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The importance of myo-inositol and D-chiro-inositol to support fertility and reproduction

L'importance du myo-inositol et du D-chiro-inositol pour soutenir la fertilité et la reproduction

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Abstract. 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. In addition, MI plays a pivotal role in the physiology of reproduction, and has beneficial effects on the development of oocytes, spermatozoa, and embryos. By contrast, DCI has little effect on spermatozoa, but high concentrations in the ovary can negatively affect the quality of oocytes and the blastocyst. Overall, the evidence in the literature supports the beneficial effects of MI in both female and male reproduction, warranting clinical use of MI in assisted reproductive treatment (ART).

Key words: myo-inositol, D-chiro-inositol, polycystic ovary syndrome, fertility, reproduction, IVF

Résumé. Cette revue détaille les rôles physiologiques de deux sensibilisateurs à l'insuline, le myo-inositol (MI) et le D-chiro-inositol (DCI). Dans l'ovaire humain, le MI est un second messager de l'hormone folliculostimulante (FSH) et le DCI est un inhibiteur de l'aromatase. Ces activités permettent de définir un traitement du syndrome des ovaires polykystiques (SOPK) basé sur l'administration combinée de MI et de DCI, où le meilleur rapport MI:DCI est de 40:1. En outre, le MI joue un rôle essentiel dans la physiologie de la reproduction et a des effets bénéfiques sur le développement des ovocytes, des spermatozoïdes et des embryons. En revanche, le DCI a peu d'effet sur les spermatozoïdes, mais des concentrations élevées dans l'ovaire peuvent avoir un effet négatif sur la qualité des ovocytes et du blastocyste. Dans l'ensemble, les données de la littérature confirment les effets bénéfiques du MI dans la reproduction féminine et masculine, ce qui justifie l'utilisation clinique du MI dans l'assistance médicale à la procréation.

Mots clés : myo-inositol , D-chiro-inositol , syndrome des ovaires polykystiques , fertilité , reproduction , fécondation *in vitro*

Synthesis and activities of MI and DCI

Inositols are cyclic polyols which are among the most ancient molecules on Earth. They can be found in 9 stereoisomers [1-3] and MI and DCI are the most prevalent.

In the human body, MI is actively synthesized in the kidneys, liver, testes, mammary gland, brain [4, 5].

Under insulin stimulation, a specific epimerase converts MI to DCI [6, 7].

Endogenously, the production of both stereoisomers depends on the specific tissue requirements [8]. As such, in healthy women the plasma MI:DCI ratio is 40:1 [9], whereas in ovarian follicular fluid is close to 100:1 [10].

MI and DCI activities

MI and DCI are deeply involved in insulin signaling, since insulin requires the presence of both stereoisomers to exert its functions. As inositolphosphoglycans, MI and DCI are second messengers in the insulin signaling, mediating different effects [3, 11-13]. Notably, being the two stereoisomers metabolically linked to

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each other, a drastic separation of their individual effects in vivo can be challenging. However, while MI mainly controls cellular glucose uptake, and its content is significantly higher in tissues with high-glucose utilization, like brain, heart, and ovaries [13-16], DCI is principally involved in glucose storage as glycogen.

Likely interfering with glucose intestinal uptake, MI seems to prevent glucose absorption at the duodenal level and decrease glucose rise in the blood [17]. Furthermore, MI improves insulin sensitivity in adipocytes by increasing lipid storage and glucose uptake, and by inhibiting lipolysis [18]. It also downregulates the inflammatory response, particularly in macrophages, probably through the inhibition of proinflammatory transcription factors [19].

Inositols in the ovary and in pregnancy

In the ovary MI (as InsP3) is one of the second messengers of FSH [20]. Its concentration in the mammalian female reproductive tract is higher than in blood serum, suggesting that MI plays critical roles at the ovarian level, like ensuring correct oocyte maturation [21].

Instead, DCI down-modulates the activity of aromatase enzyme, found in fat tissue, ovaries, testicles, placenta, brain, bone [22]. Indeed, DCI decreases the expression of aromatase gene dose-dependently and consequently inhibits testosterone to estrogen conversion [23]. Furthermore, as insulin mediator, DCI stimulates testosterone biosynthesis by theca cells [24]. This effect is more marked in PCOS women compared to normal ones and contributes to explain the higher amounts of testosterone produced in PCOS patients, in comparison with healthy subjects [24].

Each organ balances the intracellular levels of inositols to achieve tissue-specific intracellular MI/DCI ratios that modulate metabolic processes [10]. In the ovaries, such ratio is about 100:1 [10].

The excess of DCI in ovarian follicles is potentially detrimental in some cases, as demonstrated by Ravanos *et al.* [25]. The authors observed that MI positively correlated with quality of blastocysts, while DCI concentrations above the MI/DCI limit ratio of 70:1 in follicular fluid decreased blastocyst quality [25].

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Treatment with MI in impaired female fertility (PCOS and other conditions)

The first study (1992) on the role of MI in IVF found a positive correlation between serum concentrations of MI and successful IVF pregnancy. Serum samples with high MI content showed clear trophic properties when added to in vitro embryo cultures, and supported better post-implantation development of mouse embryos [26]. Subsequent studies showed that high MI levels in human follicular fluid correlate positively with satisfactory oocyte quality [27]. Moreover, addition of MI to the culture medium stimulated meiotic progression by mouse germinal vesicle oocytes, a process that requires intracellular calcium mobilization [28]. On the other hand, Goud and coworkers demonstrated that inositol-1,4,5-trisphosphate plays an important physiological role during in vitro maturation, fertilization, and early cleavages of human oocytes and embryos [29]. PCOS women undergoing ART are a particularly challenging target group and have been the subject of several recent studies. Wdowiak [30] investigated the activity of oral MI in PCOS women enrolled for intracytoplasmic sperm injection (ICSI). Over the 3 months before ICSI, 60 control PCOS patients received 200 µg of folic acid twice per day; the remaining 52 PCOS subjects were treated with 2 g of MI plus 200 µg folic acid, also twice per day. Further controls (no treatment) were 105 healthy women. A significant difference in the number of pregnancies was found: pregnancy was reported in 34.62% of the MI treated group but in only 20% of the PCOS controls.

In another IVF clinical trial [31], 133 PCOS and 137 non-PCOS women with preserved ovarian reserve were treated daily by the oral route for 3 months during the preconception period and ovarian stimulation. The first group (PCOS) received 1 g MI plus 400 µg folic acid, whereas the second group (non-PCOS) received 400 µg folic acid plus 2 µg cyanocobalamin. The total number of mature oocytes in the MI-treated patients was significantly higher than in the second group. Pregnancy rates per embryo transfer, 'take home baby' index, and miscarriage rates were comparable. In conclusion, MI improves oocyte quality, thus potentially improving IVF outcome [31].

MI reduces the amount of gonadotropin in IVF procedures

Emekçi Özay *et al*. [32] administered 4 g MI plus 400 µg folic acid, before and during controlled ovarian hyperstimulation (COH) with recombinant (r)FSH and intrauterine insemination (IUI), to 98 infertile PCOS women undergoing controlled ovulation induction and IUI. Controls (n = 98) received rFSH and 400 µg folic acid. Of the treated subjects, nine accomplished spontaneous pregnancy. During COH + IUI treatment three cycles were canceled in the study group and eight in the control group. In the treated patients, a significant decrease in total rFSH dose and cycle duration was reported; in addition, clinical pregnancy rate was higher (18.6%) in patients receiving MI compared with controls (12.2%) [32]. Systematic review and meta-analysis, including eight randomized controlled trials (RCTs) with a total of 812 participants [33], confirmed that oral inositol supplementation during controlled ovarian stimulation (COS) and ART can reduce the total amount of gonadotropins used and the length of COS in both PCOS and non-PCOS women undergoing IVF. According to the analysis, MI was effective in reducing gonadotropin administration in both PCOS and non-PCOS women. However, MI supplementation decreased the length of COH only in PCOS women. Although the data from the current literature do not take pharmacoeconomic aspects into account, based on the above results the authors assert that MI therapy may significantly reduce the overall cost of IVF procedures, with direct benefits for the patients [33]. Zheng and colleagues [34] performed systematic literature review and meta-analysis concerning the efficacy of MI administration to infertile (non-PCOS) patients undergoing ovulation induction for ICSI or IVF and embryo transfer (IVF-ET). Seven trials, with a total of 935 women, were taken in consideration. MI treatment was associated with a significantly higher clinical pregnancy rate and proportion of grade 1 embryos. The abortion rate in the MI group was significantly lower than in controls. Furthermore, the study group required significantly fewer total units of gonadotropins such as rFSH compared with controls. MI therefore increases both the clinical pregnancy rate in infertile women undergoing assisted motherhood and the quality of embryos, as well as reducing the number of unsuitable oocytes and the amount of stimulation drugs required [34]. Further evaluation of MI supplementation, in a larger cohort of patients, will be necessary to assess its economic advantages in IVF treatments and its effect on long-term fertility outcomes.

MI and embryo development *in vitro*

Preimplantation mouse embryos supplemented in vitro with MI exhibit (i) increased percentage of progression to the most advanced stage of development; (ii) overall

increased percentage of development to the expanded blastocyst stage; (iii) increased average number of blastomeres forming the embryos at the blastocyst stage [35]. A plausible mechanism may include rapid incorporation of MI into phosphatidylinositides (PtdIns) and increased production of intracellular second messengers that enhance proliferation [36, 37]. In particular, MI supplementation of the culture medium of late preimplantation mouse embryos induced Akt phosphorylation at serine 473 [38]. This demonstrates that, in the early stages of development, new phosphorylation of Akt occurs in the mid-to-late preimplantation stages, and this process depends on the availability of MI. Previous evidence showed that the development of preimplantation mouse embryos (8-16 cell stage) requires the activity of PI3K, an enzyme that produces PIP3 from PtdIns [39, 40]. The increased synthesis of phosphoinositides [41], and the resulting increase in PIP3, may account for these observations. In conclusion, enhanced phosphorylation of Akt in the presence of MI may be responsible for the faster development rate of cultured embryos. Reasonably, MI supplementation to dividing blastomeres enhances the pathway leading to Akt phosphorylation and accelerates development. In conclusion, the direct effects of MI on oocyte health and subsequent development may improve ART outcome.

Inositols for the treatment of PCOS

Pre-clinical evidence

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 [12, 42]. Bevilacqua *et al.* [43] 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 non-cycling 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 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 [44]. In fact, a hypertrophic theca cell layer is a hallmark of polycystic ovaries and causes a greater production of androgens [45]. 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.

Clinical evidence

Recently, a clinical trial confirmed these findings also in PCOS women [46]. Since the "ovarian paradox" [47] postulated that ovaries, unlike liver and muscles, never become insulin resistant [48-50], the hyperinsulinemia in PCOS women enhances ovarian MI to DCI epimerization, increasing DCI concentration at the expense of MI [7, 10, 47].

Indeed, while healthy women show an ovarian MI:DCI ratio around 100:1, in PCOS women it drops to 0.2:1 [10], likely affecting the FSH signaling. Therefore, restoring the physiological MI:DCI ratio in the follicular fluid may be crucial for proper ovarian function [10].

In a recent meta-analysis [51] 9 randomized controlled trials (RCTs) on PCOS patients were evaluated, with a total of 247 cases and 249 controls [32, 52-59].

The authors determined the efficacy of treatments (length: 12-24 weeks) with MI, alone, or in association with DCI in the 40:1 ratio, on fasting insulin, HOMA index and serum levels of testosterone, androstenedione, and sex hormone-binding globulin (SHBG). Inositol supplementation significantly reduced fasting insulin and HOMA index, slightly decreasing testosterone with respect to controls. After at least 24 weeks of administration, MI significantly increased SHBG levels.

Since high doses of DCI/day may be detrimental for ovaries and oocyte maturation, the authors recommend

avoiding exclusive DCI supplementation, also considering that DCI cannot be converted into MI and that deficiencies of MI correlate with insulin-resistance conditions.

On the contrary, the meta-analysis strongly supports MI supplementation for improving the metabolic profile of PCOS patients. Also, a systematic review and meta-analysis [60], including 10 RCTs and 573 patients further confirmed these results [52-55, 58, 59, 61-63].

Therefore, inositols can be recommended for managing PCOS with insulin resistance, as well as for improving symptoms caused by decreased estrogen in PCOS [60]. The best therapeutic regimen, clinically tested in women with PCOS, is the oral combination of MI and DCI in a molar ratio of 40:1.

The optimal daily amount of inositols is 4 g divided in two administrations, for at least 3 or 6 months.

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 [10], 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 [10]. In a clinical study [58], 46 obese PCOS women (BMI N 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 [58, 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 [25]. Indeed, DCI increases testosterone levels through two different pathways: in the theca cells from PCOS women as insulin mediator (inositolglycan mediator) [24] and in the granulosa cells as aromatase inhibitor [23]. 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 [13]. In addition, literature data indicate that MI signaling may regulate the AMH production induced by FSH in the granulosa cells [66]. AMH decreases oocyte sensitivity to FSH and participates in regulating follicle maturation. 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 [33] and an increase in the clinical pregnancy rate [32].

Association with CC

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 [67]. Researchers investigated inositol treatment, combined with CC, to assess possible fertility improvements in PCOS women seeking pregnancy [68]. 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 [69] 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 [70]. 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.

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) [71]. To date, SMIT2 is the only known transporter of MI located in the intestine (duodenum and jejunum). In vitro experiments identified a Km (DCI) lower than Km (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 [72, 73].

Inositol resistance

Some patients exhibit a reduced intestinal absorption of inositol and are defined "inositol-resistant". Researchers tried to overcome this problem combining MI with the whey protein alpha-lactalbumin (α-LA), already known for its propriety as carrier for metal ions and vitamin D [74, 75].

To test this association, Monastra *et al*. [76] supplemented 18 healthy volunteers with a single dose of MI (6 g) and, one week later, with 6 g of MI + 150 mg of α-LA in a single dose.

After the combined administration, the authors observed that maximum MI plasma concentration (Cmax) and area under the time course curve of MI plasma concentration (AUC) were significantly higher (+32.4% and +27.5%, respectively) in respect to when MI alone was administrated alone.

Subsequently, the authors investigated the possible mechanism underlying this effect. In particular, they evaluated the transport of MI, alone and combined with α-LA across the human intestinal Caco-2 cell monolayer [76], currently used as in vitro model of gut mucosa [77, 78].

In the presence of α-LA the passage of MI across the monolayer was increased, due to the transient opening of the tight junctions between the cells [76] and the subsequent 'passive' transport of MI.

The clinical study by Montanino Oliva *et al*. on 37 anovulatory PCOS women confirmed the beneficial effects of the combination of MI and α-LA [79].

After an orally supplementation with 2g of MI twice a day for 3 months, 23 women (62%) ovulated, whereas 14 (38%) remained anovulatory, showing hallmarks of inositol resistance. These 14 women were further supplemented with the association of 2 g MI and 50 mg α-LA, twice a day for an additional 3 months. As a result, 12 (86%) patients ovulated, featuring significantly higher serum levels of MI and better hormone and lipid profiles with respect to the baseline.

This study supports Monastra's findings [76], providing a promising option to overcome some limitations in the clinical approaches using inositol.

Future research is needed in order to confirm these results on larger cohorts of patients as well as on different PCOS phenotypes and to accurately tailor the proper administration and the best combinations of MI, DCI, and α-LA.

Conflicts of interest

The authors declare no conflict of interest concerning this article.

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