EXPERT REVIEWS

Updates on the myo-inositol plus D-chiro-inositol combined therapy in polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting women of reproductive age. It is characterized by chronic anovulation, hyperandrogenism, and insulin resistance. It is the main cause of infertility due to the menstrual dysfunction and metabolic disorders. Women with PCOS also have an increased cardiovascular risk because of dyslipidemia and insulin resistance. So far, we have a lot of information about the etiology of PCOS, and many steps forward have been made about the diagnosis of this syndrome, but there is still no certainty about the therapy. Myo-inositol (MI) and D-chiro-inositol, two inositol stereoisomers, have been proven to be effective in PCOS treatment. However, only MI has been shown to have beneficial effects on reproductive function, whereas the administration of MI/D-chiro-inositol, in the physiological plasma ratio (i.e., 40:1) ensures better clinical results, such as the reduction of insulin resistance, androgens' blood levels, cardiovascular risk and regularization of menstrual cycle with spontaneous ovulation.

KEYWORDS: infertility • inositol • insulin resistance • oocyte • polycystic ovarian syndrome

Inositol (INS) is a hexahydroxycyclohexane with nine isomeric forms among which the myo-inositol (MI) stands out for its important biological role. INS is found in many foods, particularly in cereals, nuts, fruits and animal tissues, where it is concentrated in the phospholipids. In plants, INS is generally represented in the form of hexaphosphate and phytic acid or phytates [1].

MI was classified as a component of B complex (referring to it as B7) and it is synthesized by human body from glucose [2]. Liver is the key organ for its endogenous synthesis as well as the kidneys. Similar to the B group vitamins, INS is water soluble; for that reason, each supplementation is well tolerated and devoid of toxicity. It is synthesized from glucose 6-phosphate (the first product of glycolysis) and the excess is catabolized and disposed of through the kidneys. Inside the cells, INS is present in its free form or as phosphatidylinositol. Many studies, in fact, support the notion that MI is one of the precursors for the synthesis of phosphatidylinositol polyphosphates. Phosphatidylinositol can be phosphorylated to form phosphatidylinositol phosphate and bisphosphate, which perform several relevant physiological roles [3].

INS is incorporated into cell membranes as phosphatidyl-MI, the precursor of inositol trisphosphate (InsP3), which acts as a second messenger, regulating the activities of several hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone and insulin [4]. Phosphatidylinositol polyphosphates are also key biomolecules in signal transduction pathways involved in the regulation of several cellular processes: cell membrane formation, lipid synthesis and cell growth [5].

The InsP3 is produced via hydrolysis of a specific PtdInsPs by phospholipase-C. This reaction produces two different signal transduction molecules: the InsP3, also called INS 1,4,5-trisphosphate and the diacylglycerol, both second messengers used by cells to perform signal transduction [6].

While the diacylglycerol remains within the membrane, the InsP3 is soluble and is capable of diffusing through the cell. The InsP3 is derived from phosphatidylinositol

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4,5-bisphosphate, a phospholipid localized within the cell membrane, by the action of phospholipase-C. Once produced, the InsP3 binds to and activates the INS trisphosphate receptor, a large channel protein that is located on the surface of the endoplasmic reticulum. Its connection with this protein allows its opening and the release of calcium flowing into the cytoplasm [6].

Besides MI, another notable INS stereoisomer is the D-chiro-inositol (DCI). It is a product of the epimerization of the C1 hydroxyl group of MI. Within the cell, the DCI seems to play an important role as a second messenger of insulin.

While, in many tissues, almost >99% of the intracellular INS pool is constituted by MI, significant differences have been observed in MI and DCI concentration in fat, muscle and liver. This different distribution reflects the distinct roles that these two stereoisomers have within the tissues. This proportion within the cell is maintained by means of a direct enzymatic transformation of MI to DCI through nicotinamide adenine dinucleotide-dependent epimerase, a coenzyme found in all living cells [7–9], according to tissue requirement.

Role of INS & its derivatives in oocyte biology

It is well known that INS, by itself, or through its derivatives, plays an important role in different critical biochemical pathways: indeed, it has been shown that a defect in binding and/or lipid composition results in the onset of several diseases. It plays a central role in morphogenesis, cytoskeleton rearrangement, glucose metabolism, regulation of cell proliferation and fertility. In the latter case, MI regulates gamete development, oocyte maturation, fertilization and early embryonic development [10–12].

The testes are rich in MI, as well as the prostate, epididymis and seminal vesicles in rats. The seminal fluid, for example, is one of the most abundant sources of INS with a concentration nearly threefold higher than in the plasma [13–15]. Furthermore, MI concentration increased the flow through the epididymis and the deferent duct.

Studies in rats showed that MI concentration in uterus and ovaries is under hormonal control. The concentration of MI in the reproductive organs is much higher compared with that present in the blood [16].

MI is essential in ensuring proper oocyte maturation, and phospholipase-C is involved in the regulation of luteinizing hormone (LH)/FSH activity [17,18]. MI has a central role in mammalian cell metabolism. In mammalian oocytes, Ca^{2+} oscillations play an important role in the acquisition of meiotic competence and in driving the final stages of oocyte maturation [19]. Indeed, it has been demonstrated that mouse oocytes are capable of releasing Ca^{2+} following injection of InsP3 through its interaction with the specific membrane receptor. Regarding human oocytes, it has been observed that they express InsP3-receptor (InsP-R1) involved in the release of intracellular Ca^{2+} [20,21]. This evidence demonstrated the role of INS as a second messenger of calcium signaling in oocyte growth. Moreover, supplementation with MI can promote meiotic progression into fertilization-competent eggs, while depletion of MI may desensitize InsP signal, thus leading to the interruption of oocyte maturation [20,21].

As well as in oocytes, also in the zygote, Ca^{2+} oscillations may play a relevant physiological role. The input of the MI in murine embryo is an ATP-dependent process [22]. InsP3 receptors are indeed overexpressed during the early stages of zygote development, suggesting that INS is involved in Ca^{2+} release also in the early stages of development and that the Ca^{2+} oscillations could influence the development of preimplantation embryo [23].

Moreover, it has been demonstrated that the proportion of fertilized oocytes, the number of two-cell stage embryos developed, and the percentage of normality of the postimplantation embryos were significantly higher when germinal vesicles were cultured in a maturation medium containing MI compared with control medium [23]. Therefore, we can assess that high concentration of MI in the follicular fluid has an important role in follicular maturation and in embryonic development [24].

As it is now clear, the importance of fluctuations in Ca^{2+} levels during the process of oocyte maturation, fertilization and embryogenesis is linked to bioavailable MI. We know that the presence of high concentration of MI in the follicular fluid has become a marker of good quality oocytes [25].

The phosphorylated derivatives of INS (Ins-1,4,5P3) participate in cytoskeleton regulation [26,27] and are required to promote the transport of oocytes [28].

Moreover, MI seems to act on the release of anti-Müllerian hormone (AMH) modulating its serum levels [29].

AMH belongs to the TGF-β superfamily. It is released after FSH stimulation by the granulosa cells and participates in regulating follicle maturation [30]. Indeed, poor serum AMH levels are considered a marker of diminished ovarian reserve [31]. It is worth noting that MI supplementation significantly enhances AMH serum levels in patients affected by diminished ovarian reserve and then increases the likelihood of pregnancy. For this reason and for its role in improving oocyte quality, AMH is administered to patients undergoing assisted reproductive technologies [29,32,30].

Even during pregnancy, a proper intake of INS is needed. The fetus, in fact, requires INS during gestation and is able to get it from maternal blood. In mid-gestation, the MI concentration in venous blood from the umbilical cord was fivefold higher than that detected in the maternal serum. At term, serum MI concentration of the neonates decreased, but it was still two- to threefold higher than in maternal blood [33].

We know that pregnancy has a negative effect on the activity of several antioxidant enzymes such as superoxide dismutase and glutathione peroxidase in liver and placenta. During pregnancy, women experience an increase in oxidative stress and some pregnancy disorders depend on both high levels of oxidative stress and unbalanced levels of some micronutrients in the maternal blood. MI seems to restore and maintain a healthy pregnancy and fetal development [34,35].

Yet, MI promotes the differentiation of the fetal lung and prevents neural tube defects [36]. Given that MI uptake from embryonic cells is competitively inhibited by glucose, it has been suggested that congenital malformations, especially of CNS and heart, observed with high frequency in infants born to diabetic mothers [37] could be attributed to hyperglycemia-induced tissue-specific shortage of MI.

Several studies have reported that a new therapy for folate-resistant neural tube defect is the administration of a combined treatment with folic acid and MI. In this way, it is possible to successfully prevent the majority of neural tube defects, particularly those that are folate resistant [35,38].

MI, DCI & polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is characterized by chronic anovulation, hyperandrogenism, dermatological problems and hyperinsulinemia. It is the most common cause of menstrual irregularity, ovarian dysfunction and, in many cases, infertility. It affects approximately 6–10% of women in childbearing age.

The important role of INS in human reproduction as well as in insulin signal transduction is well known [39–41].

During the last decades, INS supplementation has been proposed as a reliable treatment in women affected by PCOS [42].

A reduced glucose tolerance, resulting from a defect in the insulin-signaling pathway, seems to be implicated in the pathogenesis of insulin resistance and the metabolic syndrome that affects many patients with PCOS. An altered insulin sensitivity or compensatory hyperinsulinemia is frequently found not only in overweight women with PCOS but also in normal-weight PCOS patients [43,44]. These data further support the notion that insulin resistance in PCOS patients is weight independent. Therefore, obesity has to be considered only as an exacerbating factor.

Insulin-signaling pathways involve inositol phosphoglycans (IPGs). When insulin binds to its receptor, two distinct IPGs are released by the hydrolysis of glycosylphosphatidylinositol lipids located at the outer leaflet of the cell membrane. IPGs enter inside the cells and affect intracellular metabolic processes by activating key enzymes that control the oxidative and non-oxidative metabolism of glucose.

There are multiple mediators of insulin action and after the binding of insulin to its receptor, different pathways may be triggered depending on the mediator stimulated [45].

It is noteworthy that MI and DCI have different roles as mediators of insulin, which lead to different functions within the cells. The activation of phospholipids-containing MI by insulin increased permeability of the cell membrane to glucose, which get into the cell and is immediately available as substrate. The DCI, differently from the MI, is able to determine the intracellular accumulation of glucose, (i.e., glycogen synthesis) (Figure 1). Both the MI and the DCI are capable of exerting an insulin-sensitizing effect leading to a reduction in insulin levels in the blood [7]. In fact, it is scientifically confirmed by several studies that MI supplementation significantly improves features of the metabolic syndrome including insulin

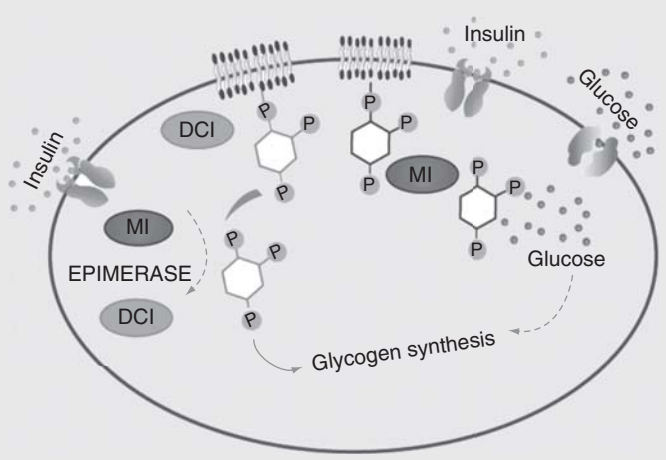

Figure 1. The picture represents a simplified model of the processes activated by MI and DCI second messengers at hepatic level. While MI second messenger (MI-phosphoglycan) is involved in glucose uptake, the DCI second messenger (DCI-phosphoglycan) regulates glycogen synthesis. In particular, the inositol trisphosphate has been used as representative of the second messengers.
AMH: Anti-Müllerian hormone; DCI: D-chiro-inositol; MI: Myo-inositol.

sensitivity, impaired glucose tolerance, lipids levels and diastolic blood pressure [42].

Studies in diabetic rats showed a reduced activity of epimerase, the enzyme that converts MI to DCI [46]. This evidence led the author to speculate that hyperinsulinemia in insulin-resistant mice could depend on a reduced or absent activity of this enzyme with a consequent lack of DCI and an alteration of the cellular response to insulin.

Indeed, insulin resistance has been associated with reduced availability of DCI, documented by decreased urinary excretion of IGP P-type in animals [47] and diabetic patients [48] and by lower DCI levels in muscle from type 2 diabetes patients [48]. Hyperglycemia was reduced in diabetic rats suffering from insulin resistance treated with IGP P-type [7].

Many studies focused on both the impaired glucose tolerance and the insulin resistance that affected many of the patients with PCOS. Insulin may have an important role in the pathogenesis of PCOS, either indirectly or directly.

Indirectly, insulin, acting at the level of the liver, leads to a reduction of circulating levels of sex hormone-binding globulin, resulting in increased circulating free testosterone. Also, insulin induces a reduction of the synthesis of insulin-like growth factors binding protein-1, giving rise to an increase of circulating IGF-1 and increasing sensitivity of the ovaries to LH [49,50]. On the other hand, the direct action of insulin takes place in the ovary where insulin promotes the synthesis of androgens, acting synergistically with LH on the theca cells, and regulates the ratio of its two messengers, promoting the direct synthesis of DCI from MI through direct action on the enzyme epimerase. Therefore, the association between PCOS and hyperinsulinemia is relevant to consider

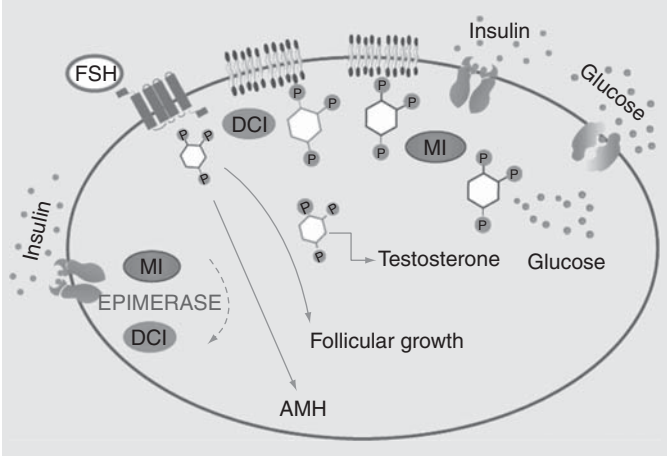

Figure 2. The picture represents a simplified model of the processes activated by MI and DCI second messengers at ovarian level. MI second messenger(s) is involved in glucose uptake and in FSH signaling; on the other hand, DCI second messenger (DCI-phosphoglycan) regulates insulin-mediated testosterone biosynthesis. In particular, the inositol trisphosphate has been used as a representative of the second messengers. DCI: D-chiro-inositol; MI: Myo-inositol.

this syndrome more a metabolic implication rather than a reproductive one.

However, it is well known that increasing insulin sensitivity in PCOS patients by means of conventional antidiabetic drugs results in an improvement of ovarian function and the decrease of serum androgen concentrations [51,52].

Metformin increases the release of insulin-stimulated DCI phosphoglycans, thus evidencing that this antidiabetic drug may enhance insulin sensitivity by restoring the INS-based signal. It was further observed that patients affected by PCOS suffer from an altered DCI urinary clearance, presumably leading to tissue depletion of IPG [53]. Once more, these data suggest a direct correlation between the availability of IPG and insulin resistance.

Starting from these results, many clinical trials have been performed in order to highlight the clinical usefulness, if any, of DCI supplementation in PCOS treatment.

Nestler *et al.* [54] demonstrated that in obese women with PCOS, DCI treatment at a dose of 1200 mg/day reduced serum testosterone level and improved ovulation rate and metabolic parameters such as blood pressure and triglycerides. Over 70% of patients, previously in amenorrhea, had their menstrual cycles occur within 45 days of treatment.

The study continued involving a larger number of patients and with increasing doses of DCI, up to 2400 mg/day [55]. Unfortunately, and unexpectedly, the authors in this study were not able to confirm the results published previously. A crucial difference from previous studies was the dose of DCI administered.

The PCOS patients treated with increasing DCI doses (from 300 to 2400 mg/day) provided a compelling confirmation that increasing DCI dosage progressively worsened both oocyte quality and ovarian response in nonobese and noninsulin-resistant PCOS women [56]. Total recombinant FSH (r-FSH) units increased significantly in the group that received the higher doses of DCI, the number of immature oocytes was significantly increased and, additionally, the number of grade I embryos was significantly reduced. It is tempting to speculate that this overdose of DCI, which in preliminary studies on PCOS gave excellent results, could be the cause of failure.

Such contradictory results could likely be explained by considering the different function each INS stereoisomer plays in distinct tissues. Indeed, a specific MI/DCI ratio has been observed within each tissue: high DCI levels (even if always lower than MI concentration) are generally observed in glycogen storing tissues (fat, liver, muscle), whereas low DCI levels are present in tissues characterized by high consumption of glucose (brain, heart) [46]. Oocytes are characterized by high glucose consumption along with the oxidative pathway and decreased or impaired sugar availability is likely to affect oocyte quality. Indeed, reduced availability of glucose in oocytes and follicular cells caused by defective transportation of glucose is suspected to occur in PCOS [57]. The energy impairment promotes alternative pathways to utilize fatty acids and amino acids to obtain energy through compensatory mechanisms to deal with the energy requirement [58]. In PCOS patients, genes involved in the glucose uptake pathway are downregulated at ovarian level [59,60]. Thus, it is important to maintain a proper glucose metabolism for oocyte development. Certainly, both DCI and MI are required to perform such function in synergy with insulin. Yet, MI seems to play a more important role in oocyte: in fact, almost 99% of intracellular INS pool is constituted by MI [61]. DCI is produced from MI through a nicotinamide adenine dinucleotide-dependent epimerase according to cells requirement. Indeed, the epimerase conversion of MI to DCI is under insulin control: in Type 2 diabetic patients, the reduced tissue insulin sensitivity leads to reduced epimerase activity and hence DCI synthesis [48]. However, unlike other tissues, the ovary never showed insulin resistance [62]. Therefore, increased insulin levels, as those recorded in insulin-resistant patients, are likely to increase the activity of ovarian epimerase, leading to an increased DCI production and MI depletion.

Thus, while DCI increase may promote androgen synthesis, MI depletion worsens the energy state of the oocytes. These events, together, impair FSH signaling and oocyte quality.

This imbalance could clarify the pathogenesis of PCOS and better explain the theory called 'the DCI paradox in the ovary', first suggested by Unfer and co-workers [63].

We have already underlined that the ovary, unlike other organs, never becomes insulin resistant, and, in cases of hyperinsulinemia, it is assaulted by high concentrations of insulin. The excess insulin causes an enhanced action of the epimerase in the ovary, resulting in excessive conversion of MI to DCI. Therefore, in PCOS, there are follicles in which DCI is abundant, while MI is present in low levels [64,65], leading to a lack of second messengers. Phosphatidyl-MI is needed to transmit the FSH signal (Figure 2). The lack of transmission of this signal

could be responsible for chronic anovulation that occurs in PCOS. There are several evidences in support of this hypothesis. Studies performed on women undergoing assisted reproductive technologies have shown that women with increased fasting insulin level required an increased number of international units of FSH in order to achieve proper ovarian stimulation [66]. Also, it was already known that pretreatment with MI, 3 months before the stimulation of PCOS patients, reduced the number of international units of FSH required to induce ovulation and the number of days needed for stimulation, positively affecting the possibility of obtaining a pregnancy [32,67,68].

In PCOS patients, MI supplementation restores spontaneous ovulation and increases progesterone release during the luteal phase [69]. It is worth noting that MI deficiency in the ovary would likely impair the FSH signaling, resulting in an increased risk of ovarian hyperstimulation syndrome in PCOS patients. MI exerts, in addition, other appreciable systemic effects by improving the reproductive axis functioning in PCOS patients through the reduction of the hyperinsulinemic state that affects LH secretion [70]. After 12 weeks of treatment with MI, serum hormone levels were normal and menstrual cycle was restored in amenorrheic patients. Also, serum and follicular fluid concentration of MI have been proven to be directly associated with oocyte maturation and fertility outcome in patients undergoing *in vitro* fertilization (IVF) treatment [25].

The usefulness of supplementation with MI has been demonstrated in numerous studies.

Morgante *et al.* have highlighted that MI supplementation in insulin-resistant PCOS patients produced significant results: the patients treated with MI showed a significant reduction in cancellation rate (0 versus 40%) and consequent improvement in clinical pregnancy rate (33.3 versus 13.3%) [71]. Gerli *et al.* conducted a randomized, double-blind, placebo-controlled trial with 283 PCOS patients treated with MI. In that study, frequency of ovulation (40%) increased almost twofold in women who received MI versus the control group [72].

In addition to studies aiming to investigate the ability of MI to improve ovarian function and fertility, a number of studies have been performed in order to clarify the systemic beneficial effects associated with MI therapy. These studies were able to demonstrate that MI treatment lowered lipids [73], insulin and androgen levels, increased insulin sensitivity, reduced diastolic blood pressure and was effective in treating acne [17] and hirsutism [74]. In general, these data demonstrated that MI is as effective as DCI in normalizing metabolic and endocrine features frequently associated with insulin resistance and PCOS.

Normalizing insulin resistance is likely not enough for restoring a proper ovulatory function, as suggested by a recent study comparing MI supplementation versus metformin [68]. 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, spontaneous ovulation activity was restored in 50%; pregnancy occurred spontaneously in 11 (36.6%) of these patients. In the MI group, spontaneous ovulation activity was restored in 65% of the patients, ovulation occurred after a mean of 14.8 days from day 1 of the menstrual cycle and pregnancy occurred spontaneously in 18 (48.4%) of these patients. Overall, these data underline that MI supplementation significantly provides higher benefits than metformin.

Evidences confirming the theory described in the manuscript by Carlomagno *et al.* [63] were published by two independent laboratories [64,65]. The first one was a study by Larner *et al.* that analyzed the epimerase activity and MI and DCI content in PCOS theca cells; the second one was reported by Unfer *et al.*, studying the concentration of MI and DCI in the follicular fluid of healthy women and women with PCOS. Both studies described similar results showing that the ovary of healthy women contains high concentrations of MI and low concentrations of DCI. On the contrary, the ovary of PCOS patients is characterized by a marked MI depletion and an increased DCI reduction. In particular, the MI/DCI ratio decreased from 100:1 in healthy women to 0.2:1 in PCOS women.

Therefore, we can assess that in the ovary of PCOS women, the increased epimerase activity leads to pathological intracellular DCI levels. Furthermore, these data clarify the clinical evidence on the link between MI and FSH and on the inconsistent data present on DCI.

Certainly, the administration of high doses of DCI determines ovarian toxicity, which progressively reduces the ovarian response to FSH and that adversely affects oocyte quality. This could explain why the promising results obtained by Nestler and co-workers during the first study have not been confirmed in the second one. Unfer and colleagues performed dose–response studies before identifying the physiological plasma ratio of the stereoisomers; the plasma ratio found was 40:1. As a next step, the clinical efficacy of a treatment based on this ratio was tested. The promising results obtained indeed showed a higher effectiveness [75,76].

PCOS being a syndrome that affects not only the ovary but also other organs, INS supplementation should preferably include both the stereoisomers: MI and DCI in a physiological ratio.

This could be considered as the first-line approach in PCOS overweight patients, able to reduce the metabolic, hormonal and clinical alteration of PCOS.

INS(s) safety

Despite the US FDA declaring INS a 'generally recognized as safe' molecule, the main data on which FDA has based its decision refer to MI. Indeed, several studies have been carried out using MI in a dosage up to 30 g/day. In these studies, the only side effects identified were reported as gastrointestinal discomfort [77].

Furthermore, there is evidence that MI is not only a safe compound to be administered during pregnancy, but it has several beneficial effects.

Indeed, it has been demonstrated that only pregnant women having certain MI concentration in the serum are capable of

carrying a healthy pregnancy. Additional evidence has shown that MI intake is able to prevent neural tube defect in folic acid-resistant patients [34].

On analyzing the beneficial effects provided by MI in the third trimester of pregnancy, it has been shown that MI can prevent the onset of gestational diabetes in high-risk patients such as PCOS women, women having a family history of diabetes and obese women [78,79].

Conversely, no data are available for DCI. Indeed, from a careful analysis of the available literature, it can be inferred that DCI administration at relatively high dosage has detrimental effects at ovarian levels. Indeed, while a first study reported ovulation induction in obese PCOS women (with no data provided on the regulation of the menstrual cycle) by using the dosage of 1.2 g/day; in a second study, by doubling the dosage, the authors were not able to confirm their previous results [55].

More recently, in an IVF setting, it has been shown that DCI administration already at 1.2 g/day worsened the ovarian response and impaired the oocyte and embryo quality [56].

Conclusion

PCOS is one of the most common endocrine diseases that affect women and related pathogenetic mechanisms are still unclear.

It is characterized by the association of hyperandrogenism, chronic anovulation and irregular menstrual cycles. It is the most common cause of female infertility.

A significant number of patients also suffer from metabolic syndrome and insulin resistance. This group of women continue to be the subject of study as many features are currently not entirely understood [80,81].

Several clinical studies have highlighted the usefulness of INS supplementation in PCOS treatment. Also, it has been proven to be safe even after high-dose consumption [77].

Overall, current evidences indicate that INS supplementation may not only improve insulin resistance and many features of the metabolic syndrome (dyslipidemia, increase in diastolic pressure and hyperglycemia), but might also efficiently modulate serum androgens and circulating LH and FSH levels [42,82]. Through these mechanisms, MI leads to a restoration of spontaneous ovulatory cycles and improves oocyte quality. Thus, MI is an excellent adjuvant in IVF treatment during ovarian stimulation with FSH [32].

Given the complexity of this syndrome, numerous studies on the use of these two INS stereoisomers have been performed. Their different concentration at the tissue level and their different role in the whole cell led to the conclusion that both of them have to be used for the purpose of better treatment efficacy.

On the contrary, DCI treatment, mostly when administered at high dosage (i.e., 600 mg or more), exerts disappointing effects on ovary functions [56].

Oocyte physiology, among other factors, is likely to be dependent on a fair balance 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 containing good quality oocytes have higher concentrations of MI in follicular fluids, probably due to the intricate relationship between MI and INS phosphates in the phosphatidylinositol cycle activation for oocyte maturation.

Additionally, by improving glucose uptake, MI ameliorates oocyte energy status, likely improving oocyte quality. Moreover, during ovarian stimulation, MI reduces the number of international units of FSH necessary for ovarian stimulation. Altogether, these results suggest that MI exerts several beneficial effects, improving the chances of pregnancy.

Given the important role of INS at the cellular level, all the studies performed on PCOS and the usefulness of both stereoisomers led to the conclusion that a synergistic action of the two stereoisomers is mandatory. Indeed, DCI reduces hyperinsulinemia and increases glycogen synthesis, thus having an indirect effect on the ovary; on the other hand, MI displaces the intrafollicular DCI in excess, allowing the signal amplification of FSH and glucose reuptake.

It is very important to focus on the altered intracellular relationship between MI and DCI in PCOS patients and identifying the right ratio between these two stereoisomers in order to find a therapeutic approach for the treatment of PCOS without incurring ovarian toxicity related to the amount of DCI administered.

Expert commentary & five-year view

In conclusion, as suggested by physiological data, the MI/DCI ratio is crucial for proper tissue function. Indeed, as shown by recent results, the association of MI/DCI, in a physiological range (i.e., 40:1) would ensure better clinical results, both at the systemic and ovary levels.

Undoubtedly, further studies are warranted to fully elucidate the molecular pathways triggered by MI and DCI, and to provide a well-grounded rationale for INS supplementation in PCOS patients.

Financial & competing interests disclosure

V Unfer is the president of LO.LI. Pharma s.r.l. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Key issues

- Polycystic ovary syndrome is one of the most common endocrine disorders affecting women in reproductive age.
- Two inositol stereoisomers, myo-inositol (MI) and D-chiro-inositol (DCI), have been proven to be effective in polycystic ovary syndrome treatment.
- Both MI and DCI are able to exert an insulin-sensitizing effect, leading to a reduction of glucose and insulin levels in the blood.
- The activation of phospholipids containing MI by insulin increases the permeability of the cell membrane to glucose, while phospholipids containing DCI mediate glycogen synthesis.
- The MI and DCI levels are crucial for a proper tissue function, and polycystic ovary syndrome patients showed an altered MI/DCI ratio.
- The combined supplementation of MI and DCI, in a physiological range (40:1), may represent a therapeutic approach that is able to ensure better clinical results both at systemic and ovary levels.

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