and free testosterone with increased serum SHBG were shown. Spontaneous ovulation was restored in up to 88% of women, and, notably, the effect of MI administration on follicular maturation was rapid because estradiol levels increased over the first week of treatment [42]. Furthermore, the ability of MI to improve ovulatory function has been tested against metformin, comparing metformin 1500 mg/day orally to 4 g MI plus 400 μg folic acid orally for 6 months or until pregnancy occurred. In this report, spontaneous ovulation was restored in 65% of patients treated with MI plus folic acid, and in 50% of the patients treated with metformin. The results were not statistically different, suggesting that treatment with MI or metformin plus folic acid are equally effective [47].

Because both MI and DCI monotherapies were beneficial in PCOS, the role of combined treatment of MI plus DCI has been investigated and compared to monotherapies. In particular, recent studies investigated the effectiveness of the combined treatment in a physiological MI: DCI serum ratio of 40:1. In these studies, the combined treatment was shown to improve ovarian function as well as hormonal and metabolic state in PCOS women more quickly than either MI or DCI treatment alone, improving the endocrine profile and IR even in obese PCOS women [48–50]. This might be due in part to synergistic actions of MI and DCI, given that MI can improve ovulatory function while DCI rapidly reduces peripheral hyperinsulinemia [28]. Based on this accumulating evidence, combined MI and DCI treatment at a physiological serum ratio of 40:1 has been proposed as an optimal and promising approach for the treatment of PCOS symptoms [28,29,51,52].

Inositol and Fertility: MI, DCI, or Both?

Regarding the role of inositols in restoring fertility in PCOS women through spontaneous ovulation (i.e., without pharmacologic induction), very few studies have investigated the spontaneous clinical pregnancy rate (i.e., without **assisted reproductive technology**, ART), and none was sufficiently powered. Furthermore, none reported data about the spontaneous live birth rate, which could be considered the most important reproductive outcome. Compared to placebo, there was no difference in the rate of spontaneous clinical pregnancy rate with MI in one study involving 92 women [relative risk (RR) 3.30; 95% confidence interval (CI) 0.40–27.13], but this study was underpowered for this outcome [43]. Furthermore, there was no difference in spontaneous clinical pregnancy rate between MI and metformin in another small study of 120 women (RR 1.64; 95% CI 0.85–3.16) [47]. Therefore, data about spontaneous improvement in clinical pregnancy rate, live birth rate, and miscarriage rate comparing inositols with placebo or other drugs are severely limited [33].

Conversely, different studies have investigated the role of inositols as a treatment associated with ART, and improvements have been reported in women with PCOS who underwent ART using inositol in different forms, combinations, or doses [53–57].

These studies found a beneficial action of MI, confirming its crucial role in FSH signaling (Figure 2), oocyte maturation, and embryo development [20,30]. Higher concentrations of MI in human follicular fluid seem to play a role in follicular maturity and have been suggested as a potential marker of good oocyte quality [30]. Treatment with MI was associated with decreased FSH levels, decreased duration of ovulation induction required for follicular development [31,58], and increased clinical pregnancy rates [58]. Conversely, the role played by DCI in ovarian physiology remains controversial [28]. In this regard, when different concentrations of DCI were administrated to non-obese PCOS women with normal insulin sensitivity undergoing ***in vitro* fertilization** (IVF), oocyte quality and ovarian response worsened as the DCI dose was progressively increased [59]. Other studies reported that MI administration, compared to DCI administration, improved oocyte quality and increased the number of oocytes collected after ovarian stimulation in PCOS patients undergoing IVF [60], and increased the number of mature oocytes in euglycemic PCOS patients undergoing ovulation induction for **intracytoplasmic sperm injection** (ICSI) [31,54,60,61].

Considering the different tissue-specific MI:DCI ratios (100:1 in the ovary) and the different physiological roles of the two inositol stereoisomers, these data suggest that DCI supplementation alone might not be the optimal or most appropriate approach to improve ART outcomes in PCOS patients. In this regard, combined MI and DCI oral supplementation at the physiological serum ratio of 40:1 was proposed as an appropriate treatment to restore normal ovarian function and improve metabolic state at the same time [28,29].

However, as far as infertility is concerned, the primary outcomes that should be considered are clinical pregnancy rate, miscarriage rate, and, above all, live birth rate. Although many studies showed improved hormonal and metabolic profile, improved ovulation rate, and a higher quality and number of oocyte retrieved in PCOS women after inositol administration during IVF, data regarding clinical pregnancy rate, live birth rate, and miscarriage rate are limited [28,33].

Regarding the role of oral inositol supplementation in PCOS woman undergoing ART, three recent systematic reviews and meta-analyses were published with partially conflicting results [55–57]. Mendoza *et al*. assessed the effectiveness of MI and DCI in improving reproductive outcomes for women with PCOS undergoing ICSI, and reported that MI supplementation, compared to folic acid, is not associated with higher number of oocytes retrieved, oocyte and embryo quality, or a higher pregnancy rate [55]. In conclusion, Mendoza *et al*. highlight a lack of evidence to justify MI supplementation as a treatment aimed to improve ART outcomes [55].

These results are consistent with those reported by Laganà *et al*. [56] who investigated MI compared to folic acid in both PCOS and non-PCOS women undergoing IVF. However, this meta-analysis found that oral MI supplementation is associated with reduced gonadotropin administration both in PCOS and non-PCOS women, but reduced duration of controlled ovarian stimulation (COS) only in PCOS women.

Different inclusion/exclusion criteria and different methodologies applied for data analysis could further explain the partially conflicting results reported by Zheng *et al*. [57]. This third meta-analysis investigated the efficacy of MI compared to folic acid during ovulation induction for ICSI and IVF. These authors reported that clinical pregnancy rate and embryo quality were significant higher in the MI group than in controls. Conversely, miscarriage rate, total amount of ovulation drugs, stimulation days, and estradiol peak were significantly reduced. No significant

differences were observed in total oocytes and meiosis II stage oocytes retrieved. Therefore, this meta-analysis concluded that MI supplement has promising benefits in PCOS women undergoing ICSI or IVF.

Although methodological differences between these meta-analyses could explain the partially conflicting results, data about inositols in IVF and ICSI cycles for PCOS women have many limitations: data on effect size were often lacking, and in many studies the sample sizes were small with low power. Furthermore, studies investigating inositol treatments often have different combinations, doses, and different investigated outcomes, which makes it difficult to compare findings [55–57].

In summary, although inositols are a promising treatment for PCOS-related infertility given their direct and indirect actions on ovarian function and metabolic state, further evidence will be necessary to confirm their efficacy to improve fertility and reproductive outcomes in PCOS women undergoing ART – including oocyte and embryo quality, clinical pregnancy rate, live birth rate, and miscarriage rate [55–57]. Furthermore, the role of inositols in improving spontaneous fertility (without ART) needs further investigation because these drugs may be a cost-effective treatment for PCOS-related infertility. In addition, inositol treatment during COS and ART could be beneficial because it reduces the total amount of gonadotropins administered and the number of days of stimulation. On that basis, current evidence may support inositol as a treatment that could reduce the costs of COS and potentially decrease the risk of ovarian hyperstimulation syndrome [56,57].

Concluding Remarks and Future Perspectives

Based on available evidence, inositols are able to improve metabolic and ovarian function in PCOS patients. Clear benefit with inositol has been shown in improving ovulation rate as well as hormonal and glycemic profiles in women with PCOS [33]. Whether this translates into clinical benefit with improved pregnancy and increased live birth rate with overall improved fertility, and reduced development of metabolic complications including gestational diabetes, T2DM, or metabolic disease remains to be confirmed. However, if demonstrated, inositol supplementation, alongside lifestyle advice, could become a first-line treatment to improve fertility in women with PCOS given the lack of significant adverse effects and safety profile, even in pregnancy [28,55,57]. Furthermore, by regularizing menstrual cycles, it has potential to reduce the burden of endometrial hyperplasia in these women.

However, efficacy data are still preliminary and are often obtained in the presence of confounders that require the results to be confirmed in other settings and on well-selected samples. Furthermore, there are so far no well-designed studies to define the best doses of DCI and MI in treating PCOS.

There is a definite need for properly controlled studies on larger cohorts of PCOS patients with greater statistical power, which would more accurately clarify post-treatment fertility outcomes associated with the different inositol isoforms, establish optimal therapeutic strategies tailored to the pretreatment phenotype of the patient (i.e., 'personalized dosage' based on the clinical or biochemical features of the patient), and evaluate the variability of the long-term outcomes on the basis of these phenotypic parameters. There is a clear need for further evaluation by large multicenter randomized controlled trials, particularly focusing on long-term fertility outcomes such as clinical pregnancy rate and live birth rate in ART and spontaneous ovarian cycles (see Outstanding Questions).

Outstanding Questions

Does oral supplementation with MI, DCI, or both significantly improve reproductive outcomes in PCOS women without ART?

Does oral supplementation with MI, DCI, or both significantly improve reproductive outcomes in healthy women without ART?

Does oral supplementation with MI, DCI, or both significantly improve the reproductive outcomes in healthy women undergoing ART?

What is the best MI:DCI ratio for oral supplementation to improve fertility in PCOS women?

Does oral supplementation with MI, DCI, or both have any epigenetic background effects in subsequent offspring?

In conclusion, although the available data have some limitations, MI and DCI are an attractive treatment option for this complex syndrome owing to their ability to modulate insulin action and orchestrate oocyte and ovarian function; MI and DCI, particularly when combined at the physiological ratio of 40:1, may indeed be cost-effective treatments for PCOS women.

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