Trends in Endocrinology & Metabolism

CellPress REVIEWS

Review

Inositols in Polycystic Ovary Syndrome: An Overview on the Advances

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This review details the physiologic roles of two insulin sensitizers, *myo*-inositol (MI) and D-chiro-inositol (DCI). In the human ovary, MI is a second messenger of follicle-stimulating hormone (FSH) and DCI is an aromatase inhibitor. These activities allow a treatment for polycystic ovary syndrome (PCOS) to be defined based on the combined administration of MI and DCI, where the best MI:DCI ratio is 40:1. Moreover, MI enhances the effect of metformin and clomiphene on the fertility of PCOS women seeking pregnancy. As impaired intestinal transport may lead to unsuccessful inositol treatment, we also discuss new data on the use of alpha-lactalbumin to boost inositol absorption. Overall, the physiological activities of MI and DCI dictate the dosages and timing of inositol supplementation in the treatment of PCOS.

Highlights

Myo-inositol (MI) and D-chiro-inositol (DCI) are two stereoisomers of inositol. These natural molecules are safe and well-tolerable and have insulin-sensitizing activity. In addition, MI mediates FSH signaling.

MI and DCI are dietary supplements with proven therapeutic activity in polycystic ovary syndrome (PCOS).

New preclinical and clinical studies support the importance of administering a combination of MI and DCI in the 40:1 ratio. Of note, this is the physiologic ratio in blood.

Inositol absorption at intestinal level and its therapeutic effect in PCOS are significantly improved with the coadministration of alpha-lactalbumin.

In conclusion, inositols represent an important therapeutic advance for PCOS treatment.

The Diverse Physiologic Role of Inositols

Inositols are cyclic polyols ($C_6H_{12}O_6$) with a molecular weight of 180.16; they are present in all living beings and participate in many metabolic pathways. These compounds exist in nine stereoisomeric forms, among them *myo*-inositol (MI) is the most important and widely distributed in nature [1]. The cellular precursor of MI is glucose-6-phosphate (G6P), which D-3-*myo*-inositol-phosphate synthase isomerizes to inositol-3-phosphate (Ins3P) [2]. Inositol monophosphatase-1 then dephosphorylates Ins3P to free MI [3]. Free inositol may also be obtained by recycling inositol-1,4,5-trisphosphate (InsP3) and inositol-1,4-bisphosphate (InsP2) [4].

In humans, inositols primarily derive from dietary sources as MI [5,6]. MI can be then converted to D-chiro-inositol (DCI) by an NAD–NADH-dependent epimerase, under the stimulus of **insulin** (see Glossary) [7,8]. As a result, every organ and tissue can balance the level of inositols and the ratio of MI/DCI in a specific manner, regulating metabolic processes [9]. Under physiological conditions, the MI/DCI ratio averages 100:1 in the follicular fluid (FF) [9] and 40:1 in plasma [10].

Inositols are present inside cells in the free form and in plasma membrane as phosphatidylinositols, which can be phosphorylated to phosphatidylinositol phosphate (PIP) and phosphatidylinositol biphosphate (PIP2). Cleavage of PIP2 by phospholipase-C (PLP-C) leads to inositol triphosphate (InsP3), a second messenger [11].

As **inositolphosphoglycans (IPGs)**, both MI and DCI (MI-IPG and DCI-IPG) are second messengers involved in insulin signaling and mediate different effects in humans [12,13]. Glycosyl-phosphatidylinositol lipids (PIP, PIP2), located at the inner leaflet of the cell membrane, undergo hydrolyzation upon insulin stimulus, releasing MI-IPG and DCI-IPG. The concentration of MI is elevated in tissues with high glucose consumption, such as the brain and the heart [12,14]. Indeed, MI-IPG participates in the cellular uptake of glucose, inducing the GLUT4 translocation to cell membrane [15], inhibits the adenylate cyclase enzyme, and reduces the release of free fatty

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acids from adipose tissues [12]. Conversely, DCI concentration is elevated in tissues that store glycogen (i.e., liver, muscles, and fat) and low in tissues with high glucose utilization. DCI-IPG primarily participates in glycogen synthesis, stimulating glycogen synthase [14]. Moreover, it stimulates pyruvate dehydrogenate (PDH), which induces glycolysis and the subsequent formation of adenosine triphosphate through the Krebs' cycle [16]. Therefore, DCI-IPG may improve the sensitivity to insulin in target tissues [17].

Recent data suggest that DCI directly regulates steroidogenic enzyme genes in human granulosa cells, reducing the mRNA expression of both **aromatase** (CYP19A1) and cytochrome P450 side-chain cleavage (P450scc) genes in a dose-response fashion [18]. Furthermore, DCI increases testosterone levels in the theca cells from polycystic ovary syndrome (PCOS) women, acting as insulin mediator (inositolglycan mediator) [19].

Such characteristics [20] are in line with the detrimental effects on blastocyst quality observed when the levels of DCI in the FF are high [21]. In particular, the best oocyte quality correlated with a MI/DCI ratio of 70–100:1 in FF.

Inositols, specifically MI, participate in the **follicle-stimulating hormone (FSH)** signaling, acting as second messengers. Through its receptor (FSHR), FSH has multiple effects via a highly complex and nonlinear signaling pathway that regulates the proliferation and the maturation of granulosa cells. In addition, MI and PLP-C mediate the release of InsP3, which regulates the intracellular Ca^{2+} levels [22]. Noteworthy, the final steps of oocyte development are calcium-dependent and the binding of InsP3 to its receptor 1 (IP3-R1) seems to have a key role in developing mature oocytes and in promoting meiotic progression, both processes characterized by high Ca^{2+} levels [23]. MI-IPG also seems to regulate the cytoskeleton [5,24] and the FSH-induced production of a**nti-Müllerian hormone (AMH)**, which modulates the sensitivity of follicles to FSH and, hence, their maturation [25]. Besides inositol, FSH activates cAMP/PKA pathways, promoting steroidogenesis by aromatase induction [6] (Figure 1). (See Box 1.)

MI concentration in mammalian female reproductive tract is substantially higher than plasma, suggesting that MI exerts specific functions at the ovarian level, like accelerating the transport of oocytes through the oviducts [26].

Inositols for the Treatment of PCOS

An important role of MI and DCI in PCOS patients is an insulin sensitizing action, mirrored by a decrease in the homeostatic model assessment (HOMA) index. Both isomers are useful in treating insulin resistance states [6,27]. (See Box 2.)

Bevilacqua *et al*. [28] recently carried out a preclinical study on an animal model of PCOS. Female mice received continuous light (L/L) for 10 weeks. At the end of this period, they developed a phenotype with several similarities to that of PCOS women. A group of mice kept under normal (12/12 h) light/dark cycle (L/D) served as control. The uteri of L/D mice had a proestrus/estrus-like appearance, as normally found in sexually mature, cycling animals. Instead, the uteri of L/L mice exhibited immature/diestrus-like features, typical of noncycling animals. Ovaries from control mice presented a corpus albicans (from recent ovulations) and a corpus luteum and showed normal primary, secondary, and tertiary follicles upon histological analysis. On the contrary, ovaries from L/L mice were smaller, without corpus albicans and, upon histological analysis, revealed paucity of primary and secondary follicles and cystic tertiary follicles that strongly resembled those in human polycystic ovaries. Such cystic follicles lacked the oocyte and presented variable amounts of granulosa cells. The early tertiary follicles with a living oocyte presented a hyperplastic theca

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cell layer and a thinner granulosa cell sheet. The ratio between the thickness of these two layers (TGR) allows reliable evaluation of the androgenic-like phenotype that typically occurs in PCOS [29]. In fact, a hypertrophic theca cell layer is a hallmark of polycystic ovaries and causes a greater production of **androgens** [30].

The study provided the first experimental evidence of the different efficacy exerted by various MI/DCI ratios (5:1; 20:1; 40:1; 80:1) in restoring a normal phenotype. Moreover, it supported the metabolic link between MI and DCI, specifically in PCOS. Mice treated daily with 420 mg/kg MI/DCI in a 40:1 molar ratio made a fast and almost full recovery from PCOS signs and symptoms. On the contrary, the other MI/DCI ratios were less effective or had even negative effects. In particular, the formulation with high content of DCI proved to worsen the PCOS pathological features. A recent clinical trial confirmed these results also in PCOS women [31].

In this regard, a very stimulating hypothesis based on the 'ovarian paradox' was formulated a few years ago [32]. Since the ovaries, unlike the liver and the muscles, never become insulin resistant [33–35], researchers suggested that the overproduction of insulin in PCOS women enhances ovarian MI to DCI epimerization, causing increased DCI concentration and corresponding decreased MI levels [7,9,32]. Ovarian MI:DCI ratio is 100:1 in healthy women, whereas it drops to 0.2:1 in PCOS patients [9], possibly impairing the FSH signaling. Hence, restoring the physiological levels of the two isomers in the FF may be crucial for proper ovarian function [9].

A recent meta-analysis [36] examined nine randomized controlled trials (RCTs) on PCOS women, including the aforementioned studies, with a total of 247 cases and 249 controls [37–45] (Table 1). The authors evaluated the efficacy of treatments (length: 12–24 weeks) with MI, alone, or combined with DCI in the 40:1 ratio, on fasting insulin concentrations as primary endpoint; and on HOMA index and serum levels of testosterone, androstenedione, and sex hormone-binding globulin (SHBG) as secondary endpoints. Inositol supplementation resulted in significant reduction of fasting insulin (standardized mean difference = −1.021 μU/ml; 95% confidence interval (CI): −1.791 to −0.251; $P = 0.009$) and HOMA index (standardized mean difference = −0.585; 95% CI: −1.145 to −0.025; $P = 0.041$). The meta-analysis demonstrates the efficacy of the therapy and highlights a slight trend towards decreased testosterone with respect to controls; androstenedione levels, however, remained unchanged. MI significantly increased SHBG levels after at least 24 weeks of administration (standardized mean difference = 0.425 nmol/L; 95% CI: 0.050 to 0.801; $P = 0.026$). Such evidence strongly suggests that the findings on the primary outcome are conclusive. The different effects obtained on androstenedione and testosterone levels should be more thoroughly investigated by dedicated studies. The authors recommend avoiding exclusive DCI supplementation for three reasons: (i) high doses of DCI/day are harmful for ovaries and oocyte maturation; (ii) DCI cannot be converted into MI, decreasing the MI:DCI ratio; and (iii) deficiencies of MI and MI-IPG are correlated with many insulin-resistance conditions. In conclusion, the meta-analysis strongly supports the supplementation of MI for improving the metabolic profile of PCOS patients.

A systematic review and meta-analysis [46], including ten RCTs (573 patients involved) further confirmed these results [37–40,43,44,47–50] (Table 1). Total testosterone, estradiol (E2), and HOMA index were the primary outcomes. Compared with the control group, inositol administration significantly improved HOMA index (weighted mean difference = −0.65; 95% CI: −1.02, −0.28; $P = 0.0005$) and raised E2 levels (weighted mean difference = 16.16; 95% CI: 2.01, 30.31; $P = 0.03$), showing only a trend in reducing total testosterone levels. The authors concluded that inositols 'may be recommended for the treatment of PCOS with insulin resistance, as well as for improving symptoms caused by decreased estrogen in PCOS' [46].

Glossary

Alpha-lactalbumin (α-LA): a whey protein that may work also as a 'carrier' for metal ions (mainly divalent, such as Ca^{2+} and Fe^{2+}) and for vitamin D. In more general terms, α-LA seems to be a facilitator of passage through biological barriers.
Androgens (mainly, testosterone, dihydrotestosterone, dehydroepiandrosterone, dehydroepiandrosterone sulfate, androstenedione, and androstenediol); steroid hormones, produced by testis, ovary, and adrenal gland.
Anti-Müllerian hormone (AMH): a glycoprotein hormone synthesized by follicle granulosa cells of the preantral and small antral follicles. It regulates their sensitivity to FSH and subsequent recruitment in the ovarian cycle.
Aromatase: an enzyme involved in the conversion of androgens to estrogens. The inhibition of its activity increases the levels of testosterone and other androgens.
Follicle-stimulating hormone (FSH): (gonadotropin), a glycoprotein, which supports and controls the growth and development of ovarian follicles. It stimulates estrogen production by granulosa cells.
Hyperandrogenism: an endocrine disorder, due to an excess of androgens in females, that causes hirsutism, acne after adolescence, alopecia, and deepening of voice.
***In vitro* fertilization (IVF):** a technique that involves extracorporeal fertilization of gametes by co-incubation of oocytes with sperm *in vitro*.
Inositolphosphoglycan (IPG): second messengers released by heterotrimeric G protein-regulated hydrolysis (phospholipase-mediated) of membrane phosphatidylinositols.
Insulin: a peptide hormone produced by β cells of pancreatic islets. Insulin resistance means that cells have a reduced response to insulin.
Luteinizing hormone (LH): (gonadotropin), a glycoprotein, which stimulates ovulation and the subsequent development of the corpus luteum. In the follicle thecal cells, LH induces androgen synthesis, then, in granulosa cells, androgens are precursor of estrogens.
LH/FSH ratio: this ratio (in mIU/ml) is higher in PCOS patients (above 2:1). Normally, women show about equal amounts of LH and FSH during the early

Based on available evidence, the optimal therapeutic regimen, clinically tested in women with PCOS, is a combination of oral MI and DCI in a molar ratio of 40:1. The total daily amount of inositols is 4 g divided in two doses, for at least 3 or 6 months.

PCOS seems to be associated with an increased risk for ovarian and endometrial cancer [51]. The reduction of DNA damage and mutation rates, due to insulin-sensitizers, may explain the decreased risk for cancer observed in PCOS patients that undergo metformin treatment [52]. Future studies should be planned to evaluate these effects at the epidemiological level and to develop customized treatments in this context [53].

The US FDA specifically included MI in the list of generally recognized as safe compounds [54]. Recent clinical trials confirmed the safety profile also during pregnancy [55–57].

MI and Metformin Alone or in Combination in PCOS Patients

Metformin has been the first-choice drug to restore ovulation in PCOS women and has an antihyperandrogenic effect in the short term [8]. Indeed, it is an insulin sensitizer that acts on different tissues, including the ovary, and reduces glycemia levels, decreasing the ovarian production of androgens and the concentration of circulating androgens. However, the use of metformin frequently leads to adverse events that include gastrointestinal symptoms (diarrhea, nausea, vomiting, and abdominal bloating) and metabolic complications [8]. Studies compared the effects of metformin and MI, proving that combined administration of them provides interesting results. A very recent meta-analysis [58] of six clinical trials, with a total of 355 patients (178 treated

part of their cycle, with a short temporary period of LH surge. Instead, many women with PCOS have their LH levels two or three times higher than the FSH levels.

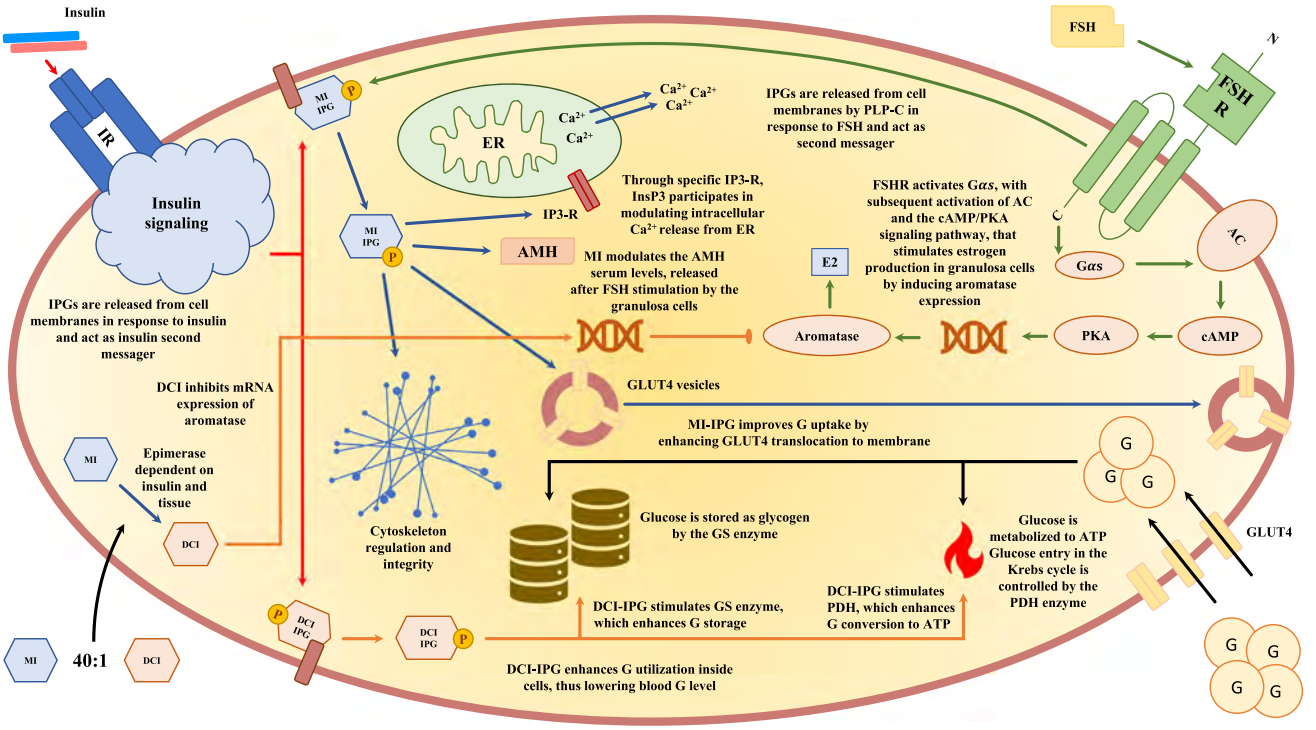

Figure 1. Intracellular Pathways of *Myo*-Inositol and D-Chiro-Inositol as Second Messengers. Abbreviations: AC, Adenylate cyclase; AMH, anti-Müllerian hormone; DCI, D-chiro-inositol; E2, estradiol; ER, endoplasmic reticulum; FSH, follicular stimulating hormone; FSHR, FSH receptor; G, glucose; Gαs, heterotrimeric G protein; GLUT-4, glucose transport type-4; GS, glycogen synthase; InsP3, inositol-3-phosphate; IPG, inositolphosphoglycan; IP3-R, InsP3 receptors; IR, insulin receptor; MI, *myo*-inositol; P, phosphate; PDH, pyruvate dehydrogenate; PKA, protein kinase A.

Box 1. Inositols

Myo-inositol (MI) and D-chiro-inositol are the most important stereoisomers of inositol [4–6]. MI is actively synthesized in testes, mammary gland, brain, kidneys, and liver. Vital organs, such as the brain, need elevated MI concentrations to function properly [4,5,8]. A specific epimerase, under insulin control, converts MI into DCI. Endogenous production of both isomers varies depending on the specific tissue needs. In normal women the plasma ratio is 40:1 [4–6,8]. Both MI and DCI are insulin second messengers. MI is primarily involved in cellular glucose uptake and its content is considerable in tissues that consume high amounts of glucose, such as brain, heart, and ovary. Conversely, DCI levels are elevated in tissues responsible for glycogen storage (e.g., liver and muscle) and low in tissues with high glucose utilization. In addition, MI in the ovary (as InsP3) is one of the second messengers of FSH [4–6,8]. The mammalian female reproductive tract has substantially higher concentrations of MI than blood serum, suggesting specific functions at the ovarian level, like ensuring proper oocyte maturation [4,5]. High concentrations of DCI in the ovaries increase testosterone levels in two different ways. As insulin mediator (inositolglycan mediator) DCI induces this rise in the theca cells of PCOS women; as aromatase inhibitor, it has the same activity in the granulosa cells. These effects can be detrimental for blastocyst quality [18,19,21]. MI and DCI are actively absorbed via specific transporters (SMIT1, SMIT2, and HMIT), which can be detected in several tissues and organs (kidney, brain, placenta, pancreas, heart, skeletal muscle, lung, liver, intestine, adipose tissue, and oocyte). Furthermore, under certain conditions, a 'passive' transport can also occur [72–74]. SMIT2 is the only MI transporter detected in the intestine (duodenum and jejunum) and has higher affinity for DCI compared with MI [74]. In light of the different affinity of SMIT2 for MI and DCI, determining their respective dosage in the therapy is essential to avoid a reduced MI intestinal absorption in favor of DCI.

with metformin, 177 with MI) [47,48,59–62] (Table 1), proved that by the end of the treatment (3–6 months) metformin and MI have comparable effects on fasting insulin, HOMA index, testosterone, androstenedione, SHBG, and body mass index (BMI). However, the authors found significant heterogeneity among the analyzed studies for changes in HOMA index, SHBG, and BMI. The subjects under MI treatment reported no adverse effects, while those receiving metformin presented increased risk for adverse events (risk ratio = 5.17; 95% CI: 2.91–9.17; $P < 0.001$).

Interestingly, MI treatment allows the use of mild metformin dosages, particularly important in patients intolerant to the normal therapeutic amounts.

A randomized clinical trial [63] included 120 infertile PCOS women, divided in two groups: group I (60 patients) received metformin (500 mg) plus MI (600 mg), three times a day; group II (60 patients) received only metformin (500 mg) three times a day. All participants tried to achieve spontaneous conception for 3 months. Those who failed received three cycles of ovulation induction and underwent intrauterine insemination. Compared with group II, patients in group I achieved a significant improvement in live birth rate, menstrual cycle (length and bleeding days), and HOMA index [63].

Effects of Inositols in the Ovary and Impact on Pregnancy in PCOS Women

As mentioned, the polycystic ovaries exhibit specific MI depletion and DCI overload [9], with impaired FSH signaling and poor quality oocytes [64]. Therefore, a probable treatment may be to restore the physiological levels of the two isomers in the FF and reestablish proper ovarian functioning [9].

Box 2. Polycystic Ovary Syndrome (PCOS) and the Therapeutic Approach with Inositols

PCOS is a common endocrine disorder, affecting 5%–15% of women of reproductive age [8]. According to the Rotterdam criteria, the diagnosis of PCOS depends on the presence of at least two of the following features: (i) oligo- or anovulation, (ii) clinical and/or biochemical signs of hyperandrogenism, and (iii) polycystic ovaries. Insulin resistance is an important disorder, frequently associated with PCOS. In the last decade, many clinical studies demonstrated that oral supplementation with inositols can significantly improve the metabolic patterns and the ovarian function in PCOS patients [36]. The best results were obtained with *myo*-inositol (MI) and D-chiro-inositol (DCI) administered in the 40:1 ratio, respectively [8]. Such ratio constitutes an appropriate approach to improve fertility outcomes, whereas excess DCI may have harmful effects on oocyte quality. The intestinal absorption of inositols significantly increases in humans coadministering alpha-lactalbumin [78]. This finding resulted in a remarkable therapeutic improvement in PCOS patients [81].

Table 1. List of the Studies with the Main Therapeutic Evidence

Study	Type of study	Patients	Treatment (daily)	Main outcomes	Results	Side effects	Refs
Artini, P.G. *et al*. (2013)	RCT	50 ow PCOS	Treated (10): MI 2 g + f.a. 200 mcg; Control (10): f.a. 200 mcg 12 weeks	Plasma LH, FSH, prolactin, E2, androstenedione, 17-hydroxyprogesterone, testosterone, glucose, insulin, C peptide concentrations, BMI, HOMA index, glucose-to-insulin ratio, menstrual cycles.	Treated: significant reduction of plasma LH, prolactin, testosterone, insulin levels, and LH/FSH. Significant improvement of glucose-to-insulin ratio and HOMA index. Restoration of menstrual cyclicity in all subjects. Controls: no changes.	No	[37]
Costantino, D. *et al*. (2009)	DBRCT	42 nw PCOS	Treated (23): MI 4 g + f.a. 400 mcg Control (19): f.a. 400 mcg 12–16 weeks	Systolic and diastolic blood pressure, triglycerides, total cholesterol, fasting insulin, fasting glucose, glucose AUC, insulin AUC, index of composite whole body insulin sensitivity.	Treated: improvement of insulin sensitivity and glucose tolerance. Decrease of glucose-stimulated insulin release, serum total and free testosterone, plasma triglycerides and total cholesterol, systolic and diastolic blood pressure. All data were significantly different with respect to controls. Ovulation: 69.5% in MI group versus 21% in controls	No	[38]
Genazzani, A.D. *et al*. (2008)	RCT	20 ow PCOS	Treated (10): MI 2 g + f.a. 200 mcg. Control (10): f.a. 200 mcg 12 weeks	Plasma LH, FSH, prolactin, E2, 17-hydroxyprogesterone, androstenedione, testosterone, glucose, insulin, C peptide concentrations, BMI, HOMA index and glucose-to-insulin ratio, menstrual cycles.	Treated: significant reduction of plasma LH, prolactin, testosterone, insulin levels, and LH/FSH. Significant improvement of insulin sensitivity expressed as glucose-to-insulin ratio and HOMA index. Restoration of menstrual cyclicity in all oligomenorrheic and amenorrheic subjects. Controls: no changes.	No	[39]
Gerli, S. *et al*. (2007)	DBRCT	92 Obese PCOS	Treated (45): MI 4 g + f.a. 400 mcg Control (47): f.a. 400 mcg 16 weeks	BMI, LH, testosterone, cholesterol (HDL and LDL), SHBG, free androgen index, fasting insulin, insulin AUC (OGTT), fasting glucose, leptin, inhibin-B	Treated: significant reduction of weight (BMI) and leptin; trend in decrease of LDL cholesterol; significant increase of HDL cholesterol; improvement of the LDL/HDL ratio; significant increase of E2 and improvement of ovulation. In the subgroup of responders to MI treatment: significant decrease of testosterone and increase of SHBG with lower free androgen index. Controls: no changes.	Yes	[40]
Pizzo, A. *et al*. (2014)	DBRCT	50 nw PCOS	Treated (25): MI 4 g + f.a. 400 mcg Control (25): 1 g DCI + f.a. 400 mcg 24 weeks	BMI, systolic and diastolic blood pressure, Ferriman–Gallwey score, Cremoncini score, serum LH, LH/FSH ratio, total and free testosterone, DHEA-S, D-4-androstenedione, SHBG, prolactin, glucose/immunoreactive insulin (IRI) ratio, HOMA index, menstrual cycles.	Both stereoisomers were effective, however MI versus DCI obtained a higher decrease of systolic arterial pressure (not significant), LH/FSH ratio (significant), total testosterone (significant), D-4-androstenedione (not significant), prolactin (not significant), HOMA index (significant). Increase of SHBG (significant) DCI versus MI: decrease of LH (not significant) and free testosterone (not significant); increase of glycemia/IRI ratio (not significant). MI and DCI improved the menstrual cycles.	No	[41]

Table 1. (continued)

Study	Type of study	Patients	Treatment (daily)	Main outcomes	Results	Side effects	Refs
Nordio & Proietti (2012)	RCT	50 ow PCOS BMI >27	Treated: (26): MI 550 mg + DCI 13.8 mg (soft gel cps) 40:1 ratio Control (24): MI alone same dosage 24 weeks	Systolic and diastolic blood pressure, BMI, glucose, insulin, HOMA-insulin resistance (IR), total and free testosterone, androstenedione, DHEA-S, SHBG.	Treated: significant reduction of glucose, insulin, HOMA-IR, total and free testosterone, androstenedione, DHEA-S, SHBG. Control: no relevant changes for glucose and insulin. Less relevant changes for sex hormones.	No	[42]
Benelli, E. *et al*. (2016)	RCT	46 Obese PCOS BMI >30	Treated: (21): MI 550 mg + DCI 13.8 mg (soft gel cps) 40:1 ratio + f.a. 400 mcg. Control (25): f.a. 400 mcg. 24 weeks	FSH, LH, 17-beta-E2, sex hormone-binding globulin, androstenedione, DHEA-S, free testosterone, HOMA index, fasting glucose and insulin.	Treated: significant reduction of LH, free testosterone, fasting insulin, and HOMA index; significant increase of 17-beta-E2 levels. Controls: no changes.	No	[43]
Pkhaladze, L. *et al*. (2016)	RCT	40 nw PCOS	MI treated (20) MI 4 g + f.a. 400 mcg; oral contraceptive pill treated (20): drospirenone 3 mg/ ethinyl estr. 30 mcg; 12 weeks	Weight, BMI, glucose, C-peptide, insulin, HOMA-IR, LH, SHBG, total and free testosterone, free androgen index, DHEA-S, AMH.	MI treated: significant reduction in weight, BMI, glucose, C-peptide, insulin, HOMA-IR, free testosterone, LH. Oral contraceptive pill treated: slight increase of weight and BMI. No changes in metabolic parameters.	No	[44]
Emekci Ozay, O. *et al*. (2017)	RCT	106 nw and ow PCOS	Treated: (52): MI 4 g + 400 mcg f.a. Control (54): f.a. 400 mcg 12 weeks	Progesterone, fasting insulin and glucose, HOMA-IR, total rFSH used, duration of the rFSH administration, trigger day endometrial thickness, >17 mm follicle number, clinical pregnancy rate, abortion rate, cancelled cycle number.	Treated compared with control: significant increase of progesterone, significant decrease of fasting insulin and glucose, HOMA-IR. Significant decrease of total rFSH dose and cycle duration; higher clinical pregnancy rates.	No	[45]
Nehra, J.K. *et al*. (2017)	RCT	60 ow PCOS	MI treated (30): 1 g twice daily Metformin treated (30): 500 mg thrice daily 24 weeks	Insulin, FSH, LH, LH/FSH ratio, testosterone, HOMA-IR, total cholesterol, triglycerides, VLDL cholesterol, LDL cholesterol, HDL cholesterol.	In both the groups: significant improvement in insulin resistance (glucose/insulin ratio, HOMA-IR); significant improvement of the lipid profile. No significant difference observed between the two treatments. MI is as effective as metformin.	No	[47]
Fruzzetti, F. *et al*. (2017)	RCT	50 ow PCOS + 30 healthy control	Metformin treated (25) 1.5 g MI treated (25): 4 g + f.a. 400 mcg. Control: no treatment 24 weeks	BMI, menstrual cycle length, 17-hydroxyprogesterone, testosterone, DHEA-S, androstenedione, HOMA-IR.	In both groups: significant improvement in BMI, insulin sensitivity, and menstrual cycle. No significant difference observed between the two treatments. MI is as effective as metformin.	No for MI	[48]
Dona, G. *et al*. (2012)	RCT	26 nw PCOS	Treated (18): MI 1.2 g Placebo (8) 12 weeks	Testosterone, androstenedione, insulin, glucose, HOMA-IR.	Treated: significant reductions of insulin resistance, androstenedione, and testosterone. Controls: no changes.	No	[49]
Gerli, S. *et al*. (2003)	DBRCT	283 Obese PCOS	Treated (136): MI 100 mg twice daily Placebo (147) 16 weeks	BMI, WHR, leptin, fasting insulin and glucose, insulin after OGTT, insulin AUC, total cholesterol, triglycerides, VLDL, LDL, and HDL cholesterol.	Treated: significant reduction of BMI, leptin; significant increase of HDL cholesterol. Trend in LDL cholesterol reduction. Significant improvement of the LDL-to-HDL cholesterol ratio. Controls: no changes.	Yes	[50]

(continued on next page)

Table 1. (continued)

Study	Type of study	Patients	Treatment (daily)	Main outcomes	Results	Side effects	Refs
Angik, R. *et al*. (2015)	RCT	100 nw PCOS	MI treated (50): 1 g twice daily. Metformin treated (50): 500 mg twice daily 24 weeks	BMI, WHR, Ferriman–Gallwey score, ovarian volume, antral follicle count, fasting insulin and glucose, postmeal insulin and glucose, HOMA-IR, testosterone, LH.	In both groups: significant reduction of Ferriman–Gallwey score, BMI, WHR, fasting insulin and glucose, postmeal insulin and glucose, HOMA (significant only in MI group), testosterone, LH, LH/FSH ratio, mean ovarian volume, and antral follicle count. No significant difference observed between the two treatments. MI is as effective as metformin.	Yes: 16% in MI and 72% in metformin	[59]
Jamilian, M. *et al*. (2017)	RCT	60 nw and ow PCOS	MI treated (30): 2 g + f.a. 200 mcg twice daily Metformin treated (30): 500 mg thrice daily 24 weeks	Total testosterone, SHBG, DHEA-S, modified Ferriman–Gallwey score, C-reactive protein, nitric oxide, gene expression of TNF-alpha, IL-1, IL-8.	MI treated: significant decrease of total testosterone, modified Ferriman–Gallwey score, C-reactive protein. Significant downregulation of IL-1 gene expression in the PBMCs in comparison with metformin.	No	[60]
De Leo, V. *et al*. (2013)	RCT	40 ow PCOS	MI treated (20): 1.5 g twice daily Metformin treated (20): 850 mg twice daily 24 weeks	BMI, FSH, LH, total and free testosterone, SHBG androstenedione, fasting glucose and insulin, total cholesterol, LDL and HDL cholesterol, triglycerides, HOMA-IR.	In both groups: significant reduction of BMI, modified Ferriman–Gallwey score, LH, total and free testosterone, androstenedione, fasting glucose and insulin, HOMA-IR, total cholesterol, LDL cholesterol, triglycerides. Significant increase of SHBG and HDL only in metformin group	Yes (mainly in metformin group)	[61]
Tagliaferri, V. *et al*. (2017)	Crossover RCT	34 Obese PCOS	Metformin treated (17): 850 mg twice daily MI (17): 1 g twice daily 24 weeks	BMI, WHR, insulin after OGTT, FSH, LH, E2, progesterone, prolactin, testosterone, androstenedione, 17-hydroxyprogesterone, SHBG, DHEA-S, AMH, menstrual cycles.	In both groups: significant reduction of insulin response to OGTT and improved insulin sensitivity. In metformin group: significant decrease of BMI, improvement of menstrual pattern and Ferriman–Gallwey score; significant decrease in LH, E2, androgens, AMH. No clinical and hormonal differences in MI group. The Authors write that the low MI dose used might have been insufficient to disclose major clinical and hormonal changes	No for MI	[62]
Nordio, M. *et al*. (2019)	RCT	56 nw PCOS	Treated (8 pt. for each group): 2 g (total MI + DCI at different ratios) twice daily 12 weeks	Ovulation, progesterone, FSH, LH, SHBG, E2, free testosterone, HOMA-IR, fasting and postmeal insulin.	The 40:1 MI/DCI ratio was the best to restore ovulation and to normalize important parameters (significant increase of progesterone, E2, SHBG; significant decrease of LH and HOMA). The other formulations were less effective or totally ineffective.	No	[31]

Abbreviations: CT, Controlled trials; DBR, double blind randomized; f.a. folic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; nw, normal weight;: ow, overweight; OGTT, oral glucose tolerance test; PBMC, peripheral blood mononuclear cell; pt, patient; VLDL, very low-density lipoprotein; WHR, waist-to-hip ratio.

In a clinical study [43], 46 obese PCOS women (BMI > 30) received combined MI and DCI (40:1 ratio) for 6 months. The authors observed improved insulin sensitivity and ovulatory function, along with decreased **luteinizing hormone (LH)** and free testosterone levels. The lower **LH/FSH ratio** subsequently reduces the observed **hyperandrogenism**. The authors also reported significantly reduced HOMA index and fasting insulin and significantly increased E2 and SHBG. The overall improved hormonal status restored the ovulation, without observed side effects. On the contrary, the placebo group reported no relevant changes in the levels of sex hormones [43,65].

The content of MI in human FF seems to play a role in follicular maturity and high concentrations represent a potential marker of good oocyte quality. As previously reported, studies have demonstrated that increased MI content improves the quality of blastocysts, while excess DCI has deleterious effects [21]. Indeed, DCI increases testosterone levels through two different pathways: in the theca cells from PCOS women as insulin mediator (inositolglycan mediator) [19] and in the granulosa cells as aromatase inhibitor [18]. Such evidence could provide a plausible explanation for the higher amounts of testosterone in women suffering PCOS, as compared with healthy individuals.

In summary, rebalancing the hormonal status and the metabolic parameters is beneficial to reproductive outcomes in humans, enhancing oocyte health and ovulatory function. The importance of preserving the balance between MI and DCI concentration in FF is also highlighted in the trials that used combined treatment of MI and DCI. Indeed, the physiologic ratio appeared to optimize the improvement of fertility [12]. In addition, literature data indicate that MI signaling may regulate the AMH production induced by FSH in the granulosa cells [11]. AMH decreases oocyte sensitivity to FSH and participates in regulating follicle maturation [66]. Treatment with MI in ***in vitro* fertilization (IVF)** allows a decrease in the amount of recombinant FSH administered and in the duration of the ovulation induction for follicular development [67] and an increase in the clinical pregnancy rate [45].

Clomiphene citrate (CC) is an antiestrogenic compound, used as first-choice drug in the therapy for oligo-anovulatory infertility. Although some patients are resistant, CC was widely used in PCOS women to induce ovulation because it increases the pituitary production of FSH and LH [68].

Researchers investigated inositol treatment, combined with CC, to assess possible fertility improvements in PCOS women seeking pregnancy [69]. In the study, 50 anovulatory PCOS patients received MI for three spontaneous cycles. If they remained anovulatory and/or failed to achieve pregnancy, they received a combination of MI and CC in the following three cycles. MI improved ovarian activity in PCOS women, as spontaneous ovulation occurred in 61.7% of patients, while 72.2% of MI-resistant subjects eventually ovulated after clomiphene treatment. A recent pilot study [70] further demonstrated that the combination of MI and CC significantly increases the ovulation rate, decreases the rate of resistance to CC, and improves the pregnancy rate. The study shows a potential benefit for MI supplementation during CC ovulation induction PCOS patients, even though the results failed to reach statistical significance for most outcomes, probably because of the small number of patients.

However, further studies are required to draw more definite conclusions [71]. A double-blinded, randomized, and controlled trial is currently recruiting patients with PCOS seeking pregnancy and eligible to simple ovulation induction by CC. Half of them will receive MI + folic acid in addition to CC, whereas the other half will receive a placebo containing only folic acid, in addition to CC. The initial results are expected by April 2020 (https://clinicaltrials.gov/ct2/show/NCT03059173).

Overcoming Issues in Inositol Therapy

Reduced Absorption of Inositol

Competition with DCI or interference of other molecules (e.g., glucose) on the transport mechanism may cause a reduced absorption of MI. Competition for the same transporter may cause insufficient MI passage across the intestinal barrier or inside the cells. This condition occurs when a competitor has affinity for the transporter greater than MI, or when a competitor has lower affinity but large concentration to displace MI. Two groups of inositol transporters exist, with different tissue distribution: sodium/*myo*-inositol cotransporter 1 and 2 (*SMIT1* and *SMIT2*), coupled with sodium ions; proton/*myo*-inositol cotransporter (*HMIT*), coupled with protons. Both of them are expressed in several tissues and organs (kidneys, brain, placenta, pancreas, heart, skeletal muscles, lungs, liver, intestine, adipose tissue, and oocytes) [72]. To date, *SMIT2* is the only known transporter of MI located in the intestine (duodenum and jejunum). *In vitro* experiments identified a K_m (DCI) lower than K_m (MI), hence DCI transport is slightly favored. To avoid enhanced intestinal absorption of DCI at the expense of MI, determining the proper MI:DCI ratio to administer to patients is essential. The abovementioned 40:1 ratio proved to be optimal. MI and DCI have a much greater affinity (more than 100 times) than glucose for the transporter [73,74].

Inositol Resistance

Inositol resistance indicates the therapeutic inefficacy of inositols in some 'resistant' patients, a condition occurring in several clinical studies [69,75]. Indeed, in 30%–40% of PCOS patients, inositols failed to significantly improve the metabolic and hormonal parameters and to restore ovulation. A well-founded hypothesis considers that unclear or unpredictable conditions (e.g., obesity, chronic intestinal diseases, dysbiosis) may cause reduced or absent absorption of inositol. To increase the absorption, researchers combined the administration of MI with **alpha-lactalbumin (α-LA)**, a whey protein that is an excellent 'carrier' for metal ions (mainly divalent, such as Ca^{2+} and Fe^{2+}) and for vitamin D [76,77]. The study [78] included 18 healthy volunteers that received a single dose of MI (6 g) in the first phase. After a week, they received 6 g of MI + 150 mg of α-LA in a single dose. Maximum MI plasma concentration (C_{max}) and area under the time-course curve of MI plasma concentration (AUC) after the combined administration were significantly higher (+32.4% and +27.5%, respectively) than after the administration of MI alone. Noteworthy, in both formulations, the time elapsed before the peak plasma concentration was the same. To understand the mechanism(s) underlying this effect, the authors tested the transport of MI, alone and combined with α-LA (as biopeptides, i.e., 'digested' protein), across the human intestinal Caco-2 cell monolayer [78], an *in vitro* model of gut mucosa commonly used [79,80]. They observed increased passage of MI in the presence of α-LA and a concomitant transient lowering of the transepithelial electrical resistance, indicating a temporary opening of the tight junctions between the cells [78] that causes a 'passive' transport of MI.

A subsequent clinical study on PCOS patients treated with MI plus α-LA (primary outcome: restoring ovulation) provided confirmation that the combined formulation is effective in human subjects [81]. Thirty-seven anovulatory PCOS women received oral MI (2 g), twice a day for 3 months. As a result, 23 subjects (62%) ovulated, whereas 14 (38%) remained anovulatory and showed signs of inositol resistance (i.e., no increase in MI plasma levels). These 14 patients received further treatment with 2 g MI + 50 mg α-LA, twice a day for an additional 3 months. Twelve (86%) patients ovulated, featuring significantly higher serum levels of MI and better hormone and lipid profiles with respect to the baseline.

These results confirm that a poor intestinal absorption of MI represents a relevant cause of inefficacy for inositol treatment in PCOS patients. Together with the study by Monastra *et al*.

[78], this study provides a promising option to overcome some limitations in inositol therapy. Future research is required to confirm these findings on larger cohorts of patients and on different PCOS phenotypes and to accurately devise the appropriate administration modes and the best combinations of MI, DCI, and α-LA.

Concluding Remarks

MI and DCI represent the most effective treatment in PCOS when administered in the 40:1 ratio, similar to the serum ratio of healthy women. Supplementation dosage of DCI solely must be carefully evaluated, considering increasing concentrations may alter the ovarian physiology. In this context, the different affinities of MI and DCI for the intestinal inositol transporter must be taken into account. New findings, based on the concurrent administration of α-LA, allow improved absorption of MI at the intestinal level. All these reports fruitfully enrich and enlarge the promising field of inositol studies (see Outstanding Questions) related to PCOS.

Outstanding Questions

Does the administration of *myo*-inositol (MI) and DCI decrease the risk of cancer in PCOS patients?

Is there an economic advantage coming from the reduction of recombinant follicle stimulating hormone (rFSH) used in IVF after MI treatment?

How beneficial is the supplementation with MI during ovulation induction with clomiphene citrate in PCOS patients?

Does alpha-lactalbumin, administered either with MI alone or with MI and DCI, exert a therapeutic effect *per se*, further improving inositol activity in PCOS?

Disclaimer Statement

Vittorio Unfer is an employee at Lo.Li. Pharma srl., Rome, Italy. Fabio Facchinetti has been a consultant of the same company. The other authors declare no potential conflicts of interest with respect to the publication of this article. The authors of this review are recognized experts in the field of PCOS and inositols. The studies presented here were selected by the authors based on their specific scientific expertise.

Supplemental Information

Supplemental information associated with this article can be found online at https://doi.org/10.1016/j.tem.2020.02.002.

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