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The Rationale of the Myo-Inositol and D-Chiro-Inositol Combined Treatment for Polycystic Ovary Syndrome

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Abstract

PCOS is one of the most common endocrine disorders affecting women and it is characterized by a combination of hyper-androgenism, chronic anovulation, and insulin resistance. While a significant progress has recently been made in the diagnosis for PCOS, the optimal infertility treatment remains to be determined. Two inositol isomers, myo-inositol (MI) and D-chiro-inositol (DCI) have been proven to be effective in PCOS treatment, by improving insulin resistance, serum androgen levels and many features of the metabolic syndrome. However, DCI alone, mostly when it is administered at high dosage, negatively affects oocyte quality, whereas the association MI/DCI, in a combination reproducing the plasma physiological ratio (40:1), represents a promising alternative in achieving better clinical results, by counteracting PCOS at both systemic and ovary level.

Keywords

embryo, infertility, inositol, oocyte, polycystic ovarian syndrome

Inositols and their derivatives (salts, phosphates, and associated lipids) are found in many foods, especially fruits and beans. In plants, inositol (INS) is generally represented in the form of hexaphosphate, and phytic acid or its salts (phytates).[1] Inositol is a hexahydroxycyclohexane, chemically represented by a stereo isomeric family of 9 inositols, among which myo-inositol (MI) is the most widely distributed in nature.

INS was once considered as a member of the vitamin B complex, however, it cannot be considered a "true" essential nutrient, in order that the human body can synthesized it from glucose.[2] Indeed, INS is synthesized by both prokaryotic and eukaryotic cells, even if in mammals it is mainly obtained from dietary sources, as well inositol-6-phosphate. Within the cells INS is put in its free form or as phosphatidylinositol (phosphoinositides, PtdIns). Phosphatidylinositol can be phosphorylated to form phosphatidylinositol phosphate (PIP) and biphosphate (PIP2), which fulfill several relevant physiological roles.[3]

INS is basically incorporated into cell membranes as phosphatidyl-myo-inositol, the precursor of inositol triphosphate (Ins-1,4,5P3, InsP3), which acts as second messenger, regulating the activities of several hormones such as FSH, TSH, and insulin.[4] In addition, INS serves as an important component of the structural lipids phosphatidyl-inositol and its various phosphorylated derivatives, the phosphatidyl-inositol phosphate lipids.[5] The INS derivative inositol-3-phosphate is a second messenger formed by phospholipase-C (PLP-C) mediated cleavage of phosphatidyl-inositol-4,5-phosphate (PI-4,5-P2, PIP2), when cells are stimulated by growth factors or other hormones.[6] Following interaction of Ins-1,4,5P3 with its mitochondria-coupled receptors, INS derivatives participate in calcium regulation and further activate several protein phosphorylation processes via protein kinase C (PKC) (Figure 1).

In addition, membrane-bound phosphoinositides (glycosyl-phosphatidylinositol, GPI) anchors various proteins to the plasma membrane. Approximately 150 different GPI-anchored proteins have been identified, they belong to different molecules' families. Two

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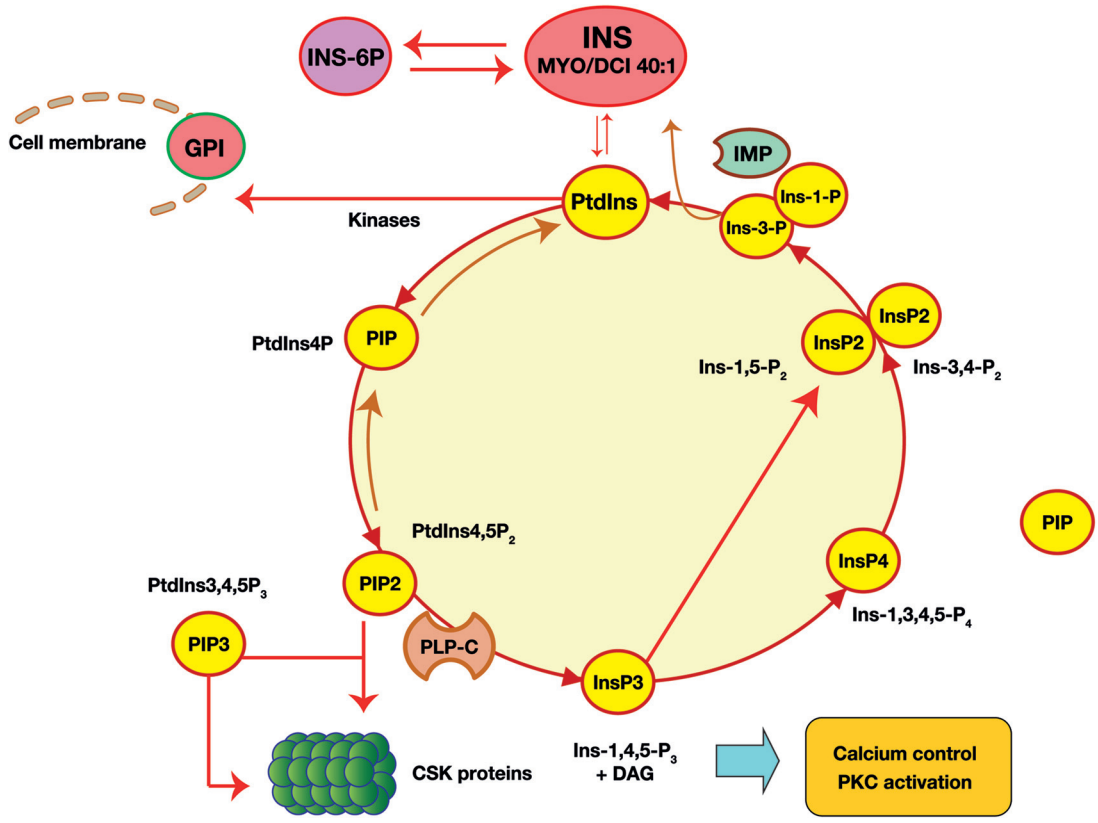

Figure 1. Intracellular inositol (INS) biochemical pathways. Ins-6-P, inositol exaphosphate (phytic acid); MYO, myo-inositol; DCI, D-chiro-inositol; PtdIns, phosphoinositides, phosphatidylinositol; PIP, PtdIns4P, phosphatidylinositol-4-phospate; PIP2, PtdIns4,5P2, phosphatidylinositol-4,5-biphospate; PIP3, PtdIns3,4,5P3, phosphatidylinositol-3,4,5-biphospate; GPI, glycosyl-phosphatidylinositol; PLP-C, phospholipase-C; CSK, cytoskeleton; InsP3, Ins-1,3,4-P3, inositol-1,3,4-phosphate; DAG, Di-acyl-glycerol; InsP4, inositol-1,3,4,5-phosphate; InsP2, inositol-1,5-phosphate and inositol-3,4-phosphate; Ins-3-P, inositol-3-phosphate; Ins-1-P, inositol-1-phosphate; IMP, inositol monophosphate phosphatise; PKC, protein kinase C.

distinct inositol phosphoglycan (IPG) putative mediators of insulin action have been separated and purified from GPI-proteins anchored on the extracellular side of cell membrane:[7,8] IPG-A (inositol phosphoglycan-AMP kinase inhibitor) containing MI and glucosamine, and IPG-P(Inositol phosphoglycan-phosphatase simulator) are constituted by D-chiro-inositol (DCI) and galactosamine.[9] Convincingly data obtained from different experiment suggest that IPGs are released in the extracellular space[10] and they hence actively re-enter the cell membrane through an ATP-dependent transport.[11]

Whereas intracellular INS pool is almost (>99%) constituted by MI in most tissues, significant differences have been noticed in the concentration of MI and DCI in fat, muscle and liver.[12] This different distribution reflects the distinct function, the two isomers are likely to play in those tissues, and their respective proportions are actively maintained as MI is enzymatically transformed into DCI through a NAD, NADH-dependent epimerase, accordingly to tissue requirement.

Biological Function of Inositol and Its Derivatives in Oocyte Biology

Increasing evidence indicates that INS, by itself or through its derivatives, plays a relevant role in several critical biochemical pathway including morphogenesis, cytoskeleton rearrangement, glucose metabolism, regulation of cell proliferation and fertility.[13,14]

MI is thought to exert a pivotal role namely in oocyte and spermatozoa development, as well as during fertility-related processes.[15]

The organs of the male reproductive tract are particularly rich in free MI.[16] High concentrations have been confirmed in the testis, epididymal, vesicular, and prostatic fluids of the rat.[17,18] Rat studies reveal that the MI concentration of the uterus and ovaries are under hormonal control. The INS concentration of female reproductive organs is much higher than in blood serum owing to their ability to concentrate MI from the blood stream.[19] Interestingly, the INS concentration in the uterine fluid was much lower than in seminal fluid of the

rat; it means that the surrounding MI concentration for the sperm is lowered when entering the uterus. These high MI concentrations in the male and female reproductive tracts suggest that inositol concentration in physiological fluids may significantly influence fertility. As far as results obtained on animal experimental models can hardly be transferred to human beings. Such data have been partially observed also in human studies and a clear-cut correlation has been found between MI serum levels and the outcome of pregnancy in vitro fertilization (IVF) treated patients: MI levels increased significantly during in IVF treatment when they are compared to natural cycle[20]; additionally, in the same setting, the embryotrophic properties of the sera were examined by a post-implantation mouse embryo culture showing a strong correlation with IVF outcome.

Indeed, MI is essential in ensuring proper oocyte maturation. MI and PLP-C mediated InsP3 release and LH/FSH activity[21,22]; furthermore, through specific Ins-1,4,5P3 receptors (IP_3-R), inositol-3-phosphate participates in modulating intracellular Ca^{2+} release from mitochondria. In oocytes that mechanism involves a specific receptor subtype (IP_3-R1),[23] and it seems to play a pivotal role in oocyte maturation, namely during the final stages of oogenesis, when oocyte sensitivity to calcium fluctuations reaches the maximal value. Indeed, calcium release from rat oocytes is triggered by Ins-1,4,5P3 injection, leading then to oocyte maturation.[24] Moreover, a supplementation with MI can promote meiotic progression into fertilization-competent eggs, whereas depletion of MI intracellular stores desensitizes inositol-related pathways, reducing $InsP_3$ and releasing[25] proper calcium. Seemingly to what happen in the oocyte, in the zygote as well Ca^{++} oscillations may play a relevant physiologic role.

MI absorption into embryos is an ATP-dependent process,[26] leading to incorporation of INS into PtdIns and inositol phosphates[27]; as such, MI and its derivatives enhance bovine blastocyst development from in vitro culture with medium supplemented with MI[28] and actively participate in embryogenesis.[29] Unfortunately, it is not possible to drawn firm clinical conclusions, since the culture media were not the one used in the clinical settings. Yet, in "Colazingari et al," a new published paper, more relevant evidence is provided on the effect of MI in improving IVF outcomes. Indeed, by employing media routinely used into clinic and applying the protocol for embryo culture used for batch release, authors were able to demonstrate that culturing embryos in media enriched with MI, embryos have a more physiological cleavage rate and an increased number of expanded blastocyst formed by an higher number of blastomeres.[30]

$InsP_3$ receptors are indeed over expressed during the early stages of zygote development, and calcium fluctuations occurring in the cleavage stage of the embryo are likely to influence the pre-implantation embryo development.[31] Furthermore, it has been demonstrated that the proportion of fertilized oocytes with 2PN, the number of 2-cell stage embryos developed and the percentage of normality of the post-implantation embryos were significantly higher when germinal vesicles were cultured in a maturation medium containing MI compared with control medium. Moreover, phosphorylated derivatives of INS (Ins-1,4,5P3) participate in cytoskeleton regulation[32,33] and are required to accelerate oviductal transport of oocytes[34] (Figure 2). Moreover, a very preliminary report indicates that MI modulates the anti-mullerian hormone (AMH) serum levels.[35] AMH belongs to the Tgf-β superfamily and it is releases after FSH stimulation by the granulosa cells. In turn AMH decrease oocyte sensitivity to FSH and participates in regulating follicle maturation.[36] Indeed, poor serum AMH levels are considered a marker of diminished ovarian reserve (DOR).[37] It is worth of noting that MI supplementation significantly enhances AMH serum levels in patients affected by DOR, and then increase the likelihood of pregnancy.

Inositol is also needed to afford normal developmental processes. Fetuses require INS during gestation and have been proven to be able in concentrating it from maternal blood. At midgestation the MI concentration in mixed-umbilical cord serum was fivefold higher than the maternal serum concentration. At term, the serum MI concentration of the neonates had decreased, but it was still two- to threefold higher than in maternal blood.[38] Yet, MI promotes differentiation of the fetal lung[39] and prevents neural tube defects.[40] Given that MI uptake from embryonic cells is competitively inhibited by glucose, it has been suggested that congenital malformations, especially of the central nervous system and the heart, observed with high frequency in infants of diabetic mothers,[41] could be ascribed to hyperglycemia-induced tissue specific shortage of MI.[42,43] On the contrary, MI supplementation has been shown to reduce the birth prevalence of neural tube defects in streptozotocin induced diabetic Sprague–Dawley rats and Curly tail mice. A similar effect could be expected in human subjects.[44]

Inositol and Glucose Metabolism in PCOS Patients

Overall such results advocate a relevant physiological role sustained by INS and its metabolites in human reproduction, as claimed by "Beemster et al" seminal paper.[45] Namely, during the last decades, INS supplementation has been proposed as a novel treatment in women affected by polycystic ovary syndrome (PCOS).

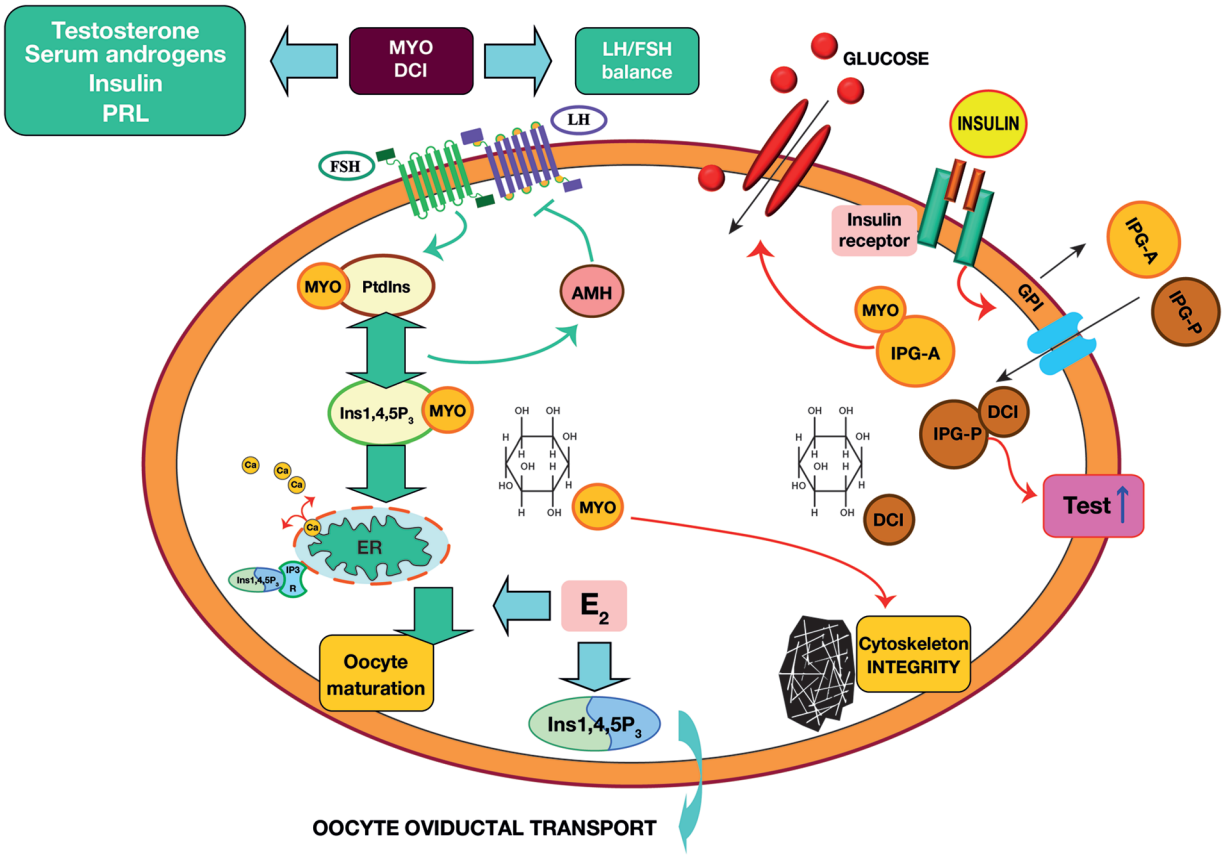

Figure 2. Inositol functions in the oocyte. E2, estradiol; Ins-1,3,4-P3, inositol-1,3,4-phosphate; IPG-P, inositol phosphoglycan-phosphatase simulator; IPG-A, inositol phosphoglycan-AMP kinase inhibitor; PtdIns, phosphoinositides, phosphatidylinositol; IP3-R, Inositol-1,3,4-phosphate-receptor; ER, endoplasmatic reticulum; CSK, cytoskeleton; MYO, myo-inositol; DCI, D-chiro-inositol; AMH, anti-mullerian hormone; GPI, glycosyl-phosphatidylinositol.

The early impetuses for these studies rely on the well-known correlation in between metabolic syndrome and PCOS, as well as the observed defects in INS metabolism in PCOS and the implication of INS in insulin signal transduction. It is widely acknowledged that both insulin insensitivity and metabolic syndrome are prominent features in a consistent proportion of patients affected by PCOS;[46–49] a disease characterized by chronic anovulation, hyperandrogenism, dyslipidemia, and infertility that affect barely 10% of women of reproductive age.[50] It is worth of noting that insulin signaling pathways involve inositol phosphoglycans. When insulin binds to its receptor, two distinct inositol phosphoglycans (IPG) are released by hydrolysis of glycosyl-phosphatidylinositol lipids located at the outer leaflet of the cell membrane. IPGs are then internalized and they affect intracellular metabolic processes, namely by activating key enzymes that control the oxidative and non-oxidative metabolism of glucose.[7]

Indeed, phosphoglycans formed by DCI (IPG-P) seem to play a relevant role in insulin signaling transduction and seems to be more effective in partially restoring insulin sensitivity and glycogen synthesis than phosphoglycan incorporating MI.[8]

Yet, MI supplementation has demonstrated to significantly improve features of dysmetabolic syndrome, including insulin sensitivity, impaired glucose tolerance, lipids levels and diastolic blood pressure.[51] In addition, it has been noticed that MI improve glucose tolerance in rhesus monkeys,[52] meanwhile Schofeld and Hackett demonstrated that MI-containing IPG from *P. falciparum* also had insulin-like effects in vitro and in vivo.*53* At least in part, such results could be explained by the transformation of MI to DCI occurring in peripheral tissues. Indeed, insulin resistance has been associated to reduced availability of DCI, documented by decreased urinary excretion of IPG-P in both animals[54] and diabetic patients,[55] and by lowered DCI levels in muscle from type 2 diabetes individuals.[55,56] In turn, hyperglycemia was reduced in diabetic rats or monkeys suffering from insulin resistance, by supplementing the diet with DCI.[7] Such discrepancies could presumably be explained considering

that while DCI is crucial for glycogen synthesis, MI increases glucose cellular uptake.[57]

On the other hand, it is well recognized that increased insulin sensitivity in PCOS patients by means of conventional antidiabetic drugs promotes a significant improvement in the ovulatory function and decreases serum androgen concentrations.[58–60] It is worth noting that Metformin increases the insulin stimulated release of DCI-phosphoglycans, thus evidencing the antidiabetic drug may enhance insulin sensitivity by restoring an inositol-based signaling.[60] In support of this hypothesis it was further documented that PCOS patients show increased DCI urinary clearance and consistent DCI urinary loss, presumably leading to a tissue depletion of DCI-phosphoglycans.[61] Once more, these data provided hints in hypothesizing a direct correlation between the availability of inositol phosphoglycans and insulin resistance, even if further studies are warranted in order to fully elucidate the mechanistic pathways linking inositol-derivatives to glucose metabolism.

Inositols and Ovary Function

Observations relating INS to glucose metabolism provided impetus to ascertain the usefulness, whether any, of DCI supplementation in PCOS clinical management. In his seminal paper, Nestler et al[62] demonstrated that in women with PCOS, DCI treatment at a dose of 1,200 mg/daily significantly reduced serum testosterone levels and improved both ovulation rate and metabolic parameters, such as blood pressure and triglycerides. Unfortunately that study provided no information on menstrual cycle regularity. Similar results have been subsequently recorded in lean PCOS women by the same group.[63] Those preliminary data prompted Nestler and colleagues to advance the results they obtained during the first trials, by increasing up to 2,400 mg the daily dose of DCI administered to PCOS patients.[64] That study was able to find a direct correlation between insulin-stimulated release of DCI-containing phosphoglycans and insulin sensitivity. Unexpectedly, that investigation was incapable in confirming the previous beneficial effects of DCI on ovary function, and the Authors did not offer any valuable hint to explain such a paradoxical outcome. A recent investigation performed on PCOS patients treated with increasing DCI doses (from 300 to 2,400 mg/day) has provided a compelling confirmation that by increasing DCI dosage paradoxically worsens oocyte quality and ovarian response in non-obese and non-insulin resistant PCOS women.[65] Namely, total r-FSH units increased significantly in the two groups receiving the higher doses of DCI, while the number of immature oocytes was significantly increased in the three groups treated with the highest doses of DCI. Additionally, the number of grade I embryos was significantly reduced by DCI supplementation.

It is intriguing that those disappointing data are in some way mirrored by results obtained by treating PCOS patients with Metformin: the antidiabetic drug decreases the follicles number and worsens their quality,[66] even if Metformin significantly increases the insulin-stimulated release of DCI-phosphoglycans.[61]

Such contradictory results could likely be explained by considering the different function in each INS isomer plays in distinct tissues. Indeed, a specific MI/DCI ratio has been observed within each tissue: high DCI (even if always lower than MI concentration) is generally observed in glycogen storage tissues (fat, liver, muscle), whereas tissues characterized by high consumption rate of glucose (brain, heart) present low DCI levels.[67] Oocytes are characterized by high glucose consumption along the oxidative pathway: thus, by impairing sugar availability oocyte quality would likely be compromised . Indeed, reduced availability of glucose in both oocytes and follicular cells caused by defective transportation of glucose it is suspected to occur in PCOS.[68] In turn, energy impairment promotes alternative pathways to utilize fatty acid and amino acids for energy as a compensatory mechanism to deal with energy requirement.[69] Moreover, in PCOS women, genes involved in the glucose uptake pathway are down-regulated at ovarian level[70,71] and energy supplementation is required in achieving higher oocyte quality and better outcome after IVF in PCOS patients.[70] Those data highlight how is important to maintain a proper glucose metabolism for oocyte development. Undoubtedly, both DCI and MI are required to fulfill such function in cooperating with insulin. Yet, MI seems to play a more important role in oocyte, as suggested by the fact that almost the 99% of intracellular INS pool is constituted by MI.[72] DCI, instead, is produced from MI through a NAD-dependent epimerase whenever it is required. The epimerase conversion of MI to DCI is under insulin control: in type 2 diabetes patients, the reduced tissue insulin sensitivity leads to reduced epimerase activity and hence DCI synthesis.[55] However, ovary never displays insulin resistance, unlike other tissues.[73] Thus, increased insulin levels as those recorded in insulin-resistant patients, are likely to increase the activity of ovary epimerase, raising in that way the DCI intracellular production, meanwhile MI levels were progressively reduced. Thereby, in hyperinsulinemic PCOS patients DCI levels paradoxically increase in the ovary. In turn, DCI increase may promote glycogen and testosterone synthesis, impairing the oocyte maturation. Eventually, that mechanism would lower oocyte's MI content, and the MI depletion will in turn negatively affect oocyte quality.

Such imbalance may shed light in the pathogenesis of PCOS at cellular level and helps elucidate the theory

known as "the DCI paradox in the ovary."[74] That hypothesis has recently received a preliminary confirmation by two independent investigations. A study authored by Larner and coworkers has found a significant increase in the epimerase activity in the theca cells obtained from ovary of PCOS women, leading to a dramatic reduction in the MI/DCI ratio.[75] Another research, by investigating the INS concentration in follicular fluid obtained from PCOS women has observed a significant decrease in the MI/DCI ratio: while normal MI/DCI ratio is nearly 100:1, in follicular fluid of PCOS women that value is only 0.2:1.[72] The remarkable abatement in MI levels may thus likely contribute in explaining at least some of the observed dysfunction of ovary in PCOS.

Indeed, whereas DCI administration has been demonstrated to improve the systemic consequences of insulin resistance, namely by modulating insulin effects of endocrinology balance in non-ovarian tissues, DCI has probably only a marginal effect on oocyte function. In turn, a DCI overload, as such obtained by administering 600 mg/day or more, may have likely worsen INS imbalance into ovary cells, determining a dramatic decrease in the MI/DCI intracellular ratio. Additionally, high release of DCI-phosphoglycans, under insulin stimulation, enhances de novo testosterone biosynthesis from ovarian theca cells, thus raising serum androgen levels.[76] Those effects would explain why the promising results obtained by Nestler and coworkers during the first study have not been confirmed in the second one.

Indeed, it is of outmost importance to ensure the proper MI concentration at the ovary level. MI-based phosphoglycans (IPG-A) are required to facilitate oocyte glucose uptake.[7] Furthermore MI improves oocyte response to FSH, as indicated by the reduced requirement in rFSH IU administered during IVF cycles.[77,78] MI supplementation restores spontaneous ovulation and increases progesterone release during the luteal phase in all but few PCOS patients.[79] Therefore, it is worth of noting that a MI deficiency in the ovary would likely impair the FSH signal, resulting in an increased risk of ovarian hyperstimulation syndrome for PCOS patients. MI exerts in addition other appreciable systemic effects, by improving the reproductive axis functioning in PCOS patients through the reduction of the hyper-insulinemic state which affects LH secretion.[80] In that study, following 12 weeks of MI treatment, serum hormone levels were normalized and menstrual cycle was restored in amenorrheic patients. Moreover, PCOS patients submitted to ICSI, the associated treatment of MI and folic acid, but not folic acid alone, significantly decreases the cancellation rate, it reduces oocyte degeneration and germinal vesicles at ovum pickup, thus increasing the success probability of the therapy.[77] Indeed, serum and follicular fluid concentration of MI has been proven to be directly associated with oocyte maturation and fertility outcome in IVF-treated patients.[81]

Inositols and PCOS Treatment

Usefulness of MI supplementation has since been assessed by several reports. Morgante et al have evidenced that MI supplementation in insulin resistant-PCOS patients produces significant results, given the significant reduction in cancellation rate (0% vs. 40%) and the consequent improvement in clinical pregnancy rate (33.3% vs. 13.3%) obtained with INS treatment.[82] Gerli et al[83] conducted a randomized, double-blind, placebo-controlled trial of 283 PCOS patients treated with MI. In that study, frequency of ovulation (40%) was increased by almost twofold in women who received MI, versus the control group. Moreover, aiming to elucidate the systemic beneficial effects associated to MI therapy, additional studies were able to show that MI treatment lowered lipids,[84] insulin and androgen levels, increased insulin sensitivity, reduced blood diastolic pressure, and was effective in treating acne[21] and hirsutism.[85] Overall, those data demonstrated that MI is equally effective than DCI in normalizing metabolic and endocrine features commonly associated to insulin resistance and PCOS.

Yet, normalizing insulin resistance is not enough for restoring a proper ovulatory function, as suggested by a recent study comparing MI supplementation versus Metformin.[86] Sixty PCOS patients were treated with MI 4 g plus Folic acid, and 60 PCOS patients with Metformin 1500 mg/day. Among the patients treated with Metformin, 50% restored spontaneous ovulation activity. Pregnancy occurred spontaneously in 11 (36.6%) of these patients. In the MI group, 65% of patients restored spontaneous ovulation activity, ovulation occurred after a mean of 14.8 days from the day 1 of the menstrual cycle; in this latter group pregnancy occurred in 18 (48.4%) patients, showing a positive trend in increase.

Overall, those data evidence that MI supplementation provides significant benefit in PCOS management (Table 1), even most papers suffer for the lack of proper randomization and/or are flawed by few statistical inconsistencies.

However, by considering both the systemic and the ovary hallmarks of PCOS, INS supplementation should preferably include both the isomers: MI and DCI. Given that physiological values of the MI/DCI ratio, evaluated both in the plasma as well as in the follicular fluid, range from 40:1 to 100:1, it seems reasonable that INS should be administered jointly respecting a proportion that should reflect the natural balance among the two stereoisomers. Therefore, as proposed by a recent paper,[87] the combined administration of MI and DCI in the physiological plasma ratio (40:1), could be considered as a first line approach in

Table 1. Eligible RCTs Where MI Have Been Evaluated for the Treatment of PCOS Patients

Ref.	Study Design	Duration	Intervation	No. of Subjects	Inclusion Criteria	Exclusion Criteria	Assessment of the Response	Results
Genazzani et al[80]	Randomized, controlled vs. folic acid	12 weeks	MYO 2 g FA 200 mg/ day	N = 20, Treatment: 10, Placebo: 10	Presence of micropolycystic ovaries at ultrasound; mild to severe hirsutism and/or acne; oligomenorrhea or amenorrhea; absence of enzymatic adrenal deficiency and/or other endocrine disease; normal PRL levels (range 5–25 ng/mL); no hormonal treatment for at least 6 months before the study	Not described	LH, FSH, PRL, E2, A, 17OHP, T, insulin, cortisol, OGTT[a] for insulin, glucose, C-peptide determinations, vaginal ultrasound examination Ferriman-Gallway score, BMI, HOMA	LH, PRL, T, insulin levels, LH/FSH resulted significantly reduced. Insulin sensitivity resulted significantly improved. Menstrual cyclicity was restored in all amenorrheic and oligomenorrheic subjects
Morgante et al[82]	Randomized, controlled trial vs. placebo	4 weeks	Inositol 1,500 mg	N = 30, Treatment:15, Placebo: 15	Clomiphene-failure patients with PCOS, with insulin resistance evaluated by Homeostatic Model Assessment index	Not described	HOMA, FSH (IU/L), LH (IU/L), PRL (ng/mL), 17-OHP, DHEAS, number of follicles >15 mm in diameter, number of follicles >18 mm in diameter, E2 levels on day of hCG administration (pg/mL), cancellation rate (%), clinical PR (%)	The total number of follicles >15 mm and >18 mm in diameter, and the peak E2 levels were significantly lower in the inositol group compared with the control group. In addition, although the cancellation rate was significantly lower in the inositol group, the clinical PR was not significantly higher, compared with the control group
Gerli et al[83]	Double-blind, placebo-controlled vs. placebo	16 weeks	Inositol 200 mg/day	N = 283, Treatment:136, Placebo:147	Age: <35 years women with oligomenorrhea and PCOS ovaries. Ovaries were described as polycystic (PCOs) about the criteria of Adams et al[b]	Patients with significant hyperprolactinemia, abnormal thyroid function tests and congenital adrenal hyperplasia	Ovarian activity was monitored using serum E2, P and LH. Ovulation frequency was calculated using the ratio of luteal phase weeks to observation weeks. Inibin-b, fasting glucose, fasting insulin, or insulin AUC, VLDL, LDL, HDL, total cholesterol, triglycerides, BMI	Ovulation frequency significantly higher in the treated group vs. the placebo. follicular maturation was rapid: the circulating concentration of E2 increased only in the inositol group during the first week of treatment. Significant weight loss was recorded in the inositol group, whereas in the placebo group was recorded an increase of the weight. A significant increase in circulating high-density

(*Continued*)

Table 1. Continued

Ref.	Study Design	Duration	Intervation	No. of Subjects	Inclusion Criteria	Exclusion Criteria	Assessment of the Response	Results
								lipoprotein was observed only in the inositol-treated group
Minozzi et al[84]	Open-label clinical study	12 months	MYO 4 g/ day	N = 155	PCOS	Women with secondary endocrine disorder, those wishing to conceive during the next 12 months and those with contradictions to oral contraceptive use	Clinical and anthropometric measurement included: age, (BMI), serum levels of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, apolipoprotein B (apoB), lipoprotein(a) [Lp(a)], fasting glucose level, fasting insulin level, insulin resistance, measured by homeostasis model assessment (HOMA-IR), testosterone (T), sex hormone-binding globulin (SHBG), D4-androstenedione (A), dehydroepiandrosterone (DHEAS), leutenizing hormone (LH), and hirsutism score, evaluated by using a modification of the Ferriman–Gallwey (FG) score	A higher reduction of FG score in OCP plus MI therapy group vs OCP alone therapy group. OCP plus MI significantly decreased hyperinsulinemia vs the OCP group. Androgens serum levels decreased in both groups, but significantly more in OCP plus MI group. The lipid profile was improved in the OCP plus MI group, by reducing low-density lipoprotein cholesterol levels and enhancing high density lipoprotein cholesterol levels
Minozzi et al[85]	Open-label clinical study	6 months	MYO 2 g/ twice a day	N = 46	Women with mild to moderate hirsutism, evaluated using a modification of the Ferriman–Gallwey score	Hyperprolactinemia, hypothyroidism, adrenal hyperplasia and Cushing's syndrome. Patients who had taken hormonal medications, including contraceptive pills, for the past 6 months	Hirsutism scores Serum concentrations of total cholesterol, HDL cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, apolipoprotein B, lipoprotein(a), glucose, insulin, testosterone, sex hormone-binding globulin (SHBG), Δ4-androstenedione, cortisol, dehydroepiandrosterone sulphate (DHEA-S), LH, FSH and E2 were measured within the first 5 days of the menstrual cycle	No changes in BMI were observed. The hirsutism decreased after therapy. Total androgens, FSH and LH concentrations decreased, oestradiol concentrations increased. There was a slight non-significant decrease in total cholesterol concentrations, an increase in HDL cholesterol concentrations and a decrease in LDL cholesterol concentrations. No significant changes were observed in serum triglyceride, apolipoprotein B and lipoprotein(a) concentrations.

(*Continued*)

Table 1. Continued

Ref.	Study Design	Duration	Intervation	No. of Subjects	Inclusion Criteria	Exclusion Criteria	Assessment of the Response	Results
								Insulin resistance, analysed by homeostasis model assessment, was reduced significantly after therapy
Raffone et al[86]	Randomized controlled vs. metformin	Until the end of the study or positive pregnancy test	MYO 4 g FA 400 mg	N = 120, Treatment: 60, Placebo: 60	PCOS <35 years defined by Rotterdam Criteria	Other medical condition causing ovulatory dysfunction, tubal defects, semen parameters defects	Restoration of spontaneous ovulation and menstrual cycles and increasing rate pregnancy, ovarian activity, serum progesterone dosage, progesterone levels, β-hCG plasma level	Among the patients treated with metformin, 50% restored spontaneous ovulation activity. Pregnancy occurred spontaneously in 11 (36.6%) of these patients. In the MI group, 65% of patients restored spontaneous ovulation activity, ovulation occurred after a mean of 14.8 days from the day 1 of the menstrual cycle; in this latter group pregnancy occurred in 18 (48.4%) patients, showing a positive trend in increase
Nordio and Proietti[87]	Randomized controlled	6 months	2 g MYO powder 550 mg of Myo plus 13.8 mg of D-chiro-inositol in soft gel capsule	N = 50, Treatment: 24, Combined therapy: 26	Overweight women with PCOS	Diabetic subjects, smokers and alcohol users were excluded from the study	Plasma glucose and insulin concentrations Serum progesterone Ovulation function	The combined administration of MI and DCI reduces the metabolic and clinical alteration of PCOS and reduces the risk of metabolic syndrome
Costantino et al[103]	Double-blind, randomized controlled vs. folic acid	12–16 weeks	MYO 4 g FA 400 mg/day	N = 42, Treatment: 23, Placebo: 19	Presence of oligomenorrhea, high serum-free testosterone level and/or hirsutism presence of micropolycystic ovaries at ultrasound	Not described	Systolic/diastolic blood pressure, triglycerides, cholesterol, BMI, plasma glucose and insulin sensitivity, total/free T, DHEAS, SHBG, A, progesterone peak value	MI increased insulin sensitivity, improved glucose tolerance and decreased glucose stimulated insulin release. There was a decrement in serum total T and serum-free T concentrations. In addition, there was a decrement in systolic and diastolic blood pressure. Plasma triglycerides and total cholesterol

(*Continued*)

Table 1. Continued

Ref.	Study Design	Duration	Intervation	No. of Subjects	Inclusion Criteria	Exclusion Criteria	Assessment of the Response	Results
								concentration decreased
Papaleo et al[104]	Prospective, randomized, controlled vs. folic acid	During ovulation induction for ICSI	MYO 4 g FA 200 mg/day	N = 60, Treatment: 30, Placebo: 30	Age: <40 years PCOS women diagnosed by oligoamenorrhea, hyperandrogenism orhyperandrogenemia and typical features of ovaries on ultrasound scan	Other medical conditions causing ovulatory disorders: hyperinsulinemia, hyperprolactinemia, hypothyroidism, or androgen excess, such as adrenal hyperplasia or Cushing syndrome	Number of morphologically mature oocytes retrieved, embryo quality, pregnancy and implantation rates. Total number of days of FSH stimulation, total dose of gonadotropin administered, E2 level on the day of hCG administration, fertilization rate per number of retrieved oocytes, embryo cleavage rate, live birth and miscarriage rate, severe ovarian hyperstimulation syndrome	Total r-FSH units and number of days of stimulation were significantly reduced in the myo-inositol group. Peak E2 levels at hCG administration were significantly lower in patients receiving myo-inositol. The mean number of oocytes retrieved did not differ in the two groups, whereas in the group cotreated with myo-inositol the mean number of germinal vesicles and degenerated oocytes was significantly reduced, with a trend for increased percentage of oocytes in metaphase II
Gerli et al[105]	Double-blind, randomized, controlled vs. folic acid	16 weeks	MYO 4 g FA 200 mg/day	N = 92, Treatment: 45, Placebo: 47	Age: <35 years Women with oligomenorrhea, amenorrhea and PCOS ovaries. Ovaries were described as polycystic (PCOs) about the criteria of Adams et al[b]	Patients with significant hyperprolactinemia, abnormal thyroid function tests, and congenital adrenal hyperplasia	Ovarian activity was monitored using serum E2, P, LH. ovulation frequency was calculated using the ratio of luteal phase weeks to observation weeks. Inibin-b, fasting glucose, fasting insulin, or insulin AUC, VLDL, LDL, HDL, total cholesterol, triglycerides, BMI	Beneficial effect of MYO treatment upon ovarian function, anthropometric measures and lipid profiles

LFA, folic acid; PRL, prolactin; E2, estradiol; A, androstenedione; 17OHP, 17-hydroxy-progesterone; T, testosterone; P, progesterone; OGTT, oral glucose tolerance; BMI, body mass index; LH, luteinizing hormone; FSH, follicle stimulatig hormone; DHEAS, dehydroepiandrosterone; SHBG, sex hormone binding globulin; EAUC, area under the curve of OGTT; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

[a]OGTT was performed sampling 15 minutes before, and 30, 60, 90, 120, and 240 minutes after the oral assumption of 75 g of glucose.

[b]Adams J, Polson JW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. Br Med J 1986; 293: 355–359.

PCOS overweight patients, being able to reduce the metabolic, hormonal and clinical alteration of PCOS.

Conclusion

PCOS is one of the most common endocrine disorders affecting women. It represents the most common cause of female infertility and it is characterized by a combination of hyperandrogenism, chronic anovulation and irregular menstrual cycle.[88] A significant number of patients suffers also from metabolic syndrome and insulin resistance, even if such features are currently not entirely understood.[89,90]

While a significant progress has recently been made in the diagnosis for PCOS,[91–93] the optimal infertility treatment in such cases remains to be determined.[94]

Recently, several clinical studies have highlighted the usefulness of INS supplementation in PCOS treatment. MI is readily taken up following oral ingestion[95] and it is has been proven to be safe even after high dose consumption.[96]

However, despite the relatively high number of reports, only few of them fulfill the criteria of randomized clinical trial. Those studies have been extensively reviewed elsewhere.[97,98] Among 70 studies focusing on PCOS treatment by means of different pharmaceutical composition incorporating INS, 21 were considered eligible as they involve MI.[97] Yet, only six of them were randomized controlled clinical trials (level of evidence I_b), involving more than 300 PCOS patients. Remarkably, in all the studies analyzed, no side effects were reported at the doses of both 2 and 4 g/day, thus resulting in a high patient compliance. The 4 g/day treatment regimen is useful to treat the symptom spectrum, resulting in a more complete and effective treatment. Overall, those studies indicate that MI supplementation improves several of the hormonal disturbances of PCOS, providing so far a level I_a evidence of MI effectiveness. MI mechanisms of action appear to be mainly based on improving insulin sensitivity of target tissues, resulting in a positive effect on the reproductive axis (MI restores ovulation and improves oocyte quality) and hormonal functions (MI reduces clinical and biochemical hyperandrogenism and dyslipidemia) through the reduction of insulin plasma levels.

These systemic hallmarks of PCOS are significantly affected by both DCI and MI supplementation. However, DCI treatment, mostly when it is administered at high dosage (ie, 600 mg or more), exerts disappointing effects on ovary functions.[65] Oocyte physiology, among other factors, is likely to be dependent on a fairy balance in between MI and DCI. Indeed, MI is an important constituent of follicular microenvironment, playing a determinant role in both nuclear and cytoplasmic oocyte development. Perhaps, the content of MI in follicular fluids may represent a more appropriate physiological indicator than follicular volume for monitoring the status of the developing follicles. Follicles that containing good quality oocytes have higher concentrations of MI in follicular fluids, probably due to the intricate relationship between MI and inositol phosphates in the PtdIns cycle activation for oocyte maturation.

Additionally, MI by improving glucose uptake may improve both oocyte energy status and oocyte quality. Moreover, during ovarian stimulation MI reduces FSH-IU necessary for ovarian stimulation. Altogether, this evidence hints that MI exerts several beneficial effects that improve the pregnancy chance.[99]

A further confirmation of the link among MI and oocyte quality has been provided by two recent studies performed on both PCOS and non-PCOS women.[100] In a prospective randomized open-label, pilot clinical trial involving one hundred non-PCOS women undergoing multiple follicular stimulation for in vitro fertilization, the addition of MI promotes the oocyte's meiotic maturation and reduces the number of gonadotropin cycles of treatment, while maintaining clinical pregnancy rate. Even if this study is underpowered to evaluate IVF outcomes like implantation and clinical pregnancy, a trend in favor of increased incidence of implantation in the group pre-treated with MI was observed. Another clinical investigation,[101] focused on one hundred PCOS women undergoing IVF-ET. Patients were treated with MI combined with DCI in the physiological ratio (40:1), or with DCI alone (500 mg). Significant better results were observed in the MI + DCI treated group, given that patients treated with this combination required lower dosages of FSH for a shorter period of time and showed an improvement in both oocyte quality and pregnancy rate.

Therefore, since the attention of the scientific community has recently been drawn on the relevance of the MI/DCI ratio for a proper ovary function, a treatment based on the association MI/DCI, in a physiological plasma range (ie, 40:1) seems to be the most effective approach, as recently stated by an international Consensus Conference held in Florence (December 2013).[102] Moreover, the recent data by Larner's group provided a molecular mechanistic basis supporting that statement, highlighting the "utility of both MI and D-chiro-inositol as effective agents in treatment. Certainly, a balance between the two inositols is required for normal physiological function and regulation of the MI to chiro-inositol epimerase opens a new avenue for upcoming studies."[75]

Undoubtedly, further studies are warranted to fully elucidate the molecular pathways triggered by myo- and D-chiro-inositol underlining their beneficial effects and to provide a well-grounded rationale for INS supplementation in PCOS patients.[103–105]

Declaration of Conflicting Interests

G.C. and V.U. are Lo.Li. Pharma employees.

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